

POSTER PRESENTATIONS

P001

IDX899; A NOVEL AND HIGHLY POTENT ANTI-HIV NON-NUCLEOSIDIC-REVERSE-TRANSCRIPTASE-INHIBITOR WITH ENHANCED BARRIER TO RESISTANCE PROFILE

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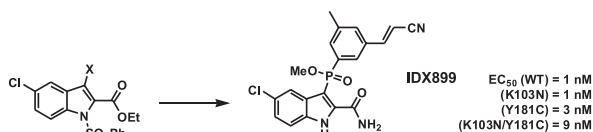
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Background: Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs) are an important part of current HIV-therapy. However, side effects, emergence of mutants and the cross-resistance of these mutants across this class of drugs require the development of a new generation of NNRTI with better tolerability and resistance profile.

Methods: Our efforts to discover new NNRTI helped by structure based design led to the identification of a new class of inhibitors: the 2-carboxamide-3-arylphosphinate indoles. Synthetic routes to those NNRTIs were investigated and the compounds evaluated for anti-HIV activity against wild type (WT), single mutant (K103N, Y181C) and double mutant (K103N/Y181C) in enzyme and cell based assay.

Results: Structure based design helped identify key protein-ligand interactions and led to the discovery of the phospho-indole scaffold. The synthesis was investigated and several scalable routes were identified and developed. The indole substitution was probed revealing that arylmethylphosphinate at position 3 of the indole and a 3,5-disubstituted phenyl ring are essential to get high activity towards mutants. The binding mode was asserted by X-ray crystallography of IDX899 complexed with the double mutant HIV1-RT. Best compounds showed low nM to sub-nM activity against WT and mutants.

Conclusion: IDX899 is a highly potent inhibitor of the HIV-1 Reverse Transcriptase with favourable preclinical profile and is currently in phase I clinical trial.



P005

DISCOVERY OF NEW INHIBITORS OF D-ALANINE D-ALANINE LIGASE

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Discovery of new inhibitors of D-alanine D-alanine ligase, a key enzyme involved in the intracellular steps of peptidoglycan synthesis, by virtual screening of the National Cancer Institute (NCI) diversity set is reported. Peptidoglycan is an essential component of the bacterial cell wall and provides the structural integrity needed to resist internal osmotic pressure. The only inhibitor of D-alanine D-alanine ligase currently used in therapy is cycloserin – a drug riddled with side effects. In vitro inhibition assay of the compounds proposed as potential inhibitors by virtual screening has revealed several inhibitors of D-alanine D-alanine ligase with micromolar inhibition. Two of the active compounds are found to have inhibiting effect on both gram+ and gram- bacteria in the microbiological evaluation. These two inhibitors serve as novel templates for development of more potent and specific D-alanine D-alanine ligase inhibitors.

P008

STRUCTURE-ACTIVITY RELATIONSHIPS AND CANCER-SELECTIVE TOXICITY OF NOVEL INHIBITORS OF GLI1 TRANSCRIPTION

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The Hedgehog (Hh) signaling pathway is implicated in many tumors including medulloblastomas. Several efforts have made to discover a specific inhibitor of this pathway by targeting Smoothened (Smo), such as cyclopamine and HhAntago (Curis/Genentech). However, since Smo is the common mediator of all three Sonic, Indian, and Desert Hh, in which Indian Hh is a key factor for bone growth, the Smo antagonist has a possible inhibitory effect in pediatric somatic growth, as we have observed in young mice. To override this problem in pediatric tumor therapeutics targeting Hh pathway, we have generated a chemical library of non-peptide drug-able small molecules and discovered inhibitor of Gli1 transcription, the key tumorigenic Hh signaling component whose knock-out mice are normal. Several lead compounds from this library significantly inhibited Gli-Luc reporter activity in C3H10T1/2 cells that are stimulated by exogenous Gli1. Concurrently, these compounds down-regulated transcription of *Gli1* and *Patched1* genes, well-known Gli1-

target. In the cell-toxicity and caspase activation assay, those compounds showed activity to cancerous cells including Rh30 rhabdomyosarcoma cells that have over-expression of Gli1, but not in non-tumorigenic BJ-*TERT* cells.

P009

SAR and BIOLOGICAL EVALUATION OF WAY-317538: NOVEL ALPHA-7 NICOTINIC RECEPTOR AGONIST

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Alpha 7 nicotinic acetylcholine receptor (nAChR) agonists are promising therapeutic candidates for the treatment of cognitive dysfunction associated with a variety of disorders including Alzheimer's disease and Schizophrenia. Alpha 7 nAChR are expressed in brain regions associated with cognitive function, regulate cholinergic neurotransmission and have been shown to be down regulated in both Schizophrenia and Alzheimer's Disease. Recently we have identified a novel, potent small molecule agonist of the alpha 7 nAChR, WAY-317538. This lead compound is a potent and selective agonist of the $\alpha 7$ nAChR with excellent in vitro and in vivo profiles. WAY-361538 shows brain penetration and oral bioavailability in rat, and demonstrates in vivo efficacy in both the auditory sensory gating and cognition models, as well as neuroprotection in a model of cholinergic degeneration. The SAR and biological evaluation of this series of compounds will be discussed.

P010

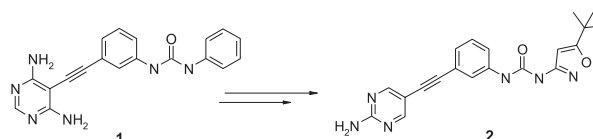
DISCOVERY OF PYRIMIDINE ALKYNYL PHENYL UREAS AS POTENT TIE-2 INHIBITORS

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The receptor tyrosine kinase Tie-2 is involved in the maturation and maintenance of blood vessels and acts in a complementary and coordinated fashion with VEGF receptor signalling. Inhibition of Tie-2 signalling is expected to prevent vessel differentiation, maturation and persistence and impair the capability for further tumour growth. A novel diaminopyrimidine alkynyl phenyl lead (**1**) was discovered, which inhibited the phosphorylation of Tie-2 in cells and was a suitable start point for further elaboration. Issues with the initial hits included poor oral bioavailability and a lack of photostability. These were improved by the development of a 2-aminopyrimidine alkyne phenyl urea series (**2**) with superior light stability and pharmacokinetic properties. Despite these improve-

ments the initial compounds in this series suffered from poor aqueous solubility which was a risk for further development due to the possibility of incomplete absorption leading to poor bioavailability at therapeutic doses. Various strategies were used to improve solubility including lowering overall lipophilicity and incorporating charged groups and groups with the potential to disrupt crystal packing. The relative success of these approaches, the SAR versus Tie-2 and the discovery of compounds with a more attractive overall profile will be described.



P012

DESIGN AND PREPARATION OF POTENT, NON-PEPTIDIC, BIOAVAILABLE RENIN INHIBITORS

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The Renin-Angiotensin-Aldosterone System (RAAS) is one of the major and most intensively studied regulating systems of the arterial blood pressure in humans. It plays a pivotal role not only in cardiovascular diseases, but also in renal diseases, and other metabolic diseases as well. The RAAS consists of a two-step cascade. First, the aspartic proteinase renin cleaves its only known substrate, angiotensinogen, in the rate-limiting step to the decapeptide angiotensin I. In a second step, angiotensin I is cleaved either by the metalloproteinase angiotensin-converting enzyme (ACE) or by the serine proteinase chymase to the tensoactive octapeptide angiotensin II. The exclusive and rate-determining function in the RAAS makes renin an ideal pharmacological drug target.

However, from the point of view of medicinal chemistry, renin proved to be a challenging target. The first generation of renin inhibitors were highly potent peptidomimetics that suffered from unacceptably low bioavailability. In the late 1990's piperidine derivatives emerged as a new class of renin inhibitors with subnanomolar IC₅₀-values (e.g. Ro-66-1168). Unfortunately, these piperidine derivatives were unsuitable for clinical development due to inadequate pharmacokinetic properties.

Starting from these piperidine renin inhibitors, we embarked on a structure-based design of new compound classes with improved pharmacokinetic properties. A new series of diazabicyclononene derivatives was rationally designed, prepared, and provided potent renin inhibitors.

After validation of the rationally designed template, three substituent positions were carefully optimized and led to subnanomolar renin inhibitors. With the help of X-ray structure analysis, the potency of these compounds was improved, and the pharmacokinetic properties were modulated. After optimization of the synthesis, multigram quantities of the most promising compounds were prepared as single enantiomers. The compounds were well absorbed in rats and efficacious orally at 10 mg/kg in double transgenic rats expressing both the human angiotensinogen and the human renin genes. When infused into sodium-depleted rhesus monkeys, the best compounds dose-proportionally decreased blood pressure.

P013

N-HYDROXYISOQUINOLINE-1,3-DIONE DERIVATIVES AS DUAL INHIBITORS OF HIV-1 INTEGRASE AND RNase H THAT BIND DIVALENT CATIONS

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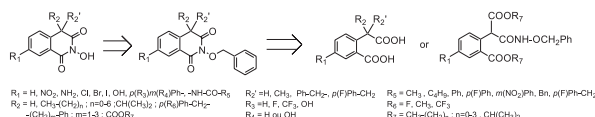
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Despite the multiple clinical benefits of the combination therapy, the emergence of HIV-1 strains resistant to current drugs highlights the need for new anti-HIV agents. Recently, HIV-1 integrase and the RNase H domain of the reverse transcriptase have been described as two novel antiviral targets that share strong structural similarities [1]. They have remarkably similar active sites with a set of three highly conserved carboxylates which are believed to hold the metal cofactors, which directly catalyze hydrolysis and/or transesterification reactions. So, compounds able to interfere in these additional essential steps of viral replication by chelating the cations could be innovative inhibitors. As examples, tropolone [2] and madurahydroxylactone [3] derivatives have been recently reported to inhibit both enzymes.

Since N-hydroxyisoquinoline-1,3-dione was previously reported as a RNase H inhibitor and a potential chelating compound, we investigated the properties of some of its derivatives. On the one hand, we describe the synthesis and biological evaluation of a new family derived from a N-hydroxyisoquinoline-1,3-dione scaffold. We have built up a series of compounds with various substituents on position 7 and/or 4 (Scheme) according to an adapted general procedure ^[4] and shown that they not only inhibit the RNase H activity but also the IN activity with IC₅₀ in low micromolar and submicromolar ranges. These results indicate that modification of the N-hydroxy-

isouquinoline-1,3-dione scaffold can influence the potency against HIV-1 integrase and RNase H and one can design a compound to favor integrase and/or RNase H inhibition. On the other hand, we have proved the ability of such compounds to interact with metal divalent cations (Mg^{2+} , Cu^{2+} , Mn^{2+}) by several spectroscopic methods including NMR, EPR, UV-VIS and fluorescence. We present here the stoichiometry, stability constants and structures of observed complexes.



References

- [1] Andréola, M.L., *Curr Pharm Des.*, 2004, 10, 3713-3723
- [2] Didierjean, J. ; Isel, C. ; Querré, F. ; Mouscadet, J.F. ; Aubertin, A.-M. ; Valnot, J.-Y. ; Piettre, S.R. ; Marquet, R., *Antimicrob. Agents Chemother.*, 2005, 49, 4884-4894
- [3] Marchand, C. ; Beutler, J.A. ; Wamiru, A. ; Budihas, S. ; Möllmann, U. ; Heinisch, L. ; Mellors, J.W. ; Le Grice, S.F. ; Pommier, Y., *Antimicrob. Agents Chemother.*, 2008, 52, 361-364
- [4] Ames, D. E.; Grey, T. F., *J. Chem. Soc.*, 1955, 3518-3521

P014

GLYCOMIMETICS AS POTENTIAL ANTI-CANCER AGENTS

Bello Claudia

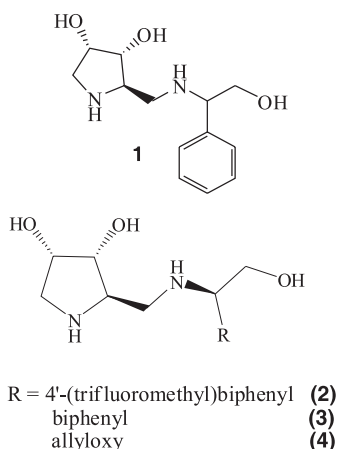
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During the past decade, research into the causes of human cancer, the molecular basis of malignant transformation and gene-environment interactions that contribute to individual cancer risks, has made significant progress. Unfortunately, cancer remains a major disease burden worldwide. Further efforts have to be done in order to improve our knowledge about the mechanisms of carcinogenesis and tumour development with the aim to find new therapies and to develop new anticancer drugs.

A library of sugar mimetics that are analogues of swainsonine has been developed by Vogel and co-workers. These new α -mannosidase inhibitors (e.g. **1**) have shown a high affinity for a target enzyme involved in cancer progression i.e.: Golgi α -mannosidase II.^{1,2} Inhibition of this key enzyme of protein glycosylation pathway has been proposed to be a useful approach to cancer treatment.³

Modification of the aromatic moiety of compound **1** has led to a series of products that show inhibitory activities toward α -mannosidase (from jack bean) similar to that of **1**. These new inhibitors⁴ (e.g. **2-4**) were tested on different cancer cell lines and were found to inhibit cancer

cell growth (e.g. **2** has $IC_{50} = 48 \mu M$ on SKBR3). Compound **3** and, to a lesser extent, **4** also exhibited growth inhibitory activities. Another series of analogues containing the same dihydroxypyrrolidine moiety, but with more differentiated substituents was synthesized and tested against several cancer cell lines. These show even better anti cancer activity than **1-4**. More extensive studies are now in progress in order to elucidate the mechanism of action of these compounds. Data suggest that the α -mannosidase inhibitory activity is not the main cause of cancer cell growth inhibitory activity.



References

- [1] Popowycz, F.; Gerber-Lemaire, S.; Schütz, C.; Vogel, P. *Helv. Chim. Acta* 2004, 87, 800-810.
- [2] Fiaux, H.; Popowycz, F.; Favre, S.; Schütz, C.; Vogel, P.; Gerber-Lemaire, S.; Jullierat-Jeanneret, L. *J. Med. Chem.* 2005, 48, 4237-4246.
- [3] Van der Elsen, J. M. H.; Kunz, D. A.; Rose, D.R. *The EMBO Journal* 2001, 20, 3008-3017.
- [4] Claudia Bello and Pierre Vogel, Novel Derivatives of Dihydroxypyrrolidine as Anti-Cancer Compounds *Eur. Pat. Appl.* No. 308210, Appl. EP07122188.1, Dec, 3.07

P015

SYNTHESIS AND EVALUATION OF NON-CLEAVABLE BIFUNCTIONAL INHIBITORS NRTI/NNRTI AS ANTI HIV

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HIV reverse transcriptase (RT) is the target of a number of important anti-AIDS drugs, as it is the enzyme responsible for catalyzing the conversion of viral RNA to (dsDNA)[1]. Drugs that inhibit RT are either nucleoside reverse transcriptase inhibitors (NRTIs) or non-nucleoside reverse transcriptase inhibitors (NNRTIs)[2].

The mechanism of inhibition of HIV-1 RT by non-nucleoside inhibitors (NNRTI) proceeds by blocking the

chemical reaction of RT but does not interfere with the nucleotide binding or the conformational change induced by the nucleotide. Thus, in saturated concentrations of the inhibitor, the nucleotide is tightly bound. This led to the conclusion that an inhibitor with the combined NRTI and NNRTI functionalities would be effective against RT and have an important application in the treatment of HIV/AIDS[3]. Bifunctional (d4T)-butyne-(HI-236) has been synthesized, and established to have anti-HIV activity with an EC_{50} of 200 nM[4].

This poster describes the synthesis of the Bifunctional [d4T]-diPEGpropyne-[HI-236] (Figure 1) and the synthesis towards the bifunctional prodrug Figure (2)

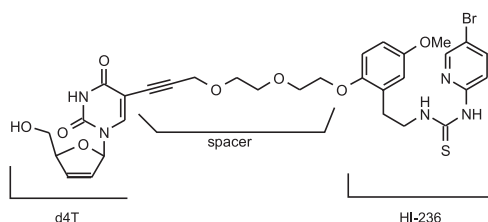


Figure (1)

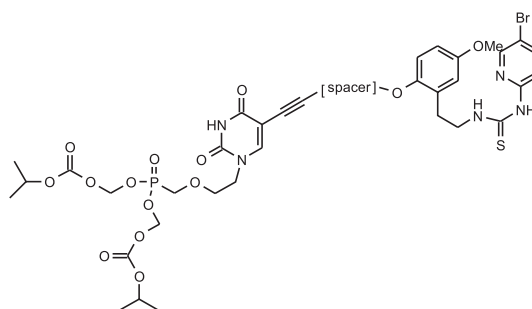


Figure (2)

References

- [1] De Clercq, E. *Curr. Med. Chem.* 2001, 8, 1543-1572.
- [2] De Clercq, E. *J. Med. Chem.* 2005, 48, 1297.
- [3] Spence, R. A.; Kati, W.M.; Anderson, K.S. and Johnson K. A.; *Science*, 1995, 267 (5200), 988-99
- [4] Hunter, R.; Muhanji, C. I.; Hale, I.; Bailey, C. M.; Basavapathruni, A.; Anderson, K. S. *Bioorg. MedChem. Lett.* 2007, 17, 2614.

P016

DESIGN AND SYNTHESIS OF SOME NEW IMIDAZOLE AND BENZIMIDAZOLE DERIVATIVES AS ANTIFUNGAL AGENTS

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In recent years the widespread use of antifungal agents has resulted in the development of resistance to these drugs by pathogenic microorganisms, causing an

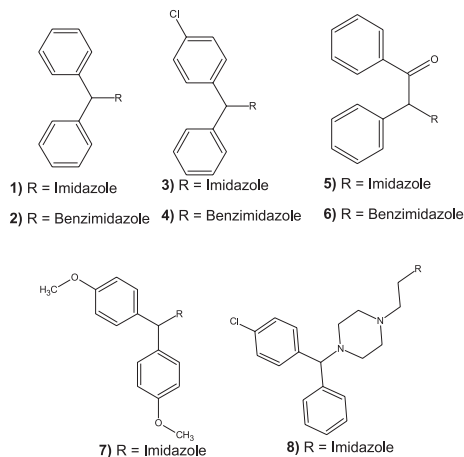
increase in morbidity and mortality. Thus, intense efforts in antifungal drug discovery are still needed to develop more promising and effective antifungal agents for use in the clinical arena.

In this study different series of Imidazole, benzimidazole and triazole derivatives were docked in the active site of the 14- α -demethylase enzyme (MT-CYP51; PDB code, 1E9X) using Auto Dock program and compared with clotrimazole and fluconazole. Then some imidazole and benzimidazole derivatives with the best binding energy were chosen for synthesis.

In order to synthesize the N-substituted heteroaromatic compounds related to clotrimazole, diphenylmethane(**1,2**), (4-chlorophenyl)(phenyl)methane(**3,4**), 1,2-diphenyl-1-ethanone(**5,6**), di(4-methoxyphenyl)methane(**7**), and 1-[(4-chlorophenyl)(phenyl)methyl]-4-ethylpiperazine(**8**) were replaced for the *ortho*-chlorotrityl moiety of clotrimazole and also the imidazole ring of clotrimazole was replaced by benzimidazole in some analogous.

Syntheses were carried out in the presence of potassium carbonate, sodium hydroxide and tetraethyl ammonium bromide under reflux temperature in acetonitrile as solvent. The crude compounds were purified by flash chromatography and identified by spectroscopic methods. They showed good affinity for the enzyme comparable to clotrimazole.

Further studies are needed to evaluate the antifungal activity of these new compounds.



P018

IDENTIFICATION OF N-ARYLPYPERIDINE OXADIAZOLES AS POTENT, SELECTIVE FULL AGONISTS OF CB₂

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The cannabinoid G-protein coupled receptors, CB₁ and CB₂, play important roles in the transduction and perception of pain. The contribution of CB₁ to the modulation of antinociception has been well established. However, agonism of CB₁, which is predominantly expressed in the CNS, results in undesirable psychotropic effects, sedation, and catalepsy. Selective agonism of CB₂, which is predominantly expressed in immune cells and tissues, presents an opportunity for pain management without the unfavorable CNS side effects. Indeed, a number of CB₂-selective agonists have shown efficacy in rodent models of inflammatory and neuropathic pain at doses which do not cause sedation or locomotor impairment. Structural modifications to the central portion of the *N*-arylpiperidine oxadiazole scaffold led to the identification of *N*-arylpiperidine oxadiazoles as conformationally constrained analogs that offered improved stability and comparable potency. The modular scaffold allowed for the use of expeditious and divergent synthetic routes, which provided two-directional SAR in parallel. Several potent, selective, full agonists from this novel ligand class are described.

P019

SYNTHESIS OF CONFORMATIONALLY RESTRICTED GLUTAMIC ACID ANALOGUES BASED ON SPIRO-[3.3]HEPTANE SCAFFOLD

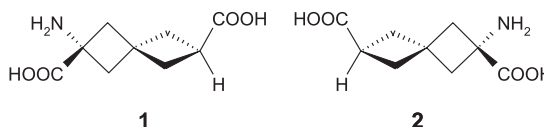
Dmytro S. Radchenko^{1,2}, Oleksandr O. Grygorenko¹, Igor V. Komarov^{1,2}

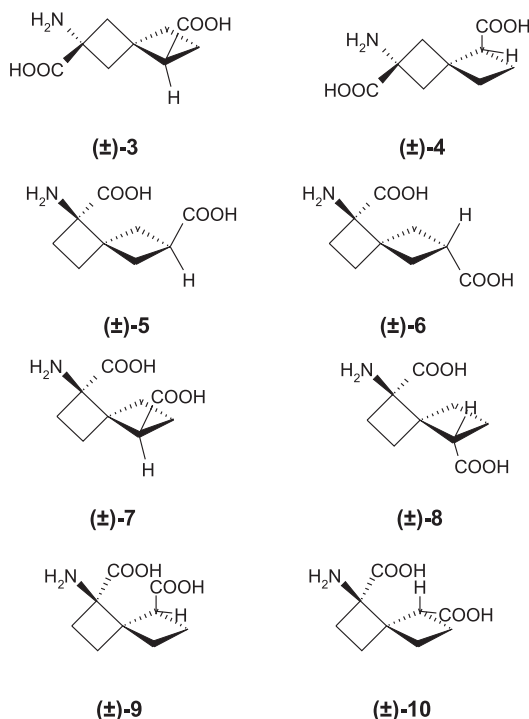
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A library of isomeric spirocyclic amino acids **1-10** – glutamic acid analogues – has been designed. Within the compounds in this library, mutual orientation of the aminocarboxylate moiety and the carboxylic group, as well as the distance between the functional groups are varied, therefore, compounds **1-10** can be used in medicinal chemistry as probes for glutamate receptors. The rigidity of the spiro[3.3]heptane scaffold might be beneficial for the biological activity: if the functional groups are fixed in space exactly as needed for efficient interaction with a receptor, the efficiency of the interaction can be higher than for the analogous conformationally flexible ligands.

Non-stereoselective and enantioselective syntheses of compounds **1** and **2** are elaborated. Studies towards synthesis of other members of the library will be presented.





P021

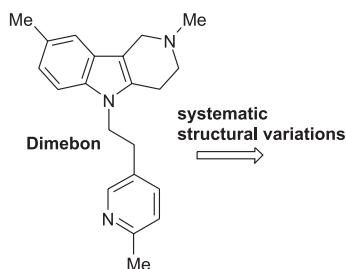
DISCOVERY AND *IN VIVO* EVALUATION OF NEW DIMEBON ANALOGS FOR COGNITION ENHANCEMENT IN TREATING ALZHEIMER'S DISEASE

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We report the development of a novel, aqueous-soluble, safe, BBB penetrant and potent series of Dimebon analogs with strong cognition enhancing properties. Recently Dimebon has been reported to have met all five efficacy endpoints in a one year randomized, double-blind, placebo-controlled Phase II study of ~200 patients with mild to moderate Alzheimer's disease (AD).

We have selected this drug as a starting point for the design of novel drug-like chemotypes. The design, synthesis, and SAR studies included tri- and bicyclic "core" and/or "decoration" variations of chemical structure in several systematic consecutive iterations.



Cognition disorders perhaps due to their unique intricacy have not benefited from rational single targeted drug design. In response to this challenge we have selected a few key biotargets for specific modulation. The "constellation" of 5-HT-2A, 5-HT2C, 5-HT6, alpha2A, DAT, NET, sigma1, AChE, Ca L-channel, NMDA, etc. was chosen as desirable for inhibition (antagonism), and hERG, 5-HT2B ("black holes") were identified as undesirable. SAR data will be demonstrated around lead series to illustrate the improvements in targeting affinities at multiple biomolecules.

Hit-to-lead optimization of this series has led to the discovery of the advanced lead compounds, which possess the desired fingerprint along with a promising *in vivo* profile. The lead compounds have demonstrated an excellent PK-PD correlation.

Administering of these agents has significantly reversed a scopolamine- and MK801-induced deficit in a rodent novel object recognition paradigm and passive avoidance test. The compounds also reversed the deficit in water maze spatial learning compared to vehicle-treated scopolamine-matched controls.

These studies provide further support for the potential therapeutic utility of small drug-like molecules possessing a desired "pharmacological fingerprint" in disorders characterized by cognitive deficits such as AD.

P022

ANALYSIS OF PROTEIN-PROTEIN INTERACTION BASED PHARMACOPHORES AS A SCREENING TOOL FOR SMALL MOLECULE PROTEIN-PROTEIN INTERACTION INHIBITORS

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Pharmacophore modelling is a well-known structure based drug design method allowing fast virtual screening of huge libraries of (commercial available) compounds. The construction of a pharmacophore model based on the protein-protein interface is a prerequisite for success.

Here we present an analysis of the amino acid composition of the protein-protein interfaces with respect to the amino acids mimicked by small molecule protein-protein interaction inhibitors (SMPPII) representing the pharmacophore features. A dataset was screened and a series of properties (interacting surface area and the Van der Waals, electrostatic and hydrogen bonding contributing to the total interaction energy) were calculated for all amino acids involved in protein-protein interaction. The obtained energy values of the mimicked amino acids were compared. Rules could be deduced allowing a more precise amino acid selection to be used in the construction of a pharmacophore model. From the superposition of the protein-protein structure and the protein-SMPPII we could deduct rules for the pharmacophore transcript of the interacting entities. This analysis shows that the phar-

macopore is based on a sub-population of the amino acids present in the total protein-protein interface. The amino acids, used as pharmacophore features, have lower interaction energy, are hydrophobic and interact via a large surface area.

In conclusion; structure based discovery (SMPPII as well as small molecules) can be facilitated by using valid pharmacophores derived from the protein-protein interfaces.

P023

ANTIPROLIFERATIVE ACTIVITY OF NATURAL BRASSINOSTEROIDS

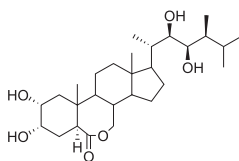
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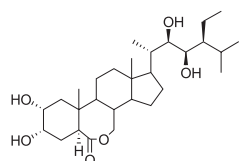
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Brassinosteroids are steroid plant hormones with important regulatory roles in various physiological processes, including growth, differentiation, root and stem elongation, disease resistance, stress responses, and vascular differentiation of many plants¹. This group of natural plant steroids includes more than 70 compounds distributed from lower to higher plants. Brassinosteroids have been detected and isolated from seeds, fruits, leaves, galls and pollen². Among plant hormones, brassinosteroids are structurally the most similar to animal steroid hormones. Like their animal counterparts, brassinosteroids regulate the expression of numerous genes, impact the activity of complex metabolic pathways, and contribute to the regulation of cell division and differentiation. The high biological activity of brassinosteroids has attracted the attention of many specialists in the field of chemistry, biology, pharmacology and agriculture. The first described brassinosteroid is brassinolide **1**, up to now the most biologically active brassinosteroid.



(1)



(2)

Some medical applications of brassinosteroids have also already been reported³, e.g. 28-homobrassinolide **2** has *in vitro* antiviral activity.

Up to now the inhibitory effects of brassinosteroids on cell division of mammalian cells are unknown. To determine basic anticancer structure-activity relationships of natural brassinosteroids on human cells, several normal and cancer cell lines have been used. We have tested some natural brassinosteroids on the viability, proliferation and cycling of hormone sensitive/insensitive breast and prostate cancer cell lines at micromolar concentrations to determine cytotoxic activity in the cancer cell lines. Our results provide the first evidence that natural brassinosteroids can inhibit the growth of several human cancer cell lines without affecting the growth of normal cells. Hence, these plant hormones are promising potential anticancer drugs.

This work was supported by project LC06077 (MSMT), and by the research project Z4 055 0506 (UOCHB).

References

- [1] Bajguz et al., *Phytochemistry* 2003, 62, 1027-1046.
- [2] Sasse J., In: *Brassinosteroids: Steroidal Plant Hormones*; Springer-Verlag, Tokyo, 1999; pp. 219-262; Khripach et al., *A new class of Plant Hormones*, Academic Press; San Diego, 1999, p. 456.
- [3] Wachsman et al.: *Antivir. Chem. Chemother.* 2000, 11, 71-77; *Chemother.* 2002, 13, 61-66.
- [4] CZ patent appl. 2007-571 (22.08.2007)

P025

NOVEL 4-ARYL-PYRIDO[1,2-c]PYRIMIDINES WITH DUAL 5-HT_{1A} AGONISM/SSRI ACTIVITIES

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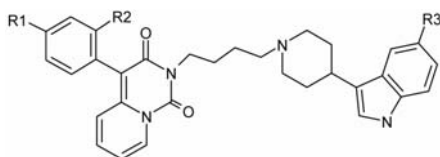
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One approach toward new antidepressants with better clinical parameters is the synthesis of compounds with dual SERT inhibitor/5-HT_{1A} activity [1]. Among many concepts in this field, the idea of incorporating a 5-HT_{1A} antagonist component into the structure of an SSRI was thought to be the most advantageous. However, there is a concern that the use of 5-HT_{1A} receptor antagonist without discriminating between its pre- and postsynaptic activity could cancel the benefits of enhancing presynaptically the 5-HT function. Therefore, an alternative method is the design of compounds with dual 5-HT reuptake

blockade/5-HT_{1A} receptor agonist action, which could desensitize presynaptic 5-HT_{1A} receptors and postinaptically enhance serotonin transmission [2-4].

The aim of this work was the design, synthesis and biological evaluation of new compounds with dual 5-HT_{1A} and 5-HT-T affinity.

Target compounds were tested for their affinity for 5-HT_{1A} receptor and 5-HT reuptake inhibition using radioligand binding assay. Compounds **7j** and **7k** revealed very high affinity for 5-HT_{1A} and 5-HT-T receptors (**7j** $K_{i\text{5-HT}_{1A}} = 4,8\text{nM}$ / $K_{i\text{5-HT-T}} = 0,7\text{nM}$, **7k** $K_{i\text{5-HT}_{1A}} = 5,8\text{nM}$ / $K_{i\text{5-HT-T}} = 0,3\text{nM}$). Other ligands showed high to moderate affinities. Compounds **7d** (20mg/kg), **7g** (10mg/kg), **7i** (20mg/kg), **7j** (20mg/kg) and **7k** (20mg/kg) were found to be presynaptic 5-HT_{1A} receptor agonists in induced hypothermia test in mice. Compounds **7g** (20mg/kg), **7i** (20mg/kg) and **7j** (10mg/kg) revealed their postsynaptic 5-HT_{1A} agonist activity in forced swimming test in mice.



	R ₁	R ₂	R ₃
7a	H	H	OCH ₃
7b	H	OCH ₃	H
7c	H	OCH ₃	OCH ₃
7d	H	Cl	H
7e	H	Cl	OCH ₃
7f	F	H	OCH ₃
7g	H	F	H
7h	H	F	OCH ₃
7i	H	H	H
7j	F	H	H
7k	H	CH ₃	H
7l	H	CH ₃	OCH ₃

References

- [1] Spinks D., Spinks G.; *Curr. Med. Chem.* 9, 799-810 (2002)
- [2] Romero L., Celada P., Martin-Ruiz R., Diaz-Mataix L., Mourelle M., Delgadillo J., Hervas I., Artigas F.; *Neuropsychopharmacology* 28, 445-456 (2003)
- [3] Zhou D., Harrison B., Shah U., Andree T., Hornby G., Scerni R., Schechter L., Smith D., Sullivan K., Mewshaw R.; *Bioorg. Med. Chem. Lett.* 16, 1338-1341 (2006)
- [4] Rocco V., Spinazze P., Kohn T., Honigschmidt N., Nelson D., Wainscott D., Ahmad L., Shaw J., Threlkeld P., Wong D., Takeuchi K.; *Bioorg. Med. Chem. Lett.* 14, 2653-2656 (2004)

P026

NOVEL 4-ARYL-5,6,7,8-TETRAHYDRO-PYRIDO-[1,2-c]PYRIMIDINES WITH DUAL 5-HT_{1A}/SSRI ACTIVITIES

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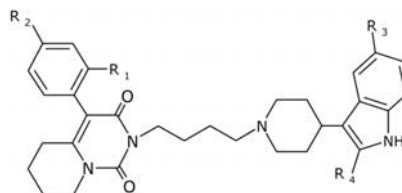
In our previous studies on the pyrido[1,2-c]pyrimidines we presented a series of new compounds with dual SSRI and 5-HT_{1A} agonist activity [1]. As a continuation of our research programme to develop faster acting antidepressants, we synthesized new derivatives of 4-aryl-5,6,7,8-tetrahydro-pyrido[1,2-c]pyrimidines with 3-(piperidin-4-yl)-1H-indole moiety.

The structures of new compounds were confirmed by ¹H and ¹³C NMR spectral data as well as by C, H, N analyses.

Target compounds were tested for their affinity for 5-HT_{1A} receptor and 5-HT reuptake inhibition using radioligand binding assay. Compounds **8a-k** revealed affinity for 5-HT_{1A} receptor which varied from high to moderate ($K_{i\text{5-HT}_{1A}}$: 10,4 – 53,8 nM), while compounds **8l-t** were almost inactive. Compounds **8a-t** displayed moderate to low affinity for 5-HT-T receptor ($K_{i\text{5-HT-T}}$: 71,6 – 943,7nM).

Compounds **8a** (20mg/kg), **8d** (20mg/kg), **8f** (10mg/kg) and **8g** (10mg/kg) were found to be presynaptic 5-HT_{1A} receptor agonists in induced hypothermia test in mice, while compound **8b** (20mg/kg) displayed presynaptic 5-HT_{1A} receptor antagonistic activity.

Compounds **8d** (20mg/kg), **8f** (10mg/kg) and **8g** (10mg/kg) revealed their postsynaptic 5-HT_{1A} agonist activity in forced swimming test in mice.



	R ₁	R ₂	R ₃	R ₄
8a	H	CH ₃	OCH ₃	H
8b	H	OCH ₃	H	H
8c	H	OCH ₃	OCH ₃	H
8d	F	H	OCH ₃	H
8e	H	Cl	OCH ₃	H
8f	H	CH ₃	H	H
8g	F	H	H	H
8h	CH ₃	H	H	H
8i	CH ₃	H	OCH ₃	H
8j	H	F	OCH ₃	H
8k	Cl	H	H	CH ₃
8l	F	H	H	CH ₃
8m	H	H	H	CH ₃
8n	OCH ₃	H	H	CH ₃
8o	CH ₃	H	H	CH ₃
8p	H	CH ₃	H	CH ₃
8q	H	OCH ₃	H	CH ₃
8r	H	Cl	H	CH ₃
8s	Cl	H	H	CH ₃
8t	H	F	H	CH ₃

References

- [1] Herold F., Chodkowski A., Izbiński L., Król M., Kleps J., Nowak G., Dawidowski M.; *XXth International Symposium on Medicinal Chemistry EFMC-ISMIC 2008, Vienna, Austria* (2008)

P028

DISCOVERY AND EVALUATION OF A SERIES OF POTENT SMALL MOLECULE MET PROTEIN RECEPTOR TYROSINE KINASE INHIBITORS

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SGX Pharmaceuticals, 10505 Roselle St, San Diego, California, 92121, USA

The role of the receptor tyrosine kinase MET in the development and progression of cancer is well established and suggests that inhibitors of MET will emerge as important novel therapeutics. We report the discovery of a series of highly ligand efficient MET inhibitors, the characterization of the bound ligand-protein conformation, the optimization of potency aided by structure based drug design and the optimization of pharmacokinetics. Our inhibitors displayed in-vivo efficacy in a mouse tumor xenograft model implanted with human gastric carcinoma cells (GTL16) and inhibition of a pharmacodynamic marker (pMET). Two phase I studies were initiated on January 2008 with SGX523 in subjects with solid tumors.

P029

CHEMICAL MODIFICATION OF ANTIBACTERIAL GLYCOPEPTIDE ANTIBIOTICS DIRECTED TO THE COMPOUNDS WITH NEW ANTIBACTERIAL OR ANTIVIRAL ACTIVITIES

Maria N. Preobrazhenskaya

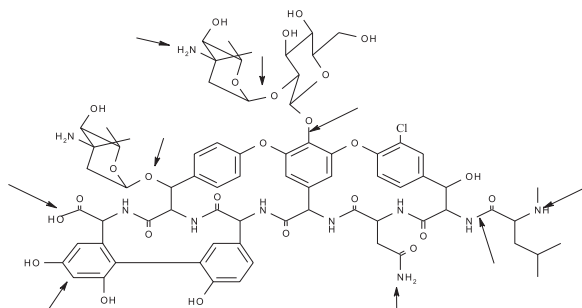
Gause Institute of New Antibiotics of the Russian Academy of Medical Sciences

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The glycopeptides (vancomycin, teicoplanin) are the antibiotics of last choice for serious infections due to Gram-positive pathogens. The search for derivatives active against multidrug-resistant bacteria resulted in the discovery of hydrophobic derivatives of glycopeptides, active against *S. aureus* (MRSA), GISA, and GRE in a manner different from that of the parent antibiotics. The introduction of both polar and hydrophobic substituent in the same molecule permitted to reduce the negative pharmacological consequences of the introduction of a hydrophobic substituent into a glycopeptide.

In cooperation with Rega Institute for Medical Research antibiotic derivatives were evaluated against the cytopathicity of HIV-1 and HIV-2 and against the transforming effect of Moloney murine sarcoma virus (MSV). Aglycon derivatives were discovered to be selec-

tively active against retroviruses including HIV-1, HIV-2 and MSV. The introduction of a hydrophobic substituent is beneficial for antibacterial and antiviral types of activity. The presence of carbohydrate moieties in glycopeptides usually results in a dramatic decrease of anti-HIV activity, whereas for antibacterial activity the presence of sugars represents a critical determinant. Inhibition of viral entry is the molecular event for the anti-HIV activity of this type of compounds.



P031

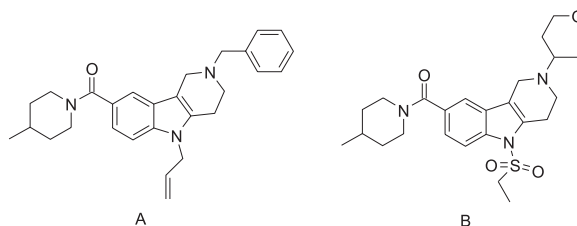
THE DISCOVERY OF γ -CARBOLINES AS HIGHLY WATER SOLUBLE AND PERIPHERALLY RESTRICTED CB1 AGONISTS

Yun-Xing Cheng, Xuehong Luo, Mehrnaz Pourashraf, Mirek Tomaszewski, Chris Walpole, Dominic Salois*, Stephane St-Onge*, Etienne Lessard#, Julie Ducharme#, Xiaohong Yu®

Department of Medicinal Chemistry, Pharmacology* and DMPK#, Bioscience®, AstraZeneca R&D Montreal, 7171 Frederick-Banting, Ville St. Laurent, Quebec H4S 1Z9 Canada

Cannabis has been used for centuries both for recreational purposes and as a medicine. The biological effects of the active ingredient of cannabis THC are mediated through two endocannabinoid receptors CB1 and CB2. However, adverse side effects of some cannabinoids including addiction, anxiety or depression have limited the clinical use of cannabinoid agonists. Most of the side effects are believed to be associated with CB1 receptors in central nervous system.

In our search for peripherally restricted CB1 agonists, we started from g-Carboline scaffold¹ identified from our GPCR focused libraries. The active to hit and hit to lead work on carboline series will be reported, including SAR and the profiles of some promising compounds in the series.



Reference

- [1] Cheng, Yun-Xing; Tomaszewski, Mirosław. (Astrazeneca AB, Swed.). PCT Int. Appl. (2006), 26pp. WO 2006101434 A1 20060928

P032

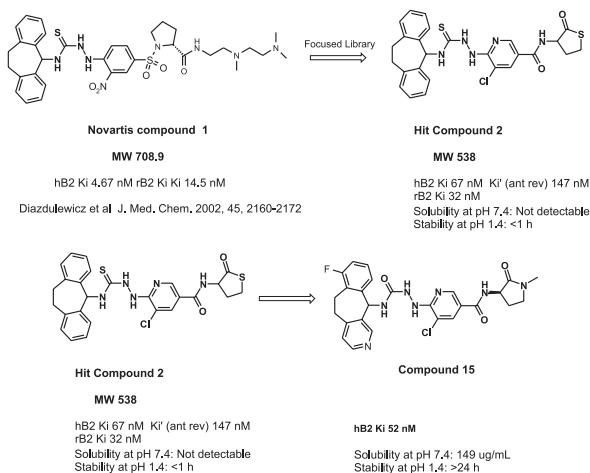
SELECTIVE BRADYKININ B₂ RECEPTOR ANTAGONISTS: DISCOVERY OF WATER SOLUBLE AND CHEMICALLY & METABOLICALLY STABLE DIBENZOSUBERANE REPLACEMENTS

Xuehong Luo¹, Mirek Tomaszewski¹, Yun-Xing Cheng¹, Mehrnaz Pourashraf¹, Christopher Walpole¹, Joanne Butterworth², Lynda Adam², Claude Godbout², Kemal Payza², Marie Roumi²

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Bradykinin (BK; Arg¹-Pro²-Pro³-Gly⁴-Phe⁵-Ser⁶-Pro⁷-Phe⁸-Arg⁹) is a peptide in the kinin family with pro-inflammatory and pro-nociceptive activity. There are many studies in animals suggesting kinins contribute to the initiation and maintenance of inflammatory pain and the B₂ receptor is involved in this process. For example, B₂ antagonists are anti-nociceptive in mice (MAC, formalin tests) and rats (carrageenan test).

Recently a Novartis team disclosed compound **1** as both a human and rat active B₂ antagonist. Compound **1** had an excellent B₂ binding affinity, but has the drawback of high MW and potential toxophores groups. In our B₂ antagonist program, compound **1** was selected as one of the starting points for B₂-directed focused libraries. In this poster, hit to lead efforts to address issues such as poor stability in acidic media, poor water solubility will be discussed. Water soluble and chemically & metabolically stable aza-dibenzosuberanes (aza-DBS) are identified to be suitable DBS replacements, with the leading compound **15** containing aza-DBS showing dramatically improved physicochemical properties and chemical & metabolic stability while retaining the binding affinity of the hit compound.



P033

NOVEL 4-MORPHOLINO-2-PHENYLQUINAZOLINES AS POTENTIAL PI3K P110 α INHIBITORS

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Phosphoinositide-3-kinases (PI3Ks) are enzymes that closely connected with the activation of the cell survival kinase PKB/Akt, leading to cell proliferation and survival. The p110 α isoform of PI3K is mutated or over-expressed in more than 30% of tumours, and great efforts have been devoted to synthesize selective p110 α inhibitors as antitumour agents. A series of 4-morpholine-2-phenylquinazolines have been designed and synthesized by our group as potential PI3K p110 α inhibitors. The docking result (Figure B and C) clearly showed that the 4-morpholine-2-phenylquinazoline moiety is deeply inserted in the ATP-binding site of p110 γ (the structure of p110 α with ligands has not yet been experimentally solved and the p110 α and p110 γ isoforms share good sequence identity, especially around the ATP-binding site) and the hydrogen bond contact of Val-882 with the morpholino group, which is reported as an essential part for p110 α inhibitory activity. The p110 γ inhibitory activities of these compounds are currently under investigation.

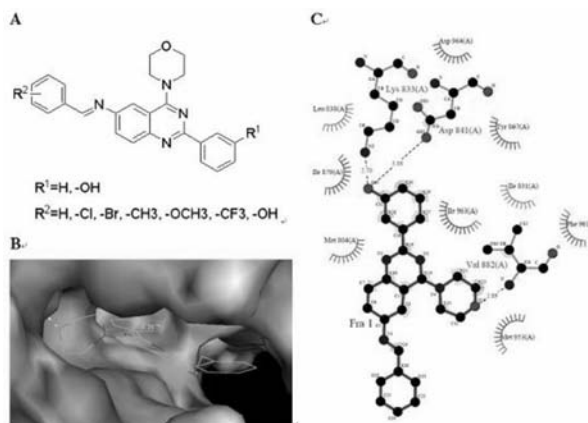


Figure (A). Target compounds. **Figure (B), (C).** Docking model of target compound (when R¹ = -OH, R² = H) within the ATP-binding site of p110 α (Dashed lines stand for hydrogen bonds).

References

- [1] Hennessy, B. T.; Smith, D. L.; Ram, P. T.; Lu, Y. L.; Mills, G. B. *Nat. Rev. Drug Disc.* 2005, 4, 988-1004.
- [2] Ruckle, T.; Schwarz, M. K.; Rommel, C. *Nat. Rev. Drug Disc.* 2006, 7, 606-619.
- [3] Hayakawa, M.; Kaizawa, H.; Moritomo, H.; Koizumi, T.; Ohishi, T.; Okada, M.; Ohta, M.; Tsukamoto, S.-I.; Parker, P.; Workman, P.; Waterfield, M. *Bioorg. Med. Chem.* 2006, 14, 6847-6858.

P036

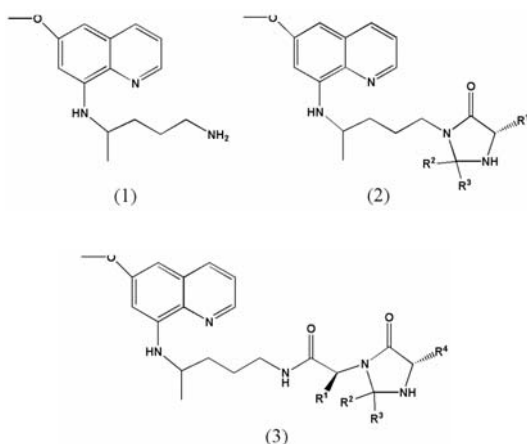
SYNTHESIS AND REACTIVITY OF DIPEPTIDE IMIDAZOLIDIN-4-ONE DERIVATIVES OF PRIMAQUINE AS POTENTIAL ANTIMALARIAL PRODRUGS

Nuno Vale,¹ Rui Moreira,² and Paula Gomes¹

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One of the main problems associated to the antimalarial drug primaquine (1, PQ) is its short half-life (~6 h) and its high extension and rate of transformation into its metabolite carboxyprimaquine.^[1] To overcome such problem, our research group developed imidazolidin-4-ones derivatives of *N*-aminoacyl-PQ (2),^[2] that displayed gametocytocidal activities against *P. berghei* comparable to that of PQ,^[3] as well as modest activities against both *P. falciparum* blood-schizonts and *Pneumocystis carinii* fungi.^[4] In contrast to peptide-based imidazolidin-4-ones, compounds 2 are remarkably stable at pH 7.4 and 37 °C, both in isotonic buffer and human plasma,^[3,5] which confirms that the compounds are active *per se* and not by virtue of releasing PQ in the course of biological assays.

Given the promising results obtained with compound 2, we have recently moved forward to their structural diversification, namely by insertion of an additional amino acid spacer between the PQ moiety and the imidazolidin-4-one pentagonal ring. This led to structures 3, whose synthesis and stability towards hydrolysis to the parent α -dipeptidylprimaquines,^[6] will be the subject of the present communication.



References

- [1] Constantino, L.; Paixão, P.; Moreira, R.; Portela, M.J.; Rosário, V.E.; Iley, J. *Exp. Toxicol. Pathol.* 1999, 51, 299.
- [2] Gomes, P.; Araújo, M.J.; Rodrigues, M.; Vale, N.; Azevedo, Z.; Iley, J.; Chambel, P.; Morais, J.; Moreira, R. *Tetrahedron* 2004, 60, 5551.

- [3] Araújo, M.J.; Bom, J.; Capela, R.; Casimiro, C.; Chambel, P.; Gomes, P.; Iley, J.; Lopes, F.; Morais, J.; Moreira, R.; Oliveira, E.; Rosário, V.; Vale, N. *J. Med. Chem.* 2005, 48, 888.

- [4] Vale, N.; Collins, M.S.; Gut, J.; Ferraz, R.; Rosenthal, P. J.; Cushion, M.T.; Moreira, R.; Gomes, P. *Bioorg. Med. Chem. Lett.* 2008, 18, 485. †

- [5] Chambel, P.; Capela, R.; Lopes, F.; Iley, J.; Morais, J.; Gouveia, L.; Gomes, J.R.B.; Gomes, P.; Moreira, R. *Tetrahedron* 2006, 62, 9883.

- [6] Vale, N.; Moreira, R.; Gomes, P. *Submitted.*

P037

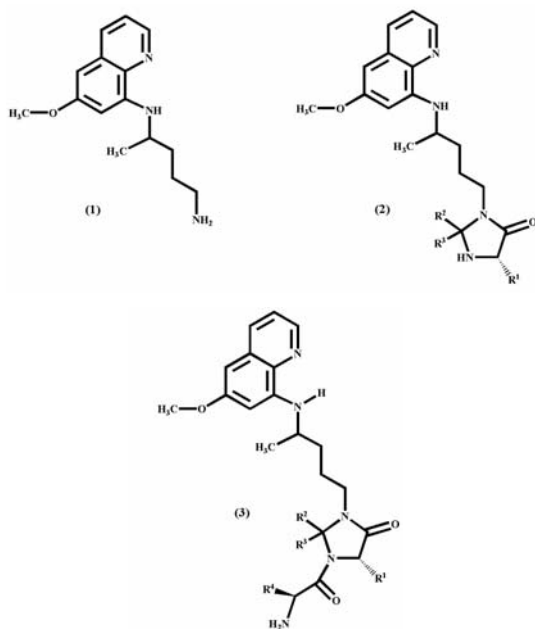
SYNTHESIS AND ANTIMALARIAL ACTIVITY OF IMIDAZOLIDIN-4-ONE PEPTIDOMIMETIC DERIVATIVES OF PRIMAQUINE

Nuno Vale,¹ Joana Matos,¹ Jiri Gutí,² Fátima Nogueira,³ Philip Rosenthal,² Virgílio do Rosário,³ Rui Moreira,⁴ and Paula Gomes¹

¹CIQUP, Departamento de Química, Faculdade de Ciências, Universidade do Porto, P-4169-007 Porto, Portugal; ²Department of Medicine, San Francisco General Hospital, University of California, San Francisco, CA 94143-0811, USA; ³CMDT, Instituto de Higiene e Medicina Tropical, P-1349-008 Lisboa, Portugal; ⁴iMed.UL, CECF, Faculdade de Farmácia, Universidade de Lisboa, P-1600-083 Lisboa, Portugal.

Primaquine (1, PQ) is the only available drug that is active against all exoerythrocytic forms of *Plasmodia*, including gametocytes and latent liver forms (hypnozoites) responsible for relapsing malaria. However, PQ has a short plasma half-life (~6h), presumably due to its rapid oxidative deamination to carboxyprimaquine.^[1] Recently, we prepared imidazolidin-4-ones (2) as potential peptidase-stable prodrugs of amino acid derivatives of PQ, which turned out to be remarkably stable and to exhibit antimalarial and anti-*Pneumocystis carinii* pneumonia activities *per se*.^[2-4]

Given the excellent results with compounds 2, we theorized that condensation of an amino acid residue to the imidazolidin-4-one's secondary amine nitrogen (N¹) would yield peptidomimetic PQ derivatives (3) with increased stability and aqueous solubility. We have prepared these novel compounds and found them to be moderately active against blood-schizonts of a chloroquine-resistant *P. falciparum* strain and also to impair the sporogonic cycle of *P. berghei* in *Anopheles* mosquitoes.^[5] Moreover, the novel imidazolidin-4-ones 3 are water-soluble and very stable at pH 7.4 and 37 °C, both in isotonic buffer and human plasma, as hypothesized. All these findings will be the subject of the present communication.



References

- [1] Constantino, L.; Paixão, P.; Moreira, R.; Portela, M.J.; Rosário, V.E.; Iley, J. *Exp. Toxicol. Pathol.* 1999, 51, 299.
- [2] Gomes, P.; Araújo, M.J.; Rodrigues, M.; Vale, N.; Azevedo, Z.; Iley, J.; Chambel, P.; Morais, J.; Moreira, R. *Tetrahedron* 2004, 60, 5551.
- [3] Araújo, M.J.; Bom, J.; Capela, R.; Casimiro, C.; Chambel, P.; Gomes, P.; Iley, J.; Lopes, F.; Morais, J.; Moreira, R.; Oliveira, E.; Rosário, V.; Vale, N. *J. Med. Chem.* 2005, 48, 888.
- [4] Vale, N.; Collins, M.S.; Gut, J.; Ferraz, R.; Rosenthal, P. J.; Cushion, M.T.; Moreira, R.; Gomes, P. *Bioorg. Med. Chem. Lett.* 2008, 18, 485.
- [5] Vale, N.; Matos, J.; Gut, J.; Nogueira, F.; Rosenthal, P.; Rosário, V.; Moreira, R.; Gomes, P. *Submitted*.

P038

MULTICOMPONENT REACTIONS AS USEFUL PLATFORMS TO EXPLORE THE CHEMICAL SPACE

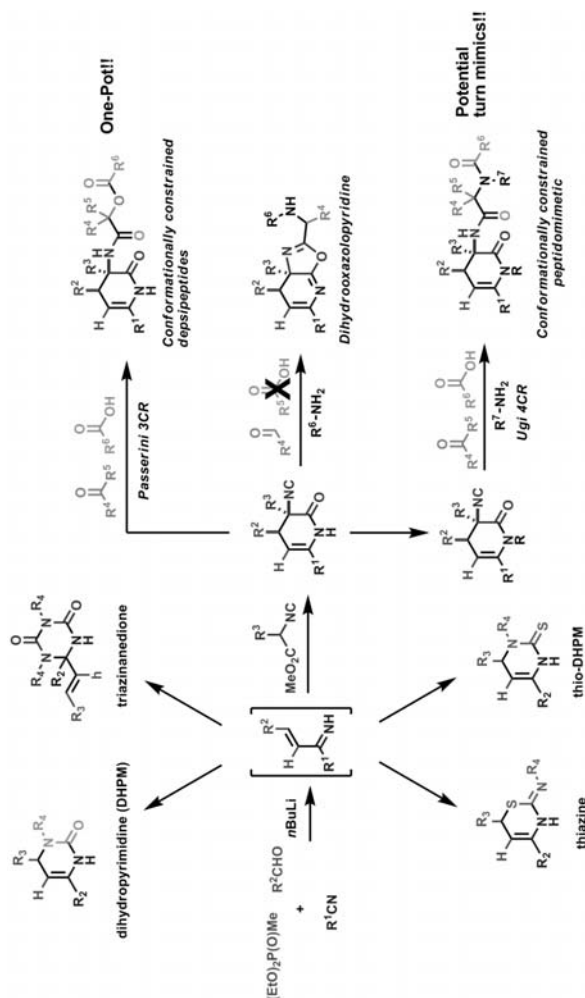
Romano V.A. Orru

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In this era of “-omics” and structural biology there is an ever-increasing need for novel small molecules that can modulate biological processes. For this, combinatorial synthesis is undisputed as an enabling tool to access the required small-molecule based compound collections. Although, the benefit for efficient biological evaluation and ultimately successful drug discovery seems obvious, the actual hit rates for new drug candidates have decreased steadily over the past decade. Structural diversity and complexity are essential to address “drug likeliness” in the design and synthesis of compound libraries. So far only a very limited number of molecules that cover the

enormous potential of the chemical space have been actually made by Man and explored for potential useful properties, like their biological relevance. This continues to drive synthetic chemists to develop novel reactions that can efficiently access unexplored regions of chemical space.

Rapid generation of diverse sets of complex molecules can be achieved by employing diversity-oriented synthetic strategies in combination with so-called complexity-generating reactions. Multicomponent reactions (MCRs), which combine in one-pot at least three simple building blocks, provide a most powerful platform to access diversity as well as complexity in a limited number of reaction steps. Here we describe novel modular reaction sequences based on our previously reported MCR chemistry in combination with other common organic reactions or even with a second MCR. Combination of our MCRs with, e.g., cycloadditions, transition-metal mediated cross-coupling reactions or with more traditional MCRs like Biginelli-3CR, Ugi-4CR and the Passerini-3CR will be shown. Examples in which the synthetic methodology was applied for the easy generation of focused libraries for the synthesis of medically relevant ligands will be discussed.



P039

DEVELOPMENT OF AMYLOID-BETA AGGREGATION INHIBITORS

Hiroaki Okuno*, Ayako Orimoto, Hideharu Suzuki, Akihito Ishigami

Toho University, Faculty of Pharmaceutical Sciences, Funabashi, Japan

Amyloid- β peptide ($A\beta$, mostly consisted of 40-42 amino acids) is major component of senile plaques in the brain of Alzheimer's disease patients, and its aggregate causes serious neurotoxicity that leads to memory loss, cognitive decline, language impairment and dementia. We previously found KLVFF (LysLeuValPhePhe) sequence ($A\beta$ 16-20) in $A\beta$ plays an important role for the aggregate/fibril formation, and developed the inhibitors composed of KLVFF and a variety of amino acid analogs with hydrophilic character, respectively, as molecular recognition and aggregation disrupting elements (Fig. 1).

Thioflavin T and Congo red pigments combine specifically with amyloid fibrils (β -sheet structure) and are extensively used for detecting the presence of $A\beta$ aggregation and their rates of formation, therefore we then focused on stilbene derivatives as a new molecular recognition moiety in order to develop alternative inhibitors with the stronger disrupting activity (Fig. 2).

Quartz-crystal microbalance (QCM) analysis was applied for evaluating their ability as the aggregation inhibitor, and the process of $A\beta$ aggregation was detected as a decrease of frequency (Hz) by $A\beta$ accumulation on the sensor. Then the reverse effect (frequency increase) was observed upon the addition of inhibitors caused by desorption of the initially aggregated $A\beta$ on the electrode.

Bioactivity evaluation was also carried out *in vitro* against neuroblastoma IMR-32 cells, and the inhibitor itself showed no cytotoxicity. However the cytotoxic activity of $A\beta$ was remarkably suppressed in the presence of the inhibitors.

Promising results *in vivo* were recently obtained, which will be also discussed.

KLVFF-(DDX)3 D: Asp
acidic amino acid & hydrophilic element
KLVFF-(EEX)3 E: Glu
acidic amino acid & hydrophilic element
KLVFF-(KKX)3 K: Lys
basic amino acid & hydrophilic element
KLVFF-(Hyl-Hyl-X)3 Hyl: Hydroxylysine
basic amino acid & OH group
KLVFF-(DDX)3 D: D-Asp
D-Asp employed for the improved stability in
physiological condition

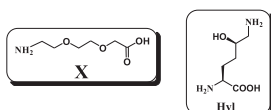
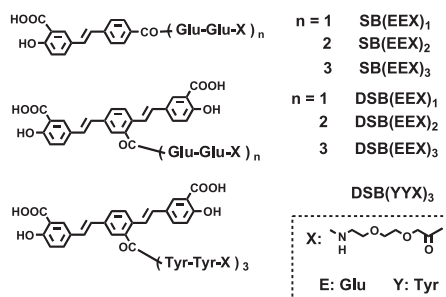


Fig. 1

Fig. 2



P042

INHIBITION OF NITRIC OXIDE SYNTHASE BY NOVEL FLUORESCENT POLYCYCLIC LIGANDS

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Nitric oxide (NO) is a molecular messenger involved in a number of mammalian physiological processes [1]. It is synthesised by nitric oxide synthase (NOS) from L-arginine and its overproduction could lead to a number of neurological disorders [2]. Selective inhibition of NOS can thus be of value in the treatment of neurodegenerative diseases. The aim of this study was to synthesise a series of novel fluorescent derivatives conjugated to polycyclic structures for evaluation in NOS assays. The enzyme is also a target system where fluorescent techniques and fluorescently labelled NOS inhibitors can be used for detecting the biophysical properties of enzyme-ligand interactions and facilitate development of novel inhibitors of neurodegeneration.

The polycyclic structures, amantadine and 3-hydroxy-4-aza-8-oxoheptacyclo-tetradecane, were conjugated to the fluorescent moieties by amination and esterification or amidation using activation chemistry. The fluorescent groups were selected on the basis of their spectroscopic properties and structure-activity relationship requirements for NOS inhibition and included *N*-methylantranilic acid, indazole-3-carboxylic acid, 1-fluoro-2,4-dinitrobenzene, 1-cyanoisindole, 1-thiocyanoisindole and 1-nitroisindole.

The oxyhaemoglobin assay [3] was employed to measure the activity of the compounds *in vitro*. IC₅₀ values of the novel fluorescent compounds were compared to that of amino guanidine (AG) and 7-nitroindazole (7-NI), two known NOS inhibitors, and showed moderate to high affinity (IC₅₀ values ranging from 0.77 μ M to 2.91 μ M) for the NOS enzyme.

In view of the increase in lipophilicity originating from the polycyclic structures [4], it is expected that the blood-brain barrier permeability of these compounds will be enhanced, thus facilitating an increase in activity and selectivity. These compounds have potential as useful pharmacological tools in the investigation of enzyme-ligand interactions.

Acknowledgement

Financial support received from the National Research Foundation of South Africa.

References

- [1] Dawson, T.M., Dawson, V.L. & Snyder, S.H. 1992. A novel neuronal messenger molecule in brain: The free radical nitric oxide. *Annals of Neurology*, 32:297– 311.
- [2] Kerwin, J.F., Lancaster, J.R. & Feldman, P.L. 1995. Nitric Oxide: A new paradigm for second messengers. *Journal of Medicinal Chemistry*, 38:4342-4362.
- [3] Salter, M. & Knowles, R.G. 1998. Assay of NOS activity by the measurement of conversion of oxyhemoglobin to methemoglobin by NO. (*In* Titheradge, M.A., ed. *Methods in molecular biology*, vol. 100. Nitric oxide protocols.) Totowa, New Jersey: Humana press. p. 61-65.
- [4] Oliver, D.W., Dekker, T.G., & Snyckers, F.O., 1991. Pentacyclo[5.4.0^{2.6}.0^{3.10}.0^{5.9}]undecylamines. Synthesis and pharmacology. *European Journal of Medicinal Chemistry*, 26:375-379.

P044

INDAZOLYLAMINO/ANILINOQUINAZOLINES BEARING A C-5 SUBSTITUTION AS erbB2 KINASE INHIBITORS: STRUCTURE ACTIVITY RELATIONSHIPS AND IDENTIFICATION OF A CANDIDATE DRUG

Bernard Barlaam,^a Peter Ballard,^b Robert H. Bradbury,^b Darren Cross,^b Richard Ducray,^a Hervé Germain,^a D. Mark Hickinson,^b Kevin Hudson,^b Jason G. Kettle,^b Teresa Klinowska,^b Françoise Magnien,^a Donald Ogilvie,^b Annie Olivier,^a Stuart E. Pearson,^b Marie-Jeanne Pasquet^a, James S. Scott,^b Abid Suleman,^b Michel Vautier,^a Robin Whittaker,^b Emma Williams,^b Robin Wood.^b

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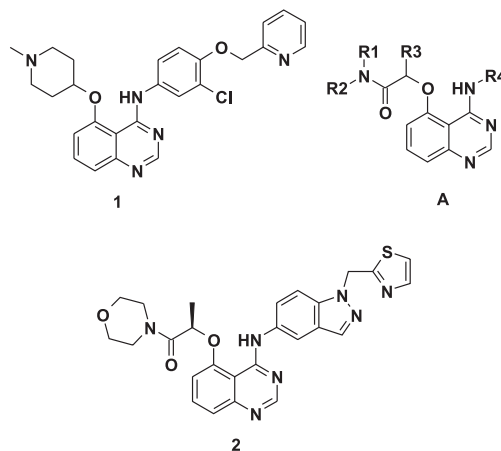
Aberrant function of the erbB family of receptor tyrosine kinases and their ligands has been described in many human cancers. Within this family, erbB2 plays a central role: mis-regulation of erbB2, for example by over-expression/gene amplification, has been observed to varying degrees in a range of tumours, particularly breast cancer.

Medicinal chemistry effort at AstraZeneca has identified certain 5-substitued anilinoquinazolines as kinase inhibitors for Src, EGF and erbB2.

We previously described **1**¹ as a potent and selective inhibitor of erbB2, showing antitumor efficacy after oral administration in mouse xenograft models. However, this compound was subsequently found to exhibit modest pharmacokinetics in other species (especially dog) and mild to severe effects in liver and lung in rat toxicological studies attributed to phospholipidosis, which precluded its progression towards clinical trials.

SAR around generic structure **A** was developed by introducing a wide range of substituents at C4 and C5

positions to optimize the potency and incorporate desirable pharmaceutical properties, including good solubility and low human microsomal clearance. We will describe the optimisation of the lead series and the identification of the lead candidate **2** which was selected from this series, based on its potent erbB2 kinase inhibition profile, its physical and pharmacokinetic properties and its antitumor efficacy after oral administration in xenograft models with erbB2 overexpression.



References

- [1] Ballard, P.; Bradbury, R.H.; Hennequin, L.F.A.; Hickinson, D.M.; Johnson, P.D.; Kettle, J.G.; Klinowska, T.; Morgentin, R.; Ogilvie, D.J.; Olivier, A. *Bioorg. Med. Chem. Lett.* 2005, 15, 4226-4229.

P045

N-(4-(4-AMINO)PYRAZOLO[3,4-D]PYRIMIDIN-3-YL)PHENYL)BENZO[D]OXAZOL-2-AMINES AS INHIBITORS OF TIE-2 RECEPTOR TYROSINE KINASE

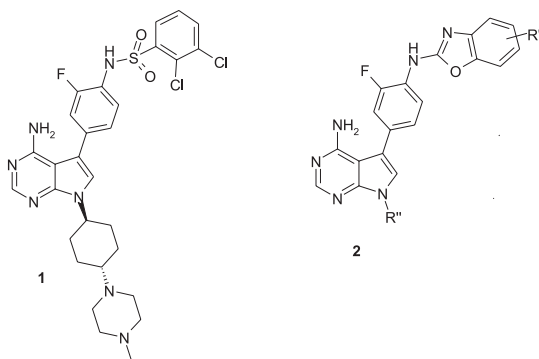
Michael Friedman,¹ Lee Arnold, Neil Wishart, Michael Michaelides, Steven Davidsen, Deborah Choquette, Bryant Yang, Matthew Martin, Shannon Fix-Stenzel, Pintipa Grongsaard, Anna Ericsson, Lu Wang, Doug Steinman, Jim Holms, David Borhani, Andreas Harsh, Peter Bousquet, Christine Nelson, Lian Rundell, Jonathan George, Xiao-Dong Qian

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Angiogenesis, the process of new blood vessel formation, is involved in many diseases such as diabetic retinopathy, macular degeneration, psoriasis, rheumatoid arthritis and cancer. It is widely accepted that the growth and metastasis of solid tumors is dependent on this process. Angiopoietins and their endogenous receptor, Tie-2, play an important role in the angiogenic process ¹. In this poster we describe the design of a class of potent Tie-2 inhibitors and demonstrate their efficacy in an *in-vivo* model of cancer.

Starting from the previously described N-(4-(4-amino-7-*trans*-4-(4-methylpiperazin-1-yl)cyclohexyl)-7H-pyrrolo[2,3-*d*]pyrimidin-5-yl)-2-fluorophenyl)-2,3-dichlorobenzenesulfonamide **1**, in conjunction with a co-crystal

structure in Tie-2 ², we adopted a structure-based drug design approach leading to the identification of a series of *N*-(4-(4-amino)pyrazolo[3,4-*d*]pyrimidin-3-yl)phenylbenzo[*d*]oxazol-2-amines (general structure **2**). In this poster, we describe the enzymological and cellular SAR, pharmacokinetic properties, PK/PD alignment and efficacy in a murine MX-1 model of cancer, for this potent class of Tie-2 inhibitors.



References

- [1] Carmeliet, P.; Jain, R.K. *Nature*, 2000, 407, 249; Jones, N.; Iljin, K.; Dumont, D.; Alitalo, K. *Nature Reviews Molecular Cell Biology*, 2001, 2(4), 257.
- [2] Wishart, N et al. *AACR 2002 Annual Meeting*, April 9, San Francisco, Abstract #4205.

P046

DESIGN AND SYNTHESIS OF PROTEIN KINASE CK2 INHIBITORS

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CK2 is a ubiquitous and constitutively active serine/threonine protein kinase. It has more than 300 substrates, the majority of which are proteins implicated in signal transduction, gene expression and other nuclear functions¹. CK2 is overexpressed in many cancer cells and seems to enhance cell proliferation and to inhibit apoptosis². It has furthermore been demonstrated that down-regulation of CK2 by treating the cells with antisense CK2 or with a small inhibitory molecule results in induction of apoptosis³. Thus, CK2 is believed to have an oncogenic potential, and so the development of potent, selective and cell-permeable inhibitors of CK2 could be of great therapeutic interest. Moreover, such inhibitors would provide a powerful tool to investigate the biological role of this constitutively active protein, still poorly understood.

Several selective ATP-competitive inhibitors of CK2 protein kinase have been described in literature^{4,5,6}. They are all planar and bind to the ATP site of the CK2 mainly by hydrophobic interactions. Selectivity in binding is dictated by a smaller ATP pocket in CK2, compared to other kinases.

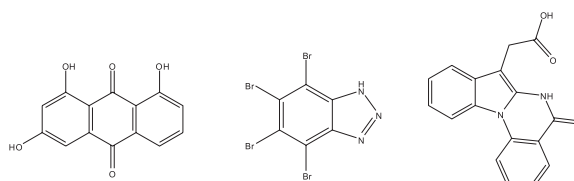


Fig. 1 Known CK2 inhibitors: emodine ($IC_{50}=0.89\mu M$, left), TBB ($IC_{50}=0.50\mu M$, middle), IQA ($IC_{50}=0.39\mu M$, right)

The biological screening of Institut Curie's small-molecule library (6560 compounds) against CK2 has led to the discovery of a new family of CK2 inhibitors, with inhibition constants already in the same range as those described in the literature. We have synthesised and evaluated analogues of these compounds, which has allowed us to find new inhibitors in the same series with IC_{50} in the nanomolar range.

To investigate the binding features of the discovered inhibitors we have performed a molecular modelling study by using the programs Gold⁷ and Charmm⁸.

References

- [1] Filhol O., Martiel J., Cochet, C., *EMBO Rep.* 2004, 5, 351–355.
- [2] Ahmed, K., Gerber, D. A., Cochet, C., *Trends in Cell Biology*, 2002, 12, 226–230.
- [3] Slaton, J. W., Unger, G. M., Sloper, D. T., Davis, A. T., Ahmed, K., *Mol. Cancer Res.* 2004, 2, 712–721.
- [4] Sarno S.; De Moliner E., Ruzzene M., Pagano M. A., Battistutta R., Bain J., Fabbro D., Schoepfer J., Elliott M., Furet P., Meggio F., Zanotti G., Pinna L. A., *Biochemical Journal* 2003, 374 Part 3, 639–646.
- [5] Battistutta R., De Moliner E., Sarno S., Zanotti G., Pinna L. A., *Protein Sci.* 2001, 10, 2200–2206.
- [6] De Moliner E., Moro S., Sarno S., Zagotto G., Zanotti G., Pinna L. A., Battistutta R., *J. Biol. Chem.* 2003, 278, 1831–1836.
- [7] GOLD, version 3.0.1; The Cambridge Crystallographic Data Centre (12 Union Road, Cambridge, CB2 1EZ, UK)
- [8] Charmm, B. R. Brooks, R. E. Brucoleri, B. D. Olafson, D. J. States, S. Swaminathan, M. Karplus, *J. Comp. Chem.* 4, 187–217 (1983)

P047

SMALL MOLECULE INHIBITORS THAT MODULATE HYPOXIA-INDUCIBLE FACTOR (HIF) PATHWAYS

Kyeong Lee,* Yinglan Jin, Yan Xia, Young-Soo Hong, Jung Jun Lee

The development of intratumoral hypoxia is a hallmark of rapidly growing solid tumors and of their metastasis. A key mediator expressed in many cell types in response to hypoxia is hypoxia-inducible factor-1 (HIF-1), which was implicated in angiogenesis, glycolysis, apopto-

sis, growth factor signaling, tumor invasion, and metastasis. Accordingly, HIF-1 represents an attractive molecular target for the development of novel cancer therapeutics. In this context, we prepared a series of small molecules and evaluated for their potential to inhibit HIF-1 activation. Herein we will disclose the identification of a novel series of potent HIF-1 inhibitors.

P049

AUTOMATED, MICROWAVE ASSISTED ALKENE SYNTHESIS VIA WITTIG REACTION

Michael Schneider, Ph.D.

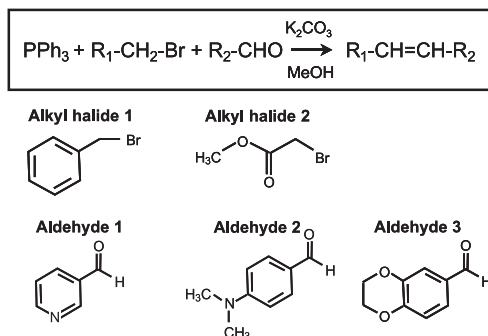
Chemspeed Technologies., Rheinstrasse 32, CH4302 Augst, Switzerland

Objective

The Wittig reaction is an efficient method for alkene synthesis, which can be completed up to ten times faster via microwave irradiation compared to conventional heating.

It was the objective of this study to compare the reactivity of different alkyl halides and aldehydes in the Wittig reaction in a fully unattended, automated synthesis approach using a set of 24 reaction vials.

In addition the existence of a "cooling while heating (cwh) effect" has been investigated.



Experimental Set Up

- 3 aldehydes, 2 alkyl halides, cooling while heating: on/off, 1 duplicate per experiment to assess reproducibility resulting in a matrix of overall 24 experiments
- Automated, sequential workflow unit operations: Dispensing of all solid and liquid reagents, capping, crimping, transport of vial to microwave cavity, heating for 5 min at 150°C with magnetic stirring, preparation of the next reaction mixture while heating, transporting vial back from microwave to the storage rack and transport of the next vial to microwave, de-cap all vials at the end of the application for analysis.
- All samples were analysed by LC/MS and yields determined by the internal standard method.

Results and Summary

- Bromoacetate is more active than benzyl bromide the nucleophilic substitution of Br⁻ by PPh₃ is facilitated by the α-carbonyl group.
- Lower yields are obtained with Aldehyde 3 compared to the other aldehydes, which can be explained by -I and -M effects.
- Cooling while heating doesn't have any significant effect.
- 24 Wittig reactions automatically have been processed in less than 4 hours.

P050

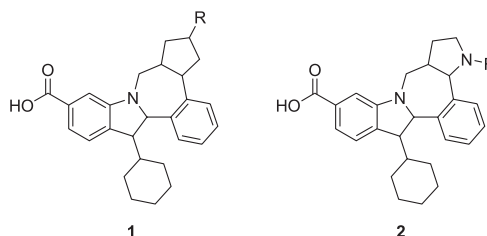
DISCOVERY OF PENTACYCLIC COMPOUNDS AS POTENT INHIBITORS OF HEPATITIS C VIRUS NS5B RNA POLYMERASE

Jörg Habermann[§], Elena Capito[§], Maria del Rosario Rico Ferreira[§], Uwe Koch[§], Nadia Gennari[†], Monica Bisbocci[†], Sergio Altamura[†] and Frank Narjes[§]

Dept. of Medicinal Chemistry[§], Dept. of Biochemistry[†], Merck Research Laboratories Rome, IRBM P. Angeletti SpA, Via Pontina km 30,600, I-00040 Pomezia (Rome), Italy

Hepatitis C is a viral infection of the liver and as such is a major cause of acute hepatitis and chronic liver disease, including cirrhosis and liver cancer. Globally, an estimated 170 million persons are chronically infected with HCV and 3 to 4 millions people are newly infected each year. HCV is primarily spread by direct contact with human blood. No vaccine is currently available to prevent hepatitis C and current treatment options for hepatitis C are very costly, often poorly tolerated and of limited success rate. Consequently, there is a need for improved therapies. The virally encoded non-structural proteins of the hepatitis C virus are attractive targets for antiviral therapy. As such, the hepatitis C virus NS5B polymerase is a key enzyme necessary for the replication of the HCV gene in infected cells.¹⁻³

Here we describe the discovery, synthesis and activity of pentacyclic indoles as potent allosteric inhibitors of HCV NS5B polymerase.



References

- [1] Koch, U.; Narjes, F. *Curr. Top. Med. Chem.* 2007, 7, 1302.
- [2] De Francesco, R.; Carfi, A. *Adv. Drug Delivery Rev.* 2007, 59, 1242.
- [3] WHO Factsheet No. 164: Hepatitis C.

P051

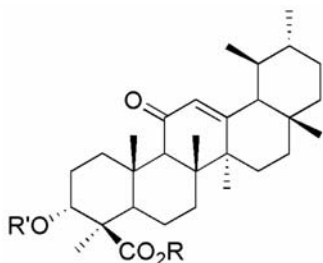
ANTITUMOR ACTIVE DERIVATIVES OF BOSWELLIC ACID

René Csuk¹, Anja Niesen¹, Harish Kommera², and Reinhard Paschke²

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Quite recently, pentacyclic triterpenes have gained increased attention due to their potential as multi-target therapeutics. These compounds were accounted for a variety of biological effects, among them significant anti-tumor activity. The anti-cancer effect especially of the *Boswellia serrata* plant has been documented from ancient times. Thus, frankincense has been part of traditional medicine for many centuries. The active constituents of the plant that have been shown to possess anti-inflammatory and anti-cancer properties are the boswellic acids. Therefore, several of these compounds (either as pure compounds or as extracts obtained from the plant) have been used (and are still in use) in the treatment of Crohn disease, ulcerative colitis, hepatitis, arthritis as well as of bronchial asthma.

During the last decade, the anti-neoplastic effects of boswellic acid derivatives came into the focus of scientific interest. During our own studies, concerning the development of antitumor active compounds we became interested in pentacyclic triterpene derived compounds especially in derivatives of boswellic acid. After having established a robust scheme for the extraction of boswellic acids from the plant material, approximately 50 different derivatives of boswellic acid were synthesized and their biological activity was determined.



Here we report the synthesis, the cytotoxicity and IC₅₀ values for these compounds; the biological data result from a sulforhodamin B assay using 16 different tumor cell lines (e.g. melanoma, lung, connective tissue, colon, breast, cervix and ovarian). From the results of an additional dye exclusion test as well as from DNA laddering techniques an apoptosis based mechanism of action was established for these compounds.

Several of these novel derivatives showed IC₅₀ values < 1 μmol.

P052

ANTITUMOR ACTIVE DERIVATIVES OF GLYCYRRHETINIC ACID

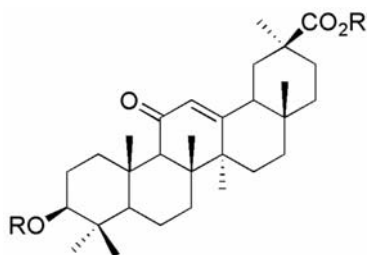
René Csuk¹, Stefan Schwarz¹, Harish Kommera², and Reinhard Paschke²

¹ Martin-Luther-Universität Halle-Wittenberg, NF II – Chemie, Organische Chemie, Kurt-Mothes-Str. 2, D-06120 Halle (Saale), Germany; ² Martin-Luther-Universität Halle-Wittenberg, Biozentrum, Weinbergweg 22, D-06120 Halle (Saale); Germany

Due to its anti-inflammatory and expectorant effect the extract of licorice has been used in traditional medicine for several hundreds of years. Glycyrrhizin and its corresponding pentacyclic terpenoid aglycone glycyrrhetic acid are the main components of these extracts. They were accounted for a variety of effects, among them antiviral activity.

Nowadays, the focus of scientific interest has shifted from the well-known and established antiviral activity to the anticarcinogenic potential of this class of compounds. During our own research in the field of pentacyclic triterpenes, we became interested in the synthesis of a variety of derivatives in order to deduce QSAR data.

Derivatives of glycyrrhetic acid were obtained by esterification, by transformation/ring opening of the A-ring, modification of the A-ring (especially by deoxygenation, inversion and fluorination) as well as by reduction of the a,b-unsaturated carbonyl moiety.



The anti-tumor activity of these compounds was evaluated utilizing a sulforhodamin B assay with up to 16 different cancer cell-lines (among them melanoma, lung, connective tissue, colon, breast, cervix and ovarian cancer). Several of these novel derivatives showed IC₅₀ values comparable to established anti-tumor drugs.

From the results of an additional dye exclusion test as well as from DNA laddering techniques an apoptosis based mechanism of action was established for these compounds. The synthesis of these compounds as well as the results of the biological screening will be presented.

P053

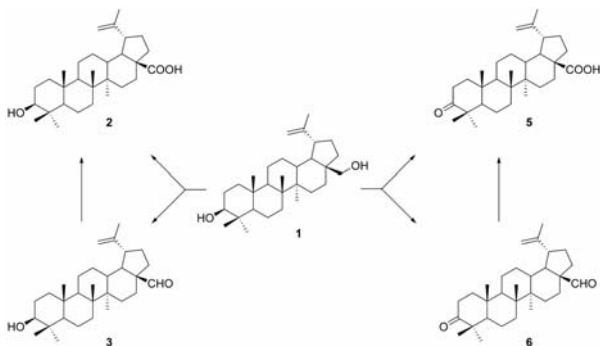
OXIDATIVE TRANSFORMATIONS OF BETULINE

René Csuk, Alexander Barthel, and Sebastian Stark

Martin-Luther-Universität Halle-Wittenberg, NF II – Chemie, Organische Chemie, Kurt-Mothes-Str. 2, D-06120 Halle (Saale), Germany.

Quite recently, several derivatives of triterpene derived compounds have gained significant interest in medicinal chemistry due to their pharmaceutical activities. Of special interest are derivatives of betuline (**1**) with a special focus on betulonic acid (**2**) since several of these compounds have shown quite promising results in the therapy of a variety of malignant cell lines in vivo; triterpenoic aldehyde or ketone derived compounds were reported to possess antimelanoma as well as anti-HIV activity.

Whereas **1** is easily accessible by extraction from natural sources in a very economic way, the isolation of **2** from plant material is rather laborious and yields material of lower purity needing either further tedious chromatographic purification or exhaustive re-crystallization at low temperatures. We could demonstrate that a 4-acetamido-TEMPO/NaClO₂/NaOCl mediated oxidation of **1** either leads to **2** or to betulinal (**3**).



Either betuline (**1**) or betulinal (**3**) are ideal starting materials for the synthesis of **2**; since yields for the direct conversion of **1** into **2** dropped slightly upon scaling-up using the 4-acetamido-TEMPO/NaClO₂/NaOCl procedure, a different approach starting from **3** was investigated in more detail. Whereas the oxidation of **3** to **2** with activated MnO₂ afforded only 18% of **2**, the oxidation of **3** with NaMnO₄ proceeded very smoothly and gave **2** in 85% isolated yield. The oxidation of **1** to betulonic acid (**5**) using the well established Jones-oxidation utilizing CrO₃/H₂SO₄ invariably gave lower yields as reported in several patents. However, higher yields of **5** were obtained when starting from betulonal (**6**) using a two-step sequence. Compound **6** was obtained in > 90% yield from **1** either by a Swern-type oxidation or with slightly diminished yields from a Jones-oxidation of **1** (thus yielding **6** in about 75% yield on a multi-gram scale).

In summary, by a combination of several selective oxidation procedures starting from betuline, betulonic or betulonic acid as well as betulinal or betulonal can be accessed very easily even on a multi-gram scale.

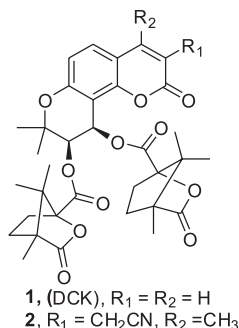
P054

DEVELOPMENT AND PRECLINICAL STUDIES OF BROAD-SPECTRUM ANTI-HIV AGENT (3'R,4'R)-3-CYANOMETHYL-3',4'-DI-O-(S)-CAMPHANOYL-4-METHYL-(+)-CIS-KHELLACTONE (3-CYANOMETHYL-DCK)

Lan Xie,^{†,*} Huanfang Guo,[†] Hong Lu,[‡] Xiaomei Zhuang,[†] Anming Zhang,^{†,#} Gang Wu,^{†,¶} Jinxiu Ruan,[†] Ting Zhou,[§] Donglei Yu,[§] Keduo Qian,[§] Kuo-Hsiung Lee^{§*}, and Shibo Jiang,^{‡*}

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In our prior studies, 3',4'-di-O-(S)-camphanoyl-(+)-cis-khellactone (DCK, **1**) and its analogs were identified as a novel class of anti-HIV agents with potent activity in H9 lymphocytes.¹⁻⁵ Mechanistic studies indicated that DCK analogs do target HIV-1 reverse transcriptase (RT) and inhibit HIV-1 replication by suppressing DNA-dependent DNA polymerase activity of HIV RT6, which is a different mechanism from that of current NNRTI drugs that block the generation of single-stranded DNA from a RNA template. This unique mechanism of action provides an opportunity to discover a novel NNRTI that remains effective against HIV-1 RT multi-drug resistant strains. Thus, we were strongly prompted to develop additional DCK analogs and discovered (3'R,4'R)-3-cyanomethyl-3',4'-di-O-(S)-camphanoyl-4-methyl-(+)-cis-khellactone (**2**, 3-cyanomethyl-DCK) as a potential drug candidate. 3-Cyanomethyl-DCK (**2**) exhibited broad-spectrum anti-HIV activity against wild-type and drug-resistant viral infection of CD4⁺ T cell lines and peripheral blood mononuclear cells by both laboratory-adapted and primary HIV-1 isolates with distinct subtypes and tropisms. Consequently, **2** was further evaluated in broad spectrum activity assays and both in vitro and in vivo pharmacokinetic studies. The latter studies indicated that **2** also has fairly good metabolic stability, cell permeability, and moderate oral bioavailability. Furthermore, a ten-step practical synthesis for scale-up preparation was developed and optimized successfully with an increased overall synthetic yield from 7.8% to 32%. These results suggest that **2** should be developed as a novel anti-HIV drug candidate with a unique mechanism of action.



Acknowledgment

Grant D0204003041631 from China awarded to Lan Xie, and NIH grants AI 33066 and AI 46221 awarded to K. H. Lee and S. Jiang, respectively.

References

- [1] Huang, L. et al. *J Med Chem* 1994, 37, 3947-3955
- [2] Xie, L. et al. *J. Med. Chem.* 1999, 42, 2662-2672
- [3] Xie, L. et al. *J. Med. Chem.* 2004, 47, 756-760
- [4] Xie, L. et al. *J. Med. Chem.* 2001, 44, 664-671
- [5] Xie, L. et al. *Bioorg. Med. Chem.* 2005, 13, 6435-6449.
- [6] Huang, L. et al. *Virology*, 2005, 332, 623-628

P055

GROWTH INHIBITION OF CANCER CELLS BY AN ACTIVE NEW VITAMIN D₃ DERIVATIVES

Shimon Ben-Shabat, Liron Berkovich, Uliana Levin and Amnon Sintov

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Vitamin D₃ analogs are useful for the treatment of hyperproliferative skin diseases, such as psoriasis vulgaris and are involved in essential cell regulatory processes such as proliferation and differentiation in a number of different cell types including cancer cells. New putative agents (BGP-013 and BGP-015), based on vitamin D₃ structure, were synthesized in our lab. Recent studies in our laboratory showed a significantly high inhibitory action of these compounds against several human cancer cell lines (Prostate carcinoma (LNCaP); breast carcinoma (MCF-7) and colon carcinoma (HT-29)) *in-vitro*. BGP-015 was also found to possess a significantly lower side effects (hypercalciuria and irritability) relatively to the endogenous vitamin D₃ (1,25-diOH vit D₃, calcitriol-CT). In addition, the molecular mechanism of cell death following treatments with these new compounds compared to calcipotriol (CPT, an antipsoriatic vitamin D₃ derivative) and CT was examined using a non-specific pancaspase inhibitor and flow-cytometry analysis

of cell-cycle condition and apoptosis. These results indicate that an apoptotic cell death mechanism is involved in the cytotoxic effect of the new compounds.

Metabolic studies in HaCaT cell (immortalized human keratinocytes) showed that BGP-013 is one of the natural metabolites of CPT that may use as quencher of singlet oxygen. In addition, BGP-013 displayed a significant antiproliferative activity on HaCaT cells, relatively to CPT, indicating its potential to serve as a topical agent for the treatment of psoriasis.

P056

SYNTHESIS OF LICOFELONE BASED ON UTILIZATION OF FENTON'S REAGENT

Stanislav Rádl, Josef Cerný, Ondrej Klecán, Jan Stach

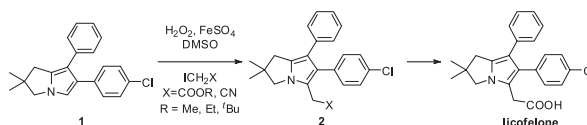
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Licofelone (ML3000),¹ a dual cyclooxygenase/5-lipoxygenase inhibitor developed by Merckle, is the first member of this new class of analgesic and anti-inflammatory drugs currently undergoing evaluation of the phase-III clinical studies for treatment of osteoarthritis.

The only known methods of transformation of key intermediate **1** into licofelone are based on reduction of the 2-oxo acid by classical Wolff-Kishner reduction² or by its modification using NaBH₃CN reduction of the corresponding *p*-toluenesulfonyl hydrazide. In order to circumvent using hydrazine derivatives, we decided to develop a new methodology of transformation of **1** into licofelone suitable for industrial application.

We have developed a new procedure using Fenton's reagent³ in combination with DMSO and an alkyl iodoacetate or iodoacetonitrile giving good yields of **2**. The best results were achieved with DMSO but the reaction proceeds also with other sulfoxides, e.g., dibutyl sulfoxide, tetrahydrothiophene 1-oxide, methyl dodecyl sulfoxide, and methyl phenyl sulfone. Though the corresponding bromo derivatives do not react, in the presence of NaI under similar conditions as with the iodo analogs the bromo esters provide also good yields of **2**. This fact is interesting since the reaction is done in the presence of relatively high amounts of water.

Both the reaction conditions and the reaction scope will be discussed, as well as the transformation of **2** to licofelone.



References

- [1] Rabasseda, X.; Mealy, N.; Castaner, J. *Drugs Fut.* 1995, 20, 1007-1009.
- [2] a) Dannhardt, G.; Kammermaier, T.; Merckle, P.; Striegel, H.G.; Laufer, S. (Merckle) *US 7,078,539* (2006); b) Dannhardt, G.; Steindl, L.; Lehr, M. (Merckle) *US 5,260,451*

(1993); c) Laufer, S.; Augustin, J.; Dannhardt, G.; Kiefer, W. *J. Med. Chem.* 1994, 37, 1894.

[3] a) Walling, C. *Acc. Chem. Res.* 1975, 8, 125; b) Gozzo, F. *J. Mol. Catal. A* 2001, 171, 1.

P057

STRUCTURE-LIPOPHILICITY RELATIONSHIPS IN SERIES OF NEW SUBSTITUTED *N*-PHENYL-PYRAZINE-2-CARBOXAMIDES AS BIOLOGICALLY ACTIVE COMPOUNDS

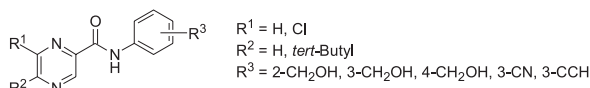
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Compounds possessing in their molecule -NHCO-moiety simulating a peptide bond show a broad-spectrum of biological effects. Various ring-substituted anilides of pyrazine-2-carboxylic acid were prepared and evaluated as potential antimicrobial and herbicidal agents [1-6]. Twenty new substituted *N*-phenylpyrazine-2-carboxamides were analysed using the RP-HPLC method for the lipophilicity measurement. Lipophilicity is one of the most important physical properties of biologically active compounds. It influences the transport of a molecule through cellular membranes, because the drugs cross biological barriers most frequently through passive transport, which strongly depends on their lipophilicity.



In the present study the correlation between RP-HPLC retention parameter $\log k$ and $\log P$ data calculated in various ways are shown, as well as the relationships between the lipophilicity and the chemical structure of the studied compounds are discussed. The distributive parameters π of various substituents are listed for the mentioned studied compounds.

The study was supported by MSM0021620822.

References

- [1] Dolezal, M.; Vicik, R.; Miletin, M.; Kralova, K. *Chem. Pap.* 2000, 54, 245.
- [2] Dolezal, M.; Hartl, J.; Miletin, M. *Folia Pharm. Univ. Carol.* 2000, 25, 15.
- [3] Dolezal, M.; Miletin, M.; Kunes, J.; Kralova, K. *Molecules* 2002, 7, 363.

[4] Dolezal, M.; Palek, L.; Vinsova, J.; Buchta, V.; Jampilek, J.; Kralova, K. *Molecules* 2006, 11, 242.

[5] Dolezal M., Tumova L., Kesetovicova D., Tuma J., Kralova K. *Molecules* 2007, 12, 2589.

[6] Dolezal, M.; Cmedlova, P.; Palek, L.; Vinsova, J.; Kunes, J.; Buchta, V.; Jampilek, J.; Kralova, K. *Eur. J. Med. Chem.* 2008, 43, in press. doi:10.1016/j.ejmech.2007.07.013.

P058

ANTIOXIDANT PROPERTIES OF 4-HYDROXYQUINOLINES

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Quinolines, possess a diversity of biological actions ranging from antimalarial and antimicrobial activity, to cardiovascular, anti-inflammatory and central nervous system effects. Clioquinol and 8-hydroxyquinolines have been reported to act as free radical scavengers and may therefore be beneficial as antioxidants [1]. Antioxidants are useful scavengers of highly reactive oxygen species, which may cause oxidative damage to essential biological structures such as DNA, proteins and lipids and consequently disrupt cellular function [2], processes linked to ageing and neurodegenerative disease. Parkinson's disease an example of a neurodegenerative disorder is highly age-dependent, and oxidative stress, impaired mitochondrial function and alteration in the antioxidant defence system within the brain have besides the destruction of the dopamine pathways also been shown to be major pathogenic factors [3].

In this regard the aim of the study was to synthesise a series of 4-hydroxyquinolines with nitro, amino and *N,N*-dibutyl substitution in the 6 or 7 position. The ability of the compounds to scavenge induced superoxide anions (NBT) and to inhibit induced lipid peroxidation (TBARS) were tested *in vitro* in rat brain homogenates and *in vivo* with MPP⁺ induced oxidative stress in rats [4].

The results of all the biological tests performed indicated that the synthesised 4-hydroxyquinolines had antioxidant activity. The *in vitro* NBT assay afforded values ranging from 4.8 – 34.6 $\mu\text{M}/\text{mg}$ protein with a value of 44.3 $\mu\text{M}/\text{mg}$ protein for the toxin (KCN). Values obtained for the *in vitro* TBARS assay ranged from 0.01 – 0.09 nM/mg tissue with a value of 0.2 nM/mg tissue for KCN. The results from the *in vivo* assays compared well to that of the *in vitro* assays suggesting that the compounds are able to cross the blood-brain barrier. Substitution in position 6 afforded compounds with potential as chain breaking antioxidants, with the aminoquinolines in general having better activity than the nitroquinolines. Substitution in position 7 favoured superoxide anion scavenging ability with 7-nitro-4-hydroxyquinoline being the most effective. The most promising compound identified from this study was 6-amino-4-hydroxyquinoline.

References

- [1] Zheng, H., Weiner, L. M. Bar-Am, O., Epsztejn, S., Cabantchik, Z. I., Warshawsky, A., Youdim, M. B. H. & Fridkin, M. 2005. Design, synthesis, and evaluation of novel bifunctional iron-chelators as potential agents for neuroprotection in Alzheimer's, Parkinson's, and other neurodegenerative diseases. *Bioorganic and Medicinal Chemistry*, 13:773-783.
- [2] Melov, S. 2002. Animal models of oxidative stress, aging and therapeutic antioxidant interventions. *The International Journal of Biochemistry and Cell Biology*, 34: 1395- 1400.
- [3] Giasson, B.I., Ischiropoulos, H., Lee, V.M.-Y. & Trojanowski, J.Q. 2002. The relationship between oxidative/nitrative stress and pathological inclusions in Alzheimer's and Parkinson's diseases. *Free Radical Biology and Medicine*, 32(12): 1264-1275.
- [4] Ottino, P. & Duncan, J.R. 1997. Effect of α -tocopherol succinate on free radical and lipid peroxidation levels in BL6 melanoma cells. *Free Radical Biology and Medicine*, 22(7): 1145-1151.

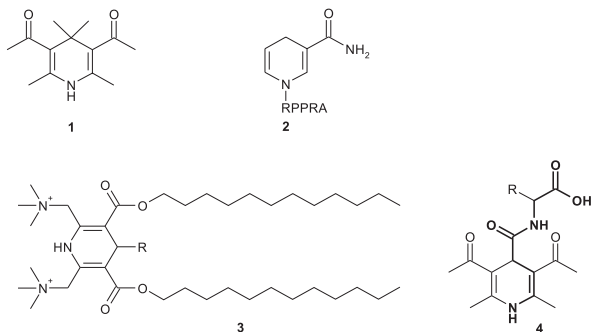
P059

PLEIOTROPIC EFFECTS OF 1,4-DIHYDROPYRIDINE DERIVATIVES - ANALOGUES OF NATURAL PRODUCTS

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Carbonyl-1,4-dihydropyridine derivatives (**1**, 1,4-DHPs) comprise moieties of several classes of natural products: 1,4-dihydronicotinamide (redox active part of coenzymes NAD(P)H **2**), amino acids (carboxy-DHPs are unsaturated cyclic amino acids), lipids (DHPs comprising lipophylic alkyl chains and cationic ammonio groups **4** are amphiphilic lipid type compounds). Appropriately, 1,4-DHPs possess pleiotropic pharmacological properties [1,2]. Besides of well-known calcium ion antagonist (agonist) properties, 1,4-DHPs have electron and hydrogen donating activities. Depending on their structure 1,4-DHPs possess anti-oxidant, radical scavenging properties, they quench active oxygen species. Accordingly, several 1,4-DHP derivatives are anti-mutagens, modifiers of free radical processes, protectors and stabilizers of lecithin liposomes and biological membranes.



N-(1,4-Dihydroisonicotinoyl)amino acid derivatives **3** (dipeptide analogues) and 4-aryl-1,4-DHPs (unsaturated cyclic amino acids) possess neuroprotective, memory-enhancing, anti-ischemic, anti-diabetic, anti-inflammatory, peptide-like long-lasting activities at low concentrations and doses. Unsaturated cyclic amino acid moiety has been used as a template to construct amphiphilic lipid-type compounds possessing self-assembling properties: liposomes are spontaneously formed and lipoplexes with DNA obtained. Such aggregates possess gene transfection properties. The above mentioned data support the opinion that 1,4-DHPs are privileged structures [3,4]; they could be regarded as multifactorial and polyvalent acting pleiotropic agents, analogues of several classes of natural products.

References

- [1] Mason RP, Marche P, Hintze TK *Arterioscl. Thromb. Vasc. Biol.* 23, 2155-2163 (2003).
- [2] Duburs G, Vigante B, Plotniece A, Krauze A, et al. *Chimica Oggi/Chemistry Today*, 2008, in press.
- [3] Triggie DJ *Mini-Reviews in Medicinal Chemistry* 3, 215-223(2003).
- [4] Jacobson KA, Kim Y-C, King BF, *J.Auton.Nerv.Syst.* 81, 152-157(2000).

P060

THE ACTIVITY OF NEW CAGE COMPOUNDS AGAINST INFLUENZA VIRUS H5N1 AND ORTHOPOX VIRUSES

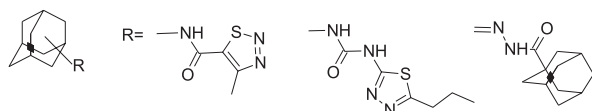
Blakhnin, S., Belanov, E., Golovin, E., Klimochkin, Y., Krasnikov, P., Kuznetsov, S., Leonova, M., Osyanin, V. & Shiraev, A.

Due to high pathogenicity influenza A (H5N1) virus have been the cause of large-scale death in poultry and death of over 140 humans. As regards orthopox viruses, discontinuation of vaccination makes population vulnerable to variola and orthopox infections.

Functional derivatives of cage compounds are as is known one of perspective classes of organic compounds for search of antiviral agents. During our investigation we have synthesized series of functional derivatives of adamantane: amides, hydrazones, amino, hydroxy, carboxy, carbamoylamino derivatives and wide range of adamantyl substituted oxygen, sulfur and nitrogen containing heterocycles. Antiviral activity of synthesized compounds was evaluated against influenza A (H5N1) virus and orthopox viruses: vaccinia, cowpox, mousepox, monkeypox in cell cultures (Vero, MK 2).

Results of biological tests show that most of synthesized compounds reveals antiviral activity to a greater or lesser extent. Among compounds having activity against poxviruses it is necessary to note cage fluoro containing amide, which inhibits reproduction of vaccinia virus in 0.06 μ M concentration and derivative of adamantyl sub-

stituted 1,3,4-thiadiazole shows good potency against cowpox ($IC_{50}=0.09 \mu M$). Also adamantane containing hydrazide has marked antiviral potency against influenza A virus (H5N1), it inhibits their reproduction at $0.5 \mu M$ concentration. Amino derivative, containing adamantylidene unit, suppresses replication of H5N1 virus at $0.7 \mu M$ concentration.



The presence of great number of high active compounds indicates some common principles of antiviral action of compounds, containing saturated cage moiety. It determines route to new virus inhibitors which block M2 ion channels. Structures of compounds having activity against poxviruses allow to suppose that their action occurs at the later stages of viral reproduction.

P061

THERMAL BEHAVIOR AND DECOMPOSITION KINETICS OF ACTIVE COMPOUNDS AND DRUGS FOR SOME CEPHALOSPORINS

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Drugs degrade to different extents on separate exposure to heat, moisture, oxidation and light. Combination of these stresses causes very complex behaviour.

In drug formulations, the presence of excipients adds further complications because the excipients may increase, have no effect on, or decrease the inherent stabilities of the drug. Thermal stability is the simplest to assess, generally by use of the techniques of thermal analysis. This methods may also be used to study possible drug-excipient interactions.¹

TG/DTG and DSC curves provide important information regarding the physical properties of the pharmaceutical compounds (stability, compatibility, polymorphism, kinetic analysis, phase transitions etc).^{2,3}

The aim of this work is to evaluate possible interactions between some pharmaceutical excipients and cephalosporins (active substance).

Thermal decomposition processes of cephalexin, cephadroxil and ceftazidime have been studied by thermogravimetry in comparison with some mixture with excipients containing the same active compounds. The

kinetic parameters, such as activation energy E, pre-exponential factor A and reaction order n, were calculated by TG-DTG techniques with the Freeman-Carroll method, Kissinger method and Ozawa method.^{5,6,7}

The principal conclusion was that the thermoanalytical techniques are very useful to study thermal behavior, compatibility with excipients and kinetic analysis of cephalosporins.

Acknowledgments

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References

- [1] F. Giordano, Cs. Novák, J. R. Moyano, *Thermochim. Acta*, 380 (2001) 123.
- [2] E. Gómez Pineda, A. Martins Ferraresi, J. Ferraresi, A. Winkler Hechenleitner, *J. Therm. Anal. Cal.*, 2005, 79, 259
- [3] F. Rodante, G. Catalani, S. Vecchio, *J. Therm. Anal. Cal.*, 2002, 68, 689
- [4] S. Vyazovkin, C. A. Wight, *Thermochim. Acta*, 1999, 340-341, 14, 53.
- [5] R. Serra, R. Nomen, J. Sempere, *J. Therm. Anal. Cal.*, 52 (1998) 933.
- [6] H.L. Friedman, *J. Polym. Sci.*, C6 (1964) 183.
- [7] T. Ozawa, *J. Therm. Anal.*, 31 (1986) 547.

P063

NEW COMBINED COUMARIN AND BENZO[B]FURAN DERIVATIVES - MICROWAVE-ASSISTED SYNTHESIS, THEIR ¹³C CP/MAS NMR/DFT DEFINED SOLID STATE STRUCTURE, AND PRELIMINARY BIOLOGICAL EVALUATION

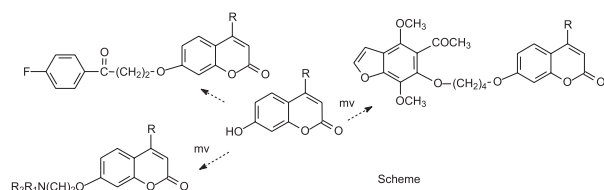
Dorota Maciejewska, Elzbieta Hejchman, Joanna Trykowska

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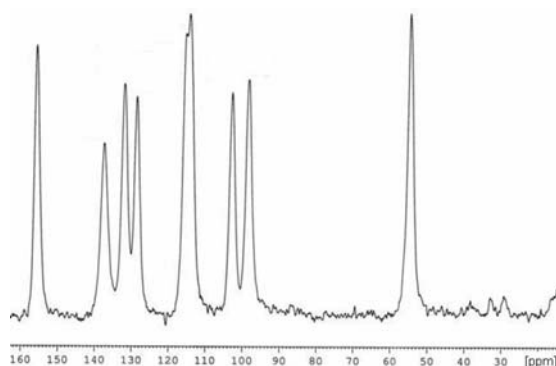
Many coumarins and benzo[b]furans have been identified from plant sources and became interesting due to their diverse biological properties such as anticancer, antioxidant, antimicrobial and antifungal activities. 7-Hydroxycoumarin has been reported to inhibit the proliferation of a number of human malignant cells *in vitro* as well as to demonstrate activity against prostate cancer, malignant melanoma and metastatic renal cell carcinoma. Derivatives of benzo[b]furan, for example 5-acetyl-6-hydroxy-4,7-dimethoxybenzofuran (khellinone), showed weak but unusually selective activity as a potassium-channel blocker, and their symmetrical dimers linked *via* the alkylation of the 7-hydroxy groups showed enhanced activity. Therefore we have adopted the substituted benzo[b]furan and 7-hydroxycoumarin systems as pharmacophores and introduced the linker to increase the

flexibility of the target compounds. We also developed conditions for O-alkylation 7-hydroxycoumarins with haloalkyl amines to create new potentially bioactive compounds.

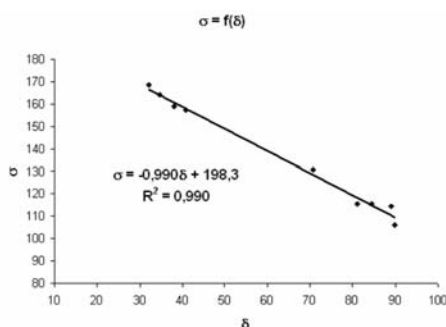
In the poster we present efficient microwave-assisted synthesis (Scheme):



and structural data based on theoretical calculations at DFT level of theory of the ¹³C atoms shielding constants for selected conformers and ¹³C CP/MAS NMR resonances.



Exemplary ¹³C CP/MAS NMR spectrum in the solid state



Linear correlation between the shielding constants and the chemical shifts for analyzed molecular conformation

Preliminary anticancer and antibacterial assay will be also presented.

Acknowledgement

The authors thank Interdisciplinary Center for Mathematical and Computational Modeling (ICM) of Warsaw University for the computational grant.

P064

THEORETICAL MODELS OF BIOLOGICAL ACTIVITY OF *BIS*-AMIDINES BASED ON INTERMOLECULAR INTERACTIONS IN THE MINOR GROOVE OF DNA

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To prevent *Pneumocystis carinii* pneumonia (PCP) in case of patients with weakened immune systems (suffering from AIDS, cancer, inherited immune deficiencies or after organ transplants), a *bis*-amidine aromatic compound – pentamidine isethionate (Figure 1) is used as a drug of second choice.

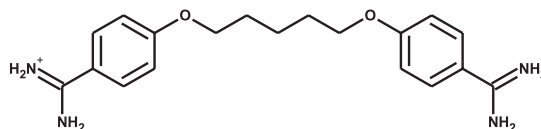


Figure 1. Structure of pentamidine.

The acute toxicity originally associated with pentamidine, have prompted investigations for new compounds with greater potency combined with lower toxicity.

The binding affinity of *bis*-amidines to poly(dA) - poly(dT) DNA fragment correlated quite well with their *in vitro* activity against PCP. The DNA binding affinity of ligands could be estimated by measurements of DNA phase transition temperature T_m . The higher this temperature is the more stable DNA-ligand complex is formed.

In order to elucidate the correlation between the theoretically estimated binding energies of *bis*-amidines-DNA complexes and the experimental differences of T_m temperature between free and bounded DNA we have investigated four models. In two of them the explicit solvent molecules were considered and in the next two models the solvent was only simulated (implicit solvent molecules).

The atomic coordinates of complex pentamidine and the DNA dodecamer d(CGCGAATTCGCG)₂ were obtained from the RCSB Data Bank. The geometries of all ligands were optimized using density functional theory (DFT) with B3LYP/6-311+G hybrid functional. The atomic partial charges on the atoms of *bis*-amidines were computed using Breneman model, reproducing the molecular electrostatic potential. The geometries of each structures were optimized using the methods of molecular mechanics (MM) and molecular dynamics (MD). All MM calculations were carried out using the AMBER force-field with energy gradients lower than 0.1 kcal/(mol·Å).

Models with implicit solvent molecules predicted melting temperatures with a better accuracy than models derived from the simulations with explicit solvent molecules. Using most accurate model ($R^2=0.939$), four derivatives of pentamidine with better binding affinities to DNA, were proposed for further investigations.

The results discussed in this work were obtained using resources of Interdisciplinary Centre for Mathematical and Computational Modelling Warsaw University.

P065

IDENTIFICATION OF SVM-BASED CLASSIFICATION MODEL, SYNTHESIS AND EVALUATION OF PRENYLATED FLAVONOIDS AS VASORELAXANT AGENTS

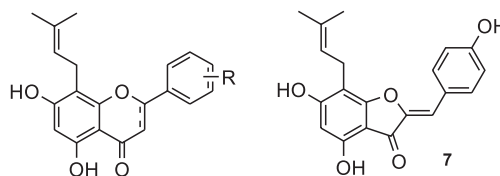
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Cardiovascular diseases are the main cause of death in most countries. One of the main reasons in cardiovascular diseases is involvement of vascular tissues through increasing tonicity or losing their capacity to relaxation. Therefore, vascular studies have been paid much attention and the vasodilator agents would be beneficial in treating cardiovascular disease.

In recently year, flavanoids have been recognized as compounds with potent biological activities that may be active in prevention of cardiovascular disease. And several flavonoids have been found to exhibit vasodilator effects in isolated vascular preparation and animal models [1-2]. Among this group of phytochemicals, prenylated flavonoids are attracting more and more attention, because prenyl groups increase the lipophilicity and confer to the molecule a strong affinity to biological membranes [3]. Despite of intensive studies for prenylated flavonoids, little studies have been reported about their vasodilator effect and the structure-activity relationships.

In the present work, a database consists of 398 compounds (training set of 278 compounds, test set of 120 compounds) taken from literatures were employed. They were divided into two classifications (active and inactive). Nine descriptors (D/D, Jhetp, TIC1, SEigp, SP20, RDF030m, Mor06u, ATS8v and JGI1) calculated by drag-on software were selected in order to establish classification model using support vector machine (SVM) technique. The model obtained in the SVM research provided correct classification in the training set of 93.8% and in the test set of 85.8%, with cross validation accuracy of 0.807, which revealed the reliability of this model. Furthermore, seven prenylated flavanoids **1-7** were synthesized (Fig. 1) and the vasodilatory activity of these compounds was subsequently evaluated using SVM based model and rat thoracic aorta rings against phenylephrine (PE) induced contraction model. The results obtained are shown in Table 1. The theoretical results are in good agreement with the experimental values. SAR analysis showed that the order of potency for vascular relaxation was as follows: flavanone > flavone > aurone.



- 1: R=3'-OMe, 4'-OH, 2, 3 sigle bond; 2: R=3', 4'-CH₂OCH₂-, 2, 3 sigle bond;
3: R=4'-OH, 2, 3 sigle bond; 4: R=3'-Br, 2, 3 sigle bond;
5: R=3'-OMe, 4'-OH, 2, 3 double bond; 6: R= 3'-Br, 2, 3 double bond

Fig. 1 The structure of synthesized prenylated flavnoids.

Table 1. The vasorelaxant activity induced by compounds **1-7**, in rat aorta rings pre-contracted with PE.

Entry	EC ₅₀ (μM)	E _{max} (%)	Classification ^a
Quercetin	244.0	91.3	active
1	101.0	95.8	active
2	24.5	74.1	active
3	77.3	106.5	Active
4	20.0	70.5	active
5	493.0	70.6	active
6	1000.0	70.6	inactive
7	7160.0	74.7%	inactive

a: The classification of compounds was provided by SVM model.

Reference

- [1] M. Cross, *Pharmaceutical Biology*, 2004, 42, 21.
- [2] S. E. Rasmussen, Functional foods, cardiovascular disease and diabetes, *woodhouse*, 2004, 157.
- [3] D. Barron, C. Baland, F. Possety, P. Ravanel. A. Desfougeres, *Acta Botanica Gallica* 1996, 143, 509.

P067

THEORETICAL STUDY OF INCLUSION COMPLEXES BETWEEN β-CYCLODEXTRIN AND GUANIDINE/IMIDAZOLINE ANALOGS

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In this research was developed new method for separation of fifteen guanidine/imidazoline derivatives, acting as antihypertensive drugs, using capillary electrophoresis (CE) with β-cyclodextrin (BCD) in running buffer. The complete CE separation of the guanidine/imidazoline derivatives was achieved by employing 30 mM phosphate background electrolyte (pH 1.5) containing 15 mM of BCD. The analytical results were used for quantitative structure-retention relationship (QSRR) study of the ligand-BCD inclusion complexes at the experimental pH 1.5. After MOPAC/PM₃ optimization of the models, was applied the CS Gaussian 98 program [2], using the B3LYP/6-31G+(d,p) and Hartree-Fock/3-21G (d) method, for molecular parameters computation. Furthermore, energy of com-

plexation (ΔE) and formation enthalpy (ΔH) of the complexation, between the ligands and BCD, were calculated and correlated with $\log(t)$ value.

Total charge of the analyzed compounds at experimental pH 1.5, HOMO (BCD-ligand) energy and SAS (BCD-ligand) area account for the electrophoretic retention time ($\log(t)$). The multiple linear regression models with three variables, $\log(t) = f(\text{Total Charge (ligand)}, \text{SAS (BCD-ligand)}, \text{HOMO (BCD-ligand)})$, was obtained with $R^2 = 0.914$ and cross-validation parameter of prediction $q^2_{\text{pre}} = 0.778$. Relatively high regression and validation parameters of the QSRR model are indicating on its good prognostic capacity. Therefore, in the early stages of the analysis of related guanidine/imidazoline analogs, it is becoming important to compute SAS (ligand-BCD) area, HOMO(ligand-BCD)/HF/3-21G (d)^{gas} energies, total charge of the ligand at pH 1.5, and to use the developed QSRR model for evaluation of their electrophoretic migration and separation behaviors.

P068

SYNTHESIS OF NOVEL POTENT ANTIPLATELET AND ANTI-INFLAMMATORY DIARYLPYRRAZOL DERIVATIVES

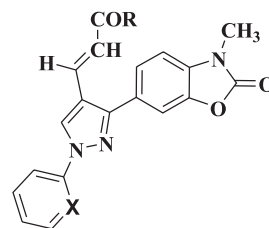
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It is well known that inhibition of the enzyme cyclooxygenase (COX) is the principal mechanism for the efficacy of NSAIDs. Among all the COX isomers selective inhibition of COX-2 which is being induced during inflammation is of interest since the resulting drugs lack the gastric side effects associated with classical NSAIDs [1].

Thromboxane A₂ (TXA₂), a short-live lipidic mediator generated by the cyclooxygenase pathway, is mainly produced by platelets, macrophages and lung parenchyma. TXA₂ is a potent platelet activator and constrictor of vascular and bronchial smooth muscles. Prostacyclin (PGI₂), another product of cyclooxygenase pathway, is a potent endogenous inhibitor of platelet function and possesses a strong vasodilator effect. Furthermore, PGI₂ is currently presented as the physiologic antagonist of TXA₂. So, balance between TXA₂ and PGI₂ is crucial for the cardiovascular system [2].

The recent doubts about cardiovascular toxicity of COX-2 inhibitors prompted us to synthesize dual inhibitor of COX-2 and thromboxan synthase enzymes. We have aimed to have safer COX-2 inhibitor by suppressing formation of TXA₂ by COX-1 at platelets to protect balance between TXA₂ and PGI₂. Thus, we have designated and synthesized novel compounds which bear a central pyrazole ring whose 1,3,4-positions are substituted with phenyl/pyridyl, 3-methyl-2(3H)-benzoxazolinone moieties and substituted cinnamic acid residues, respectively. The preparation of 1,5-diarylpyrazole derivatives to screen their COX-2 and thromboxan synthase inhibitory activities is currently under investigation in our laboratory.



References

- [1] Menozzi G, Merello L, Fossa P, Mosti L, Piana A, Mattioli F., *Il Farmaco*, 58, 795-808, 2003.
- [2] De Leval X, Hanson J, David JL, Masereel B, Pirotte B, Dogne JM., *Curr. Med. Chem.* 11, 1243-52, 2004.

P069

FENOFIBRATES RAISES PLASMA HOMOCYSTEINE LEVELS IN TUNISIAN HYPERLIPIDEMIC PATIENTS

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Introduction. Fibrates are an important class of drugs for the management of dyslipidemia. Recently, it has been shown that some of the most prescribed fibrates cause hyperhomocysteinemia, which itself has been recognised as a cardiovascular risk factor. In this study, we investigated the effect of fenofibrate on total homocysteine levels in Tunisian hyperlipidemic patients.

Methods. Twenty two patients (mean age 53.0 ± 5.6 years) with hypertriglyceridaemia, who had elevated plasma triglyceride levels (> 2,3 mmol/l) were studied. Patients did not receive any lipid-lowering drugs in the 6 week period before the study. During the study, they received either 200 mg micronised fenofibrate once day. At baseline and after 6 weeks of therapy, venous blood samples were taken after an overnight fast measurement of lipids and total homocysteine (by gas-chromatography mass-spectrometry with isotopic dilution).

Results. Fenofibrate treatment was associated with an increase in fasting tHcy (Baseline: 13.2 ± 3.4 µmol/l versus Fenofibrate: 17.0 ± 4.0 µmol/l, P = 0.000). The incidence of hyperhomocysteinemia (defined as tHcy level > 15 µmol/l) was at baseline: 3/22 (13%) and after treatment Fenofibrate: 10/22 (45%) (c² = 3.93, P = 0.047). There was no correlation between changes in plasma triglyceride levels and total homocysteine levels.

Conclusion. Our study confirms that the induction of elevations in plasma homocysteine by fenofibrate treatment. It may, for example, be reasonable to counteract the increase of total homocysteine by concurrent application of agents known to lower homocysteine, such as folate or vitamins B6 and B12.

P070

DESIGN AND SYNTHESIS OF SELECTIVE IAG-NUCLEOSIDE HYDROLASE INHIBITORS AS POTENTIAL ANTIPARASITIC AGENTS

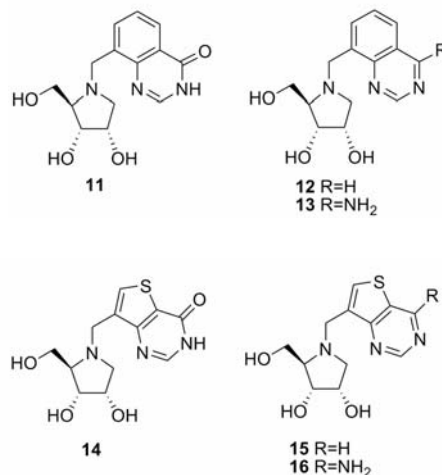
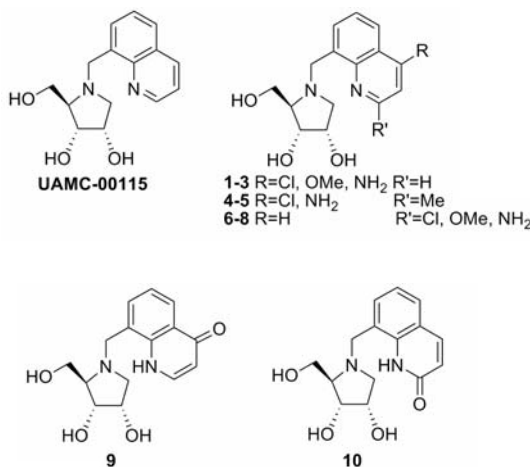
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Trypanosomiasis (sleeping sickness, Chagas' disease) is caused by parasites of the genus *Trypanosoma* and is in tropical and subtropical regions still a big medical and social problem. The existing medicines have limited activity, are toxic and lose efficacy because of growing resistance. This urges the search for new drugs against trypanosomiasis.¹ The *Trypanosoma* parasites, unlike mammals, are not able to synthesise purines *de novo* and are dependent on the *purine salvage pathway*. Purine bases are obtained by cleavage of the *N*-glycosidic bond of nucleosides by *nucleoside hydrolase* (NH).² This enzyme is absent in mammals and therefore NH might be a good target for the development of new anti-trypanosomal compounds.

In our laboratory we are interested in inhibitors against a purine-specific enzyme (IAG-NH) from *T. vivax*.³ Previous research resulted in our lead compound, 1,4-dideoxy-1,4-imino-*N*-(8-quinolinyl)methyl-D-ribitol (UAMC-00115).⁴ Molecular modeling showed the main interactions in the active centre of IAG-NH: aromatic stacking, quinolin-*N*-Asp40 interaction and multiple interactions of the iminoribitol moiety. The goal of the here presented research was to establish a structure-activity relationship (SAR), by introducing various substituents on different positions of the quinoline ring (1 to 10) and by modifying the ring (11 to 16), and to discriminate against the human nucleoside phosphorylase (hPNP).⁵



Different substituted 8-bromomethylquinolines, 8-bromomethylquinazolines and 7-bromomethylthienopyrimidines were synthesised. These were coupled to the *O*-protected iminoribitol, followed by deprotection. The target compounds were tested on isolated *T. vivax* IAG-NH and hPNP. Final compounds 1 to 3 and 11 to 16 showed IAG-NH inhibitory activities in the nanomolar range similar to the lead compound and discriminated against hPNP.⁵

[1] Barrett, M.P. *Lancet* 2003, 362, 1469-1480.

[2] El Kouni, M.H. *Pharmacol. Ther.* 2003, 99, 283-309.

[3] Versées, W., Decanniere, K., Van Holsbeke, E., Devroede, N., Steyaert, J., et al., *J. Biol. Chem.* 2002, 277, 15938-15946.

[4] Goeminne, A., Berg, M., McNaughton, M., et al., *Bioorg. Med. Chem.* 2008, doi: 10.1016/j.bmc.2008.05.056

[5] Berg, M., Bal, G., Goeminne, A., et al., Submitted in *ChemMedChem*.

P071

DUAL INHIBITION OF MONOAMINE OXIDASE B AND ANTAGONISM OF THE ADENOSINE A_{2A} RECEPTOR BY (E,E)-8-(4-PHENYLBUTADIEN-1-YL)CAFFEINE ANALOGUES

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Abstract – The adenosine A_{2A} receptor has emerged as a target for the treatment of Parkinson's disease (PD). Antagonists of the A_{2A} receptor (A_{2A} antagonists) partially alleviate the symptoms of PD, delay the development of motor complications and may also slow the underlying

neurodegenerative process. Several members of the (*E*)-8-styrylcaffeine class of A_{2A} antagonists have been reported to also act as potent reversible inhibitors of monoamine oxidase B (MAO-B). Dual-target-directed drugs that block both MAO-B and A_{2A} receptors may have enhanced value in the management of PD since MAO-B inhibitors are employed as anti-parkinsonian agents. In an attempt to identify additional dual-acting compounds, we have, in the present study, prepared (*E,E*)-8-(4-phenylbutadien-1-yl)caffeine analogues. The (*E,E*)-8-(4-phenylbutadien-1-yl)caffeine analogues were found to be exceptionally potent reversible inhibitors with enzyme-inhibitor dissociation constants (*K_i* values) in the low nano molar range. Furthermore, they also acted as potent A_{2A} antagonists with *K_i* values, also in the low nano molar range. We conclude that (*E,E*)-8-(4-phenylbutadien-1-yl)caffeines are a promising candidate class of dual-acting compounds.

P072

SYNTHESIS AND CYTOTOXIC ACTIVITY OF NOVEL AMIDINE ANALOGUES OF BIS(2-CHLOROETHYL)-AMINE

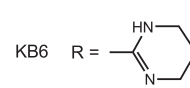
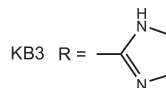
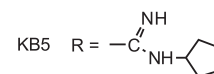
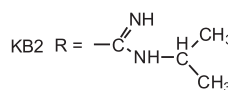
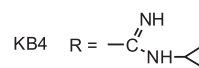
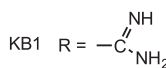
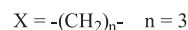
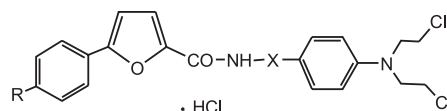
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Cytotoxic agents that act by covalent modification of DNA were the first modern anticancer chemotherapeutics and remain components of combination chemotherapy regimens. We obtained series of amidine analogues of bis(2-chloroethyl)amine differing by the nature of terminal basic side (**KB1-KB6**). Evaluation of the cytotoxicity of these compounds employing a MTT assay and inhibition of [³H]thymidine incorporation into DNA in both MDA-MB-231 and MCF-7 human breast cancer cells demonstrated that these compounds were more active than melphalan and chlorambucil. Because the antiproliferative effect of compounds **KB1-KB6** is independent of the estrogen receptor status of the breast cancer cells, these potent inhibitors are potential pharmacological agents for the treatment of both hormone responsive and nonresponsive breast cancer cells.

To test whether cytotoxic properties were related to DNA-binding, the compounds **KB1-KB6** were evaluated in a cell-free system. The binding affinities of compounds **KB1-KB6**, netropsin and distamycin to calf thymus DNA, T4 coliphage DNA, and synthetic polymers poly(dA-dT)₂ and poly(dG-dC)₂ were compared by using the ethidium displacement assay. The compounds **KB1-KB6** were found to interact with a GC base pair though the binding affinity were weak compared with that for an AT base pair. Since calf thymus DNA contains random sequences and therefore fewer AT sites than poly(dA-dT)₂, the selec-

tivities of ligands are further demonstrated by their much weaker binding to calf thymus DNA compared to poly(dA-dT)₂. The AT base pair specificity shown by these compounds could be the result of the preferential binding of highly electropositive amidines **KB1-KB6** to the most electronegative region of the DNA, the AT base pairs in the minor groove.



P073

STRUCTURE-ACTIVITY RELATIONSHIPS AMONG AMIDINE ANALOGUES OF ALKYLATING AGENTS

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A drawback common to all DNA alkylating agents is their high chemical reactivity. Other limitations, discussed particularly for mustards, are a lack of intrinsic DNA binding affinity of the core *N,N*-bis(2-chloroethyl)amine pharmacophore, and a requirement for bifunctional crosslinking of DNA to be fully cytotoxic. For these reasons there has been much interest in the concept of specifically targeting "simple" mustards and other alkylators to DNA by attaching them to DNA affine carrier molecules, since this could in principle address these limitations. Numerous nitrogen mustard have been modified during the last 30 years in order to increase their cytotoxicity and target affinity. Among them are amidine analogues of alkylating drugs [1-4]. It was found that the amidine moiety of the drugs may direct the alkylation both sequence specifically and regiospecifically [1-4]. Increasing the concentration of drug in the vicinity of the DNA would mean less chance of losing active drug by reaction with other cell components. Additionally, the use of DNA-affine carriers with their own defined binding geometry makes it possible to alter both the region and sequence specificity of alkylation compared with that of the "simple" mustards. The

present study was undertaken to extend our recent findings related to antineoplastic activity of new amidine analogues of alkylating agents [1-4]. A molecular mechanics and molecular dynamics approach was used to examine the structure of complex formed between the d(CGC-GAATTCGCG)₂ duplex and amidine analogues of alkylating agents. It is predicted that the amidine analogues of alkylating agents should have a decreased affinity for the minor groove of AT-rich regions in comparison to netropsin, and furamidine. From the energetic analysis it appears that van der Waals and electrostatic interactions are more important than specific hydrogen bonds in stabilizing the ligand-duplex DNA.

The binding of amidine analogues of alkylating agents in the minor groove of DNA may prevent binding of regulatory proteins or transcription factors to DNA promoters, as has been shown for other amidines. It is well known that sites of topoisomerase II mediated DNA cleavage are not randomly distributed in DNA fragments, hence this enzyme seems to recognize specific sequences in a given substrate. This topological enzyme binds at least in part to AT rich sequences in the minor groove of B-DNA. The ability of amidine analogues of alkylating agents to inhibit topoisomerase II activity was quantified by measuring the action on supercoiled DNA substrate as a function of increasing concentration of the ligands by the use of agarose gel electrophoresis. These results demonstrated that amidine analogues of alkylating agents have topoisomerase II (Topo II) inhibitory activity with 50% inhibitory concentrations (IC₅₀) ranging from 5 to 50 μ M. In this study, also the effects of the amidine analogues of alkylating agents on collagen and DNA biosynthesis, α 1-integrin receptor, IGF-I receptor, FAK, Shc, Grb-2, Sos-1 and phosphorylated MAP-kinases (ERK1 and ERK2) expression in human breast cancer cells were compared to those evoked by melphalan and chlorambucil.

References

- [1] Bielawska A, Bielawski K, Wolczynski S, Anchim T. Arch Pharm Pharm Med Chem 2003;336:293-299.
- [2] Bielawska A, Bielawski K, Muszynska A. Farmaco 2004; 59: 111-117.
- [3] Bielawski K, Bielawska A, Sosnowska K, Mityk W, Winnicka K, Palka J. Biochem Pharmacol 2006;72:320-331.
- [4] Bielawska A., Bielawski K., Anchim T. Arch Pharm Chem Life Sci 2007;340:251-257.

P074

A NOVEL SEROTONIN 5HT_{2C} AGONIST WITH 5HT_{2A/2B} INVERSE AGONIST ACTIVITY DEMONSTRATES ANTIPSYCHOTIC EFFICACY WITHOUT WEIGHT-GAIN LIABILITY

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The serotonin 5HT₂ receptor family consists of the 5HT_{2A}, 5HT_{2B}, and 5HT_{2C} membrane-bound G protein-coupled receptors (GPCRs) that signal primarily through G α_q protein to activate phospholipase C (PLC) and formation of inositol phosphates (IP) and diacylglycerol second messengers. The human 5HT_{2C} receptor was cloned in 1991 and is found exclusively in brain where it is putatively involved in several (patho)- physiological and psychological processes. There is compelling evidence for a pharmacotherapeutic role especially for 5HT_{2C} receptor agonists in psychosis, cocaine addiction, and several other neuropsychiatric disorders, as well as, obesity. Although the importance of the 5HT_{2C} receptor as a pharmacotherapeutic target has been apparent for about 10 years, no 5HT_{2C} receptor-specific drugs have been developed. Drug discovery targeting the 5HT_{2C} receptor is challenging because 5HT_{2C} shares transmembrane domain sequence identity of ~80% with the 5HT_{2A} receptor and ~70% with the 5HT_{2B} receptor. The highly conserved transmembrane domains and similar second messenger coupling complicates development of especially agonist ligands selective for the 5HT_{2C} receptor. Meanwhile, activation of brain 5HT_{2A} receptors produces psychomimetic effects and activation of peripheral 5HT_{2B} receptors produces cardiac valvulopathy and pulmonary hypertension. Accordingly, there is intense interest regarding development of genuinely selective serotonin 5HT_{2C} receptor agonists – all 5HT_{2C} agonists reported, so far, also activate serotonin 5HT_{2A} and/or 5HT_{2B} receptors. Results reported here, however, document that a novel small molecular weight compound synthesized in our laboratory, PNA-6 (proprietary structure revealed June 2008 and at ISMC), is a full-efficacy agonist for human serotonin 5HT_{2C} receptors, plus, it is a competitive antagonist and inverse agonist at serotonin 5HT_{2A} and 5HT_{2B} receptors. Moreover, PNA-6 penetrates rodent brain after peripheral administration to inhibit MK801- and amphetamine-induced locomotor activity, reduce food consumption, and, produce weight loss. These neurobehavioral activities are predictive of antipsychotic efficacy with concomitant anorectic activity. The affinities (K_i) of PNA-6 at recombinant human serotonin 5HT_{2A}, 5HT_{2B}, and 5HT_{2C} receptors expressed in HEK cell membranes are 410, 1200, and 37 nM, respectively. When incubated with HEK cells expressing human 5HT_{2C} receptors, PNA-6 and serotonin activate PLC/[³H]-IP formation with EC₅₀ ~ 20 and 7 nM, respectively. Relative to serotonin, PNA-6 is a full-efficacy 5HT_{2C} receptor agonist regarding activation of PLC/[³H]-IP formation. For cells expressing 5HT_{2A} and 5HT_{2B} receptors, (–)-trans-PAT demonstrates inverse agonist activity, with IC₅₀ values of 490 and 1,000 nM, respectively, for inhibition of constitutive activity of PLC/[³H]-IP formation. Moreover, (–)-trans-PAT is a competitive antagonist of serotonin activation of 5HT_{2A} and

5HT_{2B} receptor-stimulated PLC/[³H]-IP formation, with K_B values of $0.46 \pm 0.11 \mu\text{M}$ ($pA_2 = 6.3$) and $1.4 \pm 0.8 \mu\text{M}$ ($pA_2 = 5.8$), respectively. All PNA-6 concentration–response data curves will be included in poster or oral presentation at ISMC. After intraperitoneal (IP) administration to mice, PNA-6 (10 mg/kg) significantly inhibits locomotor activity (to vehicle control level) induced by MK801 and amphetamine – this antipsychotic activity of PNA-6 was comparable to the selective 5HT_{2A} antagonist M100907. In other experiments, after administration to mice, PNA-6 (10mg/kg, IP) significantly reduced food consumption (effect comparable to 6 mg/kg *d*-fenfluramine) and reduced basal body weight by ~3% in 24 hr – tolerance did not develop to the anorectic effect of PNA-6 when administered daily over 4 days. Experiments for complete dose–response data curves continue and results will be reported at ISMC. In summary, PNA-6 has multi-functional activity at multiple 5HT₂ receptor subtypes (5HT_{2C} agonism and 5HT_{2A/2B} inverse agonism) that is consistent with new pharmacotherapeutic paradigms utilizing drugs with a multi-faceted pharmacological profile that appear to be more effective than single-target drugs in treatment of psychosis and other complex neuropsychiatric disorders. Moreover, the anorectic effects of PNA-6 suggest that it will not cause weight-gain effects problematic with some currently-used antipsychotic drugs. Support for this research is from US NIH Grants to RG Booth (RO1–MH068655, MH081193, DA023928).

P075

SMALL IS BEAUTIFUL: THE DISCOVERY OF SMALL MOLECULE MOTILIN RECEPTOR AGONISTS AS GASTROPROKINETIC AGENTS

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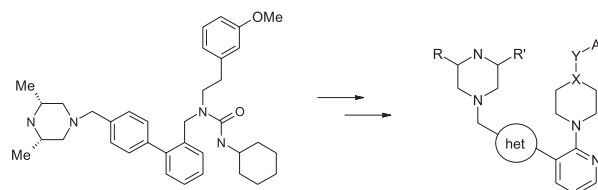
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The peptidic hormone motilin has a significant influence on gastric motility in man, through induction of antral and duodenal contractions, thus promoting gastric emptying and intestinal transit. The 22 amino acid peptide is the endogenous ligand of the class A, 7-TM receptor known as the motilin receptor (formerly known as GPR38) which is predominantly expressed on enteric neurons of the upper GI tract of humans and some other species.¹

The antibiotic erythromycin possesses agonist activity at the motilin receptor, which contributes to the gastrointestinal side effects often observed with this drug. Erythromycin has been used “off-label” to treat gastroparesis and functional dyspepsia, conditions which have

symptoms ranging from bloating to nausea and vomiting.² There is considerable interest in related macrolides devoid of antibiotic activity. However, the development of a non-peptide, non-macrolide small molecule motilin agonist would offer an alternative approach for this area of unmet medical need.

This poster will describe the journey from our HTS hit with its relatively high molecular weight and lipophilicity to several more attractive lead compounds, of the general structure shown below:



References

- [1] Feighner, S.D.; Tan, C.P.; McKee, K. K.; Palyha, O. C.; Hreniuk, D. L.; Pong, S. S.; Austin, C. P.; Figueroa, D.; MacNeil, D.; Cascieri, M. A.; Nargund, R. *et al*, *Science*, 1999, 284, 2184-2188.
- [2] Peeters, T., Potential of motilides in the treatment of hypomotility syndromes in *Problems of the Gastrointestinal Tract in Anaesthesia*, Herbert, M. K. *et al* (eds), Springer-Verlag, Berlin, Heidelberg, 1999, pp 39-51.

P078

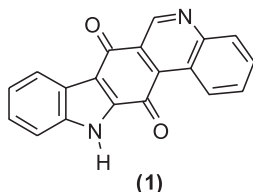
TOWARDS THE DEVELOPMENT OF THE NATURAL PRODUCTS CALOTHRIXINS AS CHEMOTHERAPEUTICS

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Secondary metabolites continue to provide a plethora of bioactive natural products as lead structures for the development of therapeutics. From these natural products, non-natural compounds are designed in order to study structure-activity-relationships so as to enable a better understanding of the pharmacophore that is required for biological activities. As part of our synthesis programme at the Institute of Chemical and Engineering Sciences and in view of the strong biomedical interest in Singapore, we are developing synthetic routes to a number of natural products and related analogues. One such natural product is calothrixin B (**1**), a secondary metabolite which was isolated from the *Calothrix* cyanobacteria in 1999.¹ Calothrixin B and the *N*-oxide, calothrixin A, have a novel pentacyclic structure comprising the indole,

quinone and quinoline moieties. Both calothrixin A and B were shown to inhibit the *in vitro* growth of the chloroquine-resistant strain of the human parasite, *Plasmodium falciparum* in a dose-dependent manner. They were also found to kill several cancer cell lines, all with excellent potency, with IC₅₀ values at nanomolar concentrations. This talk will provide an overview of our progress towards the synthesis of the calothrixins and structurally related quinones. In addition, the structure-activity studies of calothrixins and closely related quinones will also be reported.



Reference

- [1] Rickards, R. W., Rothschild, J. M., Willis, A. C., de Chazal, N. M., Kirk, J., Kirk, K., Saliba, K., J. and Smith, G. D., *Tetrahedron*, 55, 13513-20 (1999).

P079

ELECTROCHEMICAL BEHAVIOR OF MEDICAL PREPARATION - LEVOMEPRMAZINE

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Psychotropic preparation levomepromazine influencing the central nervous system have antipsychotropic effect on human emotional sphere. Study of electrochemical behaviour of medical preparation is of great importance that the penetration of substance into the human cell and its dissemination should be processed by adsorption process of the medical preparation on the boundary between cell membrane and liquid. Chemical composition of levomepromazine [10-3-dimethylamino-2-methylpropyl]-metoxiphenothiazine hydrochloride] are aliphatic derivatives of phenothiazine.

The adsorption of levomepromazine was studied using impedance method on a stationary Hg electrode by measuring the differential capacity (C) as a function of the electrode potential (E). Polarographic data were obtained using the dropping mercury electrode. All the values of potential were measured with respect to saturated calomel electrode.

Polarographic study of levomepromazine in neutral supporting electrolyte showed diffusion, single-wave, irreversible polarograms with half-wave potential $E_{1/2} = -1.72$ V.

Levomepromazine has high adsorption ability in the range of zero charge potential with maximum value. We have calculated attrucshen constand for levomepromazine.

P080

SYNTHESIS AND BIOLOGICAL EVALUATION OF ISOPHTHALATE DERIVATIVES AS T-TYPE CALCIUM CHANNEL BLOCKER

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The Ca²⁺ channel blockers have been used extensively in the treatment of hypertension and angina pectoris. Most of these Ca²⁺ channel blockers interact predominantly or exclusively with the L-type calcium channel. However, these drugs, to some extent, feature unwanted effects such as negative inotropism, atrioventricular blockade or neurohormonal activation. Thus, presently more attention has been paid on the development of selective T-type blockers for the reduction of these side effects. T-type calcium channel is also recently known as a good therapeutic target for the treatment of neuropathic pain. Since the withdrawal of mibefradil, a selective T-type calcium channel blocker, there have been a lot of efforts to develop T-type calcium channel blockers. A small molecule library of isophthalate derivatives was designed, synthesized and biologically evaluated for T-type calcium channel blocking activity. Among those compounds synthesized, a compound shows the most potent T-type calcium current blocking activity with an IC₅₀ value of 1.52 M, which is comparable to that of mibefradil. The lead compound has been optimized to improve physical properties of the compound as well as the T-type calcium current blocking activity. The process of lead optimization will be discussed in detail.

P083

INHIBITION OF HCV IRES-DEPENDENT TRANSLATION BY RNA MOLECULES

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Hepatitis C virus (HCV) infection is a serious health problem affecting to 3% of the world population. Current treatments based on the combination of generic antiviral compounds trigger a sustained virological response in only 40% of infected individuals. This is mainly due to the quasispecies structure of the RNA genome, which favors the appearance of resistant viral variants. Despite this high variability, a significant rate of conservation exists in the untranslatable regions (UTR) that flank the coding sequence and perform essential functions for HCV propagation, as viral proteins translation and genomic replication. These features make them excellent candidates for

viral targeting. The use of nucleic acids as gene silencers in combinatorial therapies is a very promising approach that is currently being investigated. We isolated a chimeric RNA molecule (HH363-50) by a previously described *in vitro* selection method. It is composed by two different inhibitors: a hammerhead ribozyme targeting position 363 of the HCV genome (HH363) and an aptamer of the essential stem-loop structure containing the translation start codon in the IRES region (Ap50). HH363-50 effectively blocks IRES-dependent translation *in vitro* up to 80%. *Ex vivo* assays confirm the inhibitory activity of HH363-50: IRES-dependent translation was significantly inhibited both in Huh-7 cells transiently transfected with a reporter construct and in a replicon system, where the combination of HH363-50 with IFN- α reduced HCV RNA levels up to 80%. The RNA inhibitor interferes with the formation of a translationally active complex, stalling its progression at the level of the 80S particle formation. This fact nicely correlates with its inhibitory effect and make it a likely explanation for the mechanism of action of HH363-50. Taken together, our results allow for proposing HH363-50 as an effective anti-HCV compound and firmly accredit the development of antiviral tools based on RNA molecules.

P084

ISOINDOLE DERIVATIVES ACTIVE AGAINST *PLASMODIUM SPP*

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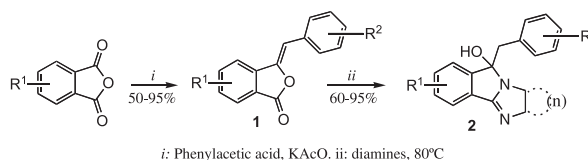
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Despite massive efforts to control malaria, still remains one of the most devastating tropical diseases, mainly affecting poor populations in developing countries. The global morbidity and mortality due to malaria have not significantly changed over the past 30 years, 300-500 million cases with 1.5-2.5 million deaths every year. The continued (or rising in some areas) rate of malaria infections is mainly attributable to the spread of drug resistant strains of *Plasmodium*, resistance of vector to insecticides, demographic growth with ensuing deterioration of living, uncontrolled migrations through endemic areas. Other facts include the failure to apply existing effective drugs in areas where they would be of most benefit. The need for new and effective antimalarial drugs is therefore an urgent priority to expand the existing monotherapy or drug combination regimens.¹

Aiming to discover new antimalarials we prepared and evaluated several series of fused isoindoles with encouraging results.² We have now prepared certain new isoindole derivatives which have shown a good *in vitro* profile against *Plasmodium falciparum*. Synthetic procedure starts from the condensation of substituted phthalic anhy-

drides with phenylacetic acids either at high temperature (215-240 °C and Dean Stark apparatus) or in microwave (5 x 30 sec) to yield benzalphthalides (**1**) in good yield. Compounds **1** treated with diamines at 80 °C gave the fused isoindoles **2**, which have been tested *in vitro* on *Plasmodium falciparum* (strain 3D7, 96 h incubation).³ IC₅₀ values around 50nM have been observed for the most potent compounds. Structure-activity relationships (SAR) have been analysed and will be presented in this communication.



SA and LD thank MAEC-AECI (Spain) and ALBAN E07/7D401988BR (Europe) for their respective fellowships. Research supported by grants MEC AGL2005-02168/GAN, FIS PI-060118 and RICET: RD06/0021/0022 (Spain); Collaborative research performed under the auspices of the Ibero-American CYTED Network RIBIOFAR.

[1] <http://www.who.int/malaria/html>

[2] del Olmo E, Armas MG, Ybarra M, López JL, Oporto P, Giménez A, Deharo E, San Feliciano A. *Bioorg Med Chem Lett.* 2003,18;13(16):2769-72.

[3] Smilkstein M, Sriwilajaroen N, Kelly JX, Wilairat P, Riscoe M. *Antimicrob. Agents Chemother* 2004, 48(5): 1803-1806.

P085

LIPIDIC AMINOCYCLOHEXANOLS AND CYCLO-HEXANEDIAMINES ACTIVE AGAINST MDR MYCOBACTERIUM TUBERCULOSIS

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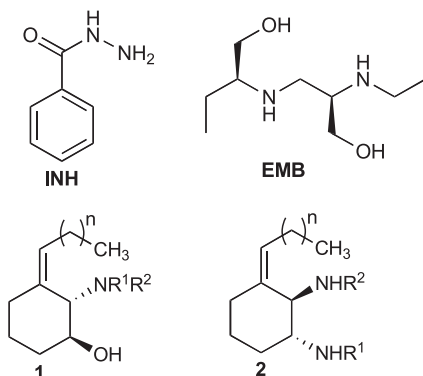
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Tuberculosis (TB), malaria and HIV/AIDS kill 6 million people every year; nearly 2 million deaths are caused by TB. 5000 people per day. There are estimated 8 million of new cases each year, 95% of which occur in developing countries². Drugs such as isoniazid (INH), Rifampicin (RMP) and ethambutol (EMB) have historically been successful in the treatment of TB infections. In the recent past, however, poor compliance with the prolonged and complicated dosage regimens currently used to treat the disease, in conjunction with the advent of the AIDS epidemic and the increased mobility of populations, has led to the emergence of numerous multidrug-resistant (MDR)

and even extremely-resistant (XDR) strains of *Mycobacterium tuberculosis*³. Resistance to the frontline agents, most notably INH and RMP, results in the treatment of patients with second-line agents. Among these second-tier drugs, thiacetazone being used in Africa and South America for the treatment of MDR-TB, has shown to be a very effective when administered in combination with INH⁴.

Our research group had previously prepared certain linear aliphatic aminoalcohols and diamines that were tested *in vitro* on virulent H37Rv and MDR strains of *M. tuberculosis*, with very good results⁵. Here we present the synthesis and the evaluation results of related compounds with conformationally restricted cyclic structures.

The synthesis starts from the cyclohexenone oxide, which is transformed into an epoxiolefin by Wittig olefination, and subsequently opened by different primary/secondary amines, to give the corresponding aminoalcohol derivatives **1**. Compounds of type **1** are transformed, through the Mitsunobu reaction, into aziridines, which are newly opened by different primary/secondary amines to give the corresponding series of diamine derivatives **2**.



Compounds of series **1** and **2** were tested on *M. tuberculosis*, H37Rv and MDR-TB strains, with MIC values of 3.12 mM.

OR thanks Banco Santander (Spain) for a research fellowship. Research supported by grants MEC AGL2005-02168/GAN and FIS PI-060118 (Spain); performed under the auspices of CYTED-Project X.11: PIBATUB.

References

- [1] World Health Organization. *WHO report* 2006.
- [2] Mehta DB, McAdam KP. *J. Intern. Med.* 2007, 261, 309-29.
- [3] Mitchison DA. *Eur Respir J.* 2005, 376-9.
- [4] Houston, S.; Fanning, A. *Drugs*, 1994, 48, 689.
- [5] Patent Applic. OPE: P200602940. Nov. 17, 2006.

P086

DESIGN OF NEW POTENT AND SELECTIVE INHIBITORS OF RAB GERANYLGERANYL TRANSFERASE

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Rab proteins, constituting the most prominent branch of small GTPases, play an important role in the regulation of intracellular trafficking.^[1] To be functionally active, these proteins have to be modified on their C-terminal cysteine residues by two geranylgeranyl groups that act as mediators of membrane association. These modifications are catalyzed by Rab geranylgeranyl transferase (RabGGTase). RabGGTase inhibition disrupts the biological functions of Rab proteins and has a direct effect on vesicular trafficking. Recent studies also indicate that inhibition of RabGGTase induces p53-independent apoptosis and validate this enzyme as a promising target for anti-cancer therapy.^[2] Potent and selective inhibitors of RabGGTase would then be of great interest as potential drugs but also to facilitate the study of the physiological role of Rab proteins.

We have recently developed a fluorescent assay that allowed us to monitor the prenylation reaction catalyzed by RabGGTase and thus to identify inhibitors of this enzyme.^[3] Using this assay, a screening of different libraries of natural products, in-house compounds and peptides led to the identification of diverse classes of RabGGTase inhibitors. Complementary studies to evaluate the selectivity of these inhibitors for RabGGTase with respect to the two other prenyltransferases FTase and GGTase-I allowed us to select lead compounds for further investigations. Thus, the next step was the synthesis of analogues of these inhibitors to study the structure-activity relationships and to find more potent and selective ones. Some of the compounds were also submitted to co-crystallisation experiments, providing X-ray crystal structures of the inhibitor bound to the enzyme that can be used to orient the design of new compounds.

Following this scheme, a first series of tripeptide inhibitors of RabGGTase has been developed, providing selective compounds that inhibit RabGGTase *in vitro* but also in a cellular assay. The same approach has then been applied to small molecule inhibitors. The synthesis of such new inhibitors of RabGGTase, their biological evaluation and the crystallographic structure of a bound inhibitor / RabGGTase complex will be presented.

References

- [1] K. F. Leung, R. Baron, M. C. Seabra, *J. Lipid Res.* 2006, 47, 467.
- [2] M. R. Lackner, R. M. Kindt, P. M. Carroll, K. Brown, M. R. Cancilla, C. Chen, H. de Silva, Y. Franke, B. Guan, T. Heuer, T. Hung, K. Keegan, L. J.M., V. Manne, C. O'Brien, D. Parry, J. J. Perez-Villar, R. K. Reddy, H. Xiao, H. Zhan, M. Cockett, G. Plowman, K. Fitzgerald, M. Costa, P. Ross-Macdonald, *Cancer Cell* 2005, 7, 325.
- [3] a) Y.-W. Wu, K. Alexandrov, L. Brunsvel, *Nat. Protocols* 2007, 2, 2704; b) Y.-W. Wu, H. Waldmann, R. Reents, F. H. Ebetino, R. S. Goody, K. Alexandrov, *ChemBioChem* 2006, 7, 1859; c) B. Dursina, R. Reents, C. Delon, Y. Wu, M. Kularia, M. Thutewohl, A. Veligodsky, A. Kalinin, V. Evstifeev, D. Ciobanu, S. E. Szedlaczek, H. Waldmann, R. Goody, K. Alexandrov, *J. Am. Chem. Soc.* 2006, 128, 2822.

P087

TOWARDS THE SYNTHESIS OF NOVEL BORONATES AS POTENTIAL HIV-1 PROTEASE INHIBITORS

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Drug discovery for HIV/AIDS has resulted in many life-saving therapies, making a great impact on modern medicine. Even though new therapies are available many drugs are highly susceptible to resistance development, have poor bioavailability, and cause several side effects. Therefore, there is an urgent need for the development of new types of inhibitory compounds, with better resistance profiles, better bioavailability, higher affinity, and lower toxicity. We are synthesizing novel boronates that were designed as compounds with potential dual-mode, both competitive and associative, of HIV-1 protease inhibitory action. Recent studies showed that boron-modified inhibitors have a higher affinity for the protease than their corresponding non-boronated analogs. Furthermore, the boron-modified structures were inhibitory to an HIV-1 protease variant that is resistant to several HIV-1 protease inhibitors. A library of both straight chain and cyclic boronates are being synthesized. The cyclic boronates, due to their structural rigidity, are expected to be better inhibitors than the straight chain compounds.

P089

DISCOVERY OF NEW INHIBITORS OF BACTERIAL LIGASES BY STRUCTURE-BASED VIRTUAL SCREENING

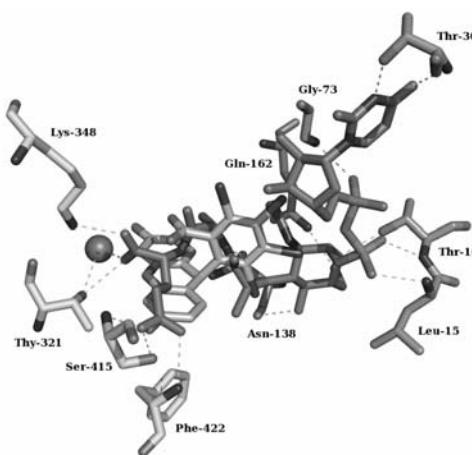
Andreja Kovac,^a Samo Turk,^a Blaž Vehar,^a Janez Konc,^b Audrey Boniface,^c Julieanne M. Bostock,^d Ian Chopra,^d Dušanka Janežič,^b Didier Blanot,^c and Stanislav Gobec^a

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Biophysique Moléculaire et Cellulaire, UMR 8619 CNRS, Univ Paris-Süd, Orsay, France; ^dInstitute of Molecular and Cellular Biology and Antimicrobial Research Centre, University of Leeds, Leeds LS 9JT, UK.

The emergence of bacterial resistance to antibiotic therapy has become a global health threat. To overcome this problem, new antibacterial agents directed toward novel targets need to be developed. The best known and most validated target for antibacterial therapy is the system of enzymes responsible for the construction of peptidoglycan. Mur ligases (MurC to MurF) and D-Alanine-D-alanine ligase (Ddl) catalyze the essential intracellular steps of peptidoglycan biosynthesis and represent under-exploited targets for the development of new antibacterial drugs.¹

Structure-based virtual screening dedicated to discovery of new enzyme inhibitors involves computational fitting the structures of compounds to the active site of an enzyme (docking) and scoring and ranking of each compound. Only the highest ranked compounds are then tested for activity in a biological or biochemical assay. In this study, the virtual screening of the National Cancer Institute "Diversity Set" against Mur ligases and DdlB was performed either with eHiTS² or AutoDock³ 4.0 program. Several new and structurally diverse hits were discovered that have IC₅₀ values in low micromolar range and also have *in vitro* antibacterial activities against both Gram-positive and Gram-negative bacteria. These initial inhibitors are promising starting points for development of new antibacterial agents.



MurD inhibitor (in blue, IC₅₀=10μM) docked into the enzyme active site (product of MurD reaction is in magenta).

References

- [1] Barreateau, H; Kovac, A.; Boniface, A.; Sova, M.; Gobec, S; Blanot, D. *FEMS Microbiol. Rev.* 2008, 32: 168.
- [2] Zsoldos, Z.; Simon, A.; Reid, D.; Sadjad, B.; Johnson, P.A. *Curr. Prot. Pept. Sci.* 2006, 7: 421.
- [3] Morris, G. M.; Goodsell, D. S.; Halliday, R. S.; Huey, R.; Hart, W. E.; Belew, R. K.; Olson, A. J. *J. Comp. Chem.* 1998; 19: 1693.

P090

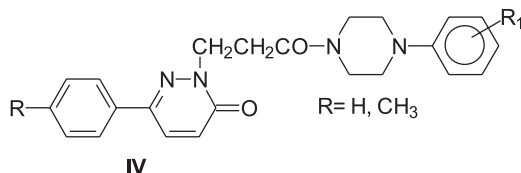
SYNTHESIS AND ANALGESIC AND ANTIINFLAMMATORY ACTIVITY OF 6-PHENYL/(4-METHYLPHENYL)-3(2H)-PYRIDAZINON-2-PROPIONAMIDE DERIVATIVES

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The ability of non steroidal anti-inflammatory drugs (NSAIDs) to modulate the pain, inflammation and fever made them one of the most used therapeutical classes in the world .

The utility of non-steroidal anti-inflammatory drugs in the treatment of inflammation and pain is often limited by gastrointestinal liabilities including ulceration and bleeding. Many papers have been reported on the analgesic anti-inflammatory and antipyretic evaluation of several pyrazolones [1-5]. It is known that some pyrazolone derivatives like dipyrone and phenylbutazone possess analgesic and anti-inflammatory activities, but several side effects have limited the clinical use of these drugs [6-9]. Pyridazinone derivatives which are related structurally to pyrazolone derivatives in the point of ring enlargement of pyrazolone to pyridazinone. A lot of 3(2H)-pyridazinone derivatives have been reported as analgesic and anti-inflammatory agents without gastrointestinal side effect [10-12]. This is agreement with in our experience in the pyridazinone field [13-16]. In the present paper, as an extension of our earlier work, we described new amide analog of 6-phenyl/(4-methylphenyl)-3(2H)-pyridazinon-2-propionamide **IV** derivatives



The chemical structures of title compounds are supported by IR and, ¹H-NMR elementary analysis. The analgesic and antiinflammatory activities were title compounds utilizing the phenylbenzoquinone-induced writhing test (PBQ test) and the carrageenan-induced foot paw edema test (CPE test) respectively. All the compounds were also tested for the irritative and ulcerogenic action on gastric mucosa.

References

- [1] Banoglu E., Akoglu C., Unlu S, Küpeli E., Yesilada E., Sahin M. F., Arzneimittelforschung 55(9), 520-527, 2005.
- [2] Cusan C., Altinier G., Sosa S, Sibilla F., Bucar F., Da Ros T., Curr Drug Discov Technol., 3(1), 67-73 , 2006.
- [3] Fahmy H. H., Eraky W., Arch Pharm Res. 24(3),171-179, 2001.

- [4] Lemke T. L., Abede E., Moore P. F., Carty T. J., J Pharm Sci. 78(4), 343-7, 1989.
- [5] Ueda T. Mase H., Furuta A., Oda N., Ito I., Yakugaku Zasshi. 102(3), 295-299, 1982.
- [6] Brogden R. N., Drug, (32) 4 60-70, 1986
- [7] Ritschar T., Filippini L, Dtsch. Med. Wochenshr.,111(41),1561-1564, 1986.
- [8] Clinch D., Waller D. G., Ir. Med. J. 82(4), 72-77, 1989.
- [9] Garcia S., Canionero M., Lopes G., Pharmacotherapy, 26(3), 440-2 2006.
- [10] Mao S., Yang S., Bi D. Biol Pharm Bull.,29(7),1355-1359, 2006.
- [11] Dal Piaz V., Vergelli C., Giovannoni M. P., Scheider M. A., Petrone G., Il Farmaco, 58, 1063-1071, 2003.
- [12] Rubat C., Coudert P., Tronche P., Bastide J., Bastide P., Privat A. M., Chem. Pharm. Bull. 37(10) 2832-2835 ,1989.
- [13] Gökçe M., Dogruer D., Sahin M. F. IL Farmaco, 56, 221-224, 2001.
- [14] Gökçe M. Sahin M. F., Küpeli E., Yesilada E. Arzneimittel-Forsch./Drug Res. 54, 396- 401, 2004.
- [15] Sahin M. F. Badiçoglu B., Gökçe M., Küpeli E., Yesilada E. Arch. Pharm. Pharm. Med. 337, 445-452, 2004.
- [16] Gökçe M. Bakir G., Sahin M. F., Küpeli E., Yesilada E.,Arzneimittel Forschung Drug Research, 55, 318-325, 2005.

P091

EXCAVATION OF 2-IMINO-1,3-THIAZOLINES AS NOVEL T-TYPE CALCIUM CHANNEL BLOCKER

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Calcium entry through T-channels mediates membrane depolarisation and increase in intracellular calcium concentration that are thought to contribute significantly to pacemaker activities in the heart and in neurons, sleep, hormone secretion, mechanosensation, epilepsy and pain. T-type calcium channels belong to the family of voltage-gated calcium channels. Three genes, a1G, a1H and a1I code for the pore-forming subunits of T-channels. Until recently, the physiological role of these channels was formulated according to the presence of T-type currents, but no selective blocker of T-channels exists to unequivocally demonstrate the roles of any of the a1 subunits. As the first selective T-channel blocker, mibefradil (Ro405967) was approved for clinical use for antihypertensive and antianginal and then launched in 1997. However, it was withdrawn from the market in 1998 due to drug interactions leading to irregular heart rhythms. Another known T-channel blocker was Kurtoxin, which was isolated from the scorpion. However, Kurtoxin also inhibits the HVA calcium channels and its peptide nature (63 amino acids) has a problem for the therapeutic agent. Therefore, new T-channel blockers having new scaffolds

are needed in understanding the exact role of T-channel in cellular functions.

In this study, an excavation of 2-imino-1,3-thiazolines as novel T-channel blocker and the preliminary screening results will be presented. We selected a hit compound, 2-imino-1,3-thiazoline from our indigenous chemical library and constructed the focused library of this scaffold for the lead optimization. Hundreds of 2-imino-1,3-thiazoline derivatives were synthesized by the condensation of halo ketones and thioureas and screened for the T-channels expressed *Xenopus* oocytes using FDSS or HEK293 cells. Structure-activity relationship will be presented also.

P092

SYNTHESIS AND ANTI-HIV ACTIVITY OF 4'-SUBSTITUTED 4'-THIOTHYMINES: A NEW ENTRY BASED ON NUCLEOPHILIC SUBSTITUTION OF 4'-ACETOXY GROUP

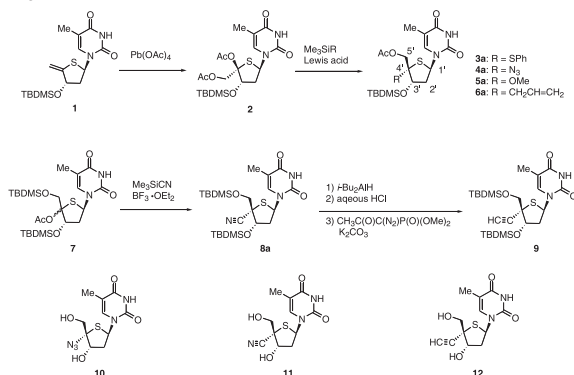
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Diacetoxylation of 1-(2,5-dideoxy-β-L-glycero-pent-4-eno-4-thiofuranosyl)thymine (**1**) with Pb(OAc)₄ allowed introduction of an acetoxy leaving group to the 4'-position (Scheme 1). Nucleophilic substitution of the resulting 4'-acetoxy derivative (**2**) with silicon reagents enabled us to prepare the 4'-phenylthio (**3a**), 4'-azido (**4a**), 4'-methoxy (**5a**), and 4'-allyl (**6a**) analogues of 4'-thiothymidine. 4'-Cyano (**8a**) and 4'-ethynyl (**9**) nucleosides were also synthesized from 3',5'-bis-O-TBDMS derivative (**7**). Among novel 4'-substituted 4'-thiothymidines, the 4'-azido (**10**), 4'-cyano (**11**) and 4'-ethynyl (**12**) derivatives were found to show potent inhibitory activity against HIV-1 and HIV-2. It is noteworthy that **11** and **12** were also inhibitory against replication of HIV variant resistant to 3TC (HIV-1_{M184V}), being as potent as against HIV-1_{IIIB}.



P093

HIGH-THROUGHPUT LOG P DETERMINATION BY ULTRAPERFORMANCE LIQUID CHROMATOGRAPHY: A CONVENIENT TOOL FOR MEDICINAL CHEMISTS

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Precise knowledge of the physicochemical properties of new chemical entities (NCEs) in early steps of drug design and discovery is of prime importance. Among these properties, lipophilicity is a key parameter involved mainly in pharmacokinetic processes such as absorption, distribution, metabolism, excretion, and toxicity (ADMET) and in ligand-target interactions. Moreover, lipophilicity is the molecular parameter of choice in numerous (quantitative) structure-activity relationships ((Q)SAR) of different classes of compounds. The advantages of reversed-phase liquid chromatography (RPLC) techniques for fast lipophilicity indices measurements are recognized. In particular, this approach includes low sample consumption, insensitivity to impurities, and automation possibilities [1]. Recently, liquid chromatography (LC) has evolved with the development of short columns packed with small particles (sub-2 μm) used in very high-pressure conditions (> 400 bar), enabling significant analysis time reduction without compromising chromatographic performance [2].

In this work, an Ultra Performance Liquid Chromatography (UPLC) system able to work up to 1000 bar was used for log P measurements. Retention mechanisms on different hybrid stationary phases packed with 1.7 μm particles were precisely characterized in terms of intermolecular forces (solvatochromic approaches) using a well-balanced set of compounds. Isocratic as well as gradient modes were evaluated, and their advantages and drawbacks pointed out. Two important features indicate that UPLC is emerging as an interesting alternative for lipophilicity measurement. (i) This approach offered a significant increase (up to a factor 12) in the throughput for lipophilicity determination. (ii) Due to the high stability of the tested columns over a large pH range (2 < pH < 11), log P of basic compounds such as β-blockers and local anaesthetics were also determined [3].

References

- [1] S. Martel, D. Guillaume, Y. Henchoz, A. Galland, J.L. Veuthey, S. Rudaz, P.A. Carrupt, *Chromatographic Approaches for Measuring log P*; In *Molecular Drug Properties: Measurement and Prediction*; R. Mannhold, Editor; Wiley-VCH: Weinheim, Germany, 2007; pp 331-356
- [2] D.T.T. Nguyen, D. Guillaume, S. Rudaz, J.L. Veuthey, *Fast Analysis in Liquid Chromatography Using Small Particle Size and High Pressure*, J. Sep. Sci. 29 (2007), 1836-1848

- [3] Y. Henchoz, D. Guilleme, S. Rudaz, J.L. Veuthey, P.A. Carrupt, High-Throughput log P Determination by Ultraperformance Liquid Chromatography: A Convenient Tool for Medicinal Chemists, *J. Med. Chem.* 51 (2008), 396-399

P094

THE ENANTIOSELECTIVE TOTAL SYNTHESIS OF AN ANTIALLERGIC β -CARBOLINE ALKALOID, (S)-(-)-DICHOTOMINE C

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Six new β -carboline alkaloids, (S)-(-)-dichotomines A, B, C and D, and dichotomide I and II, possessing antiallergic action, were isolated from *Stellaria dichotoma* in 2004.¹ We here describe the first enantioselective synthesis of (S)-(-)-dichotomine C (**1**). The required methyl *N*-methoxymethyl (MOM)- β -carboline-3-carboxylate (**5**) was synthesized in three steps starting from *N*-MOM-3-iodoindole-2-carbaldehyde (**2**) by using a microwave-assisted thermal electrocyclic reaction. Next, the synthesis of key compound **9** was performed from **5** in four steps. Finally, asymmetric dihydroxylation of **9** with AD-mix- α and - β was examined. Contrary to the Sharpless rule, (-)-dichotomine C (-)-(**1**) (43%) and its enantiomer (+)-(**1**) were obtained by AD-mix- β and AD-mix- α , respectively.²

[1] Yoshikawa M. *et al.*, *J. Nat. Prod.*, 2004, 67, 1464-1469.

[2] Hibino S. *et al.*, *Chem. Pharm. Bull.*, 2008, 56, 237-238.

P095

USE OF AMINO ACID COMPOSITION TO PREDICT LIGAND-BINDING SITES

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A novel method for predicting the binding sites for drug-like compounds on the surface of proteins was developed on the basis of the specific amino acid composition observed at the ligand-binding sites of ligand-protein complexes determined by X-ray analysis. A profile representing the preference of each of the 20 standard amino acids at the binding sites of drug-like molecules was obtained for a small set of high-quality complex structures. An index termed propensity for ligand binding (PLB) was created from these profiles. The PLB index was used to predict the propensity of binding for 804 lig-

ands at all potential binding sites on the proteins whose structures were determined by X-ray analysis. If the sites with the first two highest PLB indices are taken into consideration, the successfully predicted sites reached a high percentage of 86. The PLB prediction is relatively simple, but the validation study showed that it is both fast and accurate to detect ligand-binding sites, especially the binding sites of drug-like molecules. The present study has demonstrated that the PLB index is powerful in predicting not only ligand-binding sites of uncharacterized proteins but also novel drug-binding sites of known drug targets. Recently, we also have confirmed that the PLB index can successfully identify the druggable concavity in the protein structures constructed by homology modeling.

P096

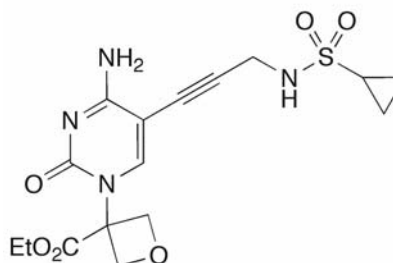
INHIBITORS OF THE KINASE ISPE: STRUCTURE-ACTIVITY RELATIONSHIPS AND CO-CRYSTAL STRUCTURE ANALYSIS

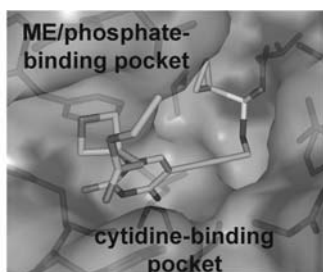
Anna K. H. Hirsch,^[a] Magnus S. Alpey,^[b] Susan Lauw,^[c] Felix Rohdich,^[c] William N. Hunter,^[b] Adelbert Bacher,^[c] and François Diederich^[a]

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Malaria and tuberculosis remain a major health concern with 300–500 million clinical cases and around one million deaths a year. In light of the rapid emergence of multi-drug-resistant parasites, the need for medicines with a novel mode of action has become increasingly important.

Plasmodium parasites, the causative agent of malaria, and the tuberculosis-causing *Mycobacterium tuberculosis* use the non-mevalonate pathway for the biosynthesis of the common isoprenoid precursors; this biosynthetic pathway is distinct from that used by humans. Hence, the enzymes of this pathway are ideal targets in the fight against these important infectious diseases.¹ The kinase IspE, at the center of the non-mevalonate pathway, was chosen as the target of a structure-based drug design project, leading to potent, competitive inhibitors with K_i values in the nanomolar range.² Herein, we report on the design, synthesis and biological evaluation of a new series of ligands for IspE protein.





These inhibitors were developed for the inhibition of *Escherichia coli* IspE. Structure–activity relationships and a co-crystal structure of *Aquifex aeolicus* IspE bound to a representative inhibitor validate the proposed binding mode: the ligand binds in the substrate- rather than the adenosine 5'-triphosphate (ATP)-binding pocket. As predicted, a cyclopropyl substituent occupies a small cavity not used by the substrate. The optimal volume occupancy of this subpocket is explored in detail. In the co-crystal structure, a diphosphate anion binds to the Gly-rich loop, which normally accepts the triphosphate moiety of ATP. This structure provides useful insights for future structure-based developments of inhibitors for the parasite enzymes.

- [1] H. Jomaa, J. Wiesner, S. Sanderbrand, B. Altincicek, C. Weidemeyer, M. Hintz, I. Türbachova, M. Eberl, J. Zeidler, H. K. Lichtenhaler, D. Soldati, E. Beck, *Science* 1999, 285, 1573–1576.
- [2] A. K. H. Hirsch, S. Lauw, P. Gersbach, W. B. Schweizer, F. Rohdich, W. Eisenreich, A. Bacher, F. Diederich, *ChemMedChem* 2007, 2, 806–810.

P098

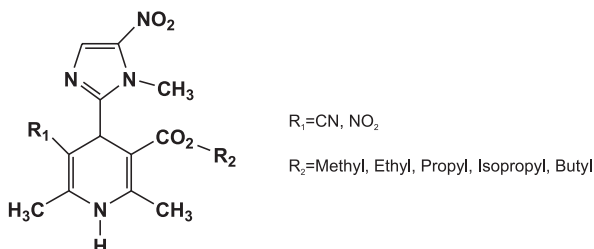
SYNTHESIS AND IN VITRO DUAL CALCIUM CHANNEL ANTAGONIST-AGONIST ACTIVITY OF SOME 1,4-DIHYDRO-2,6-DIMETHYL-3-NITRO AND CYANO-4-(1-METHYL-5-NITROIMIDAZOL-2-YL)-5-PYRIDINECARBOXYLATES

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In present study, dual cardioselective Ca²⁺ channel agonist / vascular selective smooth muscle Ca²⁺ channel antagonist as third generation of DHP drugs were synthesized using a procedure reported by Iwanami. This procedure involved the condensation of isopropyl-3-aminocrotonate with nitroacetone and 1-methyl-5-nitroimidazole-2-carboxaldehyde and condensation of alkylacetacetates with 3-aminocrotonitril and 1-methyl-5-nitroimidazole-2-carboxaldehyde for preparing 1,4-

Dihydro-2,6-dimethyl-3-nitro and cyano-4-(1-methyl-5-nitroimidazole-2-yl)-5-pyridinecarboxylates, respectively. All of compounds show calcium antagonist activity on GPILSM and some of them show agonist effect activity on guinea-pig atrium. QSAR and conformational analysis showed that conformation and charge of aryl substituent at C4 position had a main role in antagonistic activity while carbonyl group at C5 position interferes in agonistic effect.



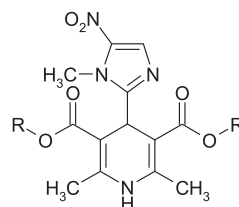
P099

CYTOTOXIC EFFECT OF SOME NEWLY SYNTHESIZED 1,4-DIHYDROPYRIDINE DERIVATIVES CONTAINING NITROIMIDAZOL MOIETY

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The 1,4-dihydropyridine (DHP) derivatives are a known class of calcium channel blockers. Some derivatives of DHP showed significant cytotoxicity. It was shown that this effect may not be the result of interaction with Ca²⁺ channels. In this study, we performed an investigation about the intrinsic cytotoxicity of some newly synthesized derivatives of DHP containing nitroimidazole moiety on their C4 position. IC₅₀ of all the compounds were more than 50 µg/ml. Therefore, IC₁₅ and IC₃₀ were calculated for each compound. The most cytotoxic effect was seen on Raji cell line while the lowest activity was observed in K562, since all of compounds had IC₃₀ of higher than 50 µg/ml. In fact, compounds showed good cytotoxicity on lower concentrations; however, their cytotoxicity was not dose dependent and their cytotoxic effect did not alter significantly in higher concentrations which might be due to their poor aqueous solubility.



R= Methyl, Propyl, Butyl, Isobutyl

P100

DESIGN AND SYNTHESIS OF NOVEL MIP INHIBITORS AS POTENTIAL COMPOUNDS AGAINST LEGIONNAIRES DISEASE

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Legionellosis is an infectious disease with mortality rate up to 20%. A common causative organism is the Gram-negative pathogen *Legionella pneumophila* which occurs in freshwater.

The major virulence factor of *L. pneumophila* is the surface protein Mip (Macrophage-infectivity-potentiator-protein) which binds to collagen IV and shows peptidyl-prolyl cis/trans isomerase activity. In consequence of these attributes the pathogen is able to spread out the human lung and causes often severe pneumonia especially in immunodeficient patients.

In addition, the surface protein belonging to the FKBP family is a receptor of the immunosuppressive drugs FK 506 and rapamycin which inhibit the enzymatic function of Mip. Because of the effect on the immune system the drugs mostly used are the respiratory quinolones like levofloxacin, moxifloxacin and gemifloxacin or macrolides like azithromycin, clarithromycin and roxithromycin, respectively. Despite of these efficient antibiotics the mortality rate of the legionnaires disease is not decreasing. In consideration of these facts Mip is a potential alternative target for novel antibiotics therapy.

The solution structure of free Mip and the Mip-rapamycin complex was solved by NMR spectroscopy [1] and a structural similarity of the Mip-rapamycin and FKBP12-rapamycin complexes was found. This emphasizes the hypothesis that FKBP12 ligands are promising starting points for the design of novel Mip inhibitors. Thus, FKBP12 ligands of the pipecolin acid type were synthesized and subjected to biological screening. Molecular modelling will accompany the study in order to give information for structural modifications.

References

- [1] Ceymann A., Horstmann M., Ehses P., Schweimer K., Paschke A., Steinert M., Faber C.; Solution structure of the *Legionella pneumophila* Mip-rapamycin complex; BMC Structural Biology; 2008 March 17; 8(1):17

P101

EVALUATION OF PRO-INFLAMMATORY CYTOKINE PATHWAY INHIBITORS FOR P38 MAPK INHIBITORY POTENTIAL

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The target for the anti-inflammatory natural products like amentoflavone which act by interfering with the pro-inflammatory cytokine pathway (e.g. TNF- α , IL-1 β and NO synthase) is not well defined till date [1]. Data obtained from molecular docking, electronic and surface analysis and bioisosterism sheds some light on steric and electronic complementarity of these molecules to p38MAPK (Figure 1), thereby suggesting a possible mechanism by which they might reduce the production of pro-inflammatory cytokines [2]

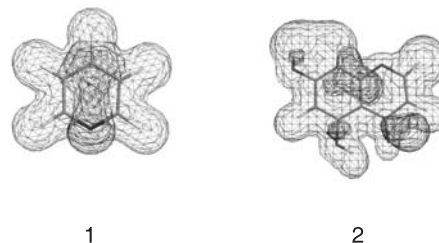


Figure 1; A representation of Molecular Electrostatic Surface Potential (MESP) of pyridine (1) and γ -benzopyrone (2) which was proposed as bioisoster for pyridine.

References

- [1]. Paul A.T. et al., *Drug Discovery Today*. 2006, 11, 725-732.
[2]. Kadam R.U. et. al., *J. Med.Chem.* 2007, 50, 6337-42.

P102

THE EFFECTS OF GLUTATHIONE AND ITS ANALOGUES ON ACTIVITY OF MANGANESE-SOD IN HUMAN BRAIN OF ALZHEIMER'S PATIENTS

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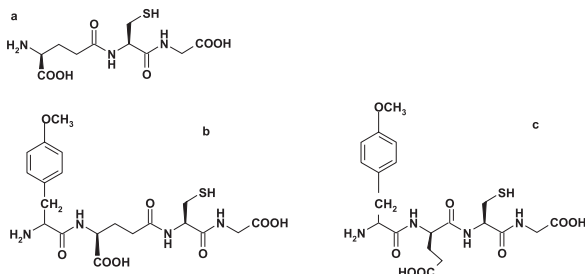
Mitochondrial alterations are involved in the neurodegeneration associated with Alzheimer's disease and oxidative damage is one of the main reasons for mitochondrial alterations. SOD is one of the most important antioxidant enzymes (MnSOD, SOD, EC 1.15.1.1.) involved in the defense systems operating against the reactive oxygen species. Glutathione (GSH, **a**) is the major intracellular antioxidant. Glutathione analogues previously developed in our lab have shown potent antioxidant activity [1,2,3].

Here, we report that GSH and its analogues UPF1(**b**) and UPF17(**c**) regulate activity of MnSOD obtained from temporal cortical regions of post mortem human brain. Activity of MnSOD in the brain of Alzheimer's patients has decreased 2.5 times as compared with healthy con-

trols. It was revealed that GSH at maximal physiological concentration has a tendency to activate MnSOD.

GSH analogues UPF1 and UPF17 increase the activity of MnSOD in a concentration dependent manner. UPF17 activated MnSOD after 5 minutes of preincubation, whereas the optimal preincubation period for UPF1 was 40 minutes. The effect of UPF17 on MnSOD activity shows a bell-shaped concentration dependence relationship, while the effect of UPF1 is increased with increasing concentrations.

MnSOD is the primary enzyme involved in the mitochondrial antioxidative defense system, therefore GSH synthetic analogues UPF1 and UPF17 might be clinically relevant agents to prevent the oxidative cellular damage.



This study was supported by grants ETF 6574 and ETF 6503 from Estonian science foundation.

References

- [1] Erlich K, Viirald S, Mahlapuu R, Saar K, Kullisaar T, Zilmer M, Langel Ü, Soomets U. Design, synthesis and properties of novel powerful antioxidants, glutathione analogues. *Free Radical Research* 2007; 1-9.
- [2] Pöder P, Zilmer M, Starkopf J, Kals J, Talonpoika A, Pulges A, Langel Ü, Kullisaar T, Viirald S, Mahlapuu R, Zarkovski A, Arend A, Soomets U. An antioxidant tetrapeptide UPF1 in rats has a neuroprotective effect in transient global brain ischemia. *Neurosci Lett* 2004;370:45-50.
- [3] Kals J, Starkopf J, Zilmer M, Pruler T, Pulges K, Hallaste M, Kals M, Pulges A, Soomets U. Antioxidant UPF1 attenuates myocardial stunning in isolated rat hearts. *Int J Cardiol* 2007;in press.

P103

DESIGN AND SYNTHESIS OF SOME CLOTRIMAZOLE DERIVATIVES AS ANTIFUNGAL AGENTS

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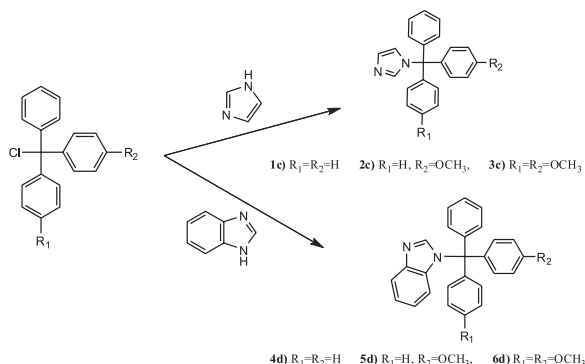
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Two different series of N-substituted heteroaromatic compounds related to clotrimazole structure were synthe-

sized. In one series *ortho*-cholortrityl moiety of clotrimazole was replaced by trityl, mono or dimethoxy trityl (series **c**). In the second series the imidazole ring of clotrimazole was also replaced by benzimidazole (series **d**). Chemical structures of all the new compounds were confirmed by spectrophotometric methods. These compounds docked into the active site of MT-CYP51 (PDB code, 1E9X) using Autodock tools software. They showed good affinity for the enzyme comparable to clotrimazole. Antifungal activities for these compounds were evaluated against *Trichophyton mentagrophytes*, *Microsporum gypseum* and *Candida albicans* using PDA as media, CHCl₃ or DMSO as solvents and agar dilution assay as method. In this method 1-triphenylmethyl-imidazole (**1c**), 1-(bis-4-methoxyphenyl)-phenylmethyl-benzimidazole (**6d**) and 1-(4-methoxyphenyl)-diphenylmethyl-imidazole (**2c**) showed 100%, 90% and 70% activity respectively. In the second step all of the derivatives also were evaluated against *Trichophyton rubrum*, *Microsporum canis* and *Epidermaphyton floccosum* using PDA medium by agar dilution method. In this method 1-triphenylmethyl-imidazole (**1c**) and 1-(bis-4-methoxyphenyl)-phenylmethyl-benzimidazole (**6d**) showed 100% and 1-(4-methoxyphenyl)-diphenylmethyl-imidazole (**2c**) and 1-(bis-4-methoxyphenyl)-phenylmethyl-imidazole (**3c**) more than 75% activity against the fungi. Then the most active analogues (**1c**, **2c** and **6d**) were tested in RPMI 1640 medium which showed desirable biological activity in comparison to clotrimazole.



P104

THE PROAPOPTOTIC EFFECTS OF PROTEIN KINASE CK2 INHIBITORS AT THE NEOPLASTIC CELLS IN VITRO

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Casein kinase 2 (CK2), the most pleiotropic protein kinase known with more than 300 protein substrates already identified is constitutively active and has been implicated in multiple cellular functions. CK2 displays

anti-apoptotic role in cancer cell lines and is more abundant in tumour as compared to normal tissues, what makes it an important target for antineoplastic drugs.

The aim of this study was the investigation of the influence of CK2 inhibitors on the cell cycle progression and apoptosis at few neoplastic cell lines. The first group were known CK2 inhibitors 4,5,6,7-tetrabromobenzotriazole (TBBt), 4,5,6,7-tetrabromobenzimidazole (TBBi) (1), and their derivatives 3-(4,5,6,7-tetrabromo-1H-benzimidazol-1-yl)propan-1-ol (MB001), 3-(4,5,6,7-tetrabromo-1H-benzotriazol-1-yl)propan-1-ol (MB002), 3-(4,5,6,7-tetrabromo-2H-benzotriazol-2-yl)propan-1-ol (MB003) (2) and 2-dimethylamino-4,5,6,7-tetrabromobenzimidazole (DMAT) (3). The second group of the tested compounds were newly synthesized hydrobromides or hydrochlorides of pentabromobenzyliothioureas variously substituted on the nitrogen atom(s).

Experiments were carried out using human neoplastic cell lines: HL-60 (human promyelocytic leukemia), KG-1 (human acute myeloid leukemia) and DTA (human colon carcinoma, phorbol esters resistant subline). All cells were in exponential growth phase, and exposed to the increasing concentrations of tested compounds for 24 and 48 h. The Annexin V/Propidium iodide apoptosis test was employed for determination of the apoptotic effect (HL-60, KG-1 cells). The cytotoxic effects on the DTA cells (cell viability) were determined using the MTT test. The cytostatic effect was estimated by measuring of cell cycle parameters using flow cytometry (propidium iodide staining, FACSCalibur cytometer, MacCycle program for the results analysis).

The tested compounds exhibited various proapoptotic efficacies. In HL-60 cells the most active of the isothioureas was N,N'-dimethyl-pentabromobenzyliothiourea inducing 60% of apoptosis at 4 μ M after 24 h incubation time and almost 100% after 48 h. The tested isothioureas and brominated heterocycles block the cell cycle in G1 and at the border of G1 and S phases. MB002 blocked HL-60 cells in G1-phase.

The most active among tetrabrominated benzimidazoles or benzotriazoles was DMAT inducing apoptosis in 50% of cells at 10 μ M concentration after 24 h exposition time (HL-60) and similarly in KG-1 cells but after 48 h. TBBi and MB001 stimulate apoptosis in 80% at the 25 μ M concentration (HL-60) and at 50 μ M in KG-1 cells. The viability of DTA cells dropped to 59% after 48 hours treatment with MB001 at concentration of 25 μ M.

- [1] Zien P., Bretner M., Zastapilo K., Szyszka R., Shugar D. *Biochem. Biophys. Res. Commun.* 306, 129 (2003).
- [2] Bretner M, Zien P, Najda A, Kulikowski T, Shugar D. (2006) patent application P 380112
- [3] Pagano M.A., Meggio F., Ruzzene M., Andrzejewska M., Kazimierzczuk Z., Pinna L. A. *Biochem. Biophys. Res. Commun.* 321, 1040 (2004).

The study was supported by the Ministry of Science and Higher Education (grant PBZ-MIN 014/P05/2004).

P105

COMPUTER-AIDED DISCOVERY OF ANTI-INFLAMMATORY THIAZOLIDINONES WITH DUAL CYCLOOXYGENASE/LIPOXYGENASE INHIBITION

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New anti-inflammatory agents possessing dual cyclooxygenase/lipoxygenase (COX/LOX) inhibition were discovered by computer-aided prediction of biological activity for 573 virtually designed chemical structures. Prediction of biological activity and selection of the most promising hits were performed by PASS and PharmaExpert software (<http://www.ibmc.msk.ru/PASS>). Nine 2-(thiazole-2-ylamino)-5-phenylidene-4-thiazolidinone derivatives differed by the phenyl group substitution were selected for synthesis and experimental testing as potential COX/LOX inhibitors. Eight tested compounds exhibited anti-inflammatory activity in the carrageenin-induced paw edema. Seven tested compounds (77.8%) were shown to be LOX inhibitors, seven compounds - COX inhibitors (77.8%), while six of them (66.7%) were shown to be both COX and LOX inhibitors. IC₅₀ values of the found hits ranged from 10⁻⁴ to 10⁻⁵ M. Analysis of lipophilicity of the compounds showed a negative correlation with inhibition of edema formation. The binding mode of the most active compounds of this series (2-(thiazole-2-ylamino)-5-(m-chlorophenylidene)-4-thiazolidinone for COX-1 and COX-2, and 2-(thiazole-2-ylamino)-5-(m-nitrophenylidene)-4-thiazolidinone for 15-LOX) were proposed on the basis of docking studies. The most active compounds dock well into the active site of the related enzymes. As gathered by our results, joint application of PASS and PharmaExpert software provides the efficient selection of dual COX/LOX inhibitors from virtual molecular libraries. Due to the computer prediction, the first anti-inflammatory agents with dual COX/LOX inhibitory mechanism of action were discovered among the thiazolidinones.

Geronikaki A.A., Lagunin A.A., Hadjipavlou-Litina D.I. et al. Computer-aided discovery of anti-inflammatory thiazolidinones with dual cyclooxygenase/lipoxygenase inhibition. *J. Med. Chem.*, 2008, **51**: 1601-1609.

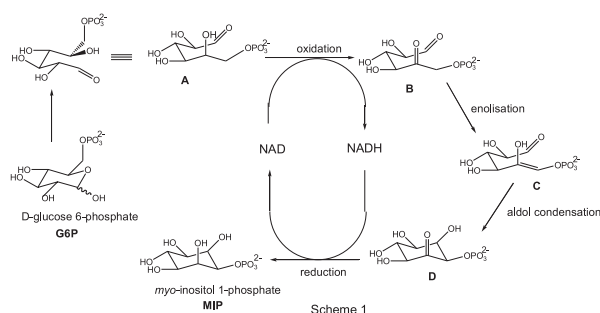
P106

PUTATIVE MULTI-SUBSTRATE ADDUCT INHIBITORS OF MIP SYNTHASE A NAD-DEPENDENT ENZYME

P.B. Le Calvez, C. Scott, M.E. Migaud

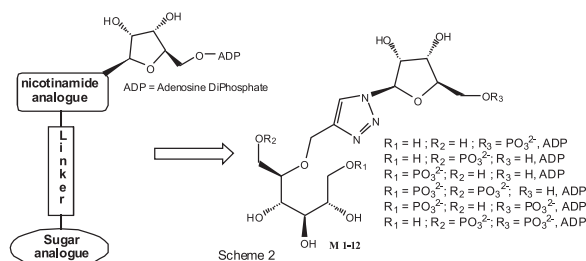
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Inositol and its various phosphate monoesters play critical biological roles including signal transduction and cell wall biosynthesis. *myo*-inositol 1-phosphate (MIP) synthase catalyses the first step in inositol biosynthesis, involving conversion of D-glucose 6-phosphate into *myo*-inositol 1-phosphate. In contrast to most NAD-dependent enzymes which use NAD as a co-substrate, MIP synthase uses it as an active-site catalytic residue.¹ For MIP synthase, each turn over of G6P into MIP requires an oxidation of an alcohol, enolization, intramolecular aldol condensation and reduction (scheme 1).² The structural gene encoding MIP synthase (INO1) has been identified, isolated and cloned from several prokaryotic and eukaryotic microorganisms³; in particular human INO1 has been cloned and proved functional when expressed in yeast⁴. The gene sequence of INO1 exhibits a high degree of homology in the case of eukaryotes but drops in the case of the prokaryotes.³ INO1 has been proved essential for the growth and virulence of *M. tuberculosis*. Stoker *et al.* have demonstrated that a mutant *M. tuberculosis* lacking INO1 is only viable in the presence of extremely high levels of exogenous inositol.⁵ Thus the development of selective inhibitors of mycobacterial MIP synthase could lead to novel antibiotics specifically against *mycobacterium* and *streptomyces* species, as both show INO1 high homology.⁶



Many transition state-based inhibitors have been previously synthesised but to our knowledge, the multi-substrate adduct inhibitors (MAI) approach was never used on the MIP synthase.

The combination of the two substrates (NAD, G6P) into a single molecule is likely to increase both the specificity and the binding affinity of the putative MAI. To investigate this idea, glucitol will be used as an analogue of G6P which will be linked to triazole moiety acting as a nicotinamide ring mimic (scheme 2). The poster presentation describes the synthetic route we employed to prepare these compounds.



References

- [1] Tian F., Migaud M.E., Frost J.W. J Am Chem Soc 1999, 121, 5796.
- [2] Migaud M.E. and Frost J.W. J Am Chem Soc 1996, 118, 495.
- [3] Majumder A.L., Johnson M.D., Henry S.A. Biochim Biophys Acta 1997, 245, 1348.
- [4] Ju S., Shaltiel G., Shamir A., Agam G., Greenberg M.L. Biol Chem 2004, 279, 21759.
- [5] Movahedzadeh F., Smith D.A., Norman R.A. et al. Mol Microbiol 2004, 51, 1003.
- [6] Majumder A.D., Chatterjee A., Dastidar K.G., Majee M., FEBS Lett 2003, 553, 3.

P107

STRUCTURE ACTIVITY RELATIONSHIP OF 6- OR 7-METHYLCHROMAN-2-CARBOXYLIC ACID N-(SUBSTITUTED)PHENYLAMIDES ON NF-κB INHIBITORY ACTIVITY

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Nuclear factor κB (NF-κB) is an inducible transcription factor which is involved in the regulation of many genes. In particular, NF-κB regulates the immune response and plays critical roles in the pathogenesis of chronic inflammatory diseases and a variety of human cancers. Therefore, it has been suggested that inhibitors of NF-κB function may be useful both as anti-inflammatory agents and as antitumor agents. We recently reported a series of substituted- or unsubstituted chroman-2-carboxylic acid N-(substituted)phenylamide derivatives as inhibitors of NF-κB. These were designed based on compound **KL-1156** which dose-dependently inhibits LPS-induced NF-κB transcriptional activity in the macrophage cell line RAW 264.7. The inhibitory activity of **KL-1156** with an IC₅₀ value of 40.4 μM was comparable to that of pyrrolidine dithiocarbamate (PDTTC) with an IC₅₀ value of 37.2 μM. PDTTC acts as an antioxidant and has been found to be a potent inhibitor of NF-κB activation.

In continuation of our effort to develop novel NF-κB inhibitory agents, a series of 6-methyl- or 7-methylchroman-2-carboxylic acid N-(substituted)phenyl amide deriv-

atives were synthesized to explore their structure-activity relationship. The previous result revealed that the phenolic OH group is not required for the good NF- κ B inhibitory activity. Except for that the compounds bearing 4-Cl on phenyl ring are the most active ones in both the 6-hydroxy-7-methoxy substituted series and the unsubstituted series, the trend for the NF- κ B inhibitory activity based on the substituents on phenyl ring are completely different between two series of chroman-2-carboxamide derivatives. This prompted us to prepare additional analogues containing 6-methyl or 7-methyl substituent. Substituents on the *N*-phenyl ring moiety of the target compounds were selected according to their electronic and hydrophobic character. Their NF- κ B inhibitory activities were evaluated in LPS-stimulated macrophage RAW 264.7 cells. The results were compared with that of **KL-1156**.

The target compounds contained various substituents (H, Cl, OMe, CH₃, CF₃, and NO₂) on phenyl ring. The positional effects of the substituents were also explored by examining the compounds with substituents at various position (2-; 3-; 4-; 2,5-; 3,4-; 3,5-). Compounds with -H, -CH₃, -CF₃ mono-substituent and -CH₃, -OCH₃ disubstituent on phenyl ring were poor inhibitors of NF- κ B. The most active NF- κ B inhibitors contained a 4-Cl and 4-OMe in 7-methyl substituted series and 2-OH and 4-Cl in 6-methyl substituted series (IC₅₀: 20.2-24.0 μ M). These were slightly more potent than the reference compound **KL-1156** (IC₅₀: 43.9 μ M). The effects of 6- or 7-methyl substituents on NF- κ B inhibitory activities are contradictory. Compound with 4-OMe substituent in 7-Me series (IC₅₀: 23.1 μ M) showed 4 times more potent activity than compound with 4-OMe substituent in 6-Me series (IC₅₀: 90.7 μ M) while compound with 2-OH substituent in 7-Me series (IC₅₀: >100 μ M) were less potent than compound with 2-OH substituent in 6-Me series (IC₅₀: 22.2 μ M). Compound with 4-Cl substituent in both 6- (IC₅₀: 24.0 μ M) and 7-Me (IC₅₀: 20.2 μ M) series were equipotent. The trend for the NF- κ B inhibitory activity based on the substituents on phenyl ring is different from the previous chroman-2-carboxamide derivatives.

P108

SYNTHESIS OF A SMALL LIBRARY OF OBOVATOL DERIVATIVES AND PRELIMINARY BIOLOGICAL EVALUATION

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Obovatol, isolated from the extract of *Magnolia obovata* leaves, has been used as a traditional medicine for neurosis and gastroenteric trouble in East Asia area. Recent result shows that obovatol has anti-inflammatory and antitumor activity through inhibition of NF- κ B, significant transcriptional factor to control of cancer cell. Moreover, it was reported that obovatol exhibits potent

anti-proliferative activities of vascular smooth muscle cell. Recently, we have developed a four-step synthesis of obovatol, based on Cu-mediated diaryl ether coupling reaction.

Taking advantage of the structural features of this natural product showing interesting biological activities, we designed and synthesized a small molecule library of obovatol, where a gallate moiety was mainly introduced due to the fact that gallic acid units play an important role in biological activities. The library was obtained following a parallel approach and using readily available synthons.

Among the synthesized derivatives, several compounds showed potent activities toward NF- κ B luciferase and anti-proliferation of vascular smooth muscle cell, respectively. Preliminary structure-activity relationship (SAR) was also revealed.

P109

INHIBITORS OF TRANSGLUTAMINASE 2: A THERAPEUTIC OPTION IN CELIAC DISEASE

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Human transglutaminase 2 (TG2), an enzyme with a variety of physiological and pathological functions, catalyzes the transamidation or deamidation of glutamine residues in peptides. Amongst others, the enzyme is involved in the pathogenesis of celiac disease, a T-cell mediated inflammatory disorder of the small intestine. Here, gluten peptides are the environmental factors required for disease activation in genetically predisposed individuals. TG2 deamidates glutamine residues from these peptides and converts them into glutamic acids, increasing the binding affinity to HLA-DQ2/DQ8-receptors. As TG2 thus increases the pathological effect of the gluten peptides, TG2 inhibitors are potential therapeutic agents to treat celiac disease and to improve the patient's quality of life: Up to now, no treatment whatsoever has been developed, patients have to follow a strict glutenfree diet.

To determine the binding mode of TG2-inhibitors, we crystallized a TG2-inhibitor complex. The crystal structure of TG2 in complex with a covalently bound inhibitor was determined at 2.5 Å resolution. The refinement of the structure is currently in progress. Additionally, the covalent binding of the inhibitor by a Michael addition reaction to the active-site cysteine 277 of TG2 was confirmed by trypsinolysis followed by LC/MS.

In parallel, we used another co-crystal structure, the only one published so far, as basis for structure-based drug design of new inhibitors using computer-aided approaches. On the basis of the observed binding mode we developed a variety of inhibitors with several new

structural features. In a docking approach, facing the obvious problems concerning docking of covalently bound inhibitors, we investigated new protein-inhibitor complexes and selected after visual sorting promising candidates for synthesis and kinetic measurements.

The crystallization of further inhibitor complexes with new TG2 inhibitors will provide information for the subsequent drug design cycles. Final goal is to develop inhibitors with the perspective for the development of therapeutics to threat celiac disease.

P110

SYNTHESIS OF TTHAM AND SEVERAL OF ITS DERIVATIVES AS NOVEL CHELATE LIGANDS FOR PARACEST MRI CONTRAST AGENTS

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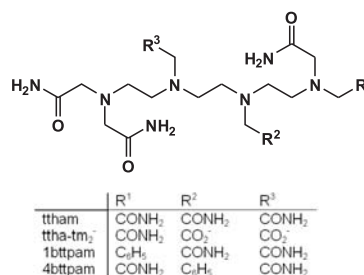
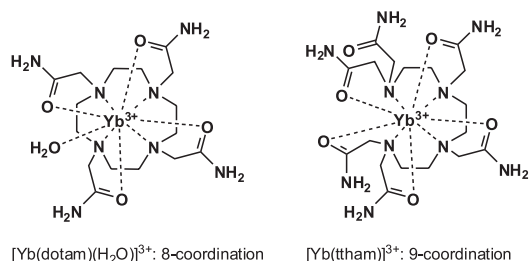
Chemical Exchange-dependent Saturation Transfer (CEST) is a novel technique for Magnetic Resonance Imaging (MRI) contrast enhancement. It is based on altering the signal intensity of the water protons by selectively saturating the resonance frequency of mildly acidic protons of a CEST contrast agent that are in an intermediately fast exchange equilibrium with bulk water protons. The CEST effect depends on pH, and pre-saturation frequency and intensity. It can, therefore, be utilized for pH-mapping, frequency-based multiplexing, as well as (almost) instantaneous pre- and post-contrast imaging, which in combination with a suitable molecular amplification strategy makes this technique very attractive for molecular imaging. An example of a well studied CEST contrast agent is [Yb(dotam)(H₂O)]Cl₃.

The paramagnetic ion (Yb³⁺) shifts the exchangeable-proton resonance of the amide moieties, avoiding direct saturation of the water signal and therefore increasing the sensitivity of the system. One of the factors that limit the sensitivity of this type of contrast agent is that it increases the T₁ relaxation of bulk water. The figure shows that the dotam ligand and its derivatives only have eight donor groups coordinated to the ytterbium ion, and the ninth coordination site is filled by a water molecule. It is anticipated that this water molecule plays an important role in the undesired lanthanide-induced relaxation enhancement of the pre-saturated water molecules also called the first sphere T₁-relaxation effect. To avoid coordination of this water molecule we designed and synthesized novel ligands that occupy all available coordination sites in complexes with Yb or other lanthanides.

The formulae of these new ligands are also presented in the figure. ttham has 10 donor groups which means that in the nona-coordinated trivalent complex ([Yb(ttham)]Cl₃) one donor group is free and exchanges with the other donor groups. Also the ligands 1btppam and 4btppam derived from ttham by substitution of one of the methylacetamide groups with a non-donating benzyl

group were synthesized. Better defined complexes are expected to be formed from these complexes containing nine donor groups resulting in sharper lines that may improve the CEST effect. In order to study the effect of stronger coordination and formation of a monovalent complex, ttha-tm⁻² was designed in which two of the acetamide groups are replaced by the stronger binding acetate groups.

In the presentation the synthetic routes towards ttham and its derivatives will be presented as well as preliminary CEST experiments of the complexes with Ytterbium.



P111

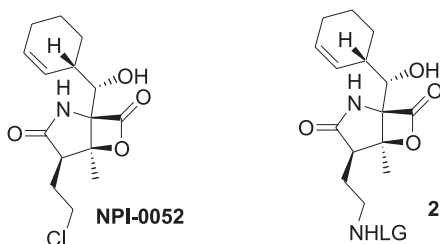
DESIGN AND SYNTHESIS OF HIGHLY POTENT NON-HALOGEN LEAVING GROUP ANALOGUES OF SALINOSPORAMIDE A (NPI-0052)

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Salinosporamide A (NPI-0052; **1**) is a potent 20S proteasome inhibitor that is currently in phase -1 clinical trials for the treatment of cancer.^{1,2} We previously reported that replacement of the chloroethyl group in **1** with unhalogenated substituents resulted in reduced potency for both proteasome inhibition and cytotoxicity, while replacement of chlorine with bromine or iodine resulted in equipotent analogues.² These data clearly indicated that the halogen contributes to the potency of this class of proteasome inhibitors. This trend could be associated with the leaving group property and/or its identity as a halogen. To elucidate the role of the halogen leaving group in **1**, we synthesized some non-halogen leaving group (NHLG) analogues (**2**) and evaluated for the inhibition of chymotrypsin-like (CT-L), trypsin-like (T-L) and caspase-like (C-L) activities of 20S proteasome. Synthesis of these

NHLG analogues (2) and their biological activities will be presented.



References

- [1] Chauhan, D.; Catley, L.; Li, G.; Podar, K.; Hideshima, T.; Velankar, M.; Mitsiades, C.; Mitsiades, N.; Yasui, H.; Letai, A.; Ovaa, H.; Berkers, C.; Nicholson, B.; Chao, T.-H.; Neuteboom, S.T.C.; Richardson, P.; Palladino, M.; Anderson, K.C. A novel orally active proteasome inhibitor induces apoptosis in multiple myeloma cells with mechanisms distinct from Bortezomib. *Cancer Cell* 2005, 8, 407-419.
- [2] Macherla, V. R.; Mitchell, S. S.; Manam, R. R.; Reed, K.; Chao, T.-H.; Nicholson, B.; Deyanat-Yazdi, G.; Mai, B.; Jensen, P. R.; Fenical, W.; Neuteboom, S. T. C.; Lam, K. S.; Palladino, M. A.; Potts, B. C. M. Structure-activity relationship studies of salinosporamide A (NPI-0052), a novel marine derived proteasome inhibitor. *J. Med. Chem.* 2005, 48, 3684-3687.

P112

PYRAZOLO[4,3-h]QUINAZOLINE-3-CARBOXAMIDES: A NEW CLASS OF ORALLY AVAILABLE INHIBITORS OF CYCLIN-DEPENDENT KINASES

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In the last years the field of kinases inhibition has seen a constant surge of interest across different therapeutic areas. CDKs have been the subject of many research projects in the pharmaceutical industry. Several chemotypes have been described leading to potent inhibitors. In an effort to explore different chemotypes which exploit the ATP kinase binding, we identified a novel series of CDKs inhibitors belonging to the pyrazolo[4,3-h]quinazoline-3-carboxamides.

Expansion of this class generated compounds with biochemical and cellular activity. Optimisation of the physical properties and pharmacokinetic profile led to the identification of highly potent and orally available compounds that exhibited good efficacy *in vivo* on A2780 human ovarian carcinoma.

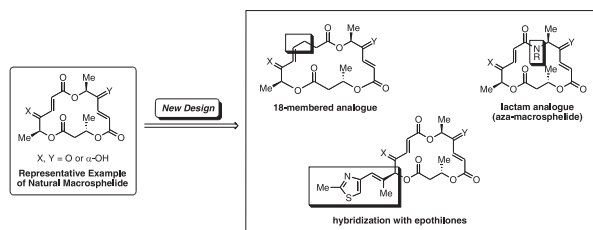
P114

SEARCH FOR A NEW ANTI-CANCER AGENT BY DESIGN, SYNTHESIS, AND BIOLOGICAL EVALUATION OF MACROSPHELIDE-BASED NON-NATURAL COMPOUNDS

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Macrosphelides are natural macrolides isolated from *Microsphaeropsis* sp. FO-5050 and *Periconia byssoides*, and characterized by a 16-membered tri-lactone framework. This novel class of macrolide compounds has been reported to exhibit potent inhibitory activity against adhesion of human leukemia HL-60 cells to human-umbilical vein endothelial cells (HUVECs). Adhesion of tumor cells to the vessel wall endothelia of distant organs is a critical step in tumor metastasis, and it has been reported that macrosphelide B can suppress the metastasis of B16-BL6 mouse melanoma cells to the lung *in vivo*. In addition, macrosphelide B and relating compounds have been found to exert inhibitory activity against tumor cell growth (colon 26-L5 adenocarcinoma cells). On the other hand, the role of apoptotic cell death has been increasingly recognized as an important aspect in the anticancer chemotherapy, and consequently, regulation of apoptosis has been a significant research target in this field. We have recently first disclosed that several natural macrosphelides can induce apoptotic cell death of human lymphoma U937 cells, and that these compounds also have a notable property as an effective sensitizer of hyperthermia-induced apoptosis. These results have revealed that macrosphelides can be a potential lead compound for development of a new anti-cancer chemotherapeutic agent. In this study, we planned design, synthesis, and biological evaluation of some types of artificial compounds based on the natural macrosphelide structure; 1) 18-membered analogue, 2) lactam analogue, and 3) thiazole-incorporating analogue (hybridization with epothilones, potent anti-tumor natural macrolide with a 16-membered framework). The syntheses of these compounds were achieved using ring-closing metathesis as a key macrocyclization, our original approach for macrosphelide synthesis, and it was found that some of these exhibited more potent apoptosis-inducing activity than the natural macrosphelides. In this symposium, we will present details of the synthesis and bioactivities.



References

- [1] Y. Matsuya and H. Nemoto, *et al.*, *Org. Lett.* 2003, 5, 2939-2941; *J. Org. Chem.* 2004, 69, 505-509; *Eur. J. Org. Chem.* 2004, 3973-3978; *Org. Lett.* 2006, 8, 4609-4612; *Chem. Biol. Interact.* 2007, 170, 86-99; *Int. J. Hyperthermia* 2007, 23, 353-361.

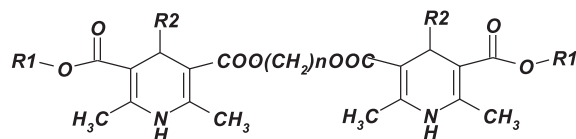
P115

DESIGN, SYNTHESIS, QSAR STUDIES OF A NOVEL GROUP OF BIS(4-NITROARYL-1,4-DIHYDROPYRIDINE) AND EVALUATION OF THEIR PHARMACOLOGICAL ACTIVITIES

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Voltage dependent calcium channels are crucial targets for a wide range of clinically active pharmacological agents. From these agents, 1,4-Dihydropyridines (1,4-DHPs) constitute a group of small organic compounds are based on a core pyridine structure which can both block and enhance calcium currents. They are considered specific for L-Type calcium channels; however other channel types, and in particular certain T-Type channels, may show sensitivity to DHP compounds. In this study, we synthesized a novel group of bis 1,4-DHPs using the procedure reported by Dagnino that involved the condensation of n-alkyl diacetoacetate (n=2-7) with methyl-3-aminocrotonate and nitro-phenylaldehyde. The synthesis was run under two conditions: 1- reflux and 2- microwave. Calcium channels antagonist activity were determined *in-vitro* using guinea-pig ileum longitudinal smooth muscle (GPILSM) assay. Synthesis of these compounds was confirmed with ¹H-NMR, IR and Mass spectrometry. Then IC₅₀% of them are calculated and compared with Nifedipine. Finally, the result of this pharmacological assay was used in QSAR studies utilizing MLR analysis. Most of these compounds are less active compared with respect to Nifedipine. Decrease in activity is the result of increase in steric hindrance. The QSAR study indicates that the activity is related to the electrostatic and topological parameters and the distance between two C5-esteric groups of DHP rings.



R₁=Ethyl, Methyl
R₂=2,3,4 nitrophenyl

P116

QSAR STUDIES ON DIFFERENT TOXICITY OF SOME DERIVATIVES OF PHENOL USING QTMS INDICES

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Quantitative structure activity relationship (QSAR) analyses using a novel type of electronic descriptors called quantum topological molecular similarity (QTMS) indices were operated to compare and to describe the mechanisms of phenols toxicity toward five different strains (i.e. *T. pyriformis*, L1210 Leukemia, *P. putida*, *R. japonica* and *C. pativus*). The appropriate QSAR models for the noted toxicity data were obtained separately employing partial least squares (PLS) regression combined with genetic algorithms (GA) as variable selection method. The resulted QSAR models were used to identify molecular fragments of phenol derivatives whose electronic properties contribute significantly to the noted toxicity. Using this information, it was possible to discriminate between the mechanisms of action of phenol toxicity to the studied strains according to the variable importance in projection (VIP) calculated for each parameter in PLS modeling. Results showed that toxicities of phenols to all strains, except L1210 Leukemia, are significantly affected by electronic features of the phenolic hydroxyl group (C-O-H) while toxicity to L1210 was affected by meta and para substituents. Also, the resulted models could describe the inductive and resonance effects of substituents on the various toxicities.

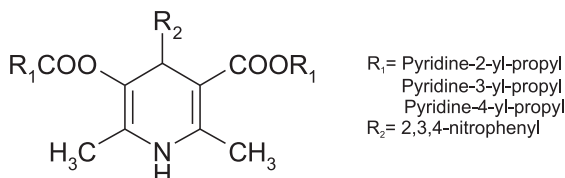
P117

DESIGN AND SYNTHESIS OF NEW SYMMETRICAL DERIVATIVES OF DIHYDROPYRIDINE WITH PYRIDYL GROUP 3,5-POSITIONS AND ARYL GROUP ON 4-POSITION AND EVALUATION OF THEIR CYTOTOXIC AND MULTIDRUG RESISTANCE REVERSAL ACTIVITIES

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Nowadays, chemotherapy plays an important role in the treatment of several kinds of cancer; however, the development of drug resistance remains one of the major obstacles in the successful chemotherapy. Several types of agents have been recognized as multiple drug resistance (MDR) inhibitors. Among these agents, 1,4-dihydropyridines (DHPs) are mostly investigated. P-glycoprotein inhibition has been reported as the main MDR reverser mechanism of DHPs; however, other mechanisms such as inhibition of topoisomerase II received less attention. Therefore, new derivatives of 1, 4-DHP were synthesized and their cytotoxic activity and their effects in reversing atypical were evaluated. Our results confirmed the appropriate effect of these compounds on atypical MDR. Although it has been revealed that these compounds had a moderate cytotoxicity effect, the cytotoxicity activity of compound 4b on K562 cell line ($IC_{50}=6.61 \mu M$) was relatively proportional to that of Doxorubicin ($IC_{50}=4.17 \mu M$). Finally, their Ca^{2+} channel antagonistic activities, an undesired effect for these compounds, were evaluated.



P118

DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION OF NEW ASYMMETRICAL 1, 4-DIHYDROPYRIDINE DERIVATIVES CONTAINING CARBAMOYL GROUPS AS ANTITUBERCULAR AGENTS

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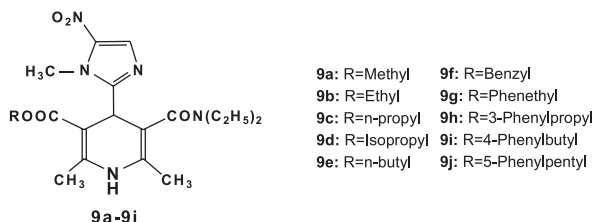
Tuberculosis is a leading infectious cause of death worldwide. The control of *Mycobacterium tuberculosis* infections is still considered of relevance, not only for the survival of AIDS patients. The recent emergence of drug-resistant *Mycobacterium tuberculosis*, in fact, also has become a serious concern.

Because of the concern of the resistance to most of the commonly used drugs displayed by the considered mycobacteria, most efforts have been done to introduce new antitubercular agents. Recently, studies showed that 1, 4-dihydropyridine-3, 5-dicarbamoyl derivatives with lipophilic groups have considerable antitubercular activity against H₃₇Rv. In view of this, in this study we synthesized new derivatives of 1, 4-dihydropyridines in which different alkyl and aryl esters and diethyl carbamoyl are substituted in C-3 and C-5 of the DHP ring respectively. In addition nitroimidazole ring (which also has antitubercular

activity especially against resistant strains) is substituted at C-4 position (**9a-j**). These asymmetric analogues were synthesized by a modified Hantzsch reaction using procedure reported by Meyer. The compounds were purified by preparative thin layer chromatography and recrystallization and were characterized by mass spectroscopy, IR and ¹H NMR.

The *in vitro* antitubercular activity of compounds against *Mycobacterium tuberculosis* H37 RV was evaluated by Agar proportion method in Middlebrook 7H₁₁ Agar sigma medium. The results indicate that the compounds containing aromatic esters are more potent than alkyl ones. The most potent aromatic compound (R= 3-phenyl-propyl) exhibits comparable antitubercular activity (MIC= 1 μmol/ml) with reference compound Isoniazide (INH) (MIC= 1 μmol/ml). In addition branching of the aliphatic chain, dramatically decrease the activity as a result of steric hindrance. Therefore, the compound containing isopropyl ester substitute is the least active derivative (MIC > 500 μmol/ml).

In addition conformational analysis, QSAR studies of these compounds show that increasing in lipophilicity and rotatable bonds of these compounds resulted in increasing antitubercular activity.



P119

TOWARDS IMPROVING PORPHYRIN TARGETING IN PHOTODYNAMIC THERAPY

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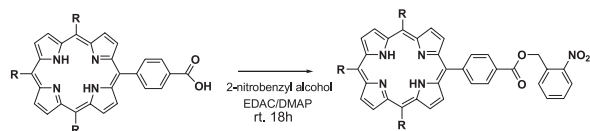
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Porphyrins have been shown to be prime photosensitizer candidates in photodynamic therapy (PDT) for cancer treatments. However, improvement in this area is currently hampered by unspecific localisation, side effects, ineffective targeting of receptors, low tissue clearance rates of the photosensitisers and inefficient *in vivo* pro-drug activation. Thus, this research aims to develop photocleavable and selective photosensitisers of porphyrins as a basis for novel applications of PDT. In order to increase the specific localisation of photoactive porphyrins in target tissue or their binding to specific receptors, appropriate effector groups need to be bound to the porphyrins. Porphyrins will act as the sensitising chromophores while photocleavable synthons will be used to

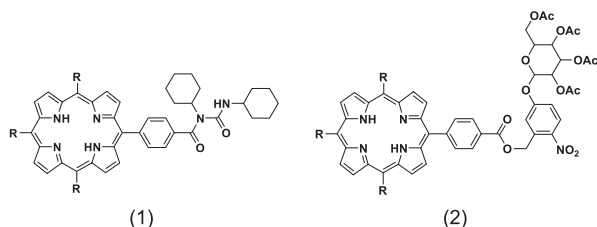
cross-link biologically relevant groups.

Our approach was to utilize the use of photolabile *o*-nitrobenzyl linkers [1]. This linker has successfully been attached to the porphyrin periphery *via* esterification between carboxylic acid porphyrins and *o*-nitrobenzyl alcohol using carbodiimide coupling method (Scheme 1) [2]. Interestingly, when using dicyclocarbodiimide (DCC) as the coupling reagent, an internal rearrangement to form the urea-type porphyrin (1) was observed.

Further studies were carried out to establish the ultimate construction of porphyrin *o*-nitrobenzyl-linked bioconjugates. Initial work has been done in attaching the simple glucose derivative (2) as an example of a sugar ligand that may be capable of binding to a receptor. The photolability of the *o*-nitrobenzyl linker will soon be investigated. Pending its success, this methodology will pave the way towards improved localisation of efficacious photosensitisers in PDT.



Scheme 1



[1] Bochet, C. G., *J. Chem. Soc., Perkin Trans 1*. 2002, 125.

[2] Nakanishi, K., Matile, S., Berova, N., *J. Am. Chem. Soc.* 1995, 117, 7021.

P120

THERMODYNAMIC STUDIES OF THE INTERACTION BETWEEN HISTIDINE, TYROSINE AND ASPARTATE (TRANSFERRIN COMPONENTS) AND COPPER ION AND TOXICITY IN BODY BY SPECTROPHOTOMETRIC, POTENTIOMETRIC AND ISO THERMAL CALORIMETRIC METHODS

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Proteins are composed of 20 essential amino acids which have donor atoms suitable for coordination to metal ions. Transferrin is one of the proteins that has an important role in biological systems. Histidine, tyrosine and aspartate are amino acids which have important roles in metabolism, absorption, repulsion, consumption and

reservation of Fe (II) through amino acids-Fe bonding. The excess amount of copper in our body will damage our biological system. In this work, the thermodynamics of the interaction of Cu (II) in invitro conditions with Histidine, tyrosine and aspartate have been investigated by spectrophotometric, potentiometric and calorimetric techniques, at various temperatures ($5^{\circ} \leq t \leq 45^{\circ}$) and two different ionic strengths. Acid dissociation constants of the ligands and the stability constants of Cu (II) chelate compound at various temperatures and ionic strength are reported. The ratio of the metal to legend was determined by UV-Vis spectrophotometric method in a buffer solution (acetate buffer PH=6.6) and ($\lambda_{\text{max}} = 620 \text{ nm}$) and ionic strength 0.1M. Potentiometric titrations of the amino acid in the absence and in the presence of the metal ion were used to determine the thermodynamic parameters at various temperatures and two ionic strengths by using Bjrrum method. The obtained results are compared with the results of the ITC method. The results have been used to describe the bonding in the Cu-His system. This is common knowledge that L- Histidine is one of the drug that be used in malaria decease, rheumatoid arthritis. Therefore mechanism of the reaction between metal ions is important which enter to human body.

References

- [1] Yizhang shreeram Akilesh, Dean E.Wilcox. *Inorg. Chem.* 2000, 39, 3057-3064.
- [2] A. A. Saboury, "Application of a new method for data analysis of isothermal titration calorimetry in the interaction between human serum albumin and Ni²⁺", *Journal of Chemical Thermodynamics*, 35, 2003, 1975-81.
- [3] A. Arabzadeh, S. Z. Bathaie, H. Farsam, M. Amanlou, A. A. Saboury and A. A. Moosavi-Movahedi, "Studies on mechanism of 8-methoxypsoralen-DNA interaction in the dark", *interactional Journal of Pharmaceutics* 237, 2002, 47-55.

P121

NEW 1,2,4-TRIAZOLE BASED GHRELIN RECEPTOR LIGANDS AS ANTI-OBESITY AGENTS

Aline Moulin,¹ Luc Demange,¹ Joanne Ryan,¹ Daniel Perrissoud,² Vittorio Locatelli,³ Antonio Torsello,³ Didier Gagne,¹ Jean-Claude Galleyrand,¹ Jean-Alain Fehrentz¹ and Jean Martinez¹

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Obesity is one of the most worrying diseases in the world [1]. Ghrelin [2] was described as an important orexigenic signal [3], thus blocking its action can appear to be a reasonable approach to treat chronic obesity.



Figure 1: human ghrelin

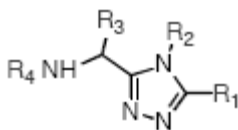


Figure 2: 1,2,4-triazole scaffold

We present here a new series of 1,2,4-triazole based compounds, which targets the ghrelin receptor (Growth Hormone Secretagogue Receptor type 1a, GHS-R1a) [4].

These new growth hormone secretagogue (GHS) analogues were evaluated for their *in vitro* binding and their ability to stimulate intracellular calcium release to the cloned *hGHS-1a* ghrelin receptor expressed in LLC PK-1 cells, and revealed potent subnanomolar range binding affinity, and agonist, as well as partial agonist, or antagonist activity.

We will also present here the first *in vivo* results obtained on food intake in rat.

[1] Bray. J. Med. Chem. 2006, 49 (14), 4001-4007

[2] Kojima et al. Nature 1999, 402 (6762), 656-660

[3] Ukkola. J. Endocrinological Invest. 2004, 27 (1), 96-98

[4] Howard et al. Science 1996, 273 (5277), 974-977

P123

CARBOHYDRATES AS SCAFFOLDS TO SYSTEMATICALLY SCAN CHEMICAL SPACE IN THE SEARCH OF NOVEL LEADS

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Libraries of drug-like molecules with very high levels of three dimensional structural diversity have been synthesized by decorating carbohydrate scaffolds with peptide-like side chains. As a proof of concept an initial library (1000 compounds) was synthesized, where the carbohydrate scaffolds were decorated with the aromatic / aromatic/ positive charge motifs in a variety of presentations, and screened against several biological targets known to recognize those motifs. Against nearly all targets screened high hit rates were observed and in a more focused program targeting somatostatins, high levels of sub-type selectivities for hits in the nanomolar range were also observed. The molecules generally have good drug-like characteristics as demonstrated by their good PK parameters. The chemistry is scalable as demonstrated by the synthesis of the lead compound for the somatostatin pro-

gram, which was prepared on a kilogram scale (9 synthetic steps) and progressed into preclinical studies.

We are currently applying the process of systematic diversity to 60 amino acid motifs decorating the carbohydrate scaffolds in 23 different presentations to produce a library of 16000 compounds. The library was designed using molecular modeling techniques to maximize the structural diversity of the peptide-like side chains on the carbohydrate scaffolds by comparison with an analysis of the structural diversity of tripeptides. Aspects of the chemical synthesis and the results for the first half (8500 compounds) of the library synthesis will also be presented.

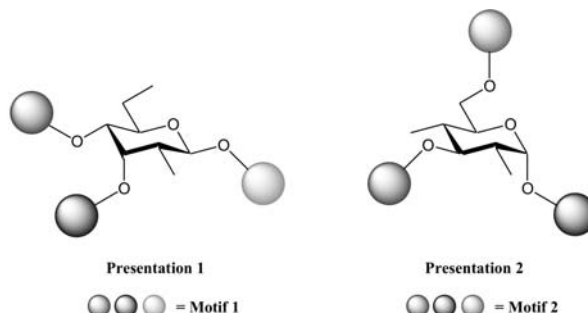


Figure 1

P124

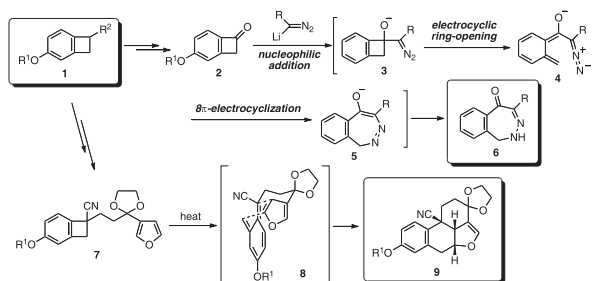
CHEMISTRY AND BIOLOGY OF BENZOCYCLOBUTENES: FACILE *o*-QUINODIMETHANE FORMATION AND TRANSFORMATION TO NEW BIOACTIVE COMPOUNDS

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Benzocyclobutenes have been a powerful tool for organic synthesis and utilized for past decades, especially for construction of poly-carbocyclic and heterocyclic systems. Versatility of this class of compounds is mainly attributed to facile formation of highly reactive *o*-quinodimethane intermediates, which can participate in tandem pericyclic reactions in an inter- or intramolecular fashion such as electrocyclization and Diels-Alder reactions. In our laboratory, in-depth studies on the reactivity of benzocyclobutenes and the utility for syntheses of various bioactive compounds have been performed on the basis of *o*-quinodimethane chemistry. We have recently reported that the electron-donating substituents such as oxy-anion and C-Si σ -bond significantly accelerate the *o*-quinodimethane formation. These studies brought about development of an extremely mild transformation of benzocyclobutenones **2** into 2,3-benzodiazepines **6** through a tandem electrocyclic reaction sequence involving diazo-*o*-quinodimethane intermediate **4**. Because 2,3-benzodiazepine skeleton **6** is an important core structure for

bioactive compounds, this novel reaction will contribute to SAR researches of 2,3-benzodiazepine compounds. Another application of the *o*-quinodimethane chemistry for the synthesis of bioactive compounds is intramolecular [4+2] cycloaddition reaction of the compounds **7** possessing a furanyl substructure. This reaction proceeded at 180°C with an exclusive *endo* stereoselectivity to provide novel dihydrofuran-fused tetracyclic compounds **9** in good yields. This class of compounds was revealed to exhibit notable bioactivities including enhancing effect of hyperthermia-induced apoptosis and anti-influenza virus activity. Interestingly, it was found that a simple benzocyclobutene derivative (e.g. **1**, R¹ = TIPS, R² = OH) also behaved as a potent enhancer of hyperthermia-induced apoptosis. In this symposium, details of syntheses and biological evaluation of benzocyclobutenes and relating compounds will be presented.



References

- [1] Y. Matsuya and H. Nemoto, *et al.*, *J. Am. Chem. Soc.* 2006, 128, 13072-13073; *J. Am. Chem. Soc.* 2006, 128, 412-413; *Bioorg. Med. Chem.* 2007, 15, 424-432; *Apoptosis* 2007, 12, 1523-1532; *Eur. J. Org. Chem.* 2008, 1426-1430; *Apoptosis* 2008, 13, 448-461; *J. Org. Chem.* 2004, 69, 7989-7993.

P125

TRIMMING THE FOLIAGE: NATURAL PRODUCT INSPIRED SCAFFOLDS FOR LEAD GENERATION

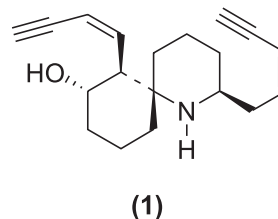
Agatha Garavelas, Ian D. Jenkins, Fabienne Lacrampe, Phuc Van Le, Priscila Almeida de Leone, Juliette E. Neve, George Nikolakopoulos, Ronald J. Quinn, Justin A. Ripper, Hasanthi P. Wijesekera

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Natural Products are constructed by biosynthetic enzymes and often display biological activities mediated by other proteins.¹ Recently, correlations have been discovered between biosynthetic enzymes which synthesise certain natural products and the therapeutic target of the natural product provide the underlying reason why natural products should be considered as validated starting points for drug discovery.¹ Similarly, natural products can be considered as a population of 'privileged' structures selected by evolutionary pressures to interact with a wide variety of proteins and other biological targets for specific purposes, a view supported by the fact that natural prod-

ucts have become effective drugs in a wide variety of therapeutic indications.

Analysis of the Dictionary of Natural Products has identified ring-scaffolds occurring in natural products has enabled the design of 'privileged' scaffolds with functionality directed in chemical space to mirror the 3D shape of the natural products. Here we report the synthesis of a scaffold based on the natural product Histronicotoxin (**1**).



References

- [1] McArdle, B. M.; Campitelli, M. R.; Quinn, R. J., A common protein fold topology shared by falvonoids biosynthetic enzymes and therapeutic targets. *J. Nat. Prod.* 2006, 69, 14-17.

P126

PHARMACOPHORE MODELING, VIRTUAL SCREENING AND BIOCHEMICAL EVALUATION OF NEW POTENT AROMATASE INHIBITORS

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Aromatase, an enzyme of the cytochrome P450 family, is involved in the conversion of androgens to aromatic estrogenic steroids such as estradiol and estrone. Suppression of estrogen biosynthesis by a mechanism of aromatase inhibition is therefore an effective approach for the endocrine treatment of hormone sensitive breast cancer. Intensive research aiming for the discovery of aromatase inhibitors identified interesting compounds, such as androstenedione analogs and azole non-steroid derivatives. In this work we have built and validated new pharmacophore models specific for each of these chemical classes, useful for a fast virtual screening of large compound databases. Small subsets of promising anti-aromatase candidates were extracted from the NCI database using this methodology, and tested on an *in vitro* assay with aromatase extracted from human term placenta. New potent aromatase inhibitors, active in the low nanomolar range, were identified. These results provide the first *in-silico* high-throughput screening methodology for potent aromatase inhibitors.

P127

SYNTHESIS AND STRUCTURE–ACTIVITY RELATIONSHIPS OF NOVEL 4-(2-HYDROXYPHENYL)-2-AMINOPYRIMIDINE DERIVATIVES AS IKK β INHIBITORS

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Nuclear Factor kappa B (NF- κ B) regulates the expression of a large number of inflammatory proteins, such as cytokines and chemokines, and is kept in an inactive state in cytoplasm by Inhibitor of κ B (I κ B). In response to diverse stimuli, I κ B kinase (IKK) complex phosphorylates I κ B, and then the phosphorylated I κ B is polyubiquitinated and led to degradation. Thus, I κ B phosphorylation by IKK complex is a trigger of the NF- κ B activation. One of the subunits of IKK complex, I κ B kinase β (IKK β), plays a major role in activation of NF- κ B.¹⁾ Therefore, it is expected that IKK β inhibitors may be useful for the treatment of inflammatory and autoimmune diseases, such as RA.

In our research for novel IKK β inhibitors, a series of 4-(2-hydroxyphenyl)-2-aminopyrimidine derivatives was found to inhibit IKK β with IC₅₀ values at the level of 10⁻⁸ M. In order to investigate the structure–activity relationships (SARs), we modified the substituents on not only the 6-position of the pyrimidine ring, but also the 2-hydroxyphenyl ring, and evaluated for their inhibitory activities against rat IKK β enzyme and TNF α -induced RANTES (Regulated upon Activation, Normal T cell Expressed and Secreted) production in A549 cells *in vitro*. In results, it was found that alkoxy groups at 6-position of the 2-hydroxyphenyl ring may be favorable for showing potent IKK β inhibitory activity *in vitro*. The compounds showing potent IKK β inhibitory activity *in vitro* were evaluated for their anti-arthritis activity in rat type II collagen-induced arthritis (CIA) model and rat adjuvant-induced arthritis (AIA) model after oral administration. Among them, (-)-2-(2-amino-6-piperidin-3-ylpyrimidin-4-yl)-3-(2-cyclopropylethoxy) phenol (AS1950930) showed potent inhibitory activity against IKK β enzyme and RANTES production with IC₅₀ values of 5.2 and 110 nM, respectively. This compound also exhibited 79% inhibition at a dose of 10 mg/kg on the CIA model and 55% inhibition at a dose of 7.5 mg/kg on the AIA model in rats. The synthesis, SARs and biological activities of the 4-(2-hydroxyphenyl)-2-aminopyrimidine derivatives, and the profile of AS1950930 will be presented.

Reference

- [1] Ebrahim Zandi and Michael Karin, *Molecular and Cellular Biology*, 1999, 19, 4547–4551.

P128

HDAC4: A POTENTIAL SMALL MOLECULE ANTI-CANCER TARGET. IDENTIFICATION OF NOVEL SELECTIVE INHIBITORS AND TARGET VALIDATION STUDIES

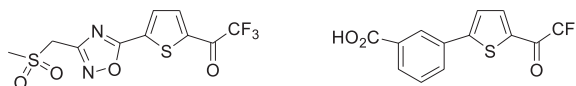
^aJesus M. Ontoria*, ^bMatthew Bottomley, ^aDanila Branca, ^bAndrea Carfi, ^cOttavia Cecchetti, ^bRaffaele De Francesco, ^cAnnalise Di Marco, ^aFederica Ferrigno, ^bPaola Lo Surdo, ^aEster Muraglia, ^aMichael Rowley, ^aRita Scarpelli, ^cSergio Serafini, ^bChristian Steinkühler and ^aPhilip Jones

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Histone deacetylases (HDACs) are a class of Zn²⁺ metalloenzymes involved in the deacetylation of histones, transcription factors and other proteins. HDAC inhibition causes hyperacetylation of histones which leads to a more open chromatin structure, thereby facilitating gene transcription and ultimately leading to differentiation, growth arrest and apoptosis representing a new strategy in cancer therapy. HDAC inhibitors (HDACi) are emerging as a new class of anti-cancer agents and with potential application in other therapeutic areas. The HDACi Vorinostat (formerly referred to as SAHA) was the first drug in this class approved two years ago by the FDA for the treatment of cutaneous manifestations in patients with cutaneous T-cell lymphoma, who have progressive, persistent or recurrent disease on or following two systemic therapies.

The HDACs are divided in two major sub-classes (Classes I and II), based on sequence homology. Most HDACi currently in the clinical inhibit multiple HDAC isoforms causing side effects of nausea, vomiting, fatigue and diarrhea. In an effort to develop a more targeted therapy with enhanced efficacy and larger therapeutic window, we were interested in developing second generation inhibitors of a restricted set of HDAC isoforms. In order to know which specific isoforms to target, RNAi knock-down studies were performed, from which HDAC 4 was identified as a promising oncology target.

Thiophene and thiazole substituted trifluoromethyl ketones will be presented as a new class of potent and selective HDAC Class II inhibitors.^{1,2,3} X-ray crystal structure of one inhibitor bound to HDAC 4 will be disclosed showing these compounds are active site binders. Biological data on this series will be disclosed showing 10-100 fold selectivity for Class II HDACs and selective inhibition of tubulin deacetylation in cells.



References

- [1] WO07/029035: Thiophene and thiazole substituted trifluoroethanone derivatives as histone deacetylase (HDAC) inhibitors.
- [2] WO07/093827: Thiophene and thiazole substituted trifluoroethanone derivatives as histone deacetylase (HDAC) inhibitors.
- [3] Jones, P. et al. *Bioorg. Med. Chem. Let.* 2008, 18, in press.

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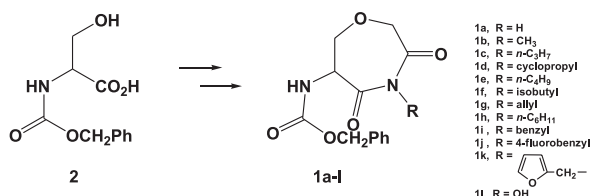
DESIGN AND SYNTHESIS OF 6-AMINO-1,4-OXAZEPANE-3,5-DIONE DERIVATIVES AS NOVEL BROAD SPECTRUM ANTICONVULSANT

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Based on the structural estimations for the typical anticonvulsant drugs, a series of 6-amino-1,4-oxazepane-3,5-dione derivatives, a novel structure of 7-membered heterocyclic imide, which was hybridized with pharmacophores such as cyclic imide and N-CO-C-N group in their molecule were designed and synthesized. Their anticonvulsant activities were evaluated by the maximal electroshock induced seizure (MES) and subcutaneous pentylenetetrazole (PTZ) test. Almost all the designed compounds except **1c** and **1f** showed comparable anticonvulsant activities in at least one of the anticonvulsant tests. Moreover, some of the tested compound exhibited moderate anticonvulsant activities in both MES and PTZ test.

From these results, 6-amino-1,4-oxazepane-3,5-dione derivatives could be recommended as novel structure of broad spectrum anticonvulsant.



P130

COMBATING ALZHEIMER'S DISEASE (AD) BY MULTITARGET MOLECULES

Prinz M.*, Hoerr V.*, Alptuzun V. #, Schreiber J. *, Holzgrabe U. *

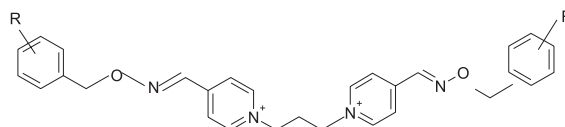
*Institute of Pharmacy and Food Chemistry, University of Würzburg, Am Hubland, 97074 Würzburg, Germany

#Faculty of Pharmacy, EGE-University, Bornova-Izmir, Turkey

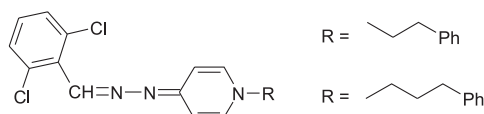
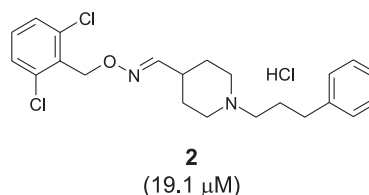
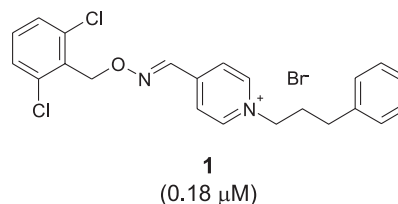
*Novartis, Boston, USA

The DUO compounds, derived from the reactivator of the poisoned acetylcholinesterase (AChE), obidoxim, and developed as allosteric modulators of muscarinic recep-

tors, were recently found to be potent ditopic inhibitors of AChE occupying both the active site and the peripheral binding site [1]. However, molecular modeling studies revealed that the compounds are too long and thus, occupying areas of the enzyme outside the catalytic gorge. Thus, the DUOs were systematically shortened to give the most active compound **1** in this series [2]. Since **1** is permanently positively charged and therefore not able to pass the blood-brain barrier (BBB), the pyridinium ring was replaced by a piperidine ring whose nitrogen is under physiological conditions partially protonated (necessary for AChE interaction) and partially non-protonated (transport form) due to a pK_A value of approximately 7. Surprisingly, this conversion leads to a 100fold loss of activity. Modeling studies revealed a worse interaction with the enzyme because of the piperidine ring [3]. Therefore the flat pyridine hydrazone ring system was employed as it is the case in compounds **3** and **4**. In **4** the inhibitor activity is slightly enhanced and due to the pK_A of about 9, the system can be charged and non-charged. Even more interestingly, compound **4** is able to inhibit the amyloid formation characterizing the compound as a new lead multitarget molecule. However, the activity has to be improved.



DUO compounds (IC₅₀ values
in a range of 0.3 to 20 μM;
by Ellman's tests)



3 (56.4 μM)
4 (5.66 μM)

References

- [1] P. Kapkova, N. Stiefl, U. Sürig, B. Engels, K. Baumann, U. Holzgrabe, *Arch. Pharm. Med. Chem.* 336, 523-540 (2003)
- [2] P. Kapkova, V. Alptüzün, P. Frey, E. Erciyas, U. Holzgrabe, *Bioorg. Med. Chem.* 14, 472-478 (2006)

P131

A NOVEL AND HIGHLY VERSATILE APPROACH TO C- AND C-AZA-NUCLEOSIDES

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C- Nucleosides form a unique class of nucleosides in which the heterocycle is connected to a sugar moiety by a C-C bond instead of the C-N bond of the natural nucleosides. As a result, the glycosidic bond is resistant to chemical and enzymatic cleavage. Naturally occurring ribose-based C-nucleosides such as formycin, tiazofurin and benzamide ribose (Fig.1) are potent anti-proliferatives with great potential as oncolytic agents. Similarly, C-aza-nucleosides and six-membered ring C-nucleosides have displayed promising anti-viral and anti-tumour activities, yet very few have been prepared.¹

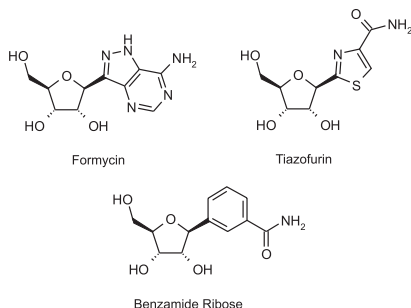


Fig. 1

The current syntheses of C-nucleosides are long and allow little access to structural diversity consequently hindering further drug development. The methodology applied in this project will challenge those problems encountered in the past and optimistically will give a simple way of obtaining the wide ranging and varied library of compounds sought after. The proposed approach is based primarily around the tandem ene-intramolecular Sakurai cyclisation, work that has already been developed by Markó² but unapplied to C-nucleoside synthesis. He has efficiently prepared a variety of diastereomerically pure exo-methylene pyrans via the condensation between a functionalized allylcarbamate or allylsilane and an aldehyde, followed by the Sakurai cyclization.³ Other work has been carried out by the Oriyama group on preparing exo-methylene furans (Fig. 2).⁴

Thus the synthetic strategy is to produce in a racemic mixture D/L C-nucleosides to maximize screening effectiveness. By varying the aromatic aldehyde early in the synthetic pathway, this will allow a range of exo-alkene

precursors unparalleled with any attempt to synthesise nucleosides before. Hence vastly increase the available library of prospective antiproliferative agents. The success of the oxygen containing pyran and furan rings will open the way for the first general approach for the syntheses of 5 and 6 membered aza-C-nucleosides (incorporating imine chemistry in the same fashion as the aldehydes in the oxygen containing rings) which have been scarcely researched and unexplored using Markó's methodology.

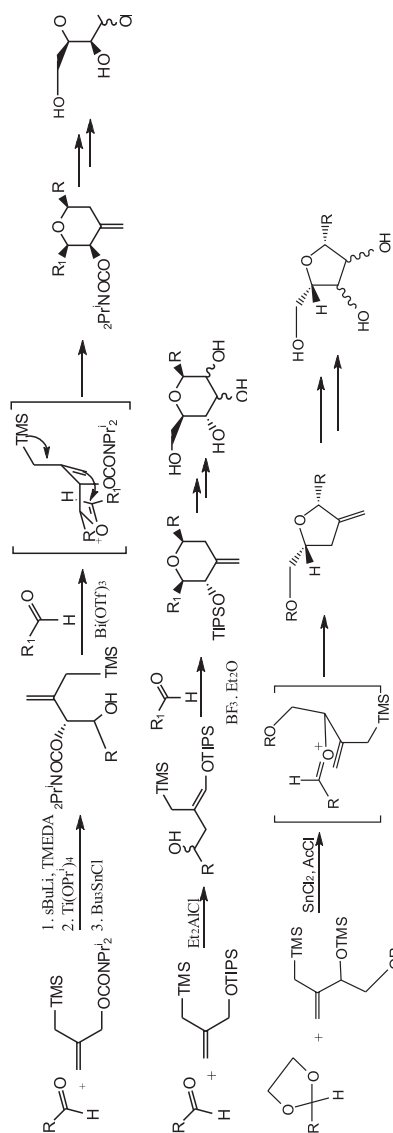


Fig. 2

[1] Sorensen, M. D.; Kvaerno, L.; Bryld, T.; Hakansson, A. E.; Verbeure, B.; Gaubert, G.; Herdewijn, P.; Wengel, J. *J. Am. Chem. Soc.* 2002, 124, 2164-2176.

[2] Markó, I. E.; Dumeunier, R.; Leclercq, C.; Leroy, B.; Plancher, J. M.; Mekhalfia, A.; Bayston, D. J. *Synthesis* 2002, 958-972.

[3] Leroy, B.; Markó, I. E. *Org. Letters* 2002, 4, 47-50.

[4] Oriyama, T.; Ishiwata, A.; Sano, T.; Matsuda, T.; Takahashi, M.; Koga, G. *Tet. Letters* 1995, 36, 5581-5584.

P132

FLEXIBLE DOCKING AND INTERACTION STUDIES OF CAPSAICIN AND CAPSIATE ANALOGUES WHICH INHIBIT ANGIOGENESIS AND VASCULAR PERMEABILITY VIA A DIRECT INHIBITION OF SRC TYROSINE KINASE

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Capsaicin is recognized for its pharmacologic and toxicological properties in the treatment of cancer and is known to inhibit the growth of or kill various types of human tumor cells. Recently, capsaicin was reported to inhibit tumor-induced or VEGF-induced new vessel formation *in vivo*. However, because of its highly pungent nature, it is rarely considered for treating angiogenesis-related diseases. In this study, we report the biological actions and underlying molecular mechanisms of capsiate, a nonpungent capsaicin analogue, on angiogenesis and vascular permeability.

Capsiate and its dihydroderivative, dihydrocapsiate, are the major capsaicinoids of the nonpungent red pepper cultivar CH-19 Sweet. *In vitro* study showed that capsiate and dihydrocapsiate inhibited vascular endothelial growth factor (VEGF)-induced proliferation, chemotactic motility, and capillary-like tube formation of primary cultured human endothelial cells. Importantly, capsiate suppressed VEGF-induced activation of Src kinase and phosphorylation of its downstream substrates, such as p125FAK and VE-cadherin, without affecting autophosphorylation of the VEGF receptor KDR/Flk-1.

Molecular modeling studies revealed that capsiate and its structurally related capsaicinoids efficiently dock to the ATP-binding region of Src tyrosine kinase through hydrogen bonding and hydrophobic interactions. Based on the docking results, we predict that capsiate efficiently docks to the ATP-binding pocket of Src kinase. Notably, capsiate's preferential binding mode in the ATP-binding pocket was similar, but not identical, to the mode of ATP binding to the Src kinase. Capsiate's phenolic group occupied the hydrophobic pocket within the catalytic cleft of Src kinase, which is not directly involved in ATP binding.

In vitro kinase assay and molecular modeling studies revealed that capsiate inhibits Src kinase activity via its preferential docking to the ATP-binding site of Src kinase. Taken together, these results suggest that capsiate could be useful for blocking pathologic angiogenesis and vascular permeability caused by VEGF. Our results indicate that capsiate has considerable potential as a novel pharmacologic model compound for the design and development of Src tyrosine kinase and angiogenesis inhibitors. This information also provides insight into future kinase inhibitor design for drug discovery.

Reference

- [1] B. J. Pyun*, S. Choi*, Y. Lee, et al. (2008). "Capsiate, a non-pungent capsaicin-like compound, inhibits angiogenesis and vascular permeability via a direct inhibition of Src kinase activity." *Cancer research* 68(1): 227-235.

P133

KANSUININE A AND B FROM *EUPHORBIA KANSUI* L. ATTENUATED STAT3 ACTIVATION BY INTERLEUKIN 6

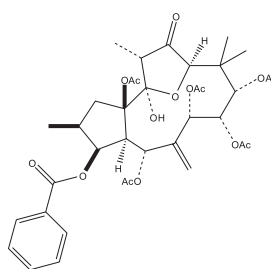
Mun-Chual Rho, Jung Ho Choi, Seung Woong Lee, Young Kook Kim

Natural Medicine Research Center, Korea Research Institute of Bioscience and Biotechnology, Daejeon, Korea

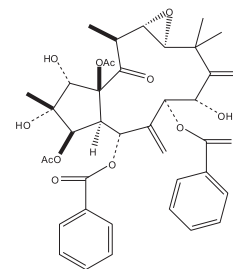
Signal transducer and activator of transcription 3 (Stat3) has been identified as the primary target transcription factor mediating interleukin 6 (IL-6)-dependent cell growth, differentiation, and survival. Evidence for this central role is derived from experiments showing that transfection of dominant negative Stat3 completely inhibits the antiapoptotic effect of IL-6 in carcinoma cells. More recent data also supports the role of IL-6 in tumor angiogenesis and the drug-resistant phenotype.

During screening for inhibitory substances in an IL-6-induced reporter gene assay using pSTAT3-Luc, methanol extract of *Euphorbia kansui* L. (*E. kansui*) showed a significant inhibition of Stat3 activation by IL-6. Via bioactivity-guided fractionation of the methanol extract of *E. kansui*, two diterpenoids were isolated. Their structures were determined as kansuine A and B by spectroscopic analysis (¹H-, ¹³C-NMR, 2D-NMR and ESI-MS).

Kansuine A and B inhibited luciferase activity and Stat3 phosphorylation in a dose dependent fashion with same IC₅₀ value of 0.4 µg/ml. On the other hand, ERK phosphorylation was activated by kansuine A and B and bisindolylmaleimide I (Inhibitor of PKC) inhibited the activity of these compounds such as Stat3 inhibition and ERK activation. The results suggest that kansuine A and B will act as PKC activator and may be applied to treatment of Stat3 related cancer.



Kansuine A



Kansuine B

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IMPROVING BINDING AFFINITY THROUGH CHARGE-ASSISTED HYDROGEN BONDS IN PROTEIN-LIGAND COMPLEXES OF TGT

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The change of pKa values of ligands upon protein binding is an often neglected aspect when discussing structure-activity-relationships. In this study we present an *in-silico* pKa calculation of different tRNA-guanine transglycosylase (TGT) complexes.[1] The results emphasize that the pKa values determined in aqueous solution can be shifted significantly when the ligand is bound to the protein.[2]

The enzyme TGT was identified as a promising target for structure-based drug design. In the anticodon loop of tRNA^{His, Asn, Asp, Tyr} TGT catalyzes the exchange of guanine against a modified base called preQ₁. The modified tRNAs are responsible for a efficient translation of pathogenic factors of *Shigella flexneri*, the causative agent of Shigellosis. Therefore successful inhibition of TGT could lead to new selective antibiotics. For the inhibition of TGT several series of structurally diverse ligands have been designed, synthesized and characterized by crystal structure analysis and enzyme kinetics. The most potent inhibitors are based on a *lin*-benzoguanine scaffold. This scaffold was further expanded to address a neighbored binding pocket where uracil 33 of the processed tRNA is recognized leading to 2-amino-*lin*-benzoguanines (Figure 1b). After this enlargement of the scaffold an increase in affinity of up to 500 times could be measured in the enzyme assay. Based on crystal structure data a charge-assisted hydrogen bond between 2-amino-*lin*-benzoguanine and the protein was assumed. The *in-silico* pKa calculation was performed and a significant upward shift was observed. The pKa value of the ligand is shifted to 8.3 (compared to 6.3 in aqueous solution) facilitating a protonation of the ligand under assay conditions (pH 7.3) (Figure 1a). Due to the created charge on the considered molecular portion, the hydrogen bond to Leu 231 is changed into a charge-assisted H-bond. The improved strength of this contact explains the strong shift in affinity.

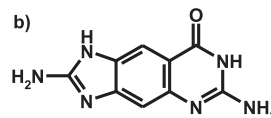
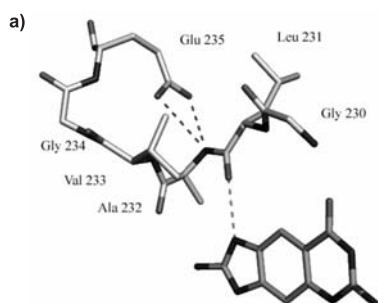


Figure 1; a) 2-amino-*lin*-benzpguanine (C: green; N: blue; O: red) in complex with TGT (C: gray; N: blue; O: red). The ligand forms a hydrogen bond to C=O of Leu 231 (green dashes). In addition Leu 231 is stabilized by the deprotonated Glu 235 (red dashes). b) Structure of 2-amino-*lin*-benzoguanine.

[1] Czodrowski, P.; Dramburg, I.; Sottriffer, C. A.; Klebe, G. Development, validation, and application of adapted PEOE charges to estimate pKa values of functional groups in protein-ligand complexes. *Proteins* 2006, 65, 424-37.

[2] Hörtner, S. R.; Ritschel, T.; Stengl, B.; Kramer, C.; Schweizer, W. B.; Wagner, B.; Kansy, M.; Klebe, G.; Diederich, F. Potent inhibitors of tRNA-guanine transglycosylase, an enzyme linked to the pathogenicity of the *Shigella* bacterium: charge-assisted hydrogen bonding. *Angew Chem Int Ed Engl* 2007, 46, 8266-9.

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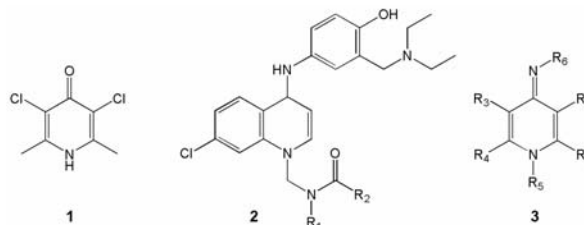
THE (1*H*-PYRIDIN-4-YLIDENE)AMINE SCAFFOLD AS POTENTIAL ANTIMALARIAL LEAD

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Malaria is one of the major diseases in the world, killing 1 to 3 million people a year [1]. The emergence of resistant strains of malaria suggests the need to discover both new drug targets and active molecules [2]. Cytochrome *bc*₁, from the mitochondrial electron transport chain, is one attractive and validated drug target, for which few efficient inhibitors are known today [3]. Clopidol, **1**, is a known *bc*₁ complex inhibitor and has been taken as a lead for structural optimization [4].



Recently we described a series of amodiaquine-like derivatives, **2**, which display good antimalarial activity [5] and a similar double bond arrangement to clopidol, **1**. Our preliminary docking study revealed that interaction of **2** with *bc*₁ complex is poor, suggesting that these compounds act in a different target. Removal of the fused

benzene ring led to the (1*H*-pyridin-4-ylidene)amine scaffold, **3**, which is easily accessible from the appropriate 4-chloropyridines. In this work, a series of (1*H*-pyridin-4-ylidene)amines, **3**, was synthesised and the antimalarial activity on the *P. falciparum* W2 strain assessed. Compounds **3** were obtained with good yields and their antimalarial activity found to be in the μM range. A docking study was designed to predict the major ligand-protein interactions. Future perspectives will be discussed.

References

- [1] Sachs, J. *et al.*, *Nature* 2002, 415, 680-685
- [2] Ersmark, K. *et al.*, *Med. Res. Rev.* 2006, 26, 625-666
- [3] Mather, M. W. *et al.*, *J. Biol. Chem.* 2005, 280, 27458-27465.
- [4] Fiandor Roman, J. M. *et al.*, WO 2006/094799, September 2006
- [5] Lopes, F. *et al.*, *Tetrahedron Lett.* 2004, 45, 7663-7666.

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GASTROPROTECTIVE DERIVATIVES OF FERRUGINOL: EFFECTS ON CELL PROLIFERATION, APOPTOSIS AND PGE₂ CONTENT IN HUMAN CELL CULTURES

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Ferruginol is an abietane diterpene occurring in plants belonging to the Podocarpaceae, Lamiaceae and Verbenaceae families among others. Ferruginol has been reported as antifungal, antibacterial, cardioactive, mitocidal, antioxidative, antiparasitic and antitumor agent. Rodríguez *et al.* (2006) have studied the gastroprotective and ulcer healing effect of ferruginol in mice and rats. Using *in vitro* models these authors have suggested that ferruginol protects the gastric mucosa by increasing the prostaglandin E₂ (PGE₂) content and preventing the lipid peroxidation of the gastric cells. Furthermore, Areche *et al.* (2008) have demonstrated that ferruginol protects the gastric mucosa by increasing the PGE₂ content, inhibiting the acid secretion and maintaining the gastric reduced glutathione levels in mice and rats. Areche *et al.* (2007) prepared 17 semisynthetic ferruginol derivatives for assessment as gastroprotective agents in mice. These authors observed that while some derivatives displayed gastroprotective activity similar to ferruginol other were devoid of activity. In order to compare the cellular changes elicited by the compounds we assessed the effects of the same 17 semisynthetic ferruginol derivatives and the diterpene totarol on cell proliferation, PGE₂ content and caspase 3 activity in human cell cultures.

The activity of the compounds on cell proliferation was assessed in fibroblasts MRC-5 and gastric AGS cell cultures. The proliferation of MRC-5 cells was stimulated only by ferruginol (**2**) (4 and 8 μM). The AGS cell proliferation was stimulated by the compounds abieta-8,11,13-triene (**1**) (25 and 50 μM), **2** (3 and 6 μM), 12-methoxyabieta-8,11,13-triene (**3**) (25 and 50 μM), abieta-8,11,13-trien-12-yl formate (**5**) (10 μM), abieta-8,11,13-trien-12-yl acetate (**6**) (11.5 μM), abieta-8,11,13-trien-12-yl propionate (**7**) (2.75 and 5.5 μM), abieta-8,11,13-trien-12-yl-2-chloropropanoate (**8**) (6 μM) and 12-(β -D-galactopyranosyloxy)-abieta-8,11,13-triene (**18**) (5 μM).

The effect of the derivatives on PGE₂ content was determined in gastric AGS cell cultures. Compounds **1** (122.5 and 245 μM), **2** (6.75 and 13.5 μM), **3** (68.7 μM), abieta-8,11,13-trien-12-yl-benzoate (**10**) (250 and 500 μM), abieta-8,11,13-trien-12-yl-3'-nitrobenzoate (**14**) (250 and 500 μM) and 12-(2,3,4,6-tetra-*O*-acetyl- β -D-glucopyranosyloxy)-abieta-8,11,13-triene (**17**) (500 μM) displayed a significant increase of PGE₂ content.

The effect of the compounds on caspase 3 activity was assessed in HL-60 cell cultures. At 50 μM the compounds **2**, **7** and totarol (**20**) increased the caspase activity by 100-150 %, derivatives **5** and **6** by 250-270 % and abieta-8,11,13-trien-12-yl propenoate (**9**) by 500 %.

Our findings further suggest that ferruginol and its derivatives could act as gastroprotective compounds *in vitro* by different and complementary mechanisms. These promising results encourage further pharmacological studies of these compounds as potential gastroprotective drugs.

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References

- [1] Rodríguez J.A., Theoduloz C., Yáñez T., Becerra T., Schmeda-Hirschmann C. *Life Sci.* 2006, 78, 2503-2509.
- [2] Areche C., Rodríguez J.A., Razmilic I., Yáñez T., Theoduloz C., Schmeda-Hirschmann G. *J. Pharm. Pharmacol.* 2007, 59, 289-300.
- [3] Areche C., Theoduloz C., Yáñez T., Souza-Brito A., Barbastefano V., de Paula D., Ferreira A., Schmeda-Hirschmann G., Rodríguez J.A. *J. Pharm. Pharmacol.* 2008, 60, 245-251.

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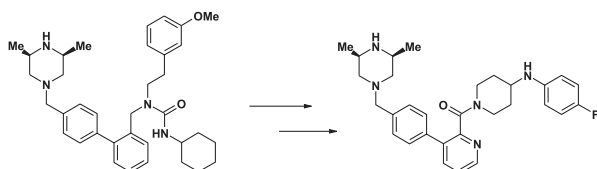
THE DESIGN OF NON-PEPTIDE MOTILIN AGONISTS AS GASTRIC MOTILITY AGENTS

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Motilin is a 22 amino acid peptide and endogenous ligand of the Motilin receptor, a class A, 7-TM GPCR receptor (also known as GPR38) predominantly expressed in the upper GI tract.¹ In man, motilin increases gastrointestinal motility, promoting gastric emptying and intestinal propulsion in an anal direction, during both fasting and fed conditions. The antibiotic erythromycin also possesses agonist activity at GPR38 and is frequently used "off-label" as a prokinetic agent in the clinic for relief of symptoms associated with delayed gastric emptying, such as enteral feeding intolerance, gastroparesis and functional dyspepsia.² Currently, there is no motilin agonist marketed specifically as a prokinetic for the treatment of these conditions.

The design and discovery of small molecule non-peptide agonists for peptide ligand receptors represents a significant challenge for medicinal chemistry. This poster will describe the medicinal chemistry programme from HTS through lead optimisation to the identification of small molecule motilin agonists.



References

- [1] Feighner, S.D.; Tan, C. P.; McKee, K. K.; Palyha, O. C.; Hreniuk, D. L.; Pong, S. S.; Austin, C. P.; Figueroa, D.; MacNeil, D.; Cascieri, M. A.; Nargund, R.; *et al.*. *Science*, 1999, 284, 2184-2188.
- [2] Peeters, T. in *Problems of the Gastrointestinal Tract in Anaesthesia*, Ed., Herbert, M. K. et al., Springer-Verlag, Berlin, Heidelberg 1999. pp 39–51.

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DISCOVERY OF POTENT AND SELECTIVE SMALL-MOLECULE PAR-2 AGONISTS

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Here we report the discovery, synthesis, and initial SAR of potent and selective small-molecule non-peptide PAR-2 agonists. The proteinase activated receptors (PARs) are a subfamily of four G-protein coupled receptors. PAR-2 is widely expressed throughout the body and has been shown to mediate pro-nociceptive and pro-inflammatory effects but does also display protective properties depending on the experimental setting and there-

fore the receptor may be considered an attractive drug discovery target.^[1] Furthermore, the discovery of metabolically stable small-molecule PAR-2 agonists would provide a useful pharmacological tool for elucidating the complex physiological functions of PAR-2 receptors.

Two structurally different compounds, AC-55541 and AC-264613 have been characterized in a variety of *in vitro* and *in vivo* functional assays. Both compounds displayed high potency, efficacy, and selectivity for PAR-2 receptors. They are metabolically stable and display pro-nociceptive and pro-inflammatory effects typical of PAR-2 agonists. Thus both ligands will be useful in further probing the physiological roles of PAR-2 receptors.

References

- [1] De Campo, B. A.; Henry, P. J. Protease-Activated Receptors (PARs) are Partly Pro-Inflammatory and Partly Anti-Inflammatory: Will PAR Agonists or Antagonists Participate in Future Drug Therapies? *Curr. Drug Targets* 2006, 7(5), 629-637.

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COMBINING VIRTUAL AND BIOLOGICAL SCREENING TO IDENTIFY NOVEL LEAD STRUCTURES FOR EPIGENETIC TARGETS

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A variety of structure- and ligand-based techniques for automated virtual library screening have been developed over the last ten years. Pharmacophore and docking tools are often applied in a hierarchical manner in order to reduce the number of potential hits from millions of compounds to a small subset of manageable size. The growing number of successful virtual screening studies have shown the potential of this approach. If the three-dimensional structure of the biological target is not available, protein models represent an alternative for ligand docking. It is widely accepted that docking to homology models is more challenging than docking to X-ray structures of proteins.

Chromatin modifications have emerged as new fundamental regulatory mechanisms for the control of gene transcription and are associated with many cellular processes. It is increasingly recognized that epigenetic modifications constitute important regulatory mechanisms for the pathogenesis of malignant transformations. The present talk will highlight the results obtained for several epigenetic targets where we used a combination of homology modelling and virtual screening methods in order to find novel lead structures. For the analyzed protein targets HAT, PRMT1^{1,2} and Sirt2³, we were able to

identify novel bioactive molecules. Good results were obtained applying a multi step approach including pharmacophore search, structure-based virtual screening and free energy calculations with subsequent biological evaluation of selected compounds.

References

- [1] Spannhoff, A., et al. Target-based approach to inhibitors of histone arginine methyltransferases. *J. Med. Chem.* 2007.
- [2] Spannhoff, A. et al.. A novel arginine methyltransferase inhibitor with cellular activity. *Bioorg. Med. Chem. Lett.* 2007.
- [3] Neugebauer, R. C. et al. Structure activity studies on splitomicin derivatives as sirtuin inhibitors and computational prediction of binding mode. *J. Med. Chem.* 2008

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TETRAHYDROBIOPTERIN ANALOGUES AS NITRIC OXIDE SYNTHASE ACTIVATORS: SOME SAR AND KINETIC STUDIES

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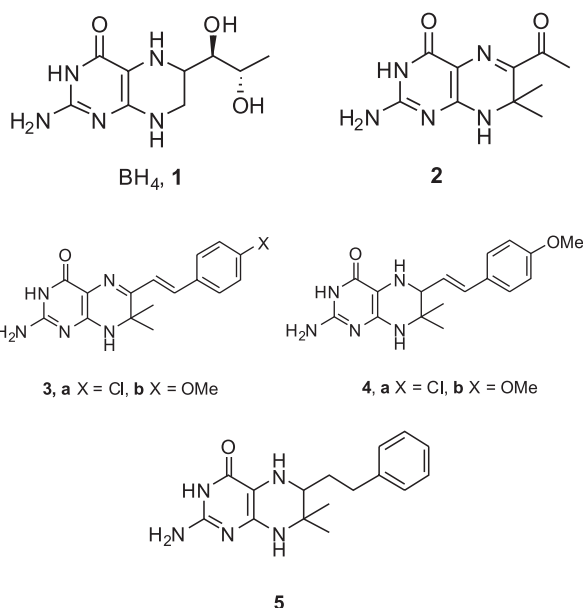
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Tetrahydrobiopterin BH₄ **1** is the naturally occurring cofactor for several oxidation reactions including aromatic amino acid biosynthesis and nitric oxide synthesis. All of these reactions have been implicated in a number of pathologies. In contrast to most medicinal chemical studies of enzymes as drug targets, we have been investigating the *activation* of nitric oxide synthases by analogues of BH₄ [*Bioorg. Med. Chem. Lett.* **2008**, *18*, 1552–1555]. We have shown that a number of blocked dihydropterins including 6-acetyl-7,7-dimethyl-7,8-dihydro pterin **2** are activators of iNOS in macrophages and eNOS in endothelial cells [US 60/242,013 (2005)]. **2** also has relevant *in vivo* activity but its bioavailability properties are poor. In order to understand better the structural properties that relate to NOS activation in this class of compounds and to seek compounds with potentially improved bioavailability, we have investigated some 6-(2-phenylethenyl) substituted pterins **3** and their reduced derivatives **4** and **5** as activators of NOS and extended our studies to nNOS. The 4-chlorophenyl and 4-methoxyphenyl compounds **3a** and **3b** were prepared by Wadsworth-Emmons alkenylation of the corresponding aldehyde; their tetrahydro derivatives **4a** and **4b** were prepared by sodium cyanoborohydride reduction. A new synthesis of the 2-phenylethyl pterin **5** was developed from α -amino-isobutyric acid using Weinreb amide methodology.

Evaluation of these compounds with iNOS and nNOS show that the introduction of the substituted aryl side chains may lead to advantages over **2**. For example in macrophages, measuring iNOS activity, **3a** was the most active compound in our set. Studies with nNOS showed that **5** was not an activator, indicating that a fully flexible side chain is not favoured, but in contrast, **4b** was an activator. Kinetic measurements comparing the behaviour of the tetrahydro derivative of **2** with **4b** and BH₄ itself showed that the k_{cat} values for the BH₄ analogues (1.8 – 5 min⁻¹) were similar to that of BH₄ itself (8 min⁻¹) but that the apparent dissociation constants were larger (18 – 150 mM compared with 1 mM). These results together with the much higher clogP values for the compounds **3** (**a** 3.06, **b** 2.28) compared with **2** (0.03) and BH₄ (-1.77) suggest that arylene substituted blocked dihydropterins may provide a suitable basis for the development of therapeutically useful compounds.



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BISQUARTERNARY NAPHTHALIMIDE DERIVATIVES – NOVEL ACTIVE COMPOUNDS AGAINST PLASMODIA AND TRYPANOSOMA

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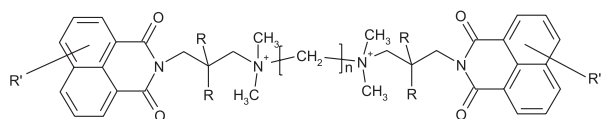
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Malaria, an infectious disease caused by Plasmodia ssp., is the world's most prevalent tropical disease. Each

year, it causes about a half billion new infections and up to three million casualties. The majority of those are young children of Sub-Sahara Africa. The currently available drugs are showing upcoming resistance and they are partially too expensive for the poor market of Africa. So new lead structures and easily accessible compounds are urgently needed.

Also the African sleeping sickness caused by *Trypanosoma* ssp. is a severe tropical disease and fatal if left untreated. For the treatment of this disease fewer agents are available than for Malaria and the currently used compounds have serious side effects.

A broad screening program identified the mono- and bisquaternary naphthalimides to be active against *Trypanosoma brucei brucei* or *Plasmodium falciparum* in the lower micromolar and nanomolar range of concentration. This class of compounds is known to have a high antitumoral effectiveness against both murine and human tumor cells. Surprisingly the cytotoxicity of these compounds studied here was evaluated and found to be very low. The structure activity relationships of cytotoxicity on the one hand and activity against Plasmodia and *Trypanosoma* on the other hand are different. Additionally these substances are easily available via a two or three step microwave-driven synthesis of high yield. Thus the bisnaphthalimides studied here are perfect lead compounds for further drug development.



General structure of the bisnaphthalimides

References

- [1] Braña, M.F.; Ramos, A.; *Naphthalimides as Anticancer Agents: Synthesis and Biological Activity*; Curr. Med. Chem. Anticancer Agents 2001, 3, 237-255
- [2] Holzgrabe, U.; *Antitrypanosomal activity of quaternary naphthalimide derivatives*; Bioorg. Med. Chem. Lett. 2007, 17, 1590-1593

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STRUCTURE-BASED DESIGN OF CERULENIN ANALOGUES AS POSSIBLE INHIBITORS OF THE FATTY ACID SYNTHESIS OF MYCOBACTERIUM TUBERCULOSIS

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Tuberculosis is a frequent and deadly disease mainly caused by *Mycobacterium tuberculosis*, which in the majority of the cases attacks the lungs. Overall, one-third of the world's population is currently infected. 10% of the

infected people become sick during their lifetime. As a result, tuberculosis is responsible for more than 1.5 million deaths worldwide every year.¹ Currently, there are several antimycobacterial antibiotics in use, but about 20% of the bacteria are already multi-drug resistant and approximately 2% are extensively drug-resistant.² This fact leads to an increasing necessity of design and development of new and highly efficient antimycobacterial substances.

Fatty acid biosynthesis, the first stage in membrane lipid biogenesis, is catalyzed in bacteria by a series of proteins which is termed the type II fatty acid synthase (FAS) system. In this pathway each enzyme has a discrete function. As the difference to the multifunctional type I synthase found in eukaryotes is significant, inhibitors of the FAS II system can be considered as effective and selective antibiotics.³

The enzyme of interest in our project is the β -keto-acyl ACP synthase (KasA) belonging to the group of enzymes that perform the elongation step in the fatty acid biosynthesis. A three-carbon substrate, malonyl-ACP, is used in a Claisen condensation to add two-carbon units to the growing acyl chain with the release of a single-carbon unit as carbon dioxide. The natural product cerulenin mimics the transition state of the condensation in a hydrophobic binding pocket, but has the disadvantage of inhibiting also the human FAS I, which makes it useless as an antibiotic. We already synthesized cerulenin derivatives with antimycobacterial activity. So the further aim of this project is to improve our substances in terms of higher activity and specificity for the mycobacterial KasA. For this purpose we are using the homologous enzyme FabB of *E.coli* since the crystal structure of KasA is not yet available. The crystal structure of FabB in complex with cerulenin provides information about the binding pocket, essential protein-ligand interactions and the mechanism of inhibition. However, because of the relatively high amount of rotatable bonds in the ligand, an uncommon binding mode and a large, mostly hydrophobic binding pocket, docking of potential cerulenin derivatives to support the design process is not straightforward. Accordingly, a reliable docking method with an appropriate scoring function had first to be defined. Subsequently, a small library of synthetically accessible cerulenin derivatives has been subjected through a docking analysis. The results provide guidelines for the optimization of the most promising candidates in terms of their interactions with the active site.

References

- [1] <http://www.who.int/mediacentre/factsheets/fs104/en/print.html>
- [2] Wright A. *et al.*, Morbidity and Mortality Weekly Report 55 (2006), 301-305
- [3] Heath R.J. *et al.*, Progress in Lipid Research 40 (2001), 467-497

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TRANSITION STATE ANALOGUES OF N-RIBOSYLTRANSFERASES: POWERFUL NEW DRUGS FOR AUTOIMMUNE DISEASE, CANCER AND MALARIA. A BLUEPRINT FOR INHIBITOR DESIGN

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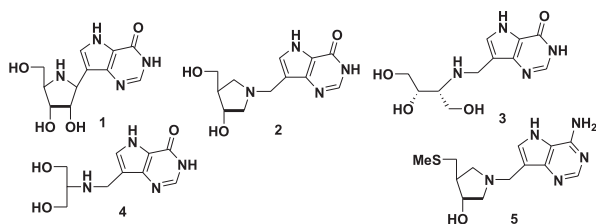
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An ideal transition state inhibitor of an enzyme will bind tighter than the substrate by a factor equal to the catalytic rate enhancement imposed by the enzyme. For many enzymes this would predict dissociation constants of 10⁻¹⁸ M or better. Detailed knowledge of the transition state structure of an enzyme catalyzed process facilitates the design and synthesis of stable molecules that capture important features of the transition state and are powerful inhibitors.

We have designed and synthesized transition state analogue inhibitors of some therapeutically relevant enzymes and in each case our inhibitors are the most potent known for that enzyme.

Purine Nucleoside Phosphorylase (PNP) is a target for the control of inappropriate T-cell proliferation and we have developed several orally available low pM transition state analogue inhibitors of this enzyme. Imm-H **1** (K_d=56 pM) is in clinical trials for T-cell cancers, while DADMe-Imm-H **2** (K_d=14 pM) has begun clinical trials for the T-cell mediated autoimmune disease psoriasis. The third generation acyclic derivatives **3** (K_d=8.5 pM) and **4** (K_d=5 pM) are the most potent.

Chemical blocking of the purine salvage enzyme Methylthioadenosine Phosphorylase (MTAP) affects polyamine biosynthesis as well as the levels of S-adenosyl methionine - and thus DNA methylation. The transition state analogue inhibitor MT-DADMe-Imm-A **5** (K_d=86 pM) is in preclinical development for the control of some cancers.



The malaria parasite *Plasmodium falciparum* is a purine auxotroph and relies on salvage enzymes to harvest its purines from the host. The enzymes PNP and Adenosine Deaminase (ADA) are essential for this salvage. We have potent and selective transition state inhibitors of the *Pf* PNP and *Pf* ADA.

The process of determining the transition state structure followed by the design and synthesis of transition

state inhibitors for these and other enzymes will be discussed. This offers a blueprint for the discovery of exceedingly potent enzyme inhibitors.

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IDENTIFICATION OF NOVEL SIRT2 INHIBITORS BY VIRTUAL SCREENING AND BINDING FREE ENERGY CALCULATIONS

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NAD⁺-dependent histone deacetylases (sirtuins) are a group of enzymes which cleave off the acetyl groups from N-acetylated lysines of histones and non-histone proteins. Sir2 proteins are conserved from bacteria to humans, and they are able to deacetylate numerous proteins in addition to histones, e.g. α -tubulin, myoD, p53, FOXO. Their ability to deacetylate such a wide range substrates has implicated them to play an important role in various biological processes, including DNA recombination, repair and longevity, and transcriptional silencing. Potent selective sirtuin inhibitors are interesting tools for investigation of the biological functions of those enzymes and may be future for the treatment of cancer.^{1,2} Here, we present detailed structure-activity relationships on thiobabitrates and their inhibition on recombinant Sirt2. Virtual screening was performed in an attempt to identify novel inhibitors for human Sirt2. Molecular docking and molecular dynamics simulations (MD) were used to relax the system and to investigate the dynamics of the binding complex. Molecular Mechanics-Poisson-Boltzman Surface Area (MM-PBSA) free energy analysis was applied to estimate the binding affinity of novel inhibitors.

[1] Heltweg, B.; Gatbonton, T.; Schuler, A. D.; Posakony, J.; Li, H. et al. Antitumor activity of a small-molecule inhibitor of human silent information regulator 2 enzymes. *Cancer Res.* 2006, 66, 4368-4377.

[2] R. C. Neugebauer, U. Uciechowska, R. Meier, H. Hraby, V. Valkov, E. Verdin, W. Sippl and M. Jung. Structure activity studies on splitomicin derivatives as sirtuin inhibitors and computational prediction of binding mode. *J. Med. Chem.*, 2008, Feb 13 [Epub ahead of print]

P149

SYNTHESES OF BORONIC ACID DERIVATIVES OF TYROPEPTIN, A PROTEASOME INHIBITOR

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Previously, we reported a SAR study of tyropeptin-based proteasome inhibitors. Among them, TP-110 turned out to be the most potent and highly selective inhibitor of the chymotrypsin-like activity of proteasome. In 2003, FDA approved the first clinical use of a proteasome antagonist (PS-341, bortezomib) for a treatment of multiple myeloma. PS-341 is a dipeptide compound with the structurally characteristic leucine-boronic acid residue at the P1 position, which is believed to be the origin of the potent anti-proteasome activity. Based on the above studies, boronic acid derivatives of tyropeptin, which were expected to be highly effective proteasome inhibitors, were synthesized. Boronic amino acid derivatives are relatively labile under usual reaction and purification conditions compared to aromatic boronic acids. The synthetic scheme to avoid decomposition of the target compounds and their synthetic intermediates will be shown in detail.

[1] I. Momose *et al.*, *Bioorg. Med. Chem. Lett.*, 15, 1867 (2005).

P150

THE STRUCTURE ACTIVITY RELATIONSHIP STUDY ON THE WATER-SOLUBLE 1,4-DIHYDROPYRIDINE DERIVATIVES BLOCKING N-TYPE CALCIUM CHANNELS

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Voltage-dependant calcium channels play an important role in the cellular functions, including muscle contraction, release of neurotransmitters, calcium dependent gene transcription and the regulation of neuronal excitability. Among voltage-gated calcium channels, N-type calcium channels are distributed on the sympathetic nerve endings and strongly associated with the neuronal cell death and neuropathic pain. In fact, several reports have suggested that a blockade or lack of N-type calcium channels can suppress the sympathetic functions or pathological processes of ischemic brain injury and pain in animal models. Although most of the efforts for discov-

ering the N-type calcium channel blocker were focused on orally-active small molecules, an injectable drug with high aqueous solubility is also in demand especially for a patient who can't take medicine via oral route. Therefore, in order to find candidates for novel neuroprotective and analgesic drugs, we have chosen APJ2708, which is a derivative of cilnidipine with dual blockage activities at the L/N-type calcium channels, as a lead compound to discover water-soluble N-type calcium channel blockers with low activity against L-type channels enough not to have an influence on the cardiovascular systems.¹ To serve this purpose, a library of 1,4-dihydropyridine-3-carboxylate derivatives was synthesized and a structure-activity relationships study was performed. From these studies, we have identified the highly water-soluble derivative APJ2929 which exerted effective blocking actions on N-type, but 170-fold lower activity for L-type channel compared to that of APJ2708. Further biological characterization of APJ2929 based on the electrophysiological as well as *in vivo* results will be described and discussed.

Reference

- [1] Yamamoto, T.; Niwa, S.; Ohno, S.; Onishi, T.; Matsueda, H.; Koganei, H.; Uneyama, H.; Fujita, S.; Takeda, T.; Kito, M.; Ono, Y.; Saitou, Y.; Takahara, A.; Iwata, S.; Shoji, M. *Bioorg. Med. Chem. Lett.*, 2006, 16(4), 798.

P151

A MOLECULAR DYNAMICS AND MM-PBSA STUDY INTO THE CYCLIN-DEPENDENT KINASE 5 INHIBITION OF PEPTIDES DERIVED FROM P25

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Cyclin Dependent Kinase 5 is a unique member in the cyclin dependent kinase family, which are mainly involved in the pathology of cell cycle related diseases. In contrast to the other members activated by cyclins, CDK5 is regulated by binding the neuron-specific noncyclin molecule, p35, or its truncated form, p25, thus, plays crucial roles in some neurodegenerative diseases such as Alzheimer's or Parkinson's Diseases. Recently, it was reported that a CDK5 inhibitory peptide (CIP) from p25 can specifically inhibit CDK5/p25 activity *in vitro*, but had no effect on endogenous cdc2 kinase activity. CIP, therefore, may develop a specific CDK5 inhibition strategy in the treatment of neurodegeneration. Mechanism of the inhibition, however, still remains unclear. In this work, molecular dynamics simulations and energy calculation models are set up to investigate the deregulation mechanism of the peptide on CDK5 activity. Both structural and energetic analyses are carried out to look into the interactions between CDK5/CIP, CDK5/CIP/substrate peptide, CDK5/p25, and CDK5/p25/substrate. Detailed comparisons of the four combinations reveal that the truncation of p25 leads to both geometrical and energetic influence on both the binding of CDK5/activator and CDK5/activator/substrate peptide and consequently introduce the inhibition deregulations.

P152

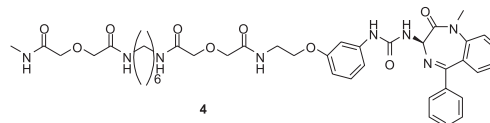
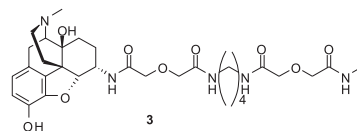
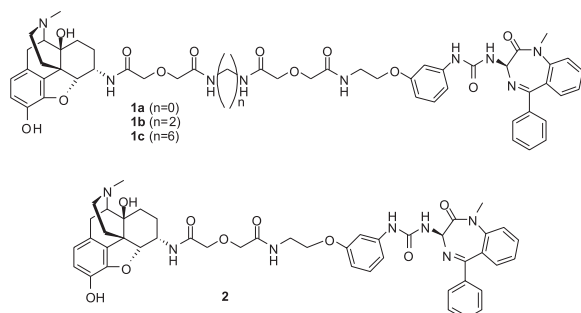
INDUCTION OF HETERODIMERIZATION OF MU OPIOID (MOP) AND TYPE 2 CHOLECYSTOKININ (CCK₂) RECEPTORS BY NOVEL BIVALENT LIGANDS

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Both mu opioid (MOP) and type 2 cholecystokinin (CCK₂) receptors are present in areas of the central nervous system involved in modulation of pain processing, and there is pharmacologic evidence for interaction between these receptors [1-3]. In order to determine whether these receptors interact through heterodimerization or by some other mechanism, bivalent ligands containing mu-opioid agonist and CCK₂ receptor antagonist pharmacophores linked through spacers containing 9 to 22 atoms (**1a-c**, **2**) and the corresponding monovalent ligands were synthesized. The ligands were evaluated using bioluminescence resonance energy transfer (BRET) on COS cells with co-expressed and singly expressed mu and CCK₂ receptors tagged with complementary donor and acceptor fluorescence dyes. In the absence of ligand a BRET signal was not observed, indicating the absence of constitutive association of coexpressed mu and CCK₂ receptors. However, a positive BRET signal was observed on exposure to bivalent ligands containing 16-22 atoms (**1a-c**), indicating that they induced heterodimerization. Significantly, both the bivalent ligand **2** with a shorter (9-atom) spacer, and a combination of monovalent ligands containing opioid agonist (**3**) and CCK₂ antagonist (**4**) pharmacophores did not produce a BRET signal, supporting the critical nature of the spacer in inducing receptor heterodimerization. The absence of constitutive heterodimerization suggests that mu/CCK heterodimers are not responsible for the interactions reported between morphine and CCK in the central nervous system. This represents the first demonstration that an appropriately designed bivalent ligand can induce heterodimerization between membrane-bound receptors. Moreover, such bivalent ligands are potential tools for determining whether there is an *in vivo* pharmacologic advantage to inducing dimerization between receptors that do not normally associate in the plasma membrane [4].



References

- [1] IUPHAR RECEPTOR DATABASE, Opioid receptors, <http://uphar-db.org/PRODIGPCR>
- [2] H.B. Gutstein and H. Akil in Goodman and Gillman's *The pharmacological Basis of Therapeutics-11th Ed.* Chapter 21 opioid analgesics (The McGraw-Hill Companies, Inc., 2006, USA).
- [3] Saito, A.; Goldfine, I.D.; Williams, J.A. Characterization of receptors for cholecystokinin and related peptides in mouse cerebral cortex. *J. Neurochem.* 1981, 37, 483-490.
- [4] Submitted to *J. Med. Chem.*

P153

THE INVESTIGATION OF INHIBITORY EFFECTS OF SOME BENZOXAZINE DERIVATIVES ON EUKARYOTIC DNA TOPOISOMERASE I

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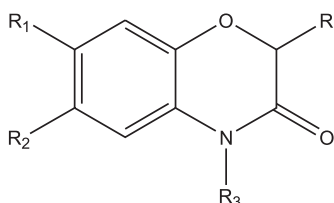
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The discovery of DNA topoisomerases has added a new dimension to the study of anticancer drugs. Role of the mammalian DNA topoisomerases as molecular targets for anticancer drugs was not recognized until 1984 [1-3]. Based on their functional mechanisms, DNA topoisomerases have been classified into two types. Type I DNA topoisomerase breaks and rejoins only one of the two strands during catalysis, while type II DNA topoisomerase acts both strands for each DNA strand-passing reaction and it requires ATP for full activity [4]. These compounds are called poisons because they convert this essential nuclear enzyme into a lethal poison. In comparison to topoisomerase II inhibitors, however, there are fewer known topoisomerase I inhibitors and camptothecin is the most extensively studied mammalian topoisomerase I poison [5,6].

In this study derivatives of 18 benzoxazine compound which were recently synthesized [7,8] by our group and investigated for their inhibitory activity on eukaryotic DNA topoisomerase I (Formula).

It has been showed that 14 compounds among the tested compounds, having IC₅₀ values between 1.61-24.84 µg/ml, were found inhibitory active on eukaryotic DNA topoisomerase I as catalytic inhibitors. Moreover, the test results indicated that ethyl(6-chloro-4-methyl-3-oxo-3,4-dihydro-2H-1,4-benzoxazin-2-yl)acetate (**BS 10**) exhibited poison effect as eukaryotic DNA topoisomerase I inhibitor, having IC₅₀ value 0.00017 µg/ml, showing more potency than the reference drug, Camptothecin (IC₅₀=0.013 µg/ml).



R = CH₂COOC₂H₅, OH ; R₁ = H, NO₂, NH₂

R₂ = H, CH₃, Cl, COOC₂H₅ ; R₃ = H, CH₃

Formula

References

- [1] K.M. Tewey, T.C. Rowe, L. Yang, B.C. Halligan, L.F. Liu, Science 226 (1984) 466_/468.
- [2] E.M. Nelson, K.M. Tewey, L.F. Liu, Proc. Natl. Acad. Sci. USA 81(1984) 1361_/1365.
- [3] K.M. Tewey, G.L. Chen, E.M. Nelson, L.F. Liu, J. Biol. Chem. 259 (1984) 9182_/9187
- [4] J.L. Nitiss, Biochem. Biophys. Acta 1400 (1998) 63–81.
- [5] C.Y. Ting, C.T. Hsu, H.T. Hsu, J.S. Su, T.Y. Chen, W.Y. Tarn, Y.H. Kuo, W.P. Jacqueline, L.F. Liu, J. Hwang, Biochem. Pharm. 66 (2003) 1981-91.
- [6] R.C. Gallo, J. Whan-Peng, R.A.H. Adamson, J. Natl. Cancer Inst. 46 (1971) 789-95.
- [7] Yalçin, I., Tekiner, B.P., Ören-Yildiz, I., Temiz-Arpaci, Ö., Aki-Sener, E., Altanlar, N., Indian Journal of Chemistry, 42B, (2003)905-909,.
- [8] Alper-Hayta, S., Aki-Sener, E., Tekiner-Gulbas, B., Yildiz, I., Temiz-Arpaci, O., Yalcin, I., Altanlar, N., Eur. J. Med. Chem., 41 (2006) 1398-1404

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INDENE-BASED SCAFFOLDS. I. DESIGN, SYNTHESIS AND IN VITRO BINDING PROFILES

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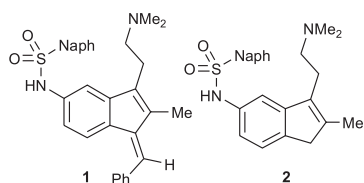
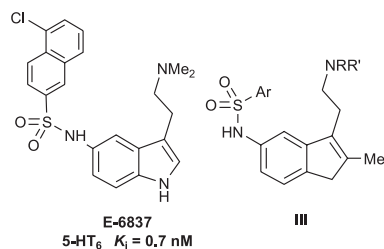
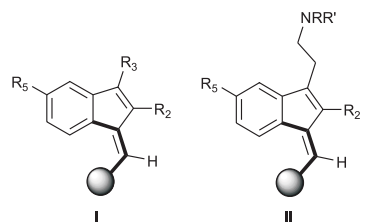
A survey of biologically active (*Z*)-stilbenes shows that (*Z*)-aryl(heteroaryl)methylideneindenenes **I** form an ensemble

of compounds with a variety of pharmacological profiles. As part of a project aimed at the study of (*Z*)-stilbenes with potential biological properties in CNS, we focused our attention on an indene core of general type **II** since indenenes constitute a source of pharmacologically active molecules and their synthesis and pharmacology have not yet been extensively explored. We thus hoped to maximise the likelihood of discovering compounds with biological properties.

On the basis of these premises, the first series in indene compounds is based on the *cis*-indene structure **I** in which the (*Z*)-stilbene moiety is embedded and the traditional *N,N*-dimethylaminoethyl CNS functionality is incorporated in the 3-position. Several indenenes of type **II** were synthesized and profiled against a panel of 64 radioligand binding assays along with the *h5*-HT₆ serotonin receptor but none of them showed significant binding affinities.

The subsequent designing step was namely the incorporation of a sulfonamide group in the 5-position of the indene ring since studies of *h5*-HT₆ serotonin receptor ligands have highlighted the relevance of a sulfonamide moiety for binding, such as the series of indole-based sulfonamides reported by Esteve Laboratories, e.g. the indolylsulfonamide agonist **E6837** (*K_i* = 0.7 nM).^{1,2}

Accordingly, (*Z*)-benzylideneindenylsulfonamide **1** and the simple indene counterpart **2** were prepared and the binding at the *h5*-HT₆ serotonin receptor was with mediocre affinity (*K_i* = 216.5 nM) for **1** and enhanced affinity for **2** (*K_i* = 50.6 nM). Selected reduced indenylsulfonamides **III** were then synthesized and tested, showing *K_i* values as low as 20.2 nM.



References

- [1] (a) R. Mercè, B. Andaluz and J. Frigola, Worl Patent WO 03/042175 A1, 2003. (b) J. Holenz, R. Mercè, J. L. Díaz, X. Guitart, X. Codony, A. Dordal, G. Romero, A. Torrens, J. Mas, B. Andaluz, S. Hernández, X. Monroy, E. Sánchez, E. Hernández, R. Pérez, R. Cubí, O. Sanfeliu and H. Buschmann, *J. Med. Chem.*, 2005, 48, 1781–1795.
- [2] D. J. Heal, S. L. Smith, A. Fisas, X. Codony and H. Buschmann, *Pharmacol. Ther.*, 2008, 117, 207–231.

Acknowledgements: This research was supported by Laboratorios Dr. Esteve, S.A. through projects FBG2002-301362, FBG2003-302131 and FBG-2004/05-2302663 Fundació Bosch i Gimpera-Universitat de Barcelona, Spain. N.M. and S.L.-P. are grateful to Laboratorios Dr. Esteve, S.A. for a post-doctoral and a graduate fellowships, respectively.

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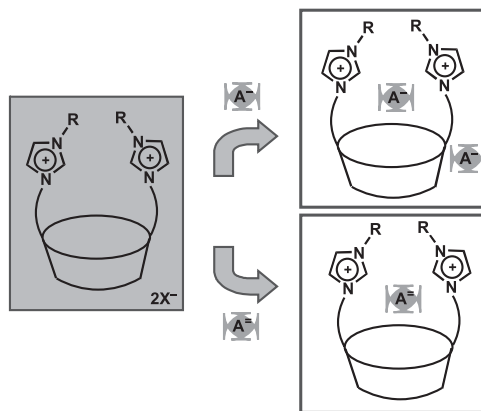
BIS(IMIDAZOLIUM)-CALIX[4]ARENE RECEPTORS FOR ANION BINDING

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Anions play a number of fundamental roles in biological and chemical processes and the development of selective anion receptors is an area of current importance. Accordingly, anion recognition chemistry includes current developments in anion-templated synthesis and selective artificial anion receptors, especially positively charged systems. The use of imidazolium salts as a recognition element for anions has already been demonstrated. In parallel, calixarenes are particularly attractive scaffolds for receptor development, the macrocyclic core being available in a variety of sizes, easily pre-organized into a number of topographies and readily selectively functionalized for the apposite introduction of ligands.¹ This circumstance has given a biological perspective in the rapidly growing field of Bionanotechnology, whose aim is to develop new tools for biology, new biomaterials, selective sensors and supramolecular devices for clinical analysis, new therapeutics and smart drug delivery systems.

As part of our ongoing research into imidazolium-based frameworks,² we herein report the anion binding properties of the dicationic bis(imidazolium) salts directly bonded to the upper rim of the calixarene structure than were studied by ¹H NMR spectroscopic methods. Efforts are currently being directed toward the use of imidazolium molecular motifs for process controlled by hydrogen-bonding networks to produce systems for anion sensing and molecular machinelike behaviour.



References

- [1] (a) L. Baldini, A. Casnati, F. Sansone and R. Ungaro. *Chem. Soc. Rev.* 2007, 36, 254-266. (b) P. A. Gale, S.E. García-Garrido and J. Garric, *Chem. Soc. Rev.* 2008, 37, 151-190.
- [2] (a) E. Alcalde, N. Mesquida, M. Vilaseca, C. Alvarez-Rúa and S. García-Granda, *Supramol. Chem.* 2007, 19, 501-509. (b) I. Dinarès, C. García de Miguel, M. Font-Bardia, X. Solans and E. Alcalde, *Organometallics* 2007, 26, 5125-5128.

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SYNTHESIS, X-RAY ANALYSIS AND THEORETICAL STUDY OF SOME NOVEL DIHYDROPYRIDINE DERIVATIVES OF COUMARIN

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Abstract: Dihydropyridines are well-known heterocyclic compounds with different pharmacological activities, such as antihypertensive, spasmolytic, anticonvulsant, muscle relaxant etc.

In this relation, we synthesized new class coumarin derivatives of 1,4-dihydropyridine (not available in literature), which were synthesized by two-step reaction, including classical Hantzsch reaction in first step.

The synthesized compounds are determined with standard analytical methods – elemental analysis, ¹H NMR, ¹³C NMR and IR. The structures of the corresponding compounds are confirmed from X-Ray crystallography data, which is presented.

The molecular geometry of the compounds was optimized with PM6 and AM1 (for comparison) semiempirical methods (MOPAC2007) and then we determine some descriptors, such as Mulliken-charges, pKa values, logP

values, which we use to explain the reaction pathway, acid-base properties and lipophilicity of the this drug-candidates.

Key Words: Calcium Channel blockers, Dihydropyridines, Coumarin, Semiempirical methods, Spectral analysis

References

- [1] Valenti P., Rampa A., Budriesi R., Bisi A., Chiarini A. *Bioorg. & Med. Chem.*, 1998; 6(6), (803-810)
- [2] MOPAC2007, James J. P. Stewart, Stewart Computational Chemistry, Version 8.032W
- [3] Hantzsch, A. (1881) *Chemische Berichte* 14: 1637-1638. doi:10.1002/cber.18810140214
- [4] Jing-Jing Xia, Guan-Wu Wang. *Synthesis* 2005: 2379-2383. doi:10.1055/s-2005-870022
- [5] Jean Jacques Vanden Eynde, Annie Mayence (2003). *Molecules* 8
- [6] *Synthesen in der Pyridinreihe. Ueber eine Erweiterung der Hantzsch'schen Dihydropyridinsynthese Berichte der deutschen chemischen Gesellschaft* 1898 (p 761-767) E. Knoevenagel, A. Fries doi:10.1002/cber.189803101157

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CHEMICAL-BIOLOGICAL KINASE SPACE

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In classical QSAR and chemometric methods relationships between a biological response and ligand parameters (1D and 2D) are expressed as linear and non-linear equations. Relationships can be derived for a single target or multiple biological responses, the protein targets of which may have no structural similarity. The major limitations to such classical QSAR methodologies are; the need for a wide range of biological responses; the difficulty in extrapolation to new candidate molecules; and the paucity of knowledge gained on the receptor target of the model.

We wished to predict the biological kinase space for a set of compounds but wanted to avoid the limitations of classical QSAR methods. Chemometrics tools were employed to correlate molecular structure to enzyme inhibition and then a proteochemometric approach was compared to this. In proteochemometrics, property descriptors on aligned amino acid sequences of the target kinases are incorporated in a statistical framework with descriptors of ligands and biological responses. The model predicts the likelihood of activity and selectivity of a compound for any of the targets from the same structural class. Further, properties of the amino acids both in

the target site and off-target amino acids can be investigated to give insights on the target kinase as well as the compound set. The derived selectivity profiles can be used to reduce the number of screening experiments and also to prioritise synthesis in virtual compound libraries.

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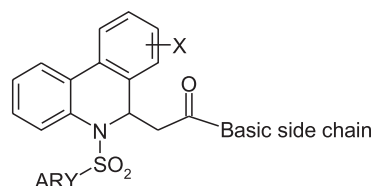
SYNTHESIS AND SAR STUDY OF PHENANTHRIDINE DERIVATIVES AS BRADYKININ B1 RECEPTOR ANTAGONISTS

Éva Bozó, Gyula Beke, István Vágó, György Domány, János Éles, György Keseru, Éva Szentirmay, Tamás Gáti, Sándor Farkas, Katalin Hornok, Éva Schmidt, Katalin Szalai

Gedeon Richter Plc, Budapest, P.O.B. 27, H-1475, Hungary

Bradykinin (BK) B1 and B2 receptors are G-protein-coupled receptors that function in pain and inflammation pathways. The BK B2 receptor is constitutively expressed in most cell types, whereas the BK B1 receptor is rapidly induced after inflammation or tissue trauma. Since BK receptors are involved both in the propagation of inflammation and in mediation of pain, BK receptor antagonists are believed to have therapeutic value in alleviating both pain and inflammation. Selective targeting of B1 receptors might have the advantage of selectively acting on sub-acute and chronic inflammatory pain states without affecting normal physiological functions. Studies with receptor knock-out mice support this notion, since B1 KO mice appeared to develop in good health.

In this poster we are going to describe the optimization process to identify novel phenanthridine derivatives as human BK B1 antagonists.



Optimized derivatives display low-nanomolar affinity for the BK B1 receptor and high selectivity against the BK B2 receptor.

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CARBONIC ANHYDRASE ACTIVATION AND THE DRUG DESIGN

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Metalloenzyme activation and inhibition constitute a widely used means for the regulation of enzymatic activity in a multitude of physiologically relevant biological systems, with important consequences for phenomena such

as biosynthetic reactions, signal transduction, secretion of electrolytes, embryonic development, nerve growth, apoptosis, inflammation, angiogenesis, cancer invasion and metastasis, tissue resorption and remodeling, bone remodeling, atherosclerosis, aneurysm, breakdown of blood-brain barrier, etc.

Recently, the activation mechanism of Carbonic Anhydrase was explained using kinetic, spectroscopic and X-ray techniques. It has been demonstrated that the activators molecules (CAAs) bind at the entrance of the enzyme active-site facilitating the rate-determining step of CA catalytic cycle. Drug design studies have been performed in order to obtain strong CAAs belonging to several chemical classes: amino acids, azoles, amine and their derivatives, etc [1,2,3,4].

Structure-activity correlations of different activators are discussed for the most studied Carbonic Anhydrase isozymes: isoform I and II.

The physiological relevance of CA activation and the possible application of CAAs in Alzheimer's disease and for other memory therapies are treated [5].

Acknowledgments

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References

- [1] Temperini C, Scozzafava A, Puccetti L, Supuran CT. "Carbonic anhydrase activators: X-ray crystal structure of the adduct of human isozyme II with L-histidine as a platform for the design of stronger activators". *Bioorg Med Chem Lett*, 2005, 15, 5136-5141.
- [2] Temperini C, Scozzafava A, Vullo D, Supuran CT. "Carbonic anhydrase activators. Activation of isoforms I, II, IV, VA, VII, and XIV with L- and D-phenylalanine and crystallographic analysis of their adducts with isozyme II: stereospecific recognition within the active site of an enzyme and its consequences for the drug design". *J Med Chem*, 2006, 49, 3019-27.
- [3] Temperini C, Scozzafava A, Vullo D, Supuran CT. "Carbonic anhydrase activators. Activation of isozymes I, II, IV, VA, VII, and XIV with L- and D-histidine and crystallographic analysis of their adducts with isoform II: engineering proton-transfer processes within the active site of an enzyme". *Chemistry*, 2006, 12, 7057-66.
- [4] Temperini C, Innocenti A, Scozzafava A, Mastrolorenzo A, Supuran CT. "Carbonic anhydrase activators: L-Adrenaline plugs the active site entrance of isozyme II, activating better isoforms I, IV, VA, VII, and XIV". *Bioorg Med Chem Lett*, 2007, 17, 628-35.
- [5] Temperini C, Scozzafava A, Supuran CT. "Carbonic anhydrase activation and the drug design". *Curr Pharm Des*, 2008, 14(7), 708-15.

P162

NOVEL TUMOR-TARGETED PEPTIDE-CAMPTOTHECIN CONJUGATES; SYNTHESIS AND BIOLOGICAL EVALUATION.

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Recent improvements in the knowledge of specific receptors overexpressed by cancer cells during their proliferation allow the exploitation of selective ligands, which, conjugated with cytotoxic agents, are able to preferentially address them to tumors, decreasing their toxicity for normal cells. $\alpha_v \beta_3$ and $\alpha_v \beta_5$ integrins, that are highly expressed receptors on tumor cells and sprouting endothelial cells but not on quiescent endothelial and normal cells, represent a promising target for bioconjugates. During the last few years, several attempts have been made, using either monoclonal antibodies or oligopeptides, but they were rewarded with random success, so far. The design of suitable conjugates represents a challenging problem, the nature of the linker connecting the targeting device with the cytotoxic agent being the prerequisite to determine the fate of the whole conjugate. A linker could be considered efficient if it does not diminish the useful biological features of the individual components, for example induce instability or conformational perturbations or ligand-receptor binding decrease. At the same time, a linker must guarantee solubility and bioavailability to the conjugate and stability in the circulation but prompt release of the cytotoxic agent inside the tumor cell. We will present the synthesis and biological evaluation of a series of bioconjugates consisting of a Camptothecin derivative connected with several α_v integrins ligands through different linkers. Cyclopeptides containing the RGD sequence were chosen as targeting devices. The selected linkers were made of small flexible glycols alternate with hydrophilic amino acids or heterocyclic structures functioning as rigid spacers, that confer solubility to the conjugate without interfering with the binding to the receptors. Between the linker and the drug, a new dipeptide sequence was introduced, enough stable in the blood, but cleavable inside the tumor cell after internalization, releasing the original cytotoxic drug. The structure of the conjugates can be schematically represented: RGD—cyclopeptide—glycol—AA—glycol—dipeptide—4-aminobenzylalcohol—camptothecin

where: AA is a hydrophilic amino acid or 1,2,3-triazole; dipeptide is Ala-Cit; 4-aminobenzylalcohol is a self-immolative group.

The presented results demonstrate that the new conjugates maintain a good binding affinity to the receptor, sufficient stability and cytotoxicity *in vitro*.

P164

DESIGN AND SYNTHESIS OF NOVEL (1R)-1-(1-NAPHTHYL)-N-[(4-ARYLPYRROLIDIN-3-YL)METHYL]ETHANAMINES AS ALLOSTERIC ENHANCERS OF CALCIUM-SENSING RECEPTOR

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The calcium-sensing receptor (CaSR) is a G protein-coupled receptor (GPCR), which senses extracellular Ca^{2+} concentration and regulates calcium ion homeostasis. Recently, it has been reported that agonistic regulatory agents of CaSR have an activity to inhibit parathyroid hormone (PTH) secretion by increasing Ca^{2+} sensitivity of CaSR of the parathyroid gland. In our research on allosteric enhancers of CaSR, a series of novel (1R)-1-(1-naphthyl)-N-[(4-arylpyrrolidin-3-yl)methyl]ethanamines derivatives was identified. However, the initial representative compounds of this class displayed CYP 2D6 inhibition. Here we describe the optimization of this series of 4-arylpyrrolidine derivatives that led to the discovery of potent allosteric enhancers of CaSR devoid of CYP 2D6 inhibition. The synthesis and structure-activity relationships of 4-arylpyrrolidine derivatives will be presented.

P165

DISCOVERY OF BIPHENYL COMPOUNDS AS S1P_1 RECEPTOR AGONISTS WITHOUT S1P_3 AGONISM

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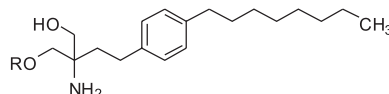
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FTY720¹ is known as a sphingosine 1-phosphate (S1P) receptor agonist with an immunomodulatory activity, and has shown promising efficacy in Phase 2 clinical trials for multiple sclerosis.² Researches on the mechanism of action of FTY720 revealed that FTY720 is converted to its phosphate (FTY720-P) by sphingosine kinase and that it acts as an agonist for four S1P receptors ($\text{S1P}_{1,3,4,5}$) among five known S1P receptors (S1P_1 , S1P_5).³ It was reported that the S1P receptor type 1 (S1P_1) plays a main role for the immunomodulatory activity by decreasing the number of peripheral lymphocytes.⁴

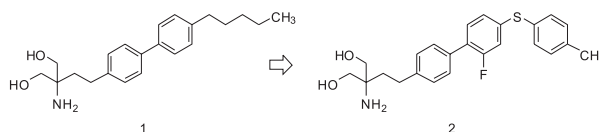
On the other hand, the administration of FTY720 was typically associated with a mild and transient reduction in heart rate as an adverse event.⁵ Furthermore, intravenous administration of FTY720-P to normal mice evoked a severe reduction in heart rate. It has been reported that the administration of FTY720-P to S1P_3 -deficient mice produced no change in heart rate.⁶ This

result indicates that S1P_3 relates to the heart rate reduction caused by FTY720. Therefore, it is widely believed that S1P_1 -selective agonist would be promising as the next generation S1P modulator following FTY720 with less effect on heart rate. Based on this presumption, the research to find selective S1P_1 agonists without agonistic activity on S1P_3 has been conducted vigorously.⁷

The phosphate of biphenyl compound **1** was identified as a compound having S1P_1 selectivity against S1P_3 by a screening of FTY720 analog library. Synthetic study of analogues of **1** based mainly on *in vivo* evaluating systems led to a unique compound **2**. The phosphate of **2** proved to be a S1P_1 agonist without agonistic activity on S1P_3 , and **2** had strong lymphocyte-decreasing effect ($\text{ED}_{50} = 0.084 \text{ mg/kg}$) and no influence on heart rate at a dose of 10 mg/kg by oral administration in rats. On the other hand, the remarkable effect on heart rate remained in almost all of the analogues except **2** although the corresponding phosphates had no S1P_3 agonistic effect. These facts strongly suggest that complicated mechanism independent of S1P_3 would contribute to heart rate reduction caused by FTY720. In this presentation, we describe the detail on the research of compounds with a reduced heart rate decreasing effect.



FTY720 : R=H
FTY720-P : R=P(O)(OH)₂



References

- [1] (a) Adachi, K.; Chiba, K. et al. *Bioorg. Med. Chem. Lett.*, 1995, 5, 853. (b) Kiuchi, M.; Adachi, K. et al. *J. Med. Chem.* 2000, 43, 2946.
- [2] Kappos, L. et al. *N. Engl. J. Med.* 2006, 355, 1124.
- [3] (a) Mandala, S. et al. *Science* 2002, 296, 346. (b) Brinkmann, V. et al. *J. Biol. Chem.* 2002, 277, 21453.
- [4] Matloubian, M. et al. *Nature* 2004, 427, 355.
- [5] Tedesco-Silva, H. et al. *Transplantation* 2004, 77, 1826.
- [6] Forrest, M. et al. *J. Pharmacol. Exp. Ther.* 2004, 309, 758.
- [7] (a) Pan, S. et al. *Chem. Biol.* 2006, 13, 1227. (b) Yan, L. et al. *Bioorg. Med. Chem. Lett.*, 2007, 17, 828.

P166

SEMI-SYNTHETIC OPTIMIZATION OF NATURAL PRODUCT LEADS

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Natural products have been the major sources of chemical diversity for starting materials for driving pharmaceutical discovery over the past century. Many natural products and their semi-synthetic derivatives have been successfully developed into drugs in almost all therapeutic areas.

Despite the initial success of combinatorial biosynthesis and genetic engineering to improve natural product lead and candidate generation, these methods are still in its industrial infancy. About 10% of all natural product derived drugs will reach the market structurally unchanged, the remaining 90% have to be optimized by synthetic approaches. While total synthesis too often is limited by poor overall yield and therefore uneconomical procedures, most tractable leads have to be optimized in respect to potency, selectivity and pharmacokinetic traits with the special armament of semi-synthesis.

The poster will cover the major challenges of semi-synthesis: the hit-to-lead platform ("What is a good natural product lead?"); up-scaling of the starting natural product; selective modification of site-specific groups by omitting yield diminishing protecting group strategies; ADMET optimization strategies. Case studies will address the aforementioned key topics.

P167

SYNTHESIS AND SAR OF THIAZOL-2(3H)-YLIDENE-1-ETHANONE DERIVATIVES AS CB₂ SELECTIVE AGONISTS

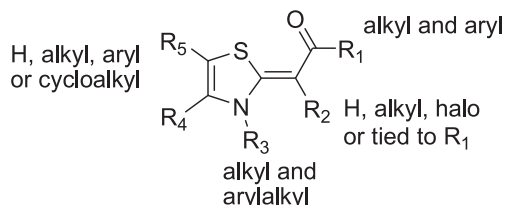
Teodozji Kolasa, Meena Patel, Xueqing Wang, William Carroll, Michael Dart, Kathy Sarris, Melissa Michmerhuizen, Anthony Daza, George Grayson, Yihong Fan, Sutapa Mukherjee, Tiffany Runyan Garrison, Loan Miller, Odile El-Kouhen, Betty Yao and Michael Meyer

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Cannabis has been utilized medicinally for centuries in a broad range of disease states due to its potential analgesic, anesthetic, anti-inflammatory and antiemetic effects. These physiological actions are mediated by two subtypes of the cannabinoid receptor, CB₁ and CB₂. CB₁ receptors are widely distributed throughout the CNS, and are expressed in the periphery in a variety of tissues of the cardiovascular and gastrointestinal systems. By contrast, CB₂ receptors are most abundantly expressed in multiple lymphoid organs and cells of the immune system, including spleen thymus, tonsils, bone marrow, pancreas, and mast cells. It is well documented that essentially all of the CNS adverse effects of cannabinoid agonists are mediated via activation of the CB₁ subtype. Targeting the CB₁ receptor for the management of pain would require the design peripherally restricted agonists to avoid the

undesired psychotropic effects associated with activation of central CB₁ receptors. Another approach to avoid undesirable CNS effects is to identify CB₂ receptor selective agonists.

A variety of structurally distinct CB₂-selective agonists has demonstrated efficacy in preclinical models of inflammatory and neuropathic pain, including HU-308, JWH-133, AM1241, GW-842166X, and A-796260. These compounds produce antinociception at doses that do not elicit CB₁-mediated side effects such as hypoactivity, hypothermia and catalepsy, thus providing support for the concept of utilizing CB₂ agonists as therapeutically effective analgesic agents. We have identified a thiazol-2(3H)-ylidene-1-ethanone based series of potent and selective CB₂ agonists for the treatment of pain. SAR studies involved evaluation of the acyl substituent (R₁), the ylidene moiety (R₂), various N(3)-side-chains (R₃), and several C(3) and C(4)-thiazole substituents (R₄ and R₅). Two synthetic approaches to this class of compounds will be described. Potency and selectivity were assessed by radioligand binding assays performed in cell lines that express recombinant human CB₂ or CB₁ receptors. Functional efficacy at the CB₂ receptor was measured in a calcium flux (FLIPR) assay. Numerous full agonists were found to possess the combination of potent CB₂ receptor activity and high selectivity versus the CB₁ receptor binding site. In addition, several compounds from this series of CB₂-selective agonists demonstrated efficacy in an incisional model of post-operative pain.



P168

2-ARYLIDENE-1-INDANONES WITH POSSIBLE ANTI-INFLAMMATORY AND ANTIPROTEOLYTIC ACTIVITY. SYNTHESIS AND PHARMACOCHEMICAL STUDY.

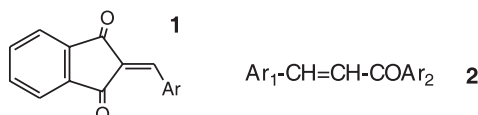
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One of the principal interests in our research is the design of antioxidant/ anti-inflammatory candidates based on the α , β unsaturated keto scaffold. A number of these molecules have been shown to alkylate thiols but not amino or hydroxyl substituents; the latter two functional groups are found in nucleic acids and thus enones may be devoid of unwanted genotoxic properties.

The 2-arylidene-1-indanones **1** may be regarded as cyclic analogues of chalcones **2**. A comparison of the shapes of representative compounds in series **1-2** was

planned which may provide information as to the structural features which influence biological activities. The diketones **1** were prepared by the general procedure [1, 2]: a mixture of 1,3-indandione, aryl aldehyde, piperidine and ethanol was heated under reflux for 2–3 h until 1,3-indandione disappeared. On cooling, the precipitate was collected and recrystallized from ethanol or flash chromatography to give the 2-arylidene-1, 3-indandiones.



The compounds have been identified using IR, ¹H-NMR, ¹³C-NMR spectra and elemental analyses. Lipophilicity as R_m values was determined using RPTLC.

The derivatives were screened for their biological activity *in vitro* on: a) soybean lipoxygenase inhibition, b) interaction with 1,1-diphenyl-2-picryl-hydrazyl (DPPH) stable free radical, c) antiradical activity as loss of ABTS[•] radical, d) inhibition of lipid peroxidation using the AAPH protocol, e) scavenging of superoxide anion radicals, f) antiproteolytic activity against trypsin g) inhibition of thrombin, h) *in vivo* for the inhibition of carrageenin induced rat paw edema.

The compounds have shown interesting biological activities as a result of their physicochemical features. The results are discussed in terms of the structural and physicochemical characteristics of the compounds.

References

- [1] Dimmock JR, Das U, Gul HI, Kawase M, Sakagami H, Barath Z, Ocsofsky I, Molnár J. *Bioorg Med Chem Lett* 2005;15:1633–1636.
- [2] Dimmock JR, Jha A, Zello GA, Allen TM, Santos CL, Balzarini J, De Clercq E, Manavathu EK, Stables JP. *Pharmazie* 2003;58:227–232.

P170

C-11 AMINO ANALOGUES OF CRYPTOLEPINE: ANTIPLASMODIAL ACTIVITY, CYTOTOXICITY AND INTERACTIONS WITH A 12 MER DNA OLIGONUCLEOTIDE (GATCCTAGGATC)₂

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Malaria is the most important infectious tropical and sub-tropical disease, with up to 660 million cases in 2002.

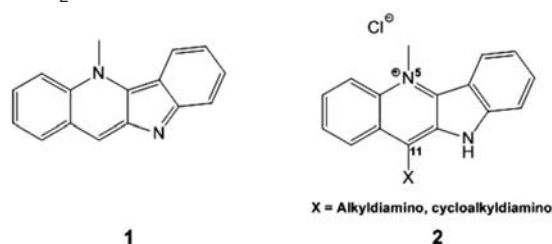
It is caused by parasites of the *Plasmodium* species, the most lethal of which is *P. falciparum*.¹

Cryptolepine (**1**), the major alkaloid of the roots of the West African climbing shrub *Cryptolepis sanguinolenta*, is a herbal drug used traditionally in the treatment of malaria. Like chloroquine and other related 4-aminoquinolines, cryptolepine's antimalarial mode of action is exerted in the acidic food vacuole (pH 5.5) of the *Plasmodium* by binding to hemozoin formed as a result of the haemoglobin digestion.²

However, cryptolepine has cytotoxic properties, possible because it is a DNA intercalator, binding to CG-rich sequences containing nonalternating CC sites. Cryptolepine interacts with the CC sites through base-stacking intercalation, enhanced by the asymmetry of the drug fitting the target in the case of nonalternating sites (CC)-(GG), because it allows different stacking on both sides.³

A basic amino side-chain is a major chemical feature required for 4-aminoquinoline accumulation in the acidic food vacuole of the parasite. Based on this structural requirement, we have designed a novel group of cryptolepine analogues with a basic side-chain at C-11(**2**), aiming to increase their accumulation in parasite food vacuole.

Their antiplasmodial activity was evaluated *in vitro* against the multidrug resistant *Plasmodium falciparum* W2, showing stronger activities than the parent **1**, with IC₅₀ values from 22 to 184 nM. The *in vitro* cytotoxicity of the new compounds on human endothelial cell line (HUVEC) revealed that compounds with branched side-chains were particularly cytotoxic while the compounds containing a cycloalkyl group were less toxic. These differences in cytotoxicity according to the side-chain structure led us to study the binding of these compounds to the self-complementary 12-mer duplex d(GATCCTAGGATC)₂.



Acknowledgments

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References

- [1] Schlitzer, M., *Arch Pharm Chem Life Sci* 2008, 341, 149-163
- [2] Onyeibor, O. *et al.*, *J Med Chem* 2005, 48, 2701-2709
- [3] Lisgarten, J.N., *et al.*, *Nature Struct Biol* 2002, 9, 57-60

P171

HISTAMINE H₃ ANTAGONISTS WITH SEROTONIN REUPTAKE INHIBITOR ACTIVITY

Letavic, Michael A.; Aluisio, Leah; Barbier, Ann J.; Boggs, Jamin D.; Bonaventure, Pascal; Carruthers, Nicholas I.; Keith, John M.; Gomez, Leslie A.; Jablonowski, Jill A.; Lord, Brian; Lovenberg, Timothy W.; Ly, Kiev S.; Miller, Kirsten L.; Shah, Chandra; Stocking, Emily M.; Wilson, Sandy J

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Depression is a serious disease that affects millions of people worldwide. Numerous treatments are now available for this disease, including the serotonin reuptake inhibitors (SSRI's), which are among the most popular drugs for the treatment of this disease. SSRI's are effective in many cases, however improved therapies are warranted due to the facts that many patients report sleep disorders and mild cognitive impairments associated with their depression. One approach that has been utilized to overcome these issues is to combine a serotonin reuptake inhibitor with a wake-promoting agent. This has proven somewhat effective in the clinic. As an example, the wake-promoting agent modafinil has been successfully used in combination with an SSRI to improve symptoms in depressed patients. Histamine H₃ antagonists have also been shown to increase waking in pre-clinical trials and our approach towards the discovery of novel antidepressants has been to combine SSRI activity with histamine H₃ antagonism in order to attempt to improve the efficacy of SSRI's. We have recently disclosed a number of novel histamine H₃ antagonists and dual histamine H₃ antagonists/SSRI's and we have been able to demonstrate that these agents effectively occupy the histamine H₃ receptor in pre-clinical studies, while simultaneously increasing the levels of serotonin in the pre-frontal cortex. We now report our efforts to improve the pharmacokinetic profiles of these compounds in order to identify compounds suitable for pre-clinical safety evaluation.

P172

FROM 11 β -HSD-1 INHIBITORS TO I_{KS} BLOCKERS: IDENTIFICATION OF ANOTHER CARDIOVASCULAR LIABILITY BESIDES HERG

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Johnson & Johnson Pharmaceutical Research and Development, Research and Early Development Europe, Beerse; J&J Center of Excellence for Cardiovascular Safety, Beerse.

In recent years, drug-induced long QT syndrome (d-LQTS) has become a major concern of drug safety, and consequently an important focus in the preclinical testing of new compounds. Although several ion channels are responsible for depolarisation and repolarisation of car-

diac cells, a central role in the development of d-LQTS has been attributed to the blockage of the hERG (human ether-à-go-go) K⁺ channel, which is responsible for the rapid delayed-rectifier current (I_{Kr}). Several strategies have been worked out to detect possible hERG interactions in an early stage. Also, based on a wealth of structure-activity data and the determination of the detailed 3-D structure of several bacterial potassium channels, medicinal chemistry strategies have been defined in order to prevent compounds from blocking hERG.

Recently, we advanced two 11 β -hydroxysteroid inhibitors to the preclinical stage. The first compound, an aminoadamantane derivative, was dropped quickly from further study due to a very pronounced effect in the Langendorff rabbit heart and the occurrence of QT prolongation in the anesthetized guinea pig. This compound was shown to inhibit both the hERG channel and a sodium channel. The second compound, an adamantane methanesulfonamide, did not block the hERG or sodium channels, and proved to be clean in the Langendorff rabbit heart. However, upon testing in anesthetized guinea pig, it exhibited a pronounced QT prolongation effect. The effect was corroborated by the observation of spontaneous arrhythmias (Torsades de Pointes) in both the awake and anaesthetized dog. Further study showed that this compound is a potent inhibitor of KvLQT1/mink which, in contrast to hERG, mediates the slowly-activating delayed-rectifier K⁺ current (I_{Ks}). Testing of a series of analogs demonstrated a strong SAR, the activity being dependent both on the presence of the adamantyl sulfonamide and certain substitution patterns of the aromatic substituent. Using a tritiated analog, we also developed a binding assay for I_{Ks}, which predicted well for activity observed in the patch clamp assay.

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INDENE-BASED SCAFFOLDS. III. SYNTHETIC APPROACHES TO ADVANCED KEY INTERMEDIATES: A CONVENIENT ENTRY TO INDENYLACETAMIDES

Sara López-Pérez^a, Neus Mesquida,^a Immaculada Dinarès,^a Jordi Frigola,^b Ramon Mercè,^b and Ermitas Alcalde^a

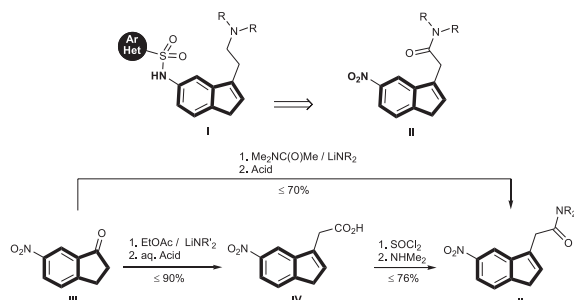
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A scaffold selection based on an indole-indene switch led to the identification of a series of indenylsulfonamides **I** that act as h5-HT₆ serotonin receptor agonists with notable pharmacological binding profiles, showing h5-HT₆ K_i values as low as 4.5 nM (E_{max} 98.5%).¹ In this presentation, we examine and summarize the synthetic peculiarities and limitations of different multi-step synthetic approaches leading to the indenylsulfonamides **I**.

Several parameters play a crucial role in a scaffold selection process, a relevant one being the chemical tractability of the scaffold.² Despite the utility of indenenes in drug discovery and development, along with metallocene-

based catalysis, their complexity means that synthetic approaches have been far less explored than in the case of heteroaromatic compounds such as indoles.^{3,4}

There are several multi-step synthetic routes to indenylsulfonamides **I** and a reasonable pathway appeared to involve indenylacetamides **II** as the key intermediates. We identified the optimal conditions for the transformation of 5-nitroindan-1-ones **III** to indenylacetic acids **IV**, which led to the key acetamides **II**. To shorten the sequential protocol, an alternative and more efficient conversion of indanones **III** to the key acetamides **II** was examined based on an aldol-type condensation using the lithium salt of disubstituted acetamides, followed immediately by dehydration with acid.



References

- [1] See: Indene-based scaffolds. II.
- [2] H. Zhao, *Drug Discovery Today*, 2007, 12, 149-155.
- [3] H. Tsukamoto, T. Ueno and Y. Kondo, *Org. Lett.*, 2007, 9, 3033-3036.
- [4] P. V. Ivchenko, I. E. Nifant'ev, Y. N. Luzikov, S. G. Mkoyan, *Synthesis*, 2007, 1038-1046.

Acknowledgements.

This research was supported by Laboratorios Dr. Esteve, S.A. through project FBG2004/05-302663 Fundació Bosch i Gimpera-Universitat de Barcelona, Spain. Thanks are also due to the DGI-MEC (CTQ2006-11821/BQU) and the AGAUR (*Generalitat de Catalunya*), *Grup de Recerca Consolidat* 2005SGR00158. S.L.-P. is grateful to Laboratorios Dr. Esteve, S.A. for a graduate fellowship and the AGAUR for a F.P.I. fellowship.

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THE SYNTHESIS OF 6-DEAZAFORMYCIN A

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The potential applications of C-nucleosides in nucleic acid chemistry attract wide interest. A number of C-nucleosides are important antibiotics, which possess interesting chemotherapeutic properties. Some of the most interesting C-nucleoside antibiotics are the purine-like

C-nucleosides formycin A and formycin B which closely mimic the isosteric naturally occurring N-nucleosides adenosine and inosine and substitute for them in many enzymatic reactions.

As a continuation of an ongoing project towards the design and synthesis of C-nucleoside antibiotics and structurally related analogs we present here details on the preparation of 7-amino-3-(β-D-ribofuranosyl)pyrazolo[4,3-*b*]pyridine. This novel nucleoside can be viewed as single modification of formycin A (6-deaza) and thus provides a very useful probe for chemical and biological studies on the importance of the 6-nitrogen of formycin A.

The synthesis features the condensation of a suitably substituted, lithiated 2-picoline with 2,3,5-*tri-O*-benzyl-D-ribonolactone, borohydride reduction of the resulting hemiacetal followed by Mitsunobu cyclization, isolation of the desired β-riboside from the anomeric mixture and subsequent ring closure to the corresponding pyrazolo[4,3-*b*]pyridine. Finally, removal of the protecting groups afforded the target C-nucleoside.

Acknowledgements

The study was supported by a Ministry of Industry, Energy and Technology of Greece, Research Grant (Program ENTER)

P175

INDENE-BASED SCAFFOLDS. II. DISCOVERY OF NOVEL INDENYLSULFONAMIDES AS POTENT AND SELECTIVE SEROTONIN 5-HT₆ RECEPTOR AGONISTS

Neus Mesquida,^a Sara López-Pérez,^a Jordi Frigola,^b Ramon Mercè^b and Ermitas Alcalde^a

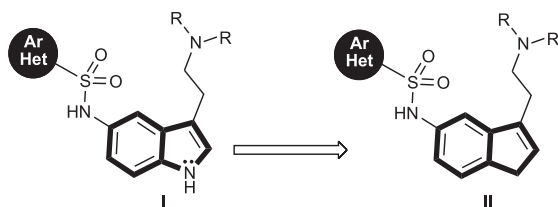
^a *Laboratori de Química Orgànica, Facultat de Farmàcia, Universitat de Barcelona, Avda. Joan XXIII s/n, E-08028 Barcelona, Spain.* ^b *Laboratorios Dr. Esteve S.A., Av. Mare de Déu de Montserrat 221, E-08041 Barcelona, Spain.*

An array of highly potent and selective *h*5-HT₆ ligands have been reported to date, the majority of which have been identified as antagonists whereas agonists have been far less explored.^{1,2} A variety of indole-based ligands targeting the *h*5-HT₆ serotonin receptor have been reported, including early lead compounds and pharmacological tools.^{1,2} Among them, we have focused our attention on the potent and selective *h*5-HT₆ serotonin receptor indolylsulfonamides **I** developed by Esteve Laboratories.^{2,3} In an interesting study carried out concurrently with our work, Glennon and co-workers have examined the binding of several isotriptamines and indenes at the *h*5-HT₆ serotonin receptor, e.g. 1-benzyl-3-(*N,N*-dimethylaminoethyl) indene (*K*_i = 3 nM), revealing that the indolic nitrogen atom was not essential for binding.⁴

We wish to disclose, for the first time, our successful efforts in the discovery of novel indenylsulfonamides based on a scaffold selection. The application of an *indole-indene* switch led to the identification of a series of

indenylsulfonamides **II** that act as $h5\text{-HT}_6$ serotonin receptor agonists with excellent pharmacological *in vitro* binding profiles and showing $h5\text{-HT}_6$ K_i values as low as 4.5 nM ($E_{\max}$ 98.5%). Notably, the scaffold was modified by satisfactory replacing an indole (a π -excessive heteroaromatic ring) with an indene (a non-aromatic carbocyclic system).

The presentation will include: (i) synthetic difficulties encountered due to the fact that the indenylsulfonamides **II** are several sets of compounds, each with their own synthetic peculiarities and limitations; (ii) *in vitro* primary binding profiles of preferred compounds.



References

- [1] R. A. Glennon, *J. Med. Chem.*, 2003, 46, 2795-2812.
- [2] D. J. Heal, S. L. Smith, A. Fisas, X. Codony, H. Buschmann. *Pharmacol. Ther.*, 2008, 117, 207-231.
- [3] (a) R. Mercè, B. Andaluz and J. Frigola, Worl Patent WO 03/042175 A1, 2003. (b) J. Holenz, R. Mercè, J. L. Díaz, X. Guitart, X. Codony, A. Dordal, G. Romero, A. Torrens, J. Mas, B. Andaluz, S. Hernández, X. Monroy, E. Sánchez, E. Hernández, R. Pérez, R. Cubí, O. Sanfeliu, H. Buschmann, *J. Med. Chem.*, 2005, 48, 1781-1795.
- [4] R. Kolanos, U. Siripurapu, M. Pullagurli, M. Riaz, V. Setola, B. L. Roth, M. Dukat, R. A. Glennon, *Bioorg. Med. Chem. Lett.* 2005, 15, 1987-1991.

Acknowledgements

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P176

TOWARDS THE OPTIMIZATION OF AZETIDINE-CONTAINING DIPEPTIDES AS HUMAN CYTOMEGALOVIRUS INHIBITORS

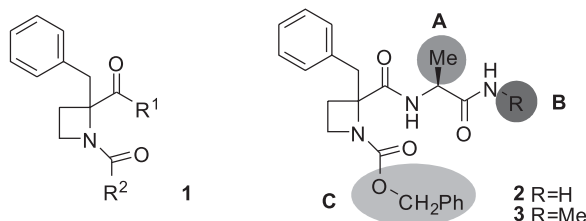
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Human cytomegalovirus (HCMV) is a member of the β -herpes virus family that causes severe infections in immunocompromised individuals. The HCMV pathogen encodes a serine protease essential for capsid assembly

and viral maturation.² Due to its critical role this enzyme is an attractive target for the development of anti-HCMV drugs. Starting from some β -lactam covalent inhibitors,² in a previous work we evolved to a series of phenylalanine-derived azetidines (**1**), as non-covalent HCMV inhibitors.³ In an attempt to optimize both the inhibitory potency and the therapeutic index, we designed three series of structural analogues including modifications at the **A-C** regions of prototypes **2** and **3**. First, we substituted Ala residue (**A**) by different representative amino acids. Second, since we have previously observed that an increase in the hydrophobic character at C-terminus seems to be related with an enhancement of the antiviral activity, bulky aliphatic groups were incorporated at the amide moiety (**B**). Finally, the benzyloxycarbonyl group at the azetidine nitrogen (**C**) was replaced by other urethane groups, or by related acyl, urea, and sulfonyl moieties. Solid-phase methodologies were used for the preparation of compounds modified at **A** and **C** level, while a solution strategy was followed for the synthesis of amide-modified analogues. All derivatives were obtained as couples of diastereoisomers that, in most cases, were chromatographically resolved.

This communication deals with the synthesis, conformational behavior and biological evaluation against HCMV in cell cultures of these three small libraries of azetidine-containing dipeptides.



- [1] Waxman, L.; Darke, P.L. *Antiviral Chemistry & Chemotherapy*, 2000, 11, 1-22.
- [2] Yoakim, C.; Ogilvie, W.; Cameron, D. R.; Chabot, C.; Guse, I.; Haché, B.; Naud, J.; O'Meara, J. A.; Plante, R.; Déziel, R. *J. Med. Chem.* 1998, 41, 2882.
- [3] Gerona-Navarro, G.; Pérez de Vega, M.J.; García-López, M.T.; Andrei, G.; Snoeck, R.; Balzarini, J.; De Clercq, E.; González-Muñoz, R. *J. Med. Chem.* 2005, 48, 2612-2621.

P177

INVESTIGATION OF MurD LIGASE INHIBITORS: BINDING FREE ENERGIES CALCULATED BY LIE (LINEAR INTERACTION ENERGY) APPROACH

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The growing occurrence of bacterial resistance to most of the available antibiotics has underlined an urgent

need for the discovery of novel efficacious antibacterial agents. The biosynthesis of bacterial peptidoglycan, where MurD¹ enzyme is involved in the intracellular phase of UDP-MurNAc-pentapeptide formation, represents a collection of highly selective targets for novel antibacterial drug design. Structural studies of *N*-sulfonyl-glutamic acid inhibitors of MurD with a general structure presented in Figure 1A enabled the possibility of examining the binding modes of this class of compounds providing valuable information to the lead optimization phase of the drug discovery cycle.²

Binding free energy for a series of eight MurD *N*-sulphonyl-Glu inhibitors with determined inhibitory activity was evaluated by using Linear Interaction Energy (LIE) method (Figure 1B).³ Analysis of the interaction energy behavior during the 20ns MD trajectories revealed non-polar van der Waals interactions as the driving force for the binding of these inhibitors. Considering this observation excellent agreement with the experimental free energies was attained. Calculations of binding free energies for the selected compound moieties provided even deeper insight into the source of binding activity. These results constitute new valuable information to assist the lead optimization process of MurD inhibitors leading to better antibacterial drugs.

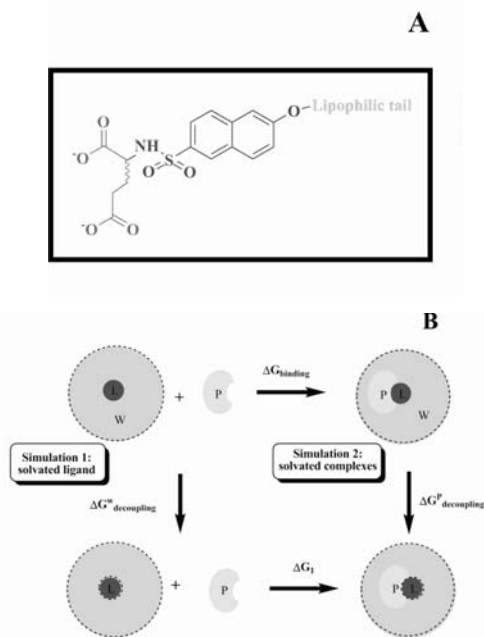


Figure 1. (A) General structure of MurD *N*-sulfonyl-glutamic acid inhibitors. 4 colored sections represent structural moieties for which binding free energy contributions were evaluated. (B) Thermodynamic cycle applied in LIE method. L represents the ligand while P represents the protein that binds it. Blue circle symbolizes water molecules surrounding free ligand or bound protein-ligand complex. Marked circle surrounding the ligand denotes a decoupled ligand that has all its non-bonded interactions (electrostatic and non polar) switched off.

References

- [1] Perdih, A.; Kotnik, M.; Hodoscek, M.; Solmajer, T. Targeted Molecular Dynamics Simulation Studies of Binding and Conformational Changes in *E. Coli* MurD. *Proteins: Structure Funct. Bioinf.*, 2007, 68, 243-254.
- [2] Kotnik, M.; Humljan, J.; Contreras-Martel, C.; Oblak, M.; Kristan, K.; Hervé, M.; Blanot, D.; Urleb, U.; Gobec, S.; Dessen, A.; Solmajer, T. Structural and Functional Characterization of Enantiomeric Glutamic Acid Derivatives as Potential Transition State Analogue Inhibitors of MurD Ligase. *J. Mol. Biol.* 2007, 370, 107-115.
- [3] Åqvist, J.; Medina, C.; Samuelson, J. E. A new method for predicting binding affinity in computer-aided drug design. *Protein Eng.* 1994, 7, 385-391.

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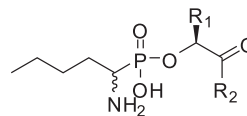
SYNTHESIS OF NORLEUCINE-DERIVED PHOSPHONOPEPTIDES

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Phosphonopeptides (1) have found important application in biology and medicine primarily as of inhibitors of metalloenzymes. The replacement of a hydrolysable peptide bond by non-hydrolysable phosphonate moiety mimics the hypothetical transition state of the substrate upon hydrolysis and may result in potent inhibition of the respective peptidases.

We present herein efficient synthesis of novel type of inhibitors, pseudopeptides of general formula Nle-ψ[PO₂O]⁻Gly(Ala) or Nle-ψ[PO₂O]⁻Gly(Ala)-Val (Figure 1). These compounds may represent potential inhibitors of methionine or leucine aminopeptidases. The proposed pseudopeptide sequence should fulfill the structural requirements of methionine aminopeptidase; Nle or Met in P₁ and small aliphatic residue, such Ala or Gly, in P₁' position. The C-terminal Val in the P₂' position was chosen based on our recent results with statine pseudopeptides as inhibitors of methionine aminopeptidases (2).



R₁ = H, CH₃

R₂ = OH, CH₃, NH₂NHCH(S-iPr)COOH, NHCH(S-iPr)COOCH₃, NHCH(S-iPr)CONH₂

Figure 1

[1] Kafarski P., Lejczak B. *Aminophosphonic and aminophosphinic acids. Chemistry and biological activity.* John Wiley & Sons, Ltd.: Chichester 2000

[2] Mitra S., Dygas-Holz A.M., Jiracek J., Zertova M., Zakova L. *Anal.Biochem.* 2006, 357, 43

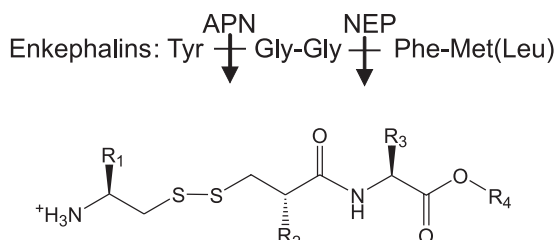
P179

PROTECTION OF ENDOGENOUS ENKEPHALINS BY PL37 A DUAL NEP/APN INHIBITOR INDUCE POTENT ANALGESIC RESPONSES P.O. IN NEUROPATHIC PAIN

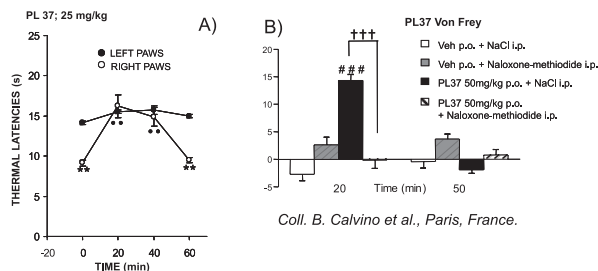
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The endogenous opioid peptides enkephalins, YGGFM(L), are rapidly inactivated at the synaptic cleft by two zinc metallopeptidases, neprylisin (NEP) and aminopeptidase N (APN). Dual inhibitors of these two enzymes strongly increase extracellular levels of endogenous enkephalins in brain structures, inducing potent analgesic responses in numerous animal models of pain (1-2). Moreover chronic treatment with these inhibitors did not produce morphine side effect, consistent with the facilitation of enkephalin-induced stimulation of opioid receptors only in pathways involved in pain control (3). All the previously synthesized inhibitors have a poor oral bioavailability hindering their possible clinical development. Introduction of esters able to be recognized by transporters located at the intestinal barriers led to PL37, an optimized dual inhibitor which is orally and dose-dependent (12.5-50 mg/kg) active in several models of neuroinflammatory and neuropathic pain : in rats intraplantar injection of carragenan, streptozotocin-induced diabetic neuropathy, vincristine-evoked neuropathic pain syndrome (Figure B); *in mice* : tibial osteosarcoma induced hyperalgesia (Figure A) and formalin-test, mechanical hyperalgesia produced by sciatic nerve constriction. The suppression of these nociceptive stimuli is completely reversed by a selective mu antagonist and by methylnaloxonium at a dose (2 mg/kg) when this antagonist is unable to enter the brain. This is consistent with an analgesic effect of PL37 occurring essentially at the peripheral level. In addition these molecules have antidepressant effects, thus reinforcing their pain alleviating effects (1,2).



Finally in several test and particularly in model of cancer-pain, the action of PL37 is synergistically potentiated by gabapentin as illustrated by isobolographic analysis. Preclinical studies have shown a remarkable safety profile and the absence of major drawbacks (e.o. no tolerance, after repeated administration). Therefore, a Phase I clinical study using the oral route is about to be initiated.



Coll. A. Baamonde et al., Oviedo, Spain.

References

- [1] Noble F and Roques BP. *Expert Opinion on Therapeutic Targets*, 2007, 11:145-59.
- [2] Jutkiewicz EM, *CNS Drug Rev.*, 2007, 13(2):192-205.
- [3] Roques BP, Noble F, Dauge V, Fournié-Zaluski MC and Beaumont A, *Pharmacological Rev.*, 1993,45:87-146.

P180

RETHINKING NATURAL PRODUCTS AS A SOURCE OF DRUG LEADS

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We have recently discovered a correlation between the biosynthetic enzymes synthesising a particular natural product and the therapeutic target of the compound. This newest correlation provides the underlying reason why natural products are validated starting points for drug design and library development.¹⁻³ Natural products are privileged structures due to their recognition of biology space (protein surfaces).

Classical bio-assay guided fractionation of extracts is too time consuming to provide a competitive lead generation option. I will describe an approach using pre-fractionated extracts to achieve competitive timelines.

Chemical tractability of natural products has often precluded development. I will describe how lead-like / drug-like properties have been achieved in the pre-fractionated library. Our work has demonstrated that natural product extracts can be tuned into a lead-like or drug-like library by a combination of isolation and computational methods.⁴

I will illustrate why all natural products, not limited to those that have evolved to have specific function in the origin species, can be lead generation starting points and how to optimise the chance of successful lead generation.

References

- [1] McArdle, B. M.; Campitelli, M. R.; Quinn, R. J., *J. Nat. Prod.* 2006, 69, 14-17.
- [2] McArdle, B. M., Quinn, R.J. *ChemBioChem*, 2007, 8, 788-798
- [3] Mitchell, J.K.; Pitcher, D.; McArdle, B.M.; Alnefelt, T.; Duffy, S.; Avery, V.; Quinn, R.J., *Bioorg. Med. Chem. Lett.* 2007, 17, 6521-6524.
- [4] Quinn, R. J.; Carroll, A. R.; Pham, N. B.; Baron, P.; Palframan, M. E; Suraweera, L.; Pierens, G. K.; Muresan, S. *J. Nat. Prod.* 2008, 71, 464-468.

P181

AN INVESTIGATION IN TO THE EFFECT OF URSODEOXYCHOLIC ACID AND DERIVATIVES ON THE GOLGI APPARATUS

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Bile Acids have traditionally been associated with their surfactant actions which enables them to aid in the absorption of lipids from the gut. However there has been much renewed interest in the field of bile acid biology because it has emerged that they activate a number of diverse cellular pathways. These include activation of tumourigenic cascades by the secondary hydrophobic bile acids Deoxycholic acid (DCA) and Chenodeoxycholic Acid (CDCA). DCA in particular has been implicated in liver disorders, colon cancer and oesophageal cancer. On the other hand the hydrophilic bile Ursodeoxycholic acid (UDCA) has been found to act as a cytoprotective agent and to antagonise the actions of DCA.

We have observed that DCA induces fragmentation of the Golgi apparatus, an organelle involved in the post-translation modification of proteins. Interestingly, UDCA has the ability to inhibit this DCA induced fragmentation.

The aims of this project are to 1) Synthesise a series of UDCA analogues to determine whether UDCA's effects are of a physicochemical nature and if not to identify the specific structural requirements for UDCA's protective effect on DCA induced Golgi fragmentation. 2) To use these analogues to elucidate a molecular mechanism of action for UDCA.

A panel of UDCA derivatives with subtle structural modifications have been synthesised and screened in an assay adapted to high content analysis to examine effects on Golgi fragmentation.

We have found that UDCA's effect are independent of physicochemical characteristics such as hydrophobicity, CMC and polar surface area but rather are specific to steroid structure indicating a biological protein interaction. UDCA has previously been shown to interact with the Glucocorticoid Receptor (GR) in rat hepatocytes¹ and we have shown that it induces translocation of the GR to the nucleus in our system. Therefore the role of the GR in

mediating the protective effects of UDCA on the Golgi was investigated. When cells were treated with the GR antagonist, Mifepristone, UDCA was no longer able to inhibit DCA induced fragmentation indicating that UDCA mediates its effects via a glucocorticoid receptor-regulated pathway.

The Golgi is involved in the post translational modification of proteins and membrane trafficking. Disruption of these processes may lead to abnormal secretion, protein glycosylation and intracellular signalling leading to carcinogenesis and represent a novel target for therapeutic intervention with UDCA.

- [1] Weitzel C et al. Ursodeoxycholic acid induced activation of the glucocorticoid receptor in primary rat hepatocytes. *Eur J Gastroenterol Hepatol* 2005; 17 (2): 169-77

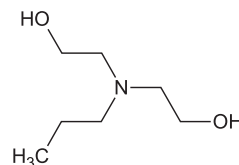
P182

VALIDATION OF A GC-FID METHOD FOR THE DETERMINATION OF N-NITROSODIETHANOLAMINE IN TROLAMINE

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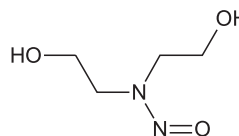
Triethanolamine, called trolamine for short, is a component of many medicines, including (among others) Lamiderm and Ketoprofen. These medicines are applied as an ointment showing soothing and alleviating properties.



Trolamine

Due to its structure, trolamine may be polluted by some nitrogen-containing compounds. Among such compounds, nitrosoamines are the most dangerous, showing several mutagenic and carcinogenic properties.

For pharmaceutical purposes, it is necessary to determine N-nitrosodiethanolamine in trolamine. However, this compound is characterized by high volatility, and low concentration in the real samples.



N-nitrosodiethanolamine

According to the 5th European Pharmacopeia, maximum concentration of N-nitrosodiethanolamine cannot exceed 25 ppb.

The poster presents the results of determination of N-nitrosodiethanolamine in trolamine by means of GC-FID. The results prove that the proposed GC-FID method is an

efficient method for the separation and quantitative determination of N-nitrosodiethanolamine. Compared with the Pharmacopeia-recommended GC-MS method, the proposed GC-FID method is better put into practice due to more common use and higher accessibility of GC-FID equipment.

P183

BLENDING TARGET- AND EFFICACY-BASED STRATEGIES IN DRUG DISCOVERY: ANTICONVULSANTS AND ANALGESICS IN THE SERIES OF NEURONAL L-TYPE CALCIUM CHANNEL ANTAGONISTS

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We have developed a *lower-risk product-focused* strategy which merges 'modern' target- and 'old-fashioned' efficacy-based approaches on the early stages of drug discovery. Our strategy involves (1) design and synthesis of novel analogs of the compounds with known pharmacological effects, (2) *in vivo* 'proof-of-principle' for efficacy and safety in well-established animal models, (3) *in vitro* screening and detection of the *in vitro* site of action for the compounds with an appropriate *in vivo* profile, (4) optimization of the series for enhanced binding at the identified *in vitro* target, (5) building up an expanded therapeutic potential for the series.

The strategy was initially implemented for design, synthesis and *in vivo* evaluation of potential tranquilizers, novel condensed pyrrolopyridines which are the structural analogs of clinically proven GABAergics. Indeed, the lead compound in the series, MPP-021 exhibited mild *in vivo* tranquilizing effects. In addition, MPP-021 demonstrated pronounced anticonvulsant, analgesic, anti-amnesic, and antihypoxic effects, and produced high survival rate in skeletal-muscle traumatic shock animal models on i.p. or p.o. administration in mice and/or rats. Currently, MPP-021 is in preclinical development for the treatment post-operative amnesia, post-operative pain and mild to moderate traumatic brain injury, and potentiation of opioid analgesia and/or opioid withdrawal.

Although designed as a structurally relevant analog of known GABAergics, MPP-021 has no *in vitro* agonist affinity at the GABA_A receptor complex. *In vitro* profiling of MPP-021 at 40 CNS major targets discovered a selective antagonist binding at neuronal L-type calcium channels (DHP and BZT sites) coupled with a weak blockage of the GABA_A chloride channel. Optimization of *in vitro* binding in the series resulted in the compounds with modified profile, enhanced or decreased inhibition of the DHP and/or BZT neuronal L-type calcium channels, and decreased blockage of the GABA_A chloride channel.

The focus of this communication will detail two types of relationship developed within this exciting series of compounds: structure-activity (*in vitro*) and structure-effi-

cacy (*in vivo*) for anticonvulsant and analgesic effects. The mechanism of action for this series is not well understood, although the growing number of studies point out at a variety of CNS functions regulated by neuronal L-type calcium channels [1-3 and references therein].

[1] Ricci A, et al. Neuronal Populations of Rat Cerebral Cortex and Hippocampus Expressed a Higher Density of L-Type Ca²⁺ Channel Than Corresponding Cerebral Vessels. Clin. Exp. Hypertension, 2002; 7&8: 715-726.

[2] Triggie DJ, Calcium Channel Antagonists: Clinical Uses – Past, Present and Future. Biochem. Pharmacol., 2007; 74: 1-9.

[3] Lucinova L, et al. Ca_v1.2 Calcium Channels Modulate the Spiking Pattern of Hippocampal Pyramidal Cells. Life Sci., 2008; 82: 41-49.

P184

SYNTHESIS, OPIOID RECEPTOR BINDING PROFILE AND SAR STUDIES OF 14-ALKOXY-SUBSTITUTED INDOLO- AND BENZOFUROMORPHINANS

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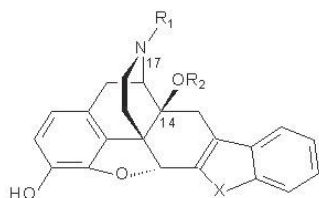
On the basis of pharmacological, behavioral and biochemical studies, opioid receptors have been classified into three major types: μ , δ and κ . Receptor type selective opioid agonists and antagonists are of interest both as pharmacological tools and as potential therapeutic agents for the treatment of pain, addiction, constipation or immunological diseases. During the past 3 decades, one of the major aims of medicinal chemistry and pharmacology has been to develop opioids with high affinity and/or selectivity for each of the three receptor types.

In this study, 14-alkoxy analogues of naltrindole (NTI), naloxindole (NLX) and naltriben (NTB) (Figure) with different benzyloxy substitution in position 14 have been investigated to elaborate on structure-activity relationships (SAR) of the morphinan class of compounds [1-4]. To this aim, several 14- benzyloxy substituted indolo- and benzofuromorphinans were prepared by multi-step synthesis using naloxone and naltrexone as starting materials. Binding affinities and selectivities of the synthesized compounds to opioid receptors (μ , δ and κ) were determined using *in vitro* binding assays with rodent brain membranes. SAR studies relating to the substitution pattern in positions 14 and 17 within this series have been pursued.

Examination of the chemical structure and opioid binding profile i.e. affinities and selectivities reveals certain SAR for the investigated compounds. Opioid binding affinity was sensitive to the character and length of the substituent in position 14. Introduction of 14-benzyloxy groups resulted in reduced affinity and selectivity for the δ

receptor, similar to chain elongation of the 14-alkoxy group. The replacement of the N-allyl by a N-cyclopropylmethyl group in position 17 resulted in increased δ affinity without affecting δ receptor selectivity.

The results of the present study indicate that the position 14 of indolo- and benzofuromorphinans represents a critical site that could be a trigger to develop opioid compounds with increased δ affinity and/or selectivity. These findings provide further evidence that the nature of the substituent at position 14 has a major impact on the abilities of morphinans to interact with opioid receptors.



Figure

NTI: R₁ = CPM; R₂ = H; X = NH
 NLX: R₁ = Allyl; R₂ = H; X = NH
 NTB: R₁ = CPM; R₂ = H; X = O

CPM = Cyclopropylmethyl

References

- [1] Schmidhammer, H.; Schwarz, P.; Wei, Z.-Y.; *Helv. Chim. Acta*, 81, 1215-1222 (1998)
- [2] Schütz, J.; Dersch, Ch.M.; Horel, R.; Spetea, M.; Koch, M.; Meditz, R.; Greiner, E.; Rothmann, R.B.; Schmidhammer, H.; *J. Med. Chem.*, 45, 5378-5383 (2002)
- [3] Portoghese, P.S.; Sultana, M.; Takemori, A.E.; *J. Med. Chem.*, 33, 1714-1720 (1990)
- [4] Biyashev, D.; Monory, K.; Benyhe, S.; Schütz, J.; Koch, M.; Schmidhammer, H.; Borsodi, A.; *Helv. Chim. Acta*, 84, 2015-2021 (2001)

P185

GASTROPROTECTIVE DERIVATIVES OF FERRUGINOL: EFFECTS ON CELL MEMBRANE PEROXIDATION, DAMAGE INDUCED BY TAUROCHOLATE AND GSH CONTENT IN HUMAN CELL CULTURES.

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Ferruginol is an abietane diterpene occurring in plants belonging to the Podocarpaceae, Lamiaceae and Verbenaceae families among others. Ferruginol has been reported as antifungal, antibacterial, cardioactive, mitocidal, antioxidative, antiplasmodial and antitumor agent. Rodríguez et al. (2006) have studied the gastroprotective and ulcer healing effect of ferruginol in mice and rats. Using in vitro models these authors have suggested that ferruginol protects the gastric mucosa by increasing the prostaglandin E₂ (PGE₂) content and preventing the lipid peroxidation of the gastric cells. Furthermore, Areche et al. (2008) have demonstrated that ferruginol protects the gastric mucosa by

increasing the PGE₂ content, inhibiting the acid secretion and maintaining the gastric reduced glutathione (GSH) levels in mice and rats. Areche et al. (2007) prepared 17 semisynthetic ferruginol derivatives for assessment as gastroprotective agents in mice. These authors observed that while some derivatives displayed gastroprotective activity similar to ferruginol other were devoid of activity. In order to compare the cellular effects elicited by the compounds we assessed the activity of the same 17 semisynthetic ferruginol derivatives and the diterpene totarol on membrane peroxidation, damage induced by sodium taurocholate (NaT) and GSH content in human cell cultures.

The activity of the compounds on cell membrane lipid peroxidation induced by *t*-butylhydroperoxide was assessed in fresh human erythrocytes. At 10 µg/mL all the derivatives displayed inhibition of the peroxidation in values ranging from 28 - 83 %. IC₅₀ were determined in all the derivatives that displayed inhibition values higher than 55 %. The compounds and their corresponding IC₅₀ (µg/mL) were: ferruginol (**2**): 0.4; totarol (**20**): 0.5; abieta-8,11,13-trien-12-yl propionate (**7**): 1.79; abieta-8,11,13-trien-12-yl propenoate (**9**): 1.44; abieta-8,11,13-trien-12-yl-4'-methylbenzoate (**12**): 2.55 and abieta-8,11,13-trien-12-yl-3'-nitrobenzoate (**14**): 5.31, respectively.

The protective effect of the derivatives on the damage induced by NaT was assessed in the human gastric cell line AGS. The treatment with the compounds abieta-8,11,13-triene (**1**) (25 µM), abieta-8,11,13-trien-12-yl-2-chloropropanoate (**8**) (1.6; 3.2 and 6.4 µM), (**9**) (1.6 µM), abieta-8,11,13-trien-12-yl-2'-methylbenzoate (**11**) (62.5 µM), (**12**) (62.5 µM) and abieta-8,11,13-trien-12-yl-4'-nitrobenzoate (**15**) (62.5 µM) increased the viability of the AGS cells.

The effect of the derivatives at 5 µM on GSH content was determined in HL-60 cell cultures, challenged with medium containing 4 % ethanol. Compounds (**7**), (**9**) and abieta-8,11,13-trien-12-yl-4'-methoxybenzoate (**13**) prevented the decrease of the cellular GSH levels induced by ethanol in the HL-60 cells.

Our findings further suggest that ferruginol and its derivatives could act as gastroprotective compounds *in vitro* by different and complementary mechanisms. These promising results encourage further pharmacological studies of these compounds as potential gastroprotective drugs.

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References

- [1] Rodríguez J.A., Theoduloz C., Yáñez T., Becerra T., Schmeda-Hirschmann C. *Life Sci.* 2006, 78, 2503-2509.
- [2] Areche C., Rodríguez J.A., Razmilic I., Yáñez T., Theoduloz C., Schmeda-Hirschmann G. *J. Pharm. Pharmacol.* 2007, 59, 289-300.
- [3] Areche C., Theoduloz C., Yáñez T., Souza-Brito A., Barbastefano V., de Paula D., Ferreira A., Schmeda-Hirschmann G., Rodríguez J.A. *J. Pharm. Pharmacol.* 2008, 60, 245-251.

P186

DEVELOPMENT OF NOVEL PSEUDOPEPTIDE GPR54 AGONISTS WITH HIGH TOLERANCE AGAINST MMP-MEDIATED DIGESTION

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GPR54, a Gq-protein coupled receptor, is stimulated by endogeneous peptides, kisspeptins. Recent studies demonstrated that GPR54/kisspeptin system is involved in placentation and the onset of puberty. This system was originally attended to cancer science because a precursor protein of kisspeptins is encoded by metastasis suppresser gene *KiSS-1*. In early studies, it is observed that kisspeptins attenuated the mobility of several GPR54-expressed cancer cell lines, indicating the therapeutic potential for suppression of cancer metastasis. However, kisspeptins are deactivated by cleavage of the Gly-Leu peptide bond by matrix metalloproteases (MMPs),[1] which assist progression and migration of carcinomas. Since cancer cells secrete a large amount of MMPs, development of novel GPR54 agonists having tolerance against MMP-mediated degradation is desired in terms of the application to anti-metastatic drugs.

We previously identified a potent GPR54 agonist **1** [Bz(4-F)-Phe-Gly-Leu-Arg-Trp-NH₂; Bz(4-F) = 4-fluorobenzoyl] from SAR study on kisspeptin-10, which exerts equipotent agonistic activity to that of kisspeptin-10.[2,3] Pentapeptide **1** shares the C-terminal sequence with kisspeptins and is deactivated by MMPs. We envisioned that the replacement of Gly-Leu dipeptide in **1** with a dipeptide isostere provides the tolerance to enzymatic degradation as well as the inhibitory activity on MMPs with maintenance of GPR54 agonistic activity. For efficient identification of novel GPR54 agonists, we utilized several Gly-Leu type dipeptide mimetics (Gly ψ Leu), which were obtained by modification of Gly-Leu type *E*-alkene dipeptide isostere.[4] The resulting dipeptide mimetics were applied to Fmoc-based solid-phase peptide synthesis for preparation of the mimics of pentapeptide **1**.

Some of the pseudopeptides [Bz(4-F)-Phe-Gly ψ Leu-Arg-Trp-NH₂] reproduced the GPR54 agonistic activity of the original peptide **1**, while inhibitory activity against MMP-2 and MMP-9 was not observed in *in vitro* assay. The degradation of the pseudopeptides by MMP-2 and -9 was not observed. In murine serum, moreover, all of them are more stable compared with the parent peptide **1**.

References

- [1] Takino, T.; Koshikawa, N.; Miyamori, H.; Tanaka, M.; Takuma, S.; Okada, Y.; Seiki, M.; Sato, H. *Oncogene*, 2003, 22, 4617-4626.
- [2] Niida, A.; Wang, Z.; Tomita, K.; Oishi, S.; Tamamura, H.; Otaka, A.; Navenot, J.-M.; Broach, J. R.; Peiper, S. C.; Fujii, N. *Bioorg. Med. Chem. Lett.* 2006, 16, 134-147.

[3] Tomita, K.; Oishi, S.; Cluzeau, J.; Ohno, H.; Navenoto, J.-M.; Wang, Z.; Peiper, S. C.; Akamatsu, M.; Fujii, N. *J. Med. Chem.* 2007, 50, 3222-3228.

[4] Fujii, N.; Nakai, K.; Tamamura, H.; Otaka, A.; Mimura, N.; Miwa, Y.; Taga, T.; Yamamoto, Y.; Ibuka, T. *J. Chem. Soc. Perkin Trans. 1*, 1995, 1359-1371.

P187

NEW INHIBITORS OF HUMAN BETAINE-HOMOCYSTEINE S-METHYLTRANSFERSE

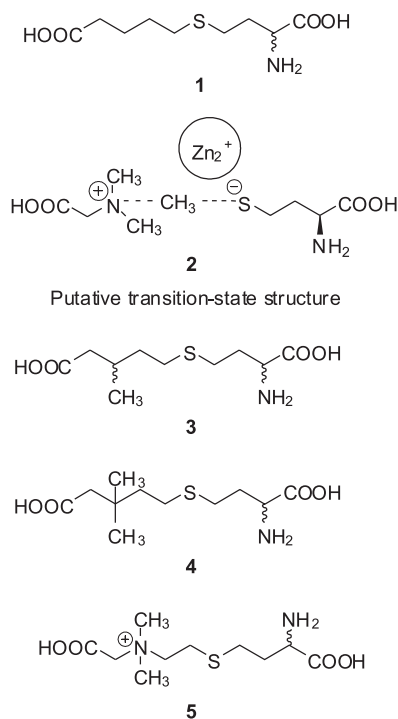
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Betaine-homocysteine S-methyltransferase (BHMT) is an enzyme involved in the metabolism of sulfur amino acids. BHMT is an abundant enzyme but only in the liver and kidney of mammals. It was shown to be Zn-metalloenzyme and the metal ion is essential for catalytic activity. BHMT catalyzes a methyl transfer from betaine to L-Hcy to form dimethylglycine and L-Met, respectively.

During past several years, we developed potent and selective inhibitors of human recombinant BHMT, phosphinic pseudopeptides (**1**) and *S*-alkylated derivatives of Hcy (**2**). The potent inhibitor **1** was co-crystallized with BHMT and the structure of the complex was determined by X-ray crystallography (**3**).



Recently we prepared and tested new series of BHMT inhibitors mimicking the hypothetical transition-state **2**. The best inhibitors **3** and **4** inhibited BHMT in more strongly than previously developed compound **1**. We found that BHMT surprisingly does not accept the presence of nitrogen atom in the alkyl chain attached to the sulphur atom of inhibitors (e.g. compound **5** is a very weak BHMT inhibitor).

- [1] Collinsova, M., Castro, C., Garrow, T. A., Yiotakis, A., Dive, V., and Jiracek, J. *Chem. Biol.* 2003, 10, 113-122.
- [2] Jiracek, J., Collinsova, M., Rosenberg, I., Budesinsky, M., Protivinska, E., Netusilova, H., and Garrow, T. A. *J. Med. Chem.* 2006, 49, 3982-3989.
- [3] Evans, J.C., Huddler, D.P., Jiracek, J., Castro, C., Millian, N.S., Garrow, T.A., Ludwig, M.L. *Structure* 2002, 10, 1159-1171.

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QSAR MODELS BASED ON LIPOPHILICITY FOR QUINOXALINE 1,4-DI-N-OXIDES AS ANTITUBERCULAR CANDIDATES

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Tuberculosis (TB), an infection of *Mycobacterium tuberculosis*, still remains the leading cause of worldwide death among infectious diseases. The statistics indicate that 1.6 million people throughout the world die from Tuberculosis, and there were an estimated 8.8 million new cases in 2005. The current frontline therapy for tuberculosis consists of administering three or more different drugs during an extended period of time. Consequently, problems due to multidrug-resistant TB arise and it is necessary to develop new therapeutic agents in order to treat drug resistant forms of the disease.

As a result of the anti-tuberculosis research project, our group published several studies in which the synthesis and biological evaluation of a large amount of quinoxalines have been described [1-3], thus leading to several compounds that showed important antitubercular activity *in vitro*.

This study resorted to the Quantitative Structure-Activity Relationships (QSAR) formalism [4] for predicting the anti-TB activity on a set including more than 300 quinoxaline derivatives compiled from recent publications. For this purpose, we analyzed the effect of lipophilicity values on the activity of compounds through QSAR models, expressing the lipophilicity contribution with the octanol/water partition coefficient ($\log K_{ow}$) as calculated via different modelling techniques reported in the specialized literature [5]. It is known that the different estimation methods of $\log K_{ow}$ would not yield to the same numerical value for characterizing this quantity for a specific organic chemical, and thus this study discovered the most suitable method. The best linear regression models established with our variable subset selection algorithm [6] were predictive. The application of the QSAR equations developed now enables the proposal of new candidate structures that still do not have experimentally assigned biological data.

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Antimycobacterial data was provided by the Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF) through a research and development contract with the U.S. National Institute of Allergy and Infectious Diseases (NIAID). E. Vicente is grateful to the Ministerio de Educación y Ciencia (Spain) for the grant AP2003-2175. PRD is a researcher from the National Council of Scientific and Technological Research (CONICET).

References

- [1] Zarranz, B.; Jaso, A.; Aldana, I.; Monge, A., Synthesis and antimycobacterial activity of new quinoxaline-2-carboxamide 1,4-di-N-oxide derivatives. *Bioorg. Med. Chem.* 2003, 11, 2149-2156.
- [2] Jaso, A.; Zarranz, B.; Aldana, I.; Monge, A., Synthesis of new 2-acetyl and 2-benzoyl quinoxaline 1,4-di-N-oxide derivatives as anti-*Mycobacterium tuberculosis* agents. *Eur. J. Med. Chem.* 2003, 38, 791-800.
- [3] Jaso, A.; Zarranz, B.; Aldana, I.; Monge, A., Synthesis of new quinoxaline-2-carboxylate 1,4-dioxide derivatives as anti-*Mycobacterium tuberculosis* agents. *J. Med. Chem.* 2005, 48, 2019-2025.
- [4] Hansch, C.; Leo, A., Exploring QSAR. Fundamentals and Applications in Chemistry and Biology. American Chemical Society: Washington, D. C., 1995.
- [5] Virtual Computational Chemistry Laboratory, <http://www.vcclab.org/lab/alogps>
- [6] Duchowicz, P. R.; Mercader, A. G.; Fernández, F. M.; Castro, E. A., Prediction of Aqueous Toxicity for Heterogeneous Phenol Derivatives by QSAR. *Chemom. Intell. Lab. Syst.* 2008, 90, 97-107.

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QSAR ANALYSIS OF 3-ARYLQUINOXALINE-2-CARBONITRILE 1,4-DI-N-OXIDE DERIVATIVES AS ANTIPLASMODIAL AGENTS

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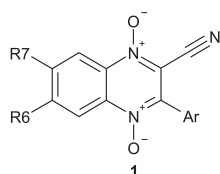
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Malaria is the most important tropical disease, remaining widespread throughout the tropics, but also occurring in many temperate regions. It causes numerous cases of illness and death - especially among children and pregnant women. It also poses a risk to travellers and immigrants, with imported cases increasing in non-endemic areas. Treatment and control have become more difficult with the spread of drug-resistant strains of parasites and insecticide-resistant strains of mosquito vectors.

In a continuing effort to identify new active compounds against *Plasmodium falciparum*, our research group synthesized sixty 3-arylquinoxaline-2-carbonitrile 1,4-di-N-oxide derivatives, **1**. Certain parameters of potency, cytotoxicity and selectivity were established in LSHTM. Four derivatives demonstrated activity against *P. falciparum* K1 strain (chloroquine-resistant) and good selectivity, making them valid new lead-compounds; they are now being more thoroughly studied [1, 2].

For the purpose of providing a rational guide for the synthesis of future compounds of this class, we established different Quantitative Structure-Activity Relationships (QSAR) with the available experimental biological data. The molecular structure of sixty quinoxaline-2-carbonitrile 1,4-di-N-oxide derivatives was appropriately represented by 1497 theoretical descriptors, calculated with Dragon software [3], and the best linear regression models established with our variable subset selection algorithm were predictive [4]. Application of the QSAR equations developed now enables the proposal of new candidate structures that still do not have experimentally assigned biological data.



R6/R7 = H/F, H/Cl, H/CH₃, H/CF₃, H/OCH₃, H/H, F/F, Cl/Cl, CH₃/CH₃

Ar = 4'-W-phenyl, 2'-furyl, 2'-thienyl

W = H, F, Cl, CH₃, OCH₃, OCF₃, COOCH₃

1

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References

- [1] Vicente, E.; Lima, L.M.; Bongard, E.; Charnaud, S.; Villar, R.; Solano, B.; Burguete, A.; Pérez-Silanes, P.; Aldana, I.; Vivas, L.; Monge, A., Synthesis and structure-activity relationship of 3-phenylquinoxaline 1,4-di-N-oxide derivatives as antimalarial agents. *Eur. J. Med. Chem.* 2008, *in press*.
- [2] Vicente, E.; Charnaud, S.; Bongard, E.; Villar, R.; Burguete, A.; Solano, B.; Ancizu, S.; Pérez-Silanes, P.; Aldana, I.; Vivas, L.; Monge, A., Synthesis and antiplasmodial activity of 3-furyl and 2-thienylquinoxaline-2-carbonitrile 1,4-di-N-oxide derivatives. *Molecules*. 2008, *13*, 69-77.
- [3] DRAGON 5.0 Evaluation Version, <http://www.disat.unimib.it/chm>
- [4] Duchowicz, P. R.; Castro, E. A.; Fernandez, F. M.; Gonzalez, M. P., A new search algorithm for QSPR/QSAR theories: Normal boiling points of some organic molecules. *Chem. Phys. Lett.* 2005, *412*, 376-380.

P190

INTERFACING NATURAL PRODUCTS: INULIN OF JERUSALEM ARTICHOKE - AFFIRMATIVE BIOLOGICAL EFFECTS

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Background. Liver plays an important part in maintenance of homeostasis. As a result of disbalance in sugar-fat metabolism, overweight and obesity become a serious health-hazard. Liver – the multifunctional biochemical laboratory of an organism – is responsible for ensuring normal functions of cardio-vascular, digestive and other systems. Since 2000, cardio-vascular diseases have happened to be 55% of all causes of death in Latvia; therefore antihyperlipidemia research is of great importance. **Aim.** Study of influence of the Latvia-produced Jerusalem artichoke concentrate (JAC) (main active substance – β -D-polyfructan inulin) on liver functions and lipid homeostasis in blood, in order to use the JAC as a component in preventive functional food. **Methods.** Influence of the JAC on various specific liver functions, studied by the use of modern hemoinformatics in three months animal experiment. **Results.** An addition of the JAC in feed of the Wistar-line white male rats maintains a constant dynamics of gross accretion, and gradually diminishes corporal gross (g) of animals involved in the experiment, not taking the nutritious content of the concentrate into consideration: the JAC, apart from 40-50% inulin, is composed of >17% protein, ~2% lipid, 7-10% sucrose, and

~1% glucose, fructose etc. The JAC prevents liver form magnification – when compared to the control, liver weight in the end of the experiment is 92%, which should be taken as a positive result, especially, if taking the parallel betterment of liver functions into consideration: the level of lipid concentration in blood – amount of triglycerides – 92-98% when compared to the control, total cholesterol 82,2%, respectively, and LDH - 51,6% - gradually diminishes, and the level of HDH advances. This facilitates transportation of cholesterol to cellular membranes and places where it biochemically turns into biliary acids, sex hormones a.o. matters important for life functions. Experimental data shows that the inulin containing JAC can prevent cardio-vascular system from pathologies, caused by hyperlipidemia. Within presence on the JAC, 98% erythrocyte (when compared to control) can be registered in blood; these contain more haemoglobin, and provide 100% enchainment and transportation of oxygen, necessary for an organism. The JAC inulin fosters pull down of waste erythrocyte in liver; biliary pigments bilirubin and biliverdin forms from the exempt haemoglobin.

Conclusions. The JAC normalizes sugar-fat metabolism in liver, fosters digestion and active and well-balanced liver functioning, maintaining homeostasis in blood. Proven biochemical and physiological effects of the JAC let us evaluate it as an advisable component in functional food, as well as a perspective stock for development of medicine.

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ANTIVIRAL ACTIVITY OF NATURAL FRUCTANS: IMMUNOBIOLOGICAL EFFECTS

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Background. Understanding mechanisms that help promote protective immune responses to pathogens is a major challenge of biomedical research. Innate immune system provides the first line of mammalian organisms' defence against microbial pathogens. Interferons (INFs) are cytokines produced by mammalian cells as a response to viral infection or other stressors. Interferon system gives an extremely powerful antiviral response: it exerts antiviral function by binding the cell surface receptors with the major effect of triggering the signalling cascade and inducing expression of interferon-stimulated genes. It has been proven that natural killer (NK) cells can directly contribute to control of viral replication and accelerate CD8 T-cells responses against viral infection *in vivo*. **The aim** of the present study was to estimate the effect of the Latvia-produced Jerusalem artichoke concentrate's (JAC) β -D-fructan inulin and the other β -D-fructan - bacterial levan, supplemented to feeds of the Wistar line white rats on the antiviral parameters of immunity: amount and activity of NK cells, common T-cells and

antiviral CD8 T-cells and synthesis of the innate immune defence regulating γ -interferon (INF γ). **Methods.** A three-month long animal experiment was carried out on male rats of the Wistar line. JAC as source of inulin was used as a feed additive for experimental animals. Method of hemoinformatics was used for analysis of immunocompetent cells, special systems and Elisa tests – for analysis of INF γ . **Results.** The JAC raised the number of NK cells in blood up till 198%, when compared to 100% in the control group where inulin was not supplemented. The number of common T-cells in blood advanced till 167,5%. Alongside with a considerable rise of T-cells' common amount, the level of antiviral CD8 T-cells in blood increased, as well. Modulation of INF γ level has been demonstrated under the influence of β -D-fructan levan, obtained in microbial synthesis. INF γ (produced by NK cells and Th cells) under influence of the bacterial β -D-fructan levan, increased by 13% in case of underdeveloped (<2000 pg/ml) synthesis of INF γ at liquid phase of immunocompetent cells' cultures. The active production of INF γ (>2000 pg/ml) was suppressed by levan till 25,5%. Hence, the immunomodulating ability of levan has been proven. Innate and adaptive immunity do not simply coexist, they are linked to one another in a complex network. The intimate interactions between immunocompetent cells noted above have been studied. Undesirable side effects have not been found and, furthermore, diverse immunostimulation and other favourable biological effects of fructans have been proven by us previously [1,2,3,4]. **Conclusion.** It has been estimated that natural β -D-fructans inulin of JAC and bacterial levan increase quantity and potentate the action of NK cells, T-cells and antiviral CD8 T-cells. Antiviral action of natural fructans has been proven.

References

- [1] Vina, I. Drugs of the Future, vol. 32, Suppl. A, 2007, p.78.
- [2] I. Vina et al. Abstract of conference "Frontiers in Medicinal Chemistry", Frankfurt am Main, Germany, 2006, p. 103.
- [3] I. Vina et al. Abstract of 6th AFMC International Medicinal Chemistry symposium, Istanbul, Turkey, 2007, p. 124.
- [4] I. Vina, R. Linde, A. Danilevich, M. Bekers, D. Sprudza. Interfacing Natural Products: Inulin of Jerusalem Artichoke - affirmative biological effects. XXth International Symposium on Medicinal Chemistry, Vienna, Austria, August 31 - September 1, 2008.

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QSRR IN INTERPRETATION OF ADSORBATE-ADSORBENT INTERACTIONS IN THE COMPLEXATION CAPILLARY GAS CHROMATOGRAPHY

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Quantitative Structure-Retention Relationship (QSRR) is one of the methods used in interpretation of the interactions in the chromatographic process. According to this method the differences in the chemical structure of compounds are described numerically by a series of measurable structural parameters called descriptors. The compounds studied are chromatographically analysed and for each of them the retention parameters are measured. Then, a mathematical equation is derived that relates the retention parameter with a group of parameters describing the structure of a certain group of compounds. On the basis of a reliable QSRR equation it is possible to predict the chromatographic behaviour of a certain group of compounds.

This paper reports results of a study on the interactions taking place in the charge-transfer chromatography. This chromatographic technique is based on the use of the fast and reversible process of reaching the coordination equilibrium in the dynamic conditions, between the molecules of the substances subjected to chromatography and the packing of the chromatographic columns. The packing must have centres capable of electron-donor-acceptor interactions. It can be an individual functional group or a metal complex of such properties. The measurements presented were performed on the capillary columns covered with the adsorption layer containing free 3-benzylketimine groups and on the capillary columns containing copper (II) and nickel (II) complexes bonded through these groups. A large number of data have been collected for a wide gamut of organic compounds divided into groups according to similarity of structure and physico-chemical properties. The study was performed on the compounds having unsaturated bonds or aromatic character bonds, and those having one or more heteroatom in certain groups in the hydrocarbon chain.

For each group of compounds a model was proposed, described by the equation derived on the basis of the multiple linear regression (MLR). The direction coefficients permitted a quantitative description of the effect of a given element of the molecule structure on its retention.

The calculations of particular descriptors on the basis of the molecular structure and the statistical analysis were performed using the program AdmeWorks ModelBuilder, version 3. Molecular modelling of the molecules studied needed in the above program was made by the CAChe, version 7.5. The two programs were provided by the Fujitsu Kyushu System Engineering Ltd.

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3,4-DIARYLSOXAZOLES AS DUAL INHIBITORS OF p38 MAPK AND CK1

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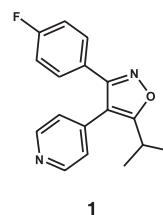
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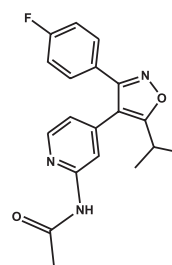
In this study we describe the synthesis and biological evaluation of 3,4-diarylsioxazoles as novel inhibitor of Casein kinase 1 (Ck1) with compound **2** possessing an IC₅₀ of 300 nM for Ck1δ.

Ck1 is a protein serine/threonine kinase family and is known to consist of multiple isoforms in eukaryotic cells (Ck α, β, γ1, γ2, γ3, δ, ε). Ck1 family members are involved in the regulation of key cellular processes such as DNA repair (2), differentiation, proliferation, and therefore play key roles in oncogenesis. Furthermore, Ck1δ was found to be cumulated in a high degree in neurodegenerative lesions of Alzheimer's patients (3). Thus, inhibitors of Ck1 may have therapeutic potential as anti-cancer agents and for the treatment of neurodegenerative disease.

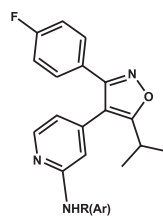
Compound **1** has been originally designed as p38^α MAPK inhibitor (IC₅₀ p38^α MAPK = 0.45 μM) (**1**) and thus possessing the typical vicinal pyridin-4-yl fluorophenyl pharmacophore system of p38^α MAPK inhibitors. However, profiling **1** in a panel of 78 protein kinases revealed also significant inhibition of Ck1δ (90% inhibition@10μM). SAR studies of this class of compounds revealed that the NHR(Ar) moiety **3** appended at C-2 locus of the pyridyl group enhances p38^α MAPK inhibition with abolished inhibition against Ck1δ. Interestingly, the acetamide group therein **2** not only enhances p38^α MAPK inhibition (IC₅₀ p38^α = 300 nM), but also has significantly inhibited Ck1δ (IC₅₀ Ck1δ = 300 nM). These results show the acetamide moiety there at **2** to be important for dual inhibition of Ck1δ and p38^α MAPK.



1



2



3

References

- [1] Peifer, Christian; Ulrich, Robert; Schattel, Verena; Abadleh, Mohammed; Roettig, Marc; Kohlbacher, Oliver; Laufer, Stefan. *Bioorganic & Medicinal Chemistry Letters* (2008), 18(4), 1431-1435.
- [2] Wolff, Sonja; Stoeter, Martin; Giamas, Georgios; Piesche, Matthias; Henne-Bruns, Doris; Banting, George; Knippschild, Uwe. *FEBS Letters* (2006), 580(27), 6477-6484.
- [3] Schwab, C.; DeMaggio, A. J.; Ghoshal, N.; Binder, L. I.; Kuret, J.; McGeer, P. L. *Neurobiology of Aging* (2000), 21(4), 503-510.

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DESIGN, SYNTHESIS AND INHIBITORY ACTIVITY OF A NOVEL STRUCTURAL CLASS OF P38 MAP KINASES INHIBITORS

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The pathogenesis of chronic inflammatory diseases, such as Chron's disease or rheumatoid arthritis, is promoted by various pro-inflammatory cytokines among interleukin-1 β and tumour necrosis factor- α play a major role. Release of these mediators is controlled by a pathway, in which the mitogen activated protein (MAP) kinase p38 represents a key regulatory enzyme. Prototype inhibitors of p38 MAP kinase like SB 203580 and second generation allosteric inhibitors like BIRB 796 are often restricted by serious drawbacks like CYP 450 interaction and lack of selectivity. Our design strategy was based on benzophenone leads by fixing the torsion angle between the two phenyl rings. In this study we describe the synthesis of 3- and 8-substituted 6,11-dihydro-dibenzo[b,e]oxepin-11-ones as a novel class of p38 MAP kinase inhibitors. Synthesis of the scaffold 3-fluoro-6,11-dihydro-dibenzo[b,e]oxepin-11-ones started by coupling phthalide with 3-fluorophenol by the use of sodium hydride and N,N-dimethylformamide to give the 2-(3-fluoro-phenoxy-methyl)-benzoic acid. Synthesis of the scaffold 8-amino-6,11-dihydrodibenzo[b,e]oxepin-11-ones started with esterification of 2-methyl-4-nitro-benzoic acid. The subsequent bromination and the following substitution with phenol give 4-nitro-2-phenoxy-methyl-benzoic acid ester. Ring closure of these benzoic acid derivatives was performed using polyphosphoric acid and tetramethylen sulfone. Coupling of the resulting halogene or amino substituted tricyclic scaffolds with an appropriate vice versa substituted phenyl moiety was performed using a base and bipolar aprotic solvent. The novel compounds were tested for their inhibitory activity in a p38 enzyme assay. The 8-substituted-6,11-dihydro-dibenzo[b,e]oxepin-11-ones resulted in an almost total loss in activity. The 3-substituted-6,11-dihydrodibenzo[b,e]oxepin-11-ones however showed moderate p38 inhibition which could be optimised down to IC₅₀ values as low as 38 nM [1].

References

- [1] Laufer *et al.*, *J. Med. Chem.*, 2006

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QSAR APPLICATION FOR IN SILICO PREDICTION OF PASSIVE TRANSPORT - FROM PAMPA PERMEABILITY TO HUMAN ORAL ABSORPTION

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In the early stage of drug discovery, a large number of diverse compounds are synthesized by the technique of combinatorial chemistry. But it is not always effective for finding new drug candidates to synthesize all compounds designed by combination of available building blocks and to evaluate their activity in high throughput screening. Appropriate filtering methods are needed for selecting only informative compounds from the whole designed library before their synthesis.

The parallel artificial membrane permeation assay (PAMPA), developed by Kansy *et al.*¹ is a model for prediction of transcellular permeation in the process of drug absorption. The PAMPA is a high throughput system and now automated by robotics. We have been interested in the PAMPA system as a permeation model through cell membranes. We have revealed that PAMPA permeability were governed by log *P* (*P*: 1-octanol/water partition coefficient), p*K*_a and hydrogen-bonding ability of compounds.² The hydrogen-bonding ability was indicated by the polar surface area (PSA) which was calculated in a stable conformation of a compound (cPSA). We used measured log *P* and p*K*_a, and cPSA calculated by Sybyl MOLPROP, but many commercially available softwares can calculate the common physicochemical descriptors, log *P*, p*K*_a and cPSA. Firstly, we developed some prediction models for the PAMPA permeability with various *in silico* descriptors to construct a new filtering method for selecting useful compounds as drug candidates from the library. By comparison of statistic indices of each model, we selected the most suitable descriptor set for practical prediction of PAMPA permeability.

In order to examine the ability of *in silico* descriptors mentioned above for predicting human oral absorption, we calculated the PAMPA permeability by the model with the most suitable descriptor set, and then predicted Caco-2 cell permeability based on the calculated PAMPA permeability. In our previous study,² we revealed that the Caco-2 cell permeability and the PAMPA permeability were well correlated for 35 compounds (7 peptide-related compounds, 20 commercial drugs and 8 chemicals) mainly permeated by the passive transcellular route. The correlation equation was used for prediction of the Caco-2 cell permeability. In addition, it was reported that human

intestinal absorption has a sigmoidal relationship with Caco-2 cell permeability.³ The plot of percent human oral absorption reported by Stenberg *et al.*³ against predicted Caco-2 permeability values is shown in Figure 1. Most of compounds were located around the sigmoidal curve as expected. Percent oral absorption of only 4 drugs, salicylic acid, acetylsalicylic acid, hydrochlorothiazide and zidovudine was underestimated. It might be because those compounds could be transported by other routes. Even if such limitation exists, it was indicated that *in silico* descriptors were useful for prediction of not only Caco-2 cell permeability but also human oral absorption.

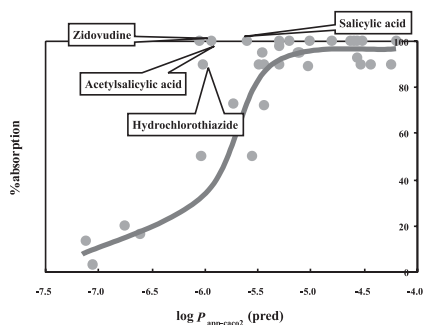


Figure 1. Correlation between predicted Caco-2 permeation and percent human oral absorption

- [1] Kansy, M.; Senner, F.; Gubernator, K.J. *Med. Chem.*, 1998, 41, 1007
- [2] Fujikawa, M.; Nakao, K.; Shimizu, R.; Akamatsu, M. *Bioorg. Med. Chem.*, 2007, 15, 3756
- [3] Stenberg, P.; Norinder, U.; Luthman, K.; Artursson, P. *J. Med. Chem.* 2001, 44, 1927

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CYCLIC XANTHONIC DERIVATIVES: A KEY APPROACH TO OPTIMIZE ANTITUMOR ACTIVITY

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The search for new and more potent antitumor agents is an area of great interest for medicinal chemists. The lack of potency and selectivity together with toxicity are the main problems of many drugs. Xanthone derivatives have been reported to mediate some important biological activities, concerning a large variety of targets with therapeutic value [1-2]. In our group we have investigated the effect of several xanthonic derivatives on the *in vitro* growth of some human tumor cell lines, with particular attention for the breast adenocarcinoma (MCF-7) [3-4]. Molecular modifications in the xanthonic “hits” **1-2** were developed in order to improve potency and/or selectivity

towards this cell line (Fig. 1). Dihydropyranoxanthones **3-5** were obtained from xanthonic building blocks **1-2**, applying the strategy of molecular rigidification. The introduction of an extra dihydropyran ring was achieved by a combined method of heterogeneous catalysis with microwave irradiation. Pyranoxanthones **6-8** were obtained applying the same strategy for dihydropyran ring, with the inclusion of an insaturation on dihydropyranoxanthones **3-5** (Fig. 1).

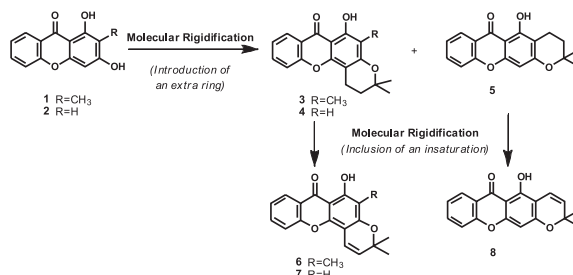


Fig.1. Strategy to improve potency and/or selectivity towards MCF-7 tumor cell line.

Compounds **3-8** were evaluated for their effect on the *in vitro* growth of human tumor cell lines: MCF-7 (breast adenocarcinoma), NCI-H460 (non-small cell lung) and SF-268 (central nervous system). Xanthone **3** exhibited a more selective and potent growth inhibitory effect against the MCF-7 cell line, comparing with respective building block **1** [4], being able to become a new “hit” compound for this series. Compounds **6-8** are under study.

References

- [1] Pinto, M., *et al. Curr. Med. Chem.* 2005, 12, 2517.
- [2] Pinto, M.; Castanheiro, R.; “Natural Prenylated Xanthones: Chemistry and Biological Activities” in *Natural Products: Chemistry, Biochemistry and Pharmacology*, Ed. Brahmachari, G., Narosa Publishing House PVT. LTD., Nova Deli, India, 2007, chap. 17, *accepted for publication*.
- [3] Pedro, M., *et al. Bioorg. Med. Chem.* 2002, 10, 3725.
- [4] Castanheiro, R., *et al. Bioorg. Med. Chem.* 2007, 15, 6080.

Acknowledgments

FCT (I&D 226/2003), FEDER, POCI for financial support and for the PhD grant to R. C. (SFRH/BD/13167/2003).

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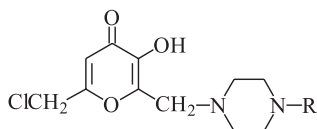
ANTIVIRAL AND ANTIMICROBIAL ACTIVITIES OF CHLOROKOJIC ACID DERIVATIVES

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Many of screening efforts have been made to find new antimicrobial and antiviral agents from natural or synthetic compounds for a variety of newly active compounds with different molecular targets that control infectious caused by bacteria, fungi and viruses. Also, during the past several years, the emergence of organisms resistant to nearly all classes of antimicrobial agents has become a serious public health concern. Hence, increasing resistance against antimicrobial agents is a major concern of medical microbiology in recent years. Alternative mechanisms of infection prevention and treatment should be included in the initial activity screenings. In our previous study, we synthesized some mannich bases of 3-hydroxy-6-methyl-4*H*-pyran-4-one derivatives and investigated their antimicrobial and anticonvulsant activities¹.



In this study, mannich bases were prepared by the reaction of appropriate substituted piperazine derivatives with 3-hydroxy-6-chloromethyl-4*H*-pyran-4-one (chlorokojic acid) and formaldehyde at the room temperature. The structures of the compounds were characterized by IR, ¹H-NMR, ESI Mass spectral and elemental analysis data. Compound 1-12 were synthesized as a mannich bases which have different substituted piperazine rings, such as 3-trifluoromethylphenyl, 4-trifluoromethylphenyl, 2-fluorophenyl, 4-fluorophenyl, 2-methoxyphenyl, 3-methoxyphenyl, 4-methoxyphenyl, 2-chlorophenyl, 3-chlorophenyl, 4-chlorophenyl, 3,4-dichlorophenyl, 4-fluorobenzyl and screened for their *in-vitro* antibacterial, antifungal and antiviral activities. Antimicrobial activity was evaluated against both standard and the isolated strains of Gram-negative (*E. coli*, *P. aeruginosa*, *P. mirabilis*, *K. pneumoniae*, *A. baumannii*) and Gram-positive (*S. aureus*, *E. faecalis*, *B. subtilis*) bacteria, as well as fungi (*Candida albicans*, *C. parapsilosis*) by microdilution method. We performed susceptibility testing according to the Clinical and Laboratory Standards Institute (CLSI; formerly NCCLS). Ampicilline, vancomisine, gentamicine, ofloxasine, levofloxacin, ketoconazole, and fluconazole were used as the control agents. All the compounds have shown more potent antibacterial activity against Gram-positive bacteria ranging between 8-32 µg/ml concentrations than Gram-negative (16-64 µg/ml) ones, while they had considerable antifungal activity at 8-16 µg/ml concentration. In particular, all of the compounds of 2-, 3- and 4-chlorophenylpiperazine- and 3,4-dichlorophenylpiperazine- were found to have a better inhibition against *S. aureus* and *E. faecalis* with MICs value of 8 µg/ml. In the mean time, all tested compounds showed notable antifungal activity against *C. albicans* and *C. parapsilosis* at a range of 8-16 µg/ml MICs values. Both, *Herpes simplex* virus Type-1 (*HSV-1*), a DNA virus, and *Parainfluenza-3* virus (*PI-3*), an RNA virus, were employed for antiviral assessment of the compounds by using Madin-Darby

Bovine Kidney and Vero cell lines. All activities were evaluated at non-toxic concentrations.

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References

- [1] Aytemir, M.D., Çalis, Ü., Özalp, M. Arch Pharm. Pharm. Med. Chem. 337, 281-288 (2004).

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SYNTHESIS, CHARACTERIZATION AND EVALUATION OF ANTIMICROBIAL ACTIVITY OF NEW HETEROCYCLIC CONDENSED SYSTEMS WITH BRIDGE-HEAD NITROGEN FROM THIAZOLO-TRIAZOLE CLASS

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Keywords: alkylation, cyclization, 1,2,4-triazole-3-thioles, thiazolo[3,2-*b*][1,2,4]triazoles, antimicrobial activity

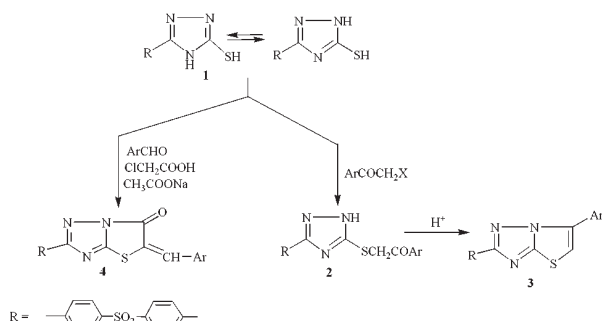
A number of bioactive molecules contain functionalized triazole and thiazole heterocycles. A 1,2,4-triazole moiety is a key component in the structure of potential antibacterial, antifungal, analgesic, anti-inflammatory, carbonic anhydrase inhibitors agents [1-3]. Thiazole derivatives have been also reported to exhibit antitumoral, antimicrobial, anti-inflammatory activity [4-6]

The thiazolo-1,2,4-triazoles are those compounds which contain in their structure two fused rings of thiazole and triazole. These condensed heterocycles can exist in both the isomeric forms: thiazolo[3,2-*b*][1,2,4]triazole and thiazolo[2,3-*c*][1,2,4]triazole. Thiazolo[3,2-*b*][1,2,4]triazoles possess a broad spectrum of biological activities: analgesic, anti-inflammatory, antipyretic, antimicrobial, anticonvulsant, anticancer [7-12]. Further, diphenylsulfone derivatives were also found to possess antibacterial activity [13].

Within this respect and in continuation of our work on the synthesis of biologically active nitrogen and sulphur containing heterocycles [4,14], we report the synthesis of new heterocyclic condensed systems with bridgehead nitrogen from thiazolo-triazole class which contains diphenylsulfone moiety. Thus, alkylation of 5-[4-(4-*X*-phenylsulfonyl)phenyl]-4*H*-1,2,4-triazole-3-thioles (*X*= H, Cl, Br) **1** [15] with α -halogenated ketones afforded *S*-substituted 1,2,4-triazoles **2**, which in acidic media yield thiazolo[3,2-*b*][1,2,4]triazoles **3**. Besides, the treatment of same 5-[4-(4-*X*-phenylsulfonyl)phenyl]-4*H*-1,2,4-triazole-3-thioles **1** with chloroacetic acid and aromatic aldehyde in the presence of sodium acetate, acetic acid and acetic

anhydride gave the thiazolo[3,2-b][1,2,4]triazole-6(5*H*)-ones **4** (Scheme 1).

The structures of all new obtained compounds were determined by IR, ¹H-NMR, ¹³C-NMR, MS spectra and elemental analysis. Some newly synthesized compounds were tested for their antimicrobial effects.



Scheme 1

References

- [1] Serdar M. Gümrükçüoğlu N., Alpay Karaoglu S., Demirbas N. – „Synthesis of some novel 3,5-diaryl-1,2,4-triazole derivatives and investigation of their antimicrobial activities”, *Turk. J. Chem.*, 2007, 31, 315-326
- [2] Tozkoparan B., Küpeli E., Yesilada E., Ertan M. – „Preparation of 5-aryl-3-alkylthio-1,2,4-triazoles and corresponding sulfones with antiinflammatory-analgesic activity”, *Bioorg. Med. Chem.*, 2007, 15, 1808-1814
- [3] Almajan G.L., Barbuceanu S.-F., Innocenti A., Scozzafava A., Supuran C. T. – „Carbonic anhydrase inhibitors. Inhibition of the cytosolic and tumor-associated carbonic anhydrase isozymes I, II and IX with some 1,3,4-oxadiazole- and 1,2,4-triazole-thiols”, *Journal of Enzyme Inhibition and Medicinal Chemistry*, 2008, 23 (1), 101-107
- [4] Bénateau V., Besson T., Guillard J., Leonce S., Pfeiffer B. – „Synthesis and in vitro antitumour evaluation of benzothiazole-2-carbonitrile derivatives”, *Eur. J. Med. Chem.*, 1999, 34, 1053-1060
- [5] Ceylan Ünlüsoy M., Bozdog Dündar O., Altanlar N., Ertan R. – „Synthesis and antimicrobial activity of some new 3-substituted benzyl-5-(4-chloro-2-piperidin-1ylthiazole-5-yl-methylene)-thiazolidine-2,4-dione derivatives”, *Turk. J. Chem.*, 2006, 30, 355-360
- [6] Geronikaki A., Hadjipavlou-Litina D. – „Synthesis of some new aryloxy/aryl-2-amino-1,3-thiazole derivatives with anti-inflammatory”, *Arzneim. Forsch./Drug Res.* 1996, 46, 1134-1138
- [7] Roy P., Leblanc Y., Ball R.G., Brideau C., Chan C.C., Chauret N., Cromlish W., Ethier D., Gauthier J.Y., Gordon R., Greig G., Guay J., Kargman S., Lau C.K., O’Neil G., Silva J., Thérien M., van Staden C., Wong E., Xu L., Prasit P. – „A new series of selective COX-2 inhibitors: 5,6-diarylthiazolo[3,2-b][1,2,4]triazoles”, *Bioorg. Med. Chem. Lett.*, 1997, 7 (1), 57-62
- [8] Pignatello R., Mazzone S., Panico A.M., Mazzone G., Pennisi G., Castana R., Matera M., Blandino G. – „Synthesis and biological evaluation of thiazolo-triazole derivatives”, *Eur. J. Med. Chem.*, 1991, 26, 929-938
- [9] Erol D.D., Çalis Ü., Demirdamar R., Yulug N., Ertan M. – „Synthesis and biological activities of some 3,6-disubstituted thiazolo-[3,2-b][1,2,4]triazoles”, *J. Pharm. Sci.*, 1995, 84, 4, 462-465
- [10] Dogan E., Tozkoparan B., Kaynak F.B., Eriksson L., Küpeli E., Yesilada E., Ertan M. – „Design and synthesis of some new thiazolo- [3,2-b]-1,2,4-triazole-5(6*H*)-ones substituted with flurbiprofen as anti-inflammatory and analgesic agents”, *Arzneim.-Forsch./Drug Res.*, 2007, 57 (4), 196-202
- [11] El-Sherif H.A.H., Mahmoud A.M., Sarhan A.A.O., Hozien Z.A., Habib O.M.A. – „One pot synthesis of novel thiazolo-[3,2-b][1,2,4]triazoles: A useful synthetic application of the acidified acetic acid method”, *J. Sulfur Chem.*, 2006, 27 (1), 65-85
- [12] Lesyk R., Vladzimirskaya O., Holota S., Zaprutko L., Gzella A. – „New 5-substituted thiazolo[3,2-b][1,2,4]-triazol-6-ones: Synthesis and anticancer evaluation”, *Eur. J. Med. Chem.*, 2007, 42, 641-648
- [13] Elslager E.F., Gavrilis Z.B., Phillips A.A., Worth D.F. – „Repository drugs. IV., 4',4'''-Sulfonylbisacetanilide (acedapsone, DADDs) and related sulfanilylanilides with prolonged antimalarial and antileprotic action”, *J. Med. Chem.*, 1969, 12 (3), 357-363
- [14] Barbuceanu S.-F., Almajan G. L., Saramet I., Draghici B. – „Sinteza si caracterizarea unor noi N¹-[(arilsulfonil)benzoil]-N⁴-3-metoxifenil-tiosemicarbazide si a produsilor lor de ciclizare dehidratanta”, *Revista de Chimie (Bucuresti)*, 2007, 58 (10), 945-947
- [15] Saramet I., Banciu M.D., Draghici C. – „Synthesis and characterization of some 5-substituted-3-mercapto-1,2,4-triazoles”, *Rev. Roum. Chim.*, 1991, 36 (1-3), 135-143

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APPLICATION OF MULTILEVEL MULTIVARIATE DATA ANALYSIS IN DRUG DESIGN.

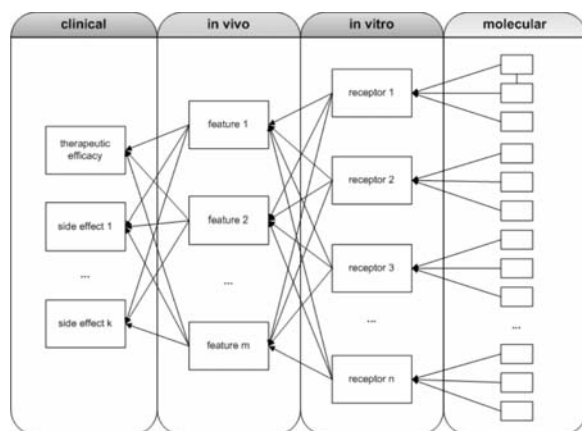
Anna Bauer-Mehren^a, Maria Isabel Loza^b, Ferran Sanz^a and Manuel Pastor^a.

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Classically, the clinical usefulness of a drug has been identified with its ability to bind a single protein. Nowadays it is widely recognized that the pharmacological effect of most drugs is far more complex and typically involves many interactions with different proteins. Some are involved in the therapeutic effects, whereas some others are related to side effects and the toxicity of the drug. Unfortunately, the awareness of the involved complexity will not help to obtain better drugs and there is a pushing need for methods able to identify the optimal multireceptorial profile. Probably the best way to address the problem is using system biology methods^{1,2}, which allow a detailed description and modelling of the relationships between biomedical entities.

In this work we present an alternative approach based on the detailed analysis of different levels of data collected for a set of clinically used drugs. For example, starting from collected binding affinity data for a set of drugs it is possible to use multivariate analysis methods (PCA, PLS-DA, OPLS) in order to identify the binding affinity profile that is associated with clinical effects. This concept can be extended by incorporating other levels of description (*in vivo* pharmacological properties or molecular descriptors). All this data can be analysed using specialised methods thus providing interesting insight that could be exploited for the rational design of drugs with improved efficacy and reduced side effects.

The study presented here will show an example of the proposed methodology for exploring antipsychotic drugs by involving the simultaneous analysis of *in vivo*, *in vitro* and molecular descriptors data.



[1] Butcher EC, Berg EL, Kunkel EJ. Systems biology in drug discovery. *Nat Biotech.* 2004; 22(10):1253-9.

[2] Keskin O, Gursoy A, Ma B, Nussinov R. Towards drugs targeting multiple proteins in a systems biology approach. *Curr Top Med Chem.* 2007; 7(10):943-51.

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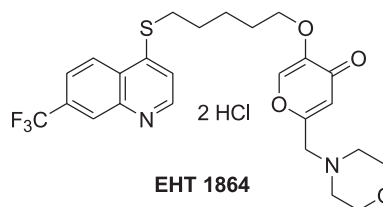
SELECTIVITY OF NUCLEOTIDE BINDING INHIBITORS ON RAC SMALL GTPASE SPLICED VARIANTS

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Rho GTPases exist in quiescent cells mainly in their inactive GDP bound state. After growth factor stimulation, Rho specific guanine nucleotide exchange factor (GEF) are activated and they catalyse guanine nucleotide exchange to produce an active GTP bound Rho. Rho GTPases such as RhoA, Rac1 and cdc42 are regulators of cell proliferation, actin cytoskeleton reorganization and

gene expression. In The Rac subfamily, Rac1 is essential for transformation caused by Ras. Alternatively, splice variant such as Rac1b is constitutively active and overexpressed in breast and colon cancers [1]. Therefore, the identification of splice variant inhibitors such as **EHT 1864** provides important pharmacological tools to elucidate the pharmacology of overexpressed Rac splice isoforms [2,3]. In order to inhibit GTPase function, the inhibitors must interact with their target and induce an inactive conformation such as the GDP bound form where their affinity with the effector is reduced. The binding sites of Rho GTPases are generally well conserved but splicing introduce sequence differences that leads to conformational effects in the binding site that can be used in the design of more potent and selective inhibitors. Using a nucleotide binding assay, we identified a series of compounds that act by inhibiting the binding of a fluorescently labeled GTP analogue, characterized their GTPase selectivities and confirmed their downstream activities in a functional cellular assay. Structure activity relationship will be presented as well as binding mode hypothesis generated by molecular docking experiments performed using the software Molegro Virtual Docker.



[1] Schnelzer A.; Prechtel, D.; Knaus U.; Dehne, K.; Gerhard, M.; Graeff, H.; Harbeck, N.; Schmitt, M. and Lengyel, E. Rac1 in Human Breast Cancer: Overexpression, Mutation Analysis, and Characterization of a New Isoform, Rac1b. *Oncogene*, 2000, 19, 3013-3020.

[2] Desiré, L.; Bourdin J.; Loiseau, N.; Peillon, H.; Picard, V.; De Oliveira, C.; Bachelot, F.; Leblond, B.; Taverne, T.; Beausoleil, E.; Lacombe, S.; Drouin, D. and Schweighoffer, F. RAC1 Inhibition Targets Amyloid Precursor Protein Processing by Gamma-Secretase and Decreases Abeta Production In Vitro and In Vivo. *J. Biol. Chem.* 2005, 280, 37516-37525.

[3] Shutes, A; Ornesto, C.; Picard, V.; Leblond, B.; Schweighoffer, F and Der, C.J. Specificity and Mechanism of Action of EHT 1864, a Novel Small Molecule inhibitor of Rac Family Small GTPases. *J. Biol. Chem.* 2007, 282, 35666-35678.

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NEW 5-HT₂ RECEPTOR ANTAGONISTS AS PUTATIVE ANTIDEPRESSANTS WITH RAPID ONSET OF ACTION

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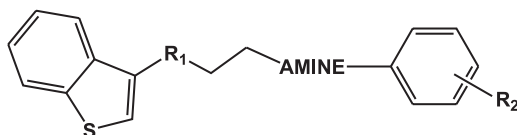
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Current antidepressants are clinically effective only after several weeks of administration. Recent research has suggested the serotonin₇ (5-HT₇) receptor as a candidate target for the treatment of depression [1,2,3]. Here we show that 5-HT₇ antagonists reduce immobility in the forced swimming test, displaying an antidepressant potential.

We have synthesized several benzo[*b*]thiophene derivatives (**Fig. 1**), searching for dual affinity towards serotonin transporter and 5-HT₇ receptor.



R1: C=O; CHOH

Amine: Aminopyrrolidine, Homopiperazine, Aminopiperidine

R2: *p*-nitro *o*-trifluoromethyl, *p*-cyano

Figure 1. General structure of the benzo[*b*]thiophene derivatives

Next, three of the compounds were selected: a 5-HT₇ antagonist, a serotonin reuptake inhibitor and a dual compound with affinity towards both the targets, for developing “*in vivo*” assays with the aim of assessing its antidepressant activity in the swimming forced test [41].

Acute treatment with the 5-HT₇ antagonist was sufficient enough to cause a statistically significant reduction in the immobility time in this test. Moreover, the three compounds studied showed antidepressant activity in mice after a 15-day regimen.

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References

- [1] Guscott M, Bristow LJ, Hadingham K, Rosahl TW, Beer, M.S, Stanton JA, et al. (2005). Genetic Knockout and pharmacological blockade studies of the 5-HT7 receptor suggest therapeutic potential in depression. *Neuropharmacology* 48: 492-502.
- [2] Hedlund PB, Huitron-Resendiz S, Henriksen SJ, Sutcliffe JG. (2005). 5-HT7 receptor inhibition and inactivation induce antidepressant-like behaviour and sleep pattern. *Biol. Psychiatry* 58: 831-837
- [3] Wesolowska A, Nikiforuk A, Stachowicz K, Tatarczynska E. (2006). Effect of the selective 5-HT(7) receptor antagonist SB 269970 in animal models of anxiety and depression. *Neuropharmacology* 51: 578-586.

- [4] Porsolt RD, Le Pichon M, and Jalfre M. (1977). Depression: a new animal model sensitive to antidepressant treatments. *Nature* 266, 730-732.

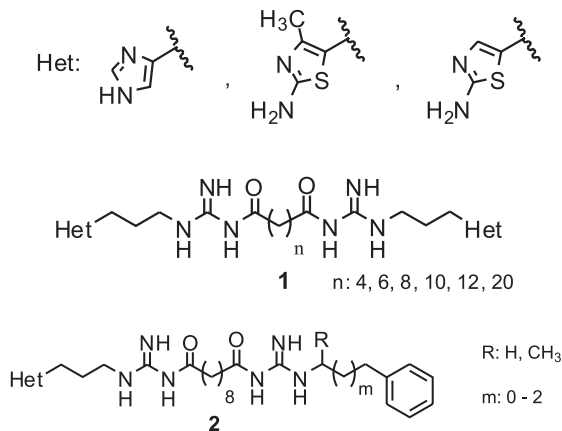
P205

BIVALENT ACYLGUANIDINE-TYPE LIGANDS AS POTENT AND SELECTIVE HISTAMINE H₂ RECEPTOR AGONISTS

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N^G -Acylated hetarylpropylguanidines represent a class of potent histamine H_2 receptor (H_2R) agonists [1, 2]. Compared to the corresponding alkylguanidines the basicity of the guanidine group is reduced by 4-5 orders of magnitude. A drastic increase in H_2R agonistic potency was achieved by pharmacophore-doubling inspired by the “bivalent ligand approach” suggested by Portoghesi for opioid receptors [3]. Compounds of general structures **1** and **2** were synthesized by connecting two (het)aryl-propylguanidines by N^G -acylation with alkanedioic acids of various chain lengths.



The resulting compounds were investigated for H₂R agonism at isolated guinea pig (gp) right atria and in a steady-state GTPase assay using fusion proteins expressed in Sf9 insect cells (H₂R-G_{saS}). The combination of two 2-amino-4-methylthiazol-5-ylpropylguanidine moieties with an octanedioyl or a decanedioyl spacer resulted in the most potent agonists at the guinea pig right atrium known so far: This compound proved to be up to 3000 times more potent than histamine [4]. This result is in good agreement with the data from the GTPase assay. Due to insufficient spacer length for interaction with both binding pockets of H₂R dimers the gain in potency is probably due to the interaction with an additional binding site at the same receptor molecule.

Investigation of the bivalent ligands on mutant H₂R-G_{saS} fusion proteins (transmembrane domains, second extracellular loop) confirmed the key role of the non-con-

served Tyr-17 and Asp-271 in TM1 and TM7 in the gpH₂R for species-selective H₂R activation and suggest that the e2 loop does not participate in direct ligand-receptor interactions [4, 5].

References

- [1] Ghorai, P., *PhD thesis*, University of Regensburg, 2005.
- [2] Xie, S.-X., et al., *J. Pharmacol. Exp. Ther.* 2006, 317, 139-146.
- [3] Portoghese, P. S., *J. Med. Chem.* 2001, 44, 2259-2269.
- [4] Kraus, A., *PhD thesis*, University of Regensburg, 2007.
- [5] Preuss, H., *PhD thesis*, University of Regensburg, 2007.

P206

NOVEL LOW MOLECULAR HSP90 INHIBITORS

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Heat-shock protein 90 (Hsp90) is an abundant cellular protein (1-2% of total protein) and one of a number of molecular chaperones. Hsp90 plays a key role in response to cellular stress and conformational maturation and activation of client proteins under normal conditions. Over 100 clients of Hsp90 have been described so far and many of the known Hsp90 clients are oncogenic including ErbB2, Raf-1, CDK4, PKB/AKT, polo1 and Met.

Inhibition of Hsp90 ATPase activity disrupts its interactions with co-chaperones and results in degradation of client proteins via the ubiquitin proteasome pathway. This leads to deregulation of many pathways crucial to cancer cell survival i.e. proliferation, cell cycle regulation, and apoptosis. In numerous reports Hsp90 inhibitors have shown promising anti-tumor activity both *in vitro* and *in vivo* and several compounds are today in clinical trials, the most advanced being in Phase III.

At TopoTarget we have developed a proprietary series of sub-micromolar inhibitors for which we will present our latest biological data.

P207

X-RAY STRUCTURE AND MECHANISM OF ACTION OF A POTENT VASODILATING COMPOUND

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Recently, we presented the SAR-guided development of benzothioamides as active vasodilating compounds (compound 2) as third generation of a lead optimisation process based on resveratrol and hypothesized that the conformation of both aromatic rings to each other was responsible for the tremendous increase of the vasodilating potency [1,2]. The following presentation demonstrates the X-ray crystal structures of compound 2 as well as of its precursor (second generation) - compound 1 (Fig.1), which were made to substantiate the hypothesis mentioned above, and compared them to the calculated results of two different force field methods (MMFF94 and TRIPOS).

Moreover, in search of clarifying the mechanism of action of compound 2 receptor-binding studies are presented.

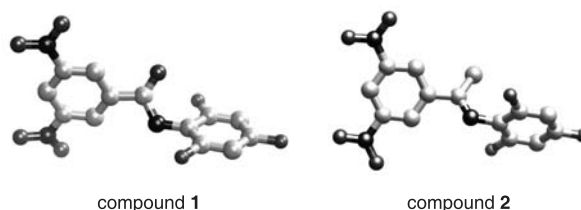


Fig.1: X-ray structure of compound 1 and compound 2.

References

- [1] Brunhofer Gerda, Handler Norbert, Leisser Klaus, Studenik Christian, and Erker Thomas: Hypertension – a novel generation of vasodilating agents. Short lecture at the DPhG – Jahrestagung, Erlangen, 10.-13.10.2007.
- [2] Handler Norbert, Brunhofer Gerda, Studenik Christian, Rawnduzi Pakiza, Erker Thomas. *PCT-Patent Application PCT/AT2007/000582*.

P208

DISCOVERY OF NOVEL SMALL MOLECULE INHIBITORS OF HSP90

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Molecular chaperones are proteins which play a key role in the conformational maturation, stability and function of other client protein substrates within the cell.^{1,2} Many of the client proteins of Hsp90³⁻⁶ are involved in signal transduction, cell cycle regulation and apoptosis, including kinases, transcription factors and hormone receptors. The dysregulation of pathways involving these proteins are commonly associated with cancer pathology.⁷ Hsp90 has attracted considerable interest as a therapeutic target for anticancer drugs since it was shown that both geldanamycin⁸ and radicicol⁹ are able to inhibit Hsp90 function by binding to an ATP binding pocket in the

N-terminal domain of the protein. Derivatives of these natural product inhibitors like 17-AAG are currently evaluated in clinical trials.¹⁰ However, these compounds have several potential limitations, including poor solubility, limited bioavailability and hepatotoxicity. These issues have led to significant efforts to identify small-molecule inhibitors. Several compounds of different chemical classes have been disclosed so far, like NVP-AUY922¹¹ or CNF-2024¹², which have entered Phase-I clinical trials.

We have identified novel small molecular weight inhibitors of Hsp90 using a fragment based approach. Fragments were selected by docking, tested in a biochemical assay and the confirmed hits were crystallized. Information gained from X-ray structures of each fragment was used to drive the fragment evolution process. Optimization of these high μ M binders resulted in 3-Benzylindazoles with significantly improved affinity. Further optimization towards drug-like molecules yielded in a compound which demonstrates anti-proliferative effects across a spectrum of cancer cell lines and down-regulates Hsp90 clients. Moreover, this derivative shows significant tumor growth inhibition after oral administration in a HCT116 xenograft model.

References

- [1] Young, J. C.; Agashe, V. R.; Siegers, K.; Hartl, F. U.; *Nat. Rev. Mol. Cell Biol.* 2004, 5, 781-789
- [2] Mosser, D. D.; Morimoto, R. I.; *Oncogene* 2004, 23, 2907-2918
- [3] Whitesell, L.; Lindquist, S. L.; *Nat. Rev. Cancer* 2005, 5, 761-772
- [4] Maloney, A.; Workman, P.; *Expert Opin. Biol. Ther.* 2002, 2, 3-24
- [5] Kamal, A.; Boehm, M. F.; Burrows, F. J.; *Trends Mol. Med.* 2004, 10, 283-290
- [6] Chiosis, G.; Vilenchik, M.; Kim, J.; Solit, D.; *Drug Discovery Today* 2004, 9, 881-888
- [7] Hanahan, D.; Weinberg, R. A.; *Cell* 2000, 100, 57-70
- [8] Neckers, L.; Schulte, T. W.; Mimnaugh, E.; *Invest. New Drugs*, 1999, 17, 361-373
- [9] Soga, S.; Shiotsu, Y.; Akinaga, S.; Sharma, S. V.; *Curr. Cancer Drug Targets*, 2003, 3, 359-369
- [10] Banerji, U. et al. *J. Clin. Oncol.* 2005, 23, 4152-4161
- [11] Brough, P. A. et al. *J. Med Chem.* 2008, 51, 196-218
- [12] Kasibhatla, S. R. et al. *J. Med Chem.* 2007, 50, 2767-2778

P209

DNA ENCODED CHEMICAL LIBRARIES: SYNTHESIS, ENCODING, DECODING AND APPLICATIONS

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The isolation of small organic binding molecules specific to proteins of interest is a central problem in chemistry, biology and medicine. The use of DNA fragments as amplifiable "bar-codes" for the identification of chemical compounds in a library represents an attractive option for the synthesis and screening of combinatorial chemical libraries. [1,2,3]

We have now developed novel orthogonal strategies for the synthesis of small molecule - DNA conjugates based on the Diels-Alder reaction and the stepwise coupling of DNA blocks after individual reaction steps. High-throughput sequencing was then used to evaluate the library before and after selection on target proteins of interest. [4]

Here we demonstrate the potential of DNA encoded chemical libraries in lead discovery by the construction of a library containing 4,000 compounds and selections against streptavidin and human serum albumin.

- [1] Brenner S., Lerner R.A., Encoded combinatorial chemistry., *Proc Natl Acad sci USA* 89 (12), 5381-5484 (1992).
- [2] Gartner Z.J., et al., DNA-templated organic synthesis and selection of a library of macrocycles. *Science* 305 (5690), 1601-5 (2004).
- [3] Melkko S., Scheuermann J., Dumelin C.E., Neri D., Encoded self-assembling chemical libraries. *Nat Biotechnol.*, 22 (5) 568-74 (2004).
- [4] Mannocci L., Melkko S., Neri D., DNA-encoded chemical libraries. US Patent Application 2008 No 61/008,249.

P211

A CONVENIENT SYNTHESIS OF BIOACTIVE CYCLO-HEXENEPHOSPHONATES

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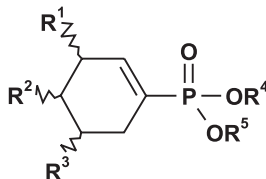
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Influenza virus infection and the shikimic acid pathway are only two of many examples for microbial biosynthetic pathways or microbe-host interactions that are interesting for interference by means of small molecules. A particularly interesting structural motif involved in both of them is the cyclohexenecarboxylic acid. In the former, it has been employed as a mimetic of the sialyl cation intermediate and forms the scaffold of the anti-influenza drug and neuraminidase inhibitor GS-4071 aka TamifluTM.¹ In the latter pathway, crucial modifications towards aromatic amino acids are being carried out via shikimic acid, a cyclohexenecarboxylic acid, as the substrate.²

We concluded that a straightforward method to replace the carboxylate in such structures by a phosphonate would give access to a wide variety of mimetics, for instance monoesters³, that still carry a negative charge under physiological conditions usually required for bioactivity.

We present an efficient synthesis of the cyclohexenephosphonate scaffold via two key steps, thus giving

access to interesting bioactive molecules with the generic structure depicted below.⁴ Wherever possible, inhibition data involving the interactions described above will be provided.



References

- [1] Abrecht, S.; Harrington, P.; Iding, H.; Karpf, M.; Trussardi, R.; Wirz, B.; Zutter, U. *Chimia* 2004, 58, 621-629.
- [2] Lindner, H. A.; Nadeau, G.; Matte, A.; Michel, G.; Menard, R.; Cygler, M. *J. Biol. Chem.* 2005, 280, 7162-7169.
- [3] H. Streicher and H. Busse, *Bioorg. Med. Chem.* 2006, 14, 885-1276.
- [4] H. Streicher and B. Carbain, in preparation.

P212

SYNTHESIS OF NOVEL HOMOSERINE ANALOGUES FOR DEVELOPMENT OF SUBSTRATE-BASED KINASE INHIBITORS

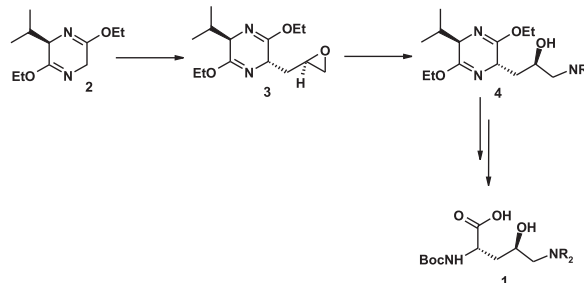
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Homoserine amino acid analogues are found in a variety of natural products showing different biological activities, *e.g.*, antibiotic, cytotoxic, and enzyme-inhibitory effects. Consequently, preparation of homoserine derivatives can be useful for development of new biologically active peptide-based compounds. The main purpose of this project is to design potential substrate-based protein kinase inhibitors, all derived from short peptides containing homoserine analogues.

At present, we focus on the synthesis of homoserine derivatives **1**. The synthetic strategy we use is shown in the figure below. By using Schöllkopf's bislactim ether **2** as a chiral auxiliary, enantiomerically pure α -amino acids can be afforded.^{1,2} We have used an improved protocol, using microwave assistance in one of the reaction steps, to prepare Schöllkopf's auxiliary (**2**) in relatively large scale.³ Alkylation of lithiated **2** with epichlorohydrin gives epoxide **3** in fairly good yield. To control the stereochemistry at the chiral center of the epoxide in the alkylation, enantiomerically pure (*R*)- and (*S*)-epichlorohydrin have been used, respectively. In the figure below, the alkylation product of (*R*)-epichlorohydrin is depicted. Ring-opening of epoxide **3** with different secondary amines in water provides β -aminoalcohol **4** in a regioselective manner for nucleophilic epoxide opening.⁴ Acidic hydrolysis of the chiral auxiliary followed by Boc-protection of the

resulting free amino groups, and ester hydrolysis, will, subsequently, generate our desired homoserine analogues (**1**). Before incorporation of **1** into short peptides using standard peptide synthesis, the hydroxyl group will be protected with a hydroxyl-protection group compatible with peptide synthesis.



- [1] Schöllkopf, U.; Groth, U.; Deng, C. *Angew. Chem. Int. Ed. Engl.* 1981, 20, 798-799.
- [2] Schöllkopf, U.; Hartwig, W.; Groth, U.; Westphalen, K.-O. *Liebigs Ann. Chem.* 1981, 696-708.
- [3] Carlsson, A.-C.; Jam, F.; Tullberg, M.; Pilotti, Å.; Ioannidis, P.; Luthman, K.; Grøtli, M. *Tetrahedron Lett.* 2006, 47, 5199-5201.
- [4] Azizi, N.; Saidi, M. R. *Org. Lett.* 2005, 7, 3649-3651.

P213

FACILE SYNTHESIS OF 2-ARYL-2,3-DIHYDRO-1,2,3-BENZOTHIADIAZOL-1,1-DIOXIDES

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Benzothiadiazole-1,1-oxides are the sulfone analogs of indazolinones. In spite of their intriguing structures and potentially biological application as isosteres of indazolinones, the synthesis of this class of compounds is hardly mentioned in the literature, most likely due to the tedious sequence and harsh reaction conditions in the early methods. We would like to report a two-step synthetic route under mild conditions to realize this class of molecules, starting from commercially available *o*-nitrophenylsulfonyl chloride. The yields of key ring formation step range from 80-95%.



P214

NEW TOPOISOMERASE INHIBITORS AS PROAPOPTOTIC AGENTS

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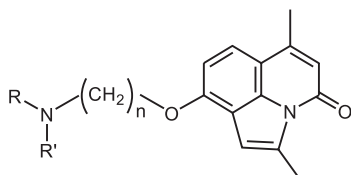
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DNA topoisomerases (topos) are nuclear enzymes that control the topological state of DNA, carrying out a series of DNA cleavage and religation reactions (1). Two major forms of topoisomerases are known, topo I and topo II. It is well known that compounds which interfere with topoisomerase activity are highly cytotoxic: the inhibition of these enzymes trigger a chain of events causing cell death. Topo inhibitors, in fact, are among the most efficient inducers of apoptosis (2). A number of drugs have been identified as dual topos inhibitors and they have shown to have advantages over single enzyme inhibitors, including sensitivity in resistant cells and a broader spectrum of activity (3). For these reasons outstanding attention is devoted to this class of compounds, not only to find more efficient anticancer agents, but also to find compounds useful to better elucidate the mechanisms on which topos activity is based. On these basis, we synthesized and evaluated a series of pyrroloquinolines functionalized with dimethylaminoalkyl chains.

We obtained a small library of compounds using both conventional synthetic methodologies and microwave assisted organic synthesis, which allowed to prepare derivatives differing in the aminoalkyl chain lenght.



$n = 2, 3, 4$

$R = H, CH_3; R' = CH_3, CH_2CH_2NMe_2$

For all the compounds the ability to inhibit the cell growth was tested in human tumor cell lines cultured *in vitro* to perform a preliminary screening. Then, the ability of the compounds to exert their activity through DNA interactions was investigated and the study of the molecular consequences on DNA was completed by evaluating the effects on the enzymatic activity of topo I and II.

At the same time, the effect of the tested compounds on the main parameters of mitochondrial functions was evaluated, since it was demonstrated that mitochondria and in particular the mitochondrial permeability transition (MPT) plays an essential role in the cellular apoptosis. So on isolated rat mitochondria the effect of the compounds on membrane potential and mitochondrial swelling was

studied, also determining the redox state of the thiols groups and glutathione to establish if the permeability transition could be the result of an oxidative stress.

Preliminary results showed that all the derivatives are topo II inhibitors and some of them could amplified MPT, depending upon the chain length, and they could be considered intrinsic apoptosis inducers with potential antiproliferative activity.

References

- [1] Wang, J.C. Cellular roles of DNA topoisomerases: a molecular perspective. *Nat. Rev. Mol. Cell Biol.* 2002, 3, 430-440.
- [2] Sordet, O.; Khan, Q.A.; Kohn, K.W.; Pommier, Y. Apoptosis induced by topoisomerase inhibitors. *Curr. Med. Chem. Anticancer Agents* 2003, 3, 271-90.
- [3] Denny, W.A.; Baguley, B.C. Dual topoisomerase I/II inhibitors in cancer therapy. *Curr. Top. Med. Chem.* 2003, 3, 339-353.

P215

ANTI-INFLAMMATORY EFFECTS OF NODAKENIN ON LIPOPOLYSACCHARIDE-INDUCED INFLAMMATION IN RAW 264.7 MACROPHAGE AND MURINE PERITONEAL MACROPHAGE CELLS

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Abstract- Herbal products are being used alternative treatments in such inflammatory conditions. Nodakenin, a coumarine isolated from *Angelicae gigantis Radix*, is an anti-inflammatory compound that inhibits nitric oxide (NO) and prostaglandin (PG) E₂ production. In this study, we investigated the suppressive activities of nodakenin on lipopolysaccharide (LPS)-induced inflammatory mediators in RAW 264.7 macrophage and murine peritoneal macrophage cells. Nodakenin inhibited LPS (1 µg/ml)-induced levels of inducible nitric oxide synthase (iNOS) and cyclooxygenase (COX)-2 at the protein, and iNOS, COX-2, tumor necrosis factor (TNF)-α, interleukin (IL)-1β and IL-6 mRNA expressions without an appreciable cytotoxic effect on RAW 264.7 macrophage cells by Western blotting and RT-PCR, respectively. Nodakenin also suppressed the LPS-induced translocation of nuclear factor-κB (NF-κB), a transcription factor pivotal necessary for pro-inflammatory mediators, such as iNOS, COX-2, TNF-α, IL-1β and IL-6 and NF-κB DNA binding activity in LUC-transfected RAW 264.7 macrophage cells. This effect was accompanied by a parallel reduction of inhibitor of κB (IκB-α) degradation and phosphorylation. To clarify the mechanisms involved, nodakenin was found to inhibit the phosphorylation of MAPKs and inhibitor of κB kinase (IKK) kinase activity. Furthermore, nodakenin also inhibited LPS (1 µg/ml)-induced levels of iNOS and COX-2 at the protein, and iNOS, COX-2, TNF-α, IL-1β and IL-6 mRNA expressions on murine peritoneal macrophage

cells by Western blotting and RT-PCR. Based on these results, we suggested that anti-inflammatory effects of nodakenin are the suppressions of the expressions of iNOS, COX-2, TNF- α , IL-1 β and IL-6 via IKK inactivation on RAW 264.7 macrophage and murine peritoneal macrophage cells.

P216

PYRAZOLE DERIVATIVES AS CALCIUM CHANNEL BLOCKERS

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Calcium channels exist in nerve, muscle, and endocrine cells, and are responsible for a number of diseases. Of the subtypes of the calcium channels, we were interested in the T-type channels that have been reported to be related to learning and memory, pain, epilepsy, etc. The first T-type calcium channel blocker, Mibefradil, was launched as an antihypertensive in 1997. The drug, however, was withdrawn in the next year due to drug-drug interaction.

Of many diseases related to the T-type calcium channels, we focused on neuropathic pain, and referring to the structure of Mibefradil and other CNS acting drugs, we designed pyrazole-containing structures that are expected to block T-type calcium channels selectively. A number of compounds were synthesized and evaluated for their biological properties.

In this presentation, we describe primary results obtained on the way to find new drug candidates for neuropathic pain by targeting T-type calcium channels (α_1G).

P217

SYNTHESIS, STRUCTURE ELUCIDATION AND MICROBIOLOGICAL ACTIVITY OF SOME NEW THIUREIDE DERIVATIVES

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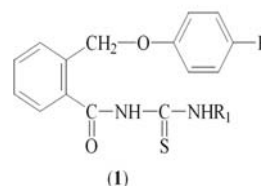
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Recent studies estimate that the mankind will remain in the future without antibiotics because the microorganisms develop rapidly crossing resistance to the existing anti-infectious drugs.

The present work reports the synthesis, structure determination and screening of new thiureides of 2-(4-methylphenoxy-methyl)-benzoic acid, with general formula (1)



where: R = CH₃

R₁ = mono- or disubstituted aryl

The new compounds were prepared in three steps:

- preparation of the 2-(4-methylphenoxy-methyl)-benzoic acid
- preparation of the 2-(4-methylphenoxy-methyl)-benzoyl chloride
- preparation of the new thiureides by the reaction of the 2-(4-methylphenoxy-methyl)-benzoyl isothiocyanate with primary aromatic amines.

The obtained compounds have been characterized by their physical properties and their chemical structures were confirmed using elemental analysis and spectral analysis. (proton and carbon NMR, IR).

The *in vitro* antimicrobial activity of new compounds was tested; our results showed that the tested compounds exhibited a specific antimicrobial activity depending on the nature of the substituents and their position on the benzene ring.

P218

RESEARCHES CONCERNING THE SYNTHESIS, STRUCTURES, PROPERTIES AND BIOREACTIVITY OF SOME ORIGINAL DIBENZO[B,E]THIEPINE SULFONES

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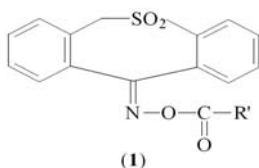
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European Center for Disease Control (ECDC) established the antibioresistance (AR) among the first 4 priorities of public health for the future years in Europe.

For this purpose new dibenzo[b,e]thiepine derivatives with the general formula (1) were synthesized.



where R' = monosubstituted aryl

The original compounds were obtained in 4 steps starting from the 6,11-dihydro-dibenzo[*b,e*]thiepin-11(6H)-one

The structures were elucidated by elemental analysis and spectral analysis. (proton and carbon NMR, IR).

The *in vitro* antimicrobial activity of some new compounds was tested; promising results were observed.

P219

NOVEL POTENTIAL NEUROPROTECTORS AND COGNITION ENHANCERS FROM NMDA-RECEPTOR ANTAGONISTS CLASS

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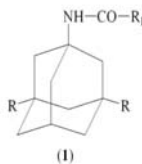
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Excitotoxicity has a main role in the pathogenesis of neurodegenerative diseases (as Alzheimer disease). An aberrant release of glutamate and a consequent overstimulation of glutamate receptors lead to brain cells death

Therefore, a major strategy has been focused on the search of agents able to antagonize the effects of glutamate (especially NMDA receptors antagonists).

Accordingly these researches, we obtained new original amino-adamantane derivatives with the general formula (1)



where R = H, CH₃

R₁ = mono- and disubstituted aryl

The new compounds were obtained by the acylation reaction of amino group from 1-aminoadamantane or 1-amino-3,5-dimethyl-adamantane (memantine) with chloride of substituted benzoic acids

The obtained compounds have been characterized by their physical properties. Their chemical structures were elucidated by elemental analysis, IR and ¹H- and ¹³C-NMR spectra.

The biological preliminary investigations by Y labyrinth test and by modified black and white box test were begun for some compounds.

P220

OPTIMIZATION OF SYNTHETIC METHODOLOGIES OF BIOLOGICAL ACTIVE PRENYLATED XANTHONES

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One of the main aims of drug optimization is to maximize interactions of the molecule with its target-binding site in order to improve activity and selectivity as well as minimize side effects.

Our research group has been focusing on the synthesis of prenylated xanthenes because of their interesting activities, namely antitumor [1-2]. Although the oxygenation pattern of these derivatives can play an important role in their biological activity, the presence of the prenyl side chains seems also to be associated with this activity. Many properties of these compounds are thought to reside in their enhanced interaction with biological membranes and with target proteins when compared with their non-prenylated analogs [3]. For that reason, we have recently obtained prenylated xanthenes through a classical prenylation method and evaluate their effect on the *in vitro* growth of human tumor cell lines [4]. In order to optimize the synthetic process to obtain these compounds, microwave assisted organic synthesis was used. Thus, we have obtained the prenylated derivatives **3-8**, by the reaction of dihydroxyxanthenes **1-2** with prenyl bromide under microwave irradiation (**Fig.1**). The dihydrofuranoxanthenes **9** and **10-11**, were also obtained by microwaves (MW), through Claisen rearrangement of monoprenylated derivatives **3** and **5**, respectively (**Fig.1**).

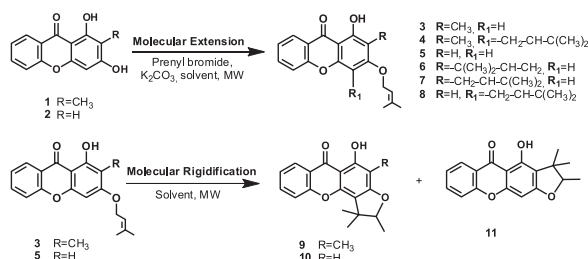


Fig. 1: General procedure for the synthesis of xanthone derivatives by microwave irradiation.

The effect of compounds **3-11** on the *in vitro* growth of three human tumor cell lines: MCF-7 (breast adenocarcinoma), NCI-H460 (non-small cell lung) and SF-268 (cen-

tral nervous system) showed for xanthenes **6** and **7** a selective and potent growth inhibitory activity against the MCF-7 cell line, comparing with respective building block **2** [4]. Compounds **9-11** are undergoing biological assays.

References

- [1] Pinto, M., *et al. Curr. Med. Chem.* 2005, 12, 2517
- [2] Pinto, M.; Castanheiro, R.; "Natural Prenylated Xanthenes: Chemistry and Biological Activities" in *Natural Products: Chemistry, Biochemistry and Pharmacology*, Ed. Brahmachari, G., Narosa Publishing House PVT. LTD., Nova Deli, India, 2007, chap. 17, *accepted for publication*
- [3] F. Epifano, *et al. Phytochemistry* 2007, 68, 939.
- [4] Castanheiro, R., *et al. Bioorg. Med. Chem.* 2007, 15, 6080.

Acknowledgments

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P221

CLASSIFICATION OF INSULIN RECEPTOR ACTIVATORS WITH SELF-ORGANIZING MAPS

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The binding of insulin to the extracellular part of the insulin receptor is a key step in the insulin signalling pathway. Upon binding, the receptor is autophosphorylated and the intracellular tyrosine kinase is activated. In 1999, Zhang *et al.* discovered a small molecule from a fungal extract which activates the human insulin receptor by binding directly to its intracellular domain [1]. This compound (L-783,281 or demethylasterriquinone B-1, DMAQ-B1) was shown to lower blood glucose levels in mouse models of type 2 diabetes mellitus.

During the last years, approximately 100 derivatives of this compound have been synthesized (e.g. [2] and [3]), most of them containing the original quinone substructure. Since this structural group might cause toxic side effects in long-term administration it would be beneficial to find active compounds with a different type of structure.

A method which was successfully used for finding new types of scaffolds is the clustering of compounds with self-organizing maps. This artificial neural network places objects with similar features into the same region of a map. The learning algorithm is unsupervised i.e. the group which the object belongs to is not known to the network at the time of training.

In this study, DMAQ-B1 and its derivatives were categorized as active or inactive according to their reported biological activity. Different sets of chemical descriptors were used as input vector for a self-organizing map.

These descriptors include general descriptors and subdivided van der Waals surface areas calculated with MOE (Chemical Computing Group) as well as 2D property-weighted autocorrelation vectors calculated with ADRIANA.Code (Molecular Networks). Varying map sizes and lengths of training were investigated. The resulting maps were examined on their ability to separate active and inactive compounds. This was done by calculating mathematical scores like the true-positive- and the true-negative-rate but also by visually inspection of the maps. Especially the 2D autocorrelation descriptors performed well in correctly classifying the compounds and may thus provide a versatile concept for virtual screening of databases for new activators of the insulin receptor.

Acknowledgement: This work was supported by the University of Vienna under the framework of the PhD programme "Molecular Drug Targets"

References

- [1] Zhang B, Salituro G, Szalkowski D, Li Z, Zhang Y, Royo I, Vilella D, Díez MT, Pelaez F, Rubi C, Kendall RL, Mao X, Griffin P, Calaycay J, Zierath JR, Heck JV, Smith RG, Moller DE. Discovery of a Small Molecule Insulin Mimetic with Antidiabetic Activity in Mice. *Science*. 1999;284(5416):974-7
- [2] Wood HB Jr, Black R, Salituro G, Szalkowski D, Li Z, Zhang Y, Moller DE, Zhang B, Jones AB. The basal SAR of a novel insulin receptor activator. *Bioorg Med Chem Lett*. 2000;10(11):1189-92
- [3] Pirrung MC, Liu Y, Deng L, Halstead DK, Li Z, May JF, Wedel M, Austin DA, Webster NJ. Methyl scanning: total synthesis of demethylasterriquinone B1 and derivatives for identification of sites of interaction with and isolation of its receptor(s). *J Am Chem Soc*. 2005;127(13):4609-24

P222

NEW ANTIMICROBIAL AGENTS: SYNTHESIS, IDENTIFICATION, AND BIOLOGICAL SCREENING

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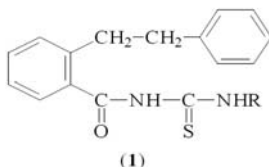
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The development of resistance to current antimicrobials continues to be a serious difficulty in the treatment of infectious diseases. Therefore, from day to day, the design and synthesis of new compounds having antimicrobial activity has become a high priority in biomedical research and continues to attract the attention of many scientists.

In this work we present the synthesis, structure determination and screening of new thiourea derivatives of 2-benzyl-carboxylic acid with general formula (1)



where R = mono- or di-substituted aryl

The new compounds were prepared starting from chloride of 2-benzyl-carboxylic acid by the reaction with isothiocyanate, followed by the reaction of the obtained substituted isothiocyanate with primary aromatic amines.

The obtained compounds have been characterized by their physical properties. Their chemical structures were elucidated by elemental analysis, IR and ¹H- and ¹³C-NMR spectra.

The biological preliminary investigations evidenced promising results

P223

NEW COMPOUNDS FROM ADAMANTANE CLASS WITH POTENTIAL ACTION ON NEURODEGENERATIVE DISEASES (ALZHEIMER DISEASE)

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The specific blockade of calcium ions influx via activated NMDA-receptor may provide efficient protection against diverse group of neurological disorders related to glutamate excitotoxicity (Alzheimer disease) and improvement in cognition function.

Memantine (1-amino-3,5-dimethyl-adamantane) is a NMDA-receptors antagonist, the only representative of this class, used in Alzheimer therapy.

New compounds were synthesized by reductive alkylation of 1-aminoadamantane and 1-amino-3,5-dimethyl-adamantane with different aromatic aldehydes in the presence of sodium triacetoxy borohydride.

The obtained compounds were characterized by elemental analysis and IR, ¹H- and ¹³C-NMR spectra.

An pharmacological evaluation of some compounds were started by Y labyrinth test and by modified black and white box test; the results are hopeful.

P224

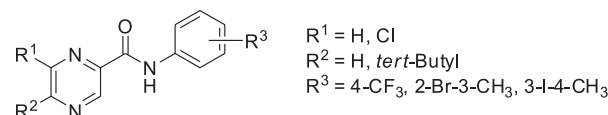
STRUCTURE-ACTIVITY RELATIONSHIPS IN SERIES OF NEW SUBSTITUTED N-PHENYLPYRAZINE-2-CARBOXAMIDES AS ANTIMYCOBACTERIAL ACTIVE COMPOUNDS

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The searching of new structures active against *Mycobacterium tuberculosis* is urgently needed to improve health of human population all over the world. In past decade the multi-drug resistant strains of tubercle bacillus appeared, thus the incidence of tuberculosis increased year by year [1]. This study describes searching for new structure derived from pyrazinamide, the first-line therapeutic agent of tuberculosis. The simple structure of pyrazinamide gives good opportunity for further modification (halogenation, alkylation, acylation, amidation of pyrazine ring) in the point of the increase of its antimycobacterial activity [2-6]. Twelve ring-substituted N-phenylpyrazinecarboxamides were prepared and evaluated for their antimycobacterial activity. All compounds were assayed *in vitro* against major *Mycobacterium* species. The anti-tuberculosis assay was provided by the Faculty Hospital in Hradec Kralove, used strains were *M. tuberculosis* H37Rv CNCTC My 331/80, *M. kansasii* CNCTC My 235/80 and *M. avium* My CNCTC 80/72. Culture was ten days old and used culture medium was Sula's medium at pH 5.6 and 37 °C. Used method was microdilution panel method [7]. Antimycobacterial data were also provided by the Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF) through a research and development contract with the U.S. National Institute of Allergy and Infectious Diseases [8]. The best activities were found for N-(4-trifluoromethylphenyl)-pyrazine-2-carboxamide, N-(2-bromo-3-methylphenyl)-pyrazine-2-carboxamide and N-(3-iodo-4-methylphenyl)-pyrazine-2-carboxamide with the values of MIC = 2 mg.L⁻¹ against *M. tuberculosis*.



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References

- [1] http://www.who.int/tb/publications/global_report/2008/chapter_2/en/index3.html (March 2008).
- [2] Dolezal, M.; Vicik, R.; Miletin, M.; Kralova, K. *Chem. Pap.* 2000, 54, 245.
- [3] Dlabal, K.; Palat, K.; Lycka, A.; Odlerova, Z. *Czech. Coll. Czech. Chem. Com.* 1990, 55, 2493.
- [4] Palek, L.; Dvorak, J.; Svobodova, M.; Buchta, V.; Jampilek, J.; Dolezal, M. *Arch. Pharm.* 2008, 341, 61.
- [5] Dolezal, M.; Palek, L.; Vinsova, J.; Buchta, V.; Jampilek, J.; Kralova, K. *Molecules* 2006, 11, 242.
- [6] Dolezal, M.; Cmedlova, P.; Palek, L.; Vinsova, J.; Kunes, J.; Buchta, V.; Jampilek, J.; Kralova, K. *Eur. J. Med. Chem.* 2008, 43, 1105.
- [7] McDermott, W.; Tompsett, R. *Am. Rev. Tuberc.* 1954, 70, 748.
- [8] <http://www.taacf.org/about-TAACF.htm> (March 2008).

P225

DESIGN, DEVELOPMENT AND SELECTIVITY PROFILE OF A NOVEL STRUCTURAL CLASS OF P38 MAP KINASE INHIBITORS

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p38 MAP kinase has become an attractive and a valid drug target for the treatment of chronic inflammatory diseases including rheumatoid arthritis, Crohn's disease and psoriasis. p38 plays a central role in the cell signalling pathway and regulates the biosynthesis of inflammatory cytokines such as TNF- α und IL-1 β both at the translational and transcriptional level. The discovery of the pyridinylimidazole class, e.g. SB-203580 initiates an ambitious pursuit of potent, selective and non-toxic p38 MAP kinase inhibitors. Although SB-203580 is quite potent, it suffers from poor selectivity. Even the second generation inhibitor BIRB-796 is far away from being selective.

Recently Ottosen et. al.^[1] reported the development of a series of aminobenzophenones described as linear binders by Lee et. al.^[2], with the two essential rings, addressing the hinge region and hydrophobic region I (HR I, selectivity pocket), connected directly. Attempts to improve both activity and selectivity implicate conformational restriction as we expect that flexible inhibitors have more options to form induced fits in various kinases. Here we describe the structural modification of benzophenones by fixing the two aromatic rings with an ethano- and etheno-linker deriving N-substituted 2-aminodibenzosuberones as a novel class of p38 MAP kinase inhibitors, potentially inhibiting in vitro p38 with IC₅₀ values as low as 87nM for 2-aminoanilindibenzosuberone.

An initial kinase profile in 17 disease related kinases from different families indicates an improved selectivity profile. Results for 2-(2-amino-phenylamino)-10,11-dihydrodibenzo[a,d]cyclohepten-5-one shows better selectivity than SB-203580 and BIRB-796. Besides the p38 MAP kinase only the closely related kinase JNK3 is inhibited which is also involved in cytokine driven inflammatory diseases.

References

- [1] Ottosen, Sørensen, Björkling, Skak-Nielsen, Fjording, Aaes, Binderup, J. *Med. Chem.*, 46, 5651 (2003)
- [2] Lee, Dominguez, *Curr. Med. Chem.*, 12, 2979 (2005)

P226

NEW CONTRAST AGENTS FOR MAGNETIC RESONANCE IMAGING TARGETING CANCER CELLS

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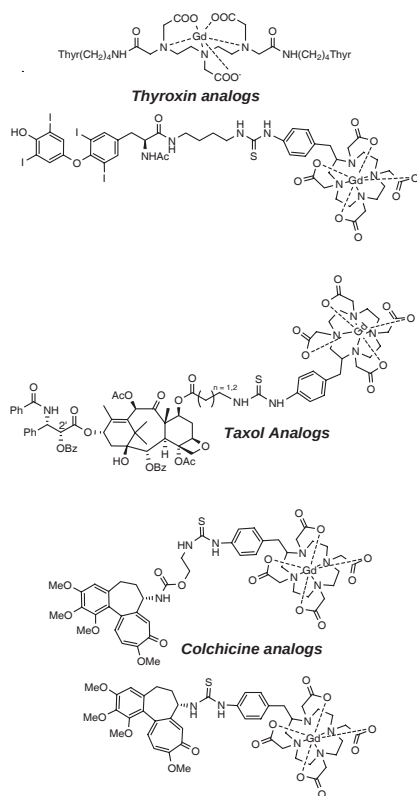
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Magnetic resonance imaging (MRI) is a non-invasive clinical imaging technique, which relies on the detection of NMR signals emitted by hydrogen protons in the body placed in a magnetic field. MRI contrast agent (CA) is a unique class of pharmaceuticals that enhances the image contrast between normal and diseased tissue, and indicates the status of organ function or blood flow after administration, by increasing the relaxation rates of water protons in tissue, in which the agent accumulates. Contrast agents are being used in MRI since 1983 when the first injection of gadolinium (Gd) DTPA was performed in men. Since then CA have gained great acceptance. They function by shortening of T1-relaxation time, thereby increasing signal intensity (SI)(positive contrast enhancement).

Conceptually, antibodies or other tissue-specific molecules may be combined with paramagnetic centers to provide disease-specific MRI agents. The challenge with regard to delivering sufficient quantity of paramagnetic label is substantial. On that front, we have synthesized a series of gadolinium conjugates with Colchicine, TaxolTM and Thyroxine, known for their tubulin- and hormone receptor-binding properties respectively, targeting the discovery of novel, cancer specific CA. Cytotoxicity and relaxation time measurements have been performed for all new complexes.



References

- [1] P. Caravan, J.J. Ellison, T.J. McMurry R.B. Lauffer, "Gadolinium(III) Chelates as MRI Contrast Agents: Structure, Dynamics, and Applications", *Chem. Rev.* 1999, 99, 2293-2352.

P227

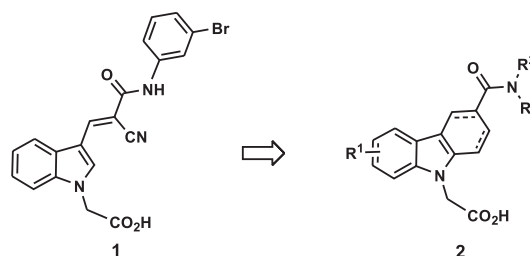
NOVEL CLASSES OF CRTH2 RECEPTOR ANTAGONISTS

Anja Fecher, Julien Pothier, Markus Gude, Dorte Renneberg, Kurt Hilpert, Markus A. Riederer, Oliver Peter, Xavier Leroy, Heidemarie Kletzl, Carmela Gnerre, Roberto Bravo, Stephane Delahaye, Daniel Bur, Luca Piali, Sylvie Froidevaux, Heinz Fretz.

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CRTH2 (Chemoattractant Receptor-homologous molecule expressed on T-Helper 2 cells, also known as DP2) is a G-protein coupled receptor expressed on the surface of eosinophils, basophils and T-helper 2 (Th2) lymphocytes.¹ It is activated by prostaglandin D₂ (PGD₂), the major prostanoid released by mast cells during allergic response, and mediates the chemotaxis of Th2 cells, eosinophils and basophils to inflammatory sites. CRTH2 antagonists may therefore be valuable compounds for treating allergic diseases.²

Screening of our in-house compound collection by means of an *in vitro* fluorescent imaging plate reader (FLIPR™) assay delivered 2-cyano-3-indolyl-acrylamide **1** as a hit compound with antagonistic activity on the CRTH2 receptor. Structure activity relationship (SAR) of this series was established in various biological assay systems. Conformational and X-ray structure analysis indicated (Z)-configuration of the cyano-acrylamide moiety in **1**. We proposed this arrangement to be preserved in tricyclic carbazole-3-carboxamide derivatives **2**.



In the following presentation we will discuss aspects like synthesis, SAR and pharmacokinetic data for CRTH2-antagonists of the 2-cyano-3-indolyl-acrylamide series and the corresponding tricyclic carbazole-3-carboxamide analogues.

References

- [1] Hirai, H. et al., *J. Exp. Med.* 2001, 193, 255-561.
[2] a) Kostenis, E.; Ulven, T., *Trends Mol. Med.* 2006, 12, 148-158 b) Pettipher, R. et al., *Nat. Rev. Drug Discovery* 2007, 6, 313-325.

P228

DESIGN AND SYNTHESIS OF PYRROLOQUINOXALINE BASED ANTICANCER AGENTS

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Salicylhydrazides represent a novel class of compounds with remarkable cytotoxicity activity in a panel of human cancer cell lines from different tumor origins. Several structural alterations were attempted in order to improve pharmacological profiles of original lead compounds. During the past several years, we have designed and synthesized series of compounds bearing a hydrazide moiety linking a nitrogen containing polycyclic system to a heteroaroyl portion. Pyrroloquinoxalinylnyl based derivatives were identified as the most promising in terms of potency and drug-like characteristics (Fig 1). Several of such derivatives showed high cytotoxicity properties, with IC₅₀ values in the low micromolar range, and promising *in vivo* activity in xenograft mice models of human ovarian cancer cell lines.

In order to further improve the physicochemical properties of these compounds and establish a more comprehensive structure-activity relationship, we planned to synthesize a new series of compounds bearing a 2,3-dihydroxypropan-1-one moiety replacing the hydrazide linker of the parent compounds (Fig. 2). This alteration should improve drug solubility while maintaining a similar overall asset of the molecule. The cytotoxic profile and the cell cycle perturbation induced by the newly synthesised compounds are presently under active investigation in our laboratories.

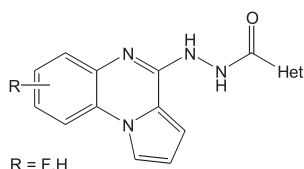


Fig. 1.

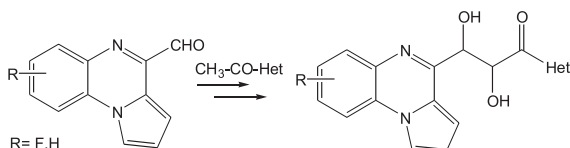


Fig. 2.

References

- [1] Grande, F.; Aiello, F.; Grazia, O. D.; Brizzi, A.; Garofalo, A.; Neamati, N. Synthesis and antitumor activities of a series of novel quinoxalinydrazides. *Bioorg Med Chem* 2007, 15, 288-294.

P229

STRUCTURE- AND LIGAND-BASED DESIGN OF HIGHLY POTENT AND SELECTIVE INHIBITORS OF 17 β -HYDROXYSTEROID DEHYDROGENASE TYPE 1 (17 β -HSD1) AS NOVEL, POTENTIAL THERAPEUTICS FOR BREAST CANCER AND ENDOMETRIOSIS

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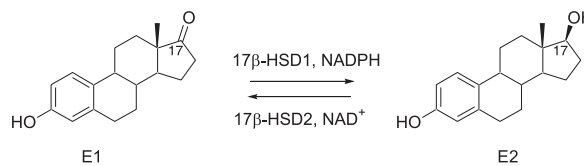
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Breast cancer is the most frequent cancer type among women and the most common cause of cancer death. Endometriosis affects approximately 20 % of the female population and can lead to infertility. Both endometriosis and a high percentage of the breast tumours are estrogen-dependent, i.e. growth and proliferation are stimulated by estrogens. Present endocrine therapies with aromatase inhibitors, GnRH-analogues or anti-estrogens result in a radical and unspecific reduction of estrogenic effects throughout the body. This lack of tissue-specificity often leads to severe side-effects like osteoporosis or induction of endometrium carcinomas.

17 β -HSD1 catalyses the reduction of the weak estrogen estrone (E1) to the highly potent estradiol (E2). The enzyme is expressed in different organs like ovaries, placenta, breast, endometrium and is often strongly overexpressed in breast cancer tissue. Inhibition of 17 β -HSD1 could selectively reduce the E2-level in specific tissues thus allowing for a novel, targeted approach in the treatment of estrogen-dependent diseases resulting in less side-effects compared to established therapies.

As 17 β -HSD2 catalyses the reverse reaction – decreasing the level of active E2 – this enzyme should not be affected by inhibitors of the type 1 enzyme. Furthermore, to avoid intrinsic estrogenic effects, 17 β -HSD1 inhibitors should not show binding affinity to the estrogen receptors (ERs) α and β .

The combination of a structure- and ligand-based drug design approach led to the development of steroidomimetics as potential inhibitors of the target enzyme. Highly potent inhibitors of 17 β -HSD1 have been synthesized with IC₅₀-values in the low nanomolar range displaying good selectivity towards 17 β -HSD2 and ERs α and β . Good pharmacokinetic profiles (rat) make them promising candidates for further drug development.



P230

NEW PHENYLETHANOL AMINE DERIVATIVES POTENTIALLY USE IN MODERN THERAPY FOR OBESITY AND/OR TYPE 2-DIABETES

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Adrenergic beta-3 receptors were first characterized in 1989 and after that their distribution in certain tissues was established: white and brown tissue, nervous central system, heart muscle, blood vessels, gastrointestinal smooth muscles, prostate, skeletal muscles. In non-clinical trials, their major involvement in glucidic metabolism and implicitly, in insulin release and also obesity demonstrated. The major advantage that the new beta-3 adrenergic receptors agonists may yield in perspective for therapeutics consists in very low predicted pharmacotoxicological potential, comparatively to existing drug arsenal in diabetes and obesity pharmacotherapy. This prediction is based on chemical structure with beta-

phenylethanol aminic nucleus, also found in catechol aminic chemical mediators.

The aim of the present study was a synthesis and characterization of some new compounds as beta-3 AR agonists, with potential antidiabetes, antiobesity action.

The synthesis way consists in the condensation of the appropriate ketone with an aryethanol amine, followed by the reduction of the obtained imine (Schiff base).

For the original compounds and the necessary intermediates, were elaborated the synthesis and physico-chemical analysis methods.

Chemical structures of the compounds have been elucidated by their IR, ¹H and ¹³C-NMR, mass and elemental analysis data, GC, TLC.

Experimental pharmacological trials were performed for six compounds (identified as C₁-C₆), structurally similar to BRL 37344, a selective agonist on beta-3-receptors.

The biological preliminary investigations evidenced promising results. Investigated compounds demonstrated a statistically significant decrease in serum triglycerides in diabetes rats (p<0.01) compared to control group (diabetic rats treated with distilled water) and the effect on baseline glycaemia in non-diabetic rat showed a statistically significant decrease of glycaemia, compared to control group (treated with distilled water).

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SYNTHESIS, CHARACTERIZATION AND MMP INHIBITORY ACTIVITY OF NEW BENZIMIDAZOLE DERIVATIVES

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In the last years the interest for the study of metalloproteinases inhibitors and their role in the regulation of enzymatic activity increased significantly all over the world. The inhibition of metalloproteinases activity has many physiological and pathological implications. The studies on the synthetic inhibitors of metalloproteinases could contributed to the development of therapeutics strategies important for the control of the diseases caused by metalloproteinases over activity.

MMPs (matrix metalloproteinases) are zinc-dependent endopeptidases that degrade both matrix and non-matrix proteins. They play an important role in morphogenesis, and in a wide range of processes including tissue repair and remodeling. Their abnormal expression contributes to pathological processes including arthritis, cancer, and cardiac and central nervous system diseases, which explains the large interest in finding specific MMP inhibitors for therapeutic use.

The matrix metalloproteinases (MMP) are an attractive target for the low molecular weight inhibitors-hydroxamate structures – which bind zinc ions like the natural products.

Our own research programme led to discovery of heterocyclic hydroxamate-based compounds, which are benzimidazole derivatives substituted at CS with various groups, as broad-spectrum MMP Is.

The new compounds were obtained starting from substituted/unsubstituted benzimidazole by a hydroxylamidation reaction of the corresponding esters.

All new synthesized compounds were characterized and their chemical structure was confirmed by spectral methods (FTIR, ¹H and ¹³C-NMR), elemental analysis.

Three of these compounds had an inhibitory effect on all MMP fractions isolated from bovine brain. The selective inhibition by C₁ compound of MMP-2, the enzyme implicated in CNS pathology [1] suggests the possible therapeutic application of this compound.

[1] Aoki, T., Kataoka, H., Morimoto, M., Nozaki, K. and Hashimoto, N. (2007) *Stroke* 38, 162-169

P232

BUILDING OF A MCH-R1 HOMOLGY MODEL AND DOCKING OF TWO NOVEL SERIES OF MCH-R1 ANTAGONISTS PROVIDES INSIGHTS INTO THE PROTEIN-DRUG CONTACTS RESPONSIBLE FOR ACTIVITY AND SELECTIVITY TO THE RECEPTOR

Nuria Cirauqui¹, Anna K. Schrey², Ronald Kühne², Silvia Galiano¹, Javier Ceras¹, Silvia Pérez-Silanes¹, Ignacio Aldana¹, Antonio Monge¹.

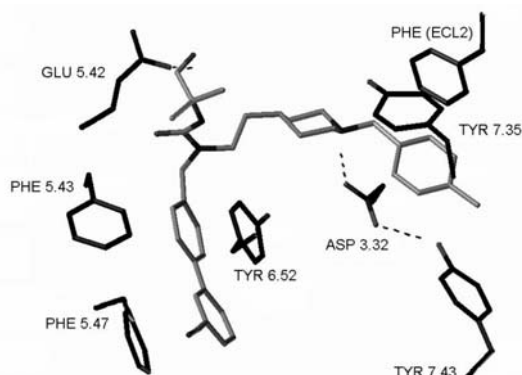
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Melanin-concentrating hormone receptor 1 (MCH-R1); also called G-protein coupled receptor 24 (GPCR24); represents a new biological target for treating obesity and is subject of research programs at many major pharmaceutical companies. The binding of the neuropeptide MCH to the receptor stimulates food intake, as confirmed by findings of lean phenotype mice with targeted deletion of the MCH and MCH-R1 whereas the overexpression of the MCH-gene gives an obese and insulin resistance phenotype [1]. The anorexigenic effects of MCH-R1 antagonist molecules reported in recent years [2] support the hypothesis that MCH-R1 provides a novel target for the treatment of obesity.

Molecular modeling and structure-activity relationship studies were performed to propose a model for the binding of two novel series of MCH-R1 antagonists [3] to the human MCH-R1. A homology model for the protein was constructed using the crystal structure of bovine

Rhodopsin as template. Induced fit docking of the most active compound (2t) [3] into the hMCH-R1 homology model resulted in several different ligand-receptor complexes which were further evaluated by automated docking of all the compounds into the resulting receptor models. Comparison of experimental binding and biological activity data for these novel series with the docking scores led to the selection of one of these binding modes as preferred. A molecular dynamics simulation in a water-vacuum-water box as a membrane model was carried out in order to relax the structure and determine the protein-ligand contacts. The model is in agreement with experimental findings concerning this receptor and can be used for rational modification of these compounds to improve activity as well as selectivity for specific GPCRs. A picture of the ligand surrounded by the main residues implied in the docking is shown down these lines.



References

- [1] Ludwig DS, Tritos NA, Mastaitis JW, Kulkarni R, Kokkotou E, Elmquist J, Lowell B, Flier JS, Maratos-Flier EJ. Melanin-concentrating hormone overexpression in transgenic mice leads to obesity and insulin resistance. *J Clin Invest* 2001;107:379-386.
- [2] Takekawa S, Asami A, Ishihara Y, Terauchi J, Kato K, Shimomura Y, Mori M, Murakoshi H, Kato K, Suzuki N, Nishimura O, Fujino M. T-226296: a novel, orally active and selective melanin-concentrating hormone receptor antagonist. *Eur J Pharm* 2002;438:129-135.
- [3] Galiano S, Ceras J, Cirauqui N, Perez S, Juanenea L., Rivera G, Aldana I, Monge A. Novel series of substituted biphenylmethyl urea derivatives as MCH-R1 antagonists for the treatment of obesity. *Bioorg Med Chem* 2007;15:3896-3911.

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NEW BIPHENYLMETHYL UREA DERIVATIVES AS MCH-R1 AND H₃ RECEPTORS ANTAGONISTS FOR THE TREATMENT OF OBESITY

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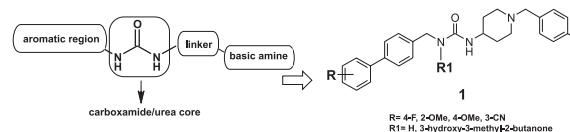
Unidad en Investigación y Desarrollo de Medicamentos. Centro de Investigación en Farmacobiología Aplicada (CIFA). University of Navarra. C/Irunlarrea, s/n, 31080 Pamplona. Spain

Obesity is set to be the world's largest single cause of mortality and morbidity in the 21st Century. The escalating problem of obesity has initiated an unprecedented search for novel anti-obesity drugs to help combat this global epidemic. An historical perspective of pharmacological interventions to treat obesity reveals several key challenges for the successful development and introduction of novel drugs in this therapeutic area. Two of these promising hypothalamic molecular targets are the melanin-concentrating hormone receptor 1 antagonists (MCH-R1) and histamine H₃ receptors antagonists.

Melanin-concentrating hormone (MCH), a cyclic 19-amino acid neuropeptide, is believed to play a critical role as a regulator of feeding behaviour based on several lines of evidence. Interestingly, mice overexpressing the MCH gene appear to give a hyperphagic and obese phenotype and display insulin-resistance, whereas transgenic MCH-R1 knockout mice are lean, resistant to diet-induced obesity and show hyperactivity¹. Consequently, selective MCH-R1 antagonism could provide a viable treatment for obesity.

A number of studies suggest that histamine can also suppress appetite and that histamine-containing neurons in the hypothalamus participate in the endogenous suppression of food intake. Intracerebroventricular injections of histamine depress feeding in rats. Several studies have shown the ability of histamine H₃ receptor antagonists to promote weight loss or prevent weight gain. Selective H₃ receptor antagonists, for example, A-331440, reduce body weight and body fat in diet-induced obese mice, and accordingly, have potential utility in the treatment of obesity².

As part of our research of new antiobesity drugs, we have designed a novel structure (1) and synthesized a new series of biphenylmethyl urea derivatives in order to evaluate their affinity as potential MCH-R1 antagonists³.



SAR was explored, suggesting that optimal binding with the receptor was achieved when the biphenylmethyl group and the linker were substituted on the same nitrogen of the urea moiety.

In addition, we tested the obtained compounds for their in vitro H₃ receptor affinity.

With the aim of evaluating the cardiotoxicity of the new compounds, we assessed the ability of these derivatives to block potassium channels encoded by hERG.

References

- [1] Della-Zuana, O.; Presse, F.; Ortola, C.; Duhault, J.; Nahon, J.L.; Levens, N. *Int. J. Obes.* 2002, 26, 1289.
- [2] Hancock AA. *Curr. Opin. Investig. Drugs* 2003, 4, 1190.
- [3] Galiano, S.; Ceras, J.; Cirauqui, N.; Pérez, S; Juanenea, L.; Rivera, G.; Aldana, I.; Monge, A. *Bioorg. Med. Chem.* 2007, 15, 3896.

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DISCOVERY OF A HIGHLY SELECTIVE SPASMOLYTIC DITHIOBENZAMIDE

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The aim of this study was to identify new spasmolytic agents. Several dithiobenzamide derivatives were synthesized and their effect on isolated guinea pig smooth muscles such as terminal ilea was investigated [1]. Some of the compounds did not show tissue selectivity or just a weak effect on the tissue preparations, while one highly selective compound (see figure 1) with an IC₅₀ value of 2.7 µM (n=5, P<0.001) was discovered. The other compound studied did not cause a significant relaxing effect with IC₅₀ values over 100 µM. A third compound tested resulted in IC₅₀ values at lower concentrations (1.77 to 43.5 µM) but did not show a selective spasmolytic profile. The synthesis of the compounds and the investigation of their pharmacological profiles are presented. We assume that for the selectivity the distance between the substituents in the molecule plays an important role.

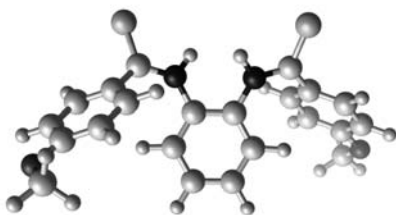


Fig.1: Dithiobenzamide derivative

References:

- [1] Reiter M (1967) Evaluation of inotropically active drugs on isolated papillary muscle. *Drug Res* 17:1249-1253

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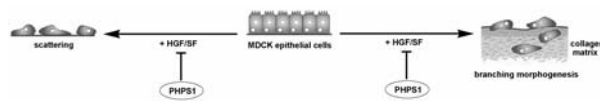
SYNTHESIS AND EVALUATION OF SPECIFIC INHIBITORS OF THE ONCOGENIC PROTEIN TYROSINE PHOSPHATASE SHP2

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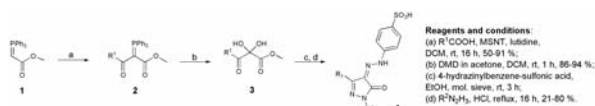
Reversible phosphorylation of tyrosine residues is a universal feature of eukaryotic intracellular signalling and provides an important mechanism for enzymatic and cellular regulation. Protein tyrosine phosphatases (PTPs) are implicated in a growing number of diseases, ranging from cancer to cardiovascular, immunological, infectious, neurological, and metabolic diseases. The design of specific PTP inhibitors may provide a powerful tool in understanding the in vivo role of these proteins in signal transduction. Furthermore, small molecule PTP inhibitors are important reagents for the evaluation of PTPs as therapeutic agents and so new classes for phosphatase inhibitors are in high demand.

The protein tyrosine phosphatase SHP2 is a positive regulator of growth factor signalling. By virtual screening of a library containing 2.7 million small molecular weight compounds the phenylhydrazonopyrazolone sulfonate PHPS1 with an inhibitory activity in the low micromolar range was identified.¹ It is also cell-permeable and specific for SHP2 over the closely related tyrosine phosphatases SHP1 and PTP1B. PHPS1 inhibits SHP2-dependent cellular events such as hepatocyte growth factor/scatter factor (HGF/SF)-induced epithelial cell scattering and branching morphogenesis (**Scheme 1**).



Scheme 1

For this new class of inhibitors a 4-step synthesis was developed in solution based on the C-acylation of phosphonylidene acetate **1** (**Scheme 2**).² This established synthesis was used to modify and optimize the lead structure of PHPS1. With the aid of this procedure a small library of potential inhibitors was designed and evaluated in an enzymatic assay. The PHSP1 class is suitable for further investigation of novel therapeutics for the treatment of SHP2-dependent diseases.



Scheme 2

References

- [1] Hellmuth, K., Grosskopf, S., Lum, C. T., Würtele, M., Röder, N., von Kries, J. P., Rosario, M., Rademann, J. and Birchmeier, W. *Proc. Natl. Acad. Sci.* 2008, 105 (20), 7275.
- [2] El-Dahshan, A.; Weik, S.; Rademann, J. *J. Org. Lett.* 2007, 9, 949.

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α_1 -ADRENOCEPTOR ANTAGONISTIC PROPERTIES OF VARIOUS PHENYLPIPERAZINE DERIVATIVES OF HYDANTOIN. PHARMACOLOGICAL STUDY AND MOLECULAR MODELLING RESULTS

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The α_1 -adrenoceptors subclass, discovered as an independent group in 1974,¹ has been divided into two types: α_{1H} (high sensitivity) and α_{1L} (low sensitivity), based on sensitivity to prazosin, a potent and selective antagonist. Three subtypes of human α_{1H} -adrenoceptors: α_{1A} , α_{1B} and α_{1D} , were identified by pharmacological and cloning studies. The α_{1L} -adrenoceptors have been recognised as original subtypes in various pharmacological assays but no discrete protein having α_{1L} -adrenoceptor characteristics has been cloned. Some lines of evidence indicated that α_{1L} -adrenoceptors represent a functional phenotype of α_{1A} -adrenoceptors. Analysis of a number of chemical structures of selective α_1 -adrenoceptor antagonists indicates that various active compounds possess arylpiperazine moieties. The rich database² of active compounds allowed to evaluate pharmacophore models of the α_1 -antagonist including a model of Barbaro,³ especially useful for phenylpiperazine derivatives. This model describes five pharmacophore features responsible for α_1 -antagonistic properties. Various α_1 -adrenoceptor antagonists can be useful in the treatment of hypertension, benign prostatic hyperplasia (BPH), lower urinary tract symptoms (LUTS) or cardiac arrhythmia. In this context, the search for selective α_1 -adrenoceptor antagonists has been, and still is, an important topic in medicinal chemistry. Thus, we designed and synthesized a series of phenylpiperazine derivatives of hydantoin (Fig.1) to

find new adrenoceptor antagonists with potential antiarrhythmic activity.

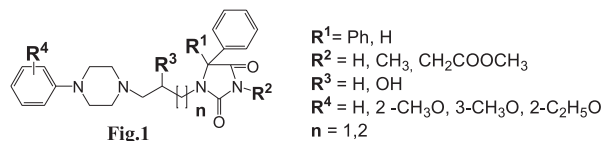


Fig.1

The compounds were evaluated on their affinity for α_1 - and α_2 -adrenoceptors in radioligand binding assays using [³H]-prazosin and [³H]-clonidine as selective radioligands. The antagonistic properties as well as activities at α_1 -adrenoceptor subtypes were assessed in functional bioassays. The molecular modelling, using conformational analysis CONFLEX and PM5-geometry optimization, was carried out to detect 3D-structure properties of the phenylpiperazine derivatives of hydantoin for comparison with ideal α_1 -antagonist features based on a pharmacophore model of Barbaro.² The SAR analysis indicated the best pharmacological properties for compounds possessing 2-methoxyphenylpiperazine moieties.

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References

- [1] Langer, S. Z. *Br. J. Pharmacol.* 1974, 60, 481.
- [2] Bremner, J. H.; Coban, B.; Griffith, R.; Groenewoud, K. M.; Yates, B. F. *Bioorg. Med. Chem.* 2000, 8, 201.
- [3] Barbaro, R.; Betti, L.; Botta, M.; Corelli, F.; Giannaccini, G.; Maccari, L.; Manetti, F.; Strappaghetti, G.; Corsano, S. J. *Med. Chem.* 2001, 44, 2118.

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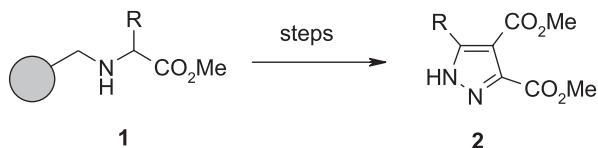
PARALLEL SOLID-PHASE SYNTHESIS OF PYRAZOLES

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Solid-phase techniques, parallel synthesis, and microwave-assisted reactions were utilized for the preparation of pyrazoles. N-Unsubstituted pyrazoles were synthesized in a traceless manner on solid support. Various amino acid methyl esters were first attached to the formyl-functionalized polymeric support with a reductive amination (1). Radleys 12 position carousel reaction station was used for parallel reactions. Esters were then hydrolyzed refluxing in basic conditions in Radleys reaction tubes, and then the acids were N-nitrosated utilizing again the Radleys carousel. N-Nitrosated polymer-bound amino acids formed mesoionic sydnones in the presence of acetic anhydride, and the sydnones reacted in 1,3-dipolar cycloaddition with dimethyl acetylene dicarboxylate giving polymer-bound pyrazoles. The 1,3-dipolar cycloaddition

was enhanced with microwave irradiation. The cleavage of the products with trifluoroacetic acid gave N-unsubstituted pyrazoles (**2**), with variety of R groups, most of them in very high purities and high overall yields.



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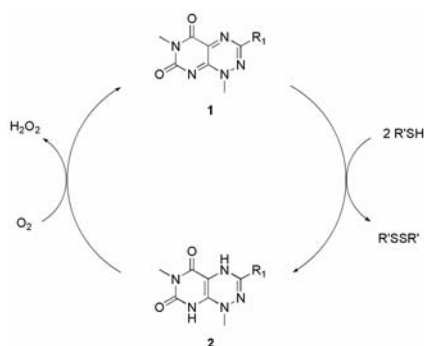
PHYSIOLOGICALLY ARMED INHIBITORS OF MYCOBACTERIUM TUBERCULOSIS PROTEIN TYROSINE PHOSPHATASE A

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Tuberculosis is a major cause of death and illness world wide. These days about one third of the world population are infected with *Mycobacterium tuberculosis* and in 2005 1.6 million people died.^[1] MtpA, a protein tyrosine phosphatase from *Mycobacterium tuberculosis* has been proposed as potential drug target against tuberculosis. Though the function of MtpA is not known yet, it was suspected to be responsible for switching off the host cell defence.^[2] We have identified pyrimidotriazines **1** as nanomolar inhibitors of MtpA and several other phosphatases. An interesting and novel mode of action of these compounds has been elucidated: Pyrimidotriazines **1** show inhibitory effects in vitro only in the presence of reducing agents such as DTT. The product of reduction, **2**, was demonstrated to undergo catalytic redox cycling as confirmed by ¹H-NMR. Dihydro-pyrimidotriazin **2** reduces elemental oxygen releasing hydrogen peroxide as the product (Scheme 1). The amount of H₂O₂ formed, however, cannot account for inhibition of the phosphatase. Thus, we have synthesized several derivatives of **1** in order to identify the intermediate of redox cycling responsible for inhibition.



Scheme 1: Redox cycle of pyrimidotriazines

Moreover, the reported redox cycling has been demonstrated to be active in infected mouse macrophages and compound **1** was capable of inhibiting mycobacterial proliferation without being toxic to the host cells.

References

- [1] WHO report on Tuberculosis 2008 "Global Tuberculosis control – surveillance, planning, financing"
- [2] Koul et al. *Nat. Rev. Microbiol.* 2004, 2, pp. 189 –202

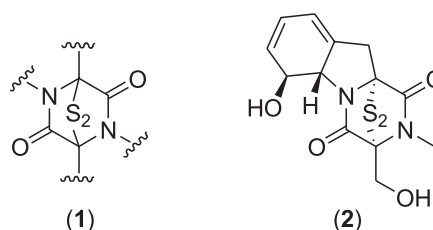
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DEVELOPMENT OF GENERAL METHODOLOGY FOR THE SYNTHESIS OF EPIDITHIOPIPERAZINEDIONES

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Epidithiopiperazinediones (ETP) are compounds which contain a piperazinedione ring bridged at the α -carbon centres by a disulfide linkage as depicted in the generic structure **1**. Naturally occurring ETP compounds comprise an important class of bioactive secondary metabolites isolated from many different species of fungi.¹ Fungal sources of ETP natural products have been found throughout the tropical and temperate regions of the world in both terrestrial and marine environments.² Perhaps the most celebrated member of this class of natural products is gliotoxin (**2**) which has been shown to possess a range of potent activities including antibacterial, antifungal, antiphagocytic, antitumor, antiviral activity and immunosuppressive properties.²⁻⁴ The previously reported syntheses of ETP compounds have drawbacks associated with generality, efficiency, yields and the use of harsh reaction conditions.⁵⁻⁸ As a result, very few total syntheses of complex ETP natural products have been reported despite the continued isolation of new and interesting members of this class of compounds.⁹⁻¹¹ Thus, there is a need to develop a novel and generally applicable methodology for the synthesis of ETP compounds. This presentation will detail our seminal synthetic work and our ongoing efforts towards the full development of such methodology.



- [1] Gardiner, D. M.; Waring, P.; Howlett, B. J. *Microbiology (Reading, U. K.)* 2005, 151, 1021-1032.
- [2] Taylor, A. *Microbial Toxins* 1971, 7, 337-376.
- [3] Waring, P.; Beaver, J. *Gen. Pharmacol.* 1996, 27, 1311-1316.
- [4] Waring, P.; Eichner, R. D.; Mullbacher, A. *Med. Res. Rev.* 1988, 8, 499-524.
- [5] Trown, P. W. *Biochem. Biophys. Res. Commun.* 1968, 33, 402-407.
- [6] Yoshimura, J.; Nakamura, H.; Matsunari, K. *Bull. Chem. Soc. Jpn* 1975, 48, 605-609.
- [7] Srinivasan, A.; Kolar, A. J.; Olsen, R. K. *J. Heterocycl. Chem.* 1981, 18, 1545-8.
- [8] Öhler, E.; Poisel, H.; Tataruch, F.; Schmidt, U. *Chem. Ber.* 1972, 105, 635-641.
- [9] Kishi, Y.; Nakatsuka, S.; Fukuyama, T.; Havel, M. *J. Am. Chem. Soc.* 1973, 95, 6493-5.
- [10] Williams, R. M.; Rastetter W. H. *J. Org. Chem.* 1980, 45, 2625-31.
- [11] Fukuyama, T.; Nakatsuka, S.; Kishi, Y. *Tetrahedron* 1981, 37, 2045-78.

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SYNTHESIS AND BIOLOGICAL EVALUATION OF NEW CYTOTOXIC PODOPHYLLOTOXIN DERIVATIVES, TARGETED FOR POLYAMINE TRANSPORTER
 Imbert T.^a, Guminski Y.^a, Grousseau M.^a, Cugnasse S.^a, Barret J.-M.^b, Kruczyński A.^b, Vispe S.^b, Gras S.^b, Annereau J.-P.^b, Vandenberghe I.^b, Stennevin A.^b, Lacastaigneratte L.^b, Novosad N.^b, Chansard N.^b, Pourtau S.^b, Roy S.^b, Castano C.^b, Blanchet J.-C.^b, Guilbaud N.^b, Bailly C.^b

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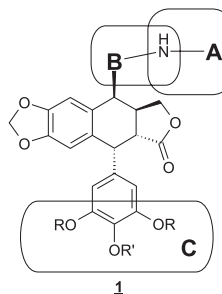
Polyamines (spermine, spermidine, putrescine) are lipophilic polycations at physiological pH, and are essential to cell proliferation. Their homeostasis is maintained by endogenous biosynthesis or uptake from external sources through the polyamine transporter system (PTS) which is activated in the case of cancer cells. This PTS has a moderate specificity for structural recognition allowing polyamine structural modifications or conjugate derivatives for internalisation.⁽¹⁾ One of the major concerns for chemotherapy is the selective targeting of drugs into highly proliferative cancer cells. Therefore, we used this mechanism, through the PTS, to target cytotoxic compounds specifically to cancer cells, and to obtain a better interaction to DNA backbone⁽²⁾ with DNA damaging agents.

Podophyllotoxin derivatives **1**, fulfilled these criteria. A series of conjugated compounds constituted by a podophyllotoxin core and a polyamine chain, with a spacer junction, was synthesized, and their *in vitro* cytotoxicity

and topoisomerase II inhibition were assessed. Demonstration was made to show their cell internalisation through PTS, by competitive experiment relative to their cytotoxicity.⁽³⁾ Lead compounds were also tested *in vivo* and proved potent antitumour activity.⁽⁴⁾

Synthetic strategies and structure-activity relationships with the framework modifications of the different parts of the molecule (A, B, C) are presented hereafter.

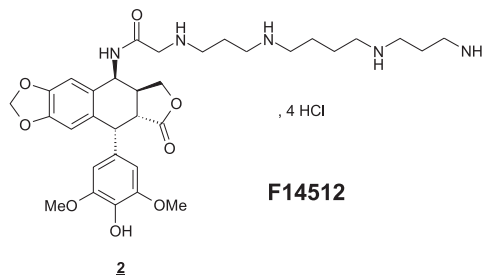
From this series of new water-soluble anticancer compounds, the podospermine derivative F14512 **2**⁽⁵⁾ was selected for further development and clinical studies.



A = mono, di, tri, tetraamine (i.e. spermine)

B = aminoalkyl-, amides, ureas, sulfonamides, carbamates, aminoacids...

C = alkoxy, phenols



References

- [1] Seiler, N. *et al. Int. J. Biochem. Cell Biol.* 1996, 28, 843.
- [2] Delcros, J.-G. *et al. Biochem. J.* 1993, 291, 269.
- [3] Barret, J.-M. *et al. AACR-NCI-EORTC International Conference. Molecular Targets and Cancer Therapeutics, Poster #A270. San Francisco oct.22-26, 2007.*
- [4] Kruczyński, A. *et al. AACR-NCI-EORTC International Conference. Molecular Targets and Cancer Therapeutics, Poster #A276. San Francisco oct.22-26, 2007.*
- [5] Imbert, T. *et al. PCT Int. Appl. WO2005/100363. Pierre Fabre Medicament.*

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SMALL, POTENT, AND SELECTIVE DIARYL PHOSPHONATE INHIBITORS FOR UROKINASE-TYPE PLASMINOGEN ACTIVATOR WITH IN VIVO ANTIMETASTATIC PROPERTIES

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Cancer is one of the leading causes of death in the Western world. Though advances in cancer therapy and diagnosis have considerably improved life expectancy, the overall survival rate of patients still remains poor. Disseminated cancer at the time of diagnosis and acquisition of tumour resistance are two main reasons. The growing knowledge of the biochemical pathways involved in a disease process increases the possibility to develop new approaches to treat this disease. One of those interesting novel targets is the urokinase plasminogen activator (uPA) system.^{1,2,3} uPA is a serine protease which is situated on the cell surface and can be bound to its receptor (uPAR). It is responsible for the formation of fibrinolytic plasmin out of plasminogen. Plasmin is a protease with strong activity towards cell-cell and cell-matrix contact proteins. Hence, the catalytic uPA activity is indirectly responsible for the breakdown of matrix proteins and fibronectin. Moreover, growth factors and other angiogenic mediators are released by the breakdown of this matrix. The function of uPAR is not only binding uPA, but it is also involved in signal transduction. This transduction is responsible for cell adhesion, cell fusion and mitogenic processes. The value of inhibitors of the uPA-system in angiogenesis and metastasis was recently demonstrated.^{2,3,4}

The Medicinal Chemistry group of the University of Antwerp (UA) designed and developed peptidic diphenyl phosphonate inhibitors of uPA. A first potent and selective lead compound was identified and a lead optimization program was started to modify the compound to a small non-peptidic diaryl phosphonate irreversible inhibitor of uPA.^{5,6,7} Potent and selective uPA inhibition was obtained ($IC_{50} < 10$ nM; selectivity toward a set of other trypsin-like serine proteases more than 1000 fold). Two compounds (**UAMC-150** and **UAMC-251**) were selected and evaluated in the BN-472 breast cancer rat metastasis model. Potent anti-metastatic effects were observed (> 70% lung metastasis inhibition) and small but significant inhibition of tumour growth (10-18%). No acute toxicity was observed after the 18 days during treatment.⁷ It is clear that this compound has an exceptional value as proof that a pure anti-metastatic compound can have a benefit in the early stage anti-cancer therapy.

References

- [1] Dano, K.; Behrendt, N.; Hoyer-Hansen, G.; Johnsen, M.; Lund, L. R.; Ploug, M.; Romer, J. Plasminogen Activation and Cancer. *Thromb. Haemostasis* 2005, 93, 676.
- [2] Mazzieri, R.; Blasi, F. The Urokinase Receptor and the Regulation of Cell Proliferation. *Thromb. Haemostasis* 2005, 93, 641
- [3] Duffy, M. J. The Urokinase Plasminogen Activator System: Role in Malignancy. *Curr. Pharm. Des.* 2004, 10, 39.
- [4] Setyono-Han, B.; Sturzebecher, J.; Schmalix, W. A.; Muehlenweg, B.; Sieuwerts, A. M.; Timmermans, M.; Magdolen, V.; Schmitt, M.; Klijn, J. G. M.; Foekens, J. A. Suppression of Rat Breast Cancer Metastasis and Reduction of Primary Tumour Growth by the Small Synthetic Urokinase Inhibitor WX-UKI. *Thromb. Haemostasis* 2005, 93, 779.
- [5] Joossens J., Van der Veken P., Lambeir A.-M., Augustyns K. and Haemers A.; Development of Irreversible Diphenyl Phosphonate Inhibitors for Urokinase Plasminogen Activator; *J. Med. Chem.*, 2004, 47; 2411
- [6] Joossens J.; Van der Veken P.; Surpateanu G.; Lambeir A.-M.; El-Sayed I.; Ali O. M.; Augustyns K.; Haemers A. Diphenyl Phosphonate Inhibitors for the Urokinase-Type Plasminogen Activator: Optimization of the P4 Position. *J. Med. Chem.* 2006, 49, 5785
- [7] Joossens J., M. Ali O., El-Sayed I., Surpateanu G., Van der Veken P., Lambeir A.-M., Setyono-Han B., Foekens J., Schneider A., Schmalix W., Haemers A., Augustyns K. Small Potent and Selective Phosphonate Inhibitors for Urokinase-type Plasminogen Activator with Potent in vivo Anti-metastatic Properties, *J. Med. Chem.*, 2007, 50, 6638

P242

SYNTHESIS AND BIOLOGICAL DATA OF A NOVEL STRUCTURAL CLASS OF P38 MAP KINASE INHIBITORS

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The p38 MAP kinase (p38) is a key enzyme in inflammatory diseases as it is involved in the biosynthesis of the proinflammatory cytokines TNF- α and IL-1 β . Thus the p38 is a promising target for the treatment of chronic inflammatory diseases such as rheumatoid arthritis and Crohn's disease.

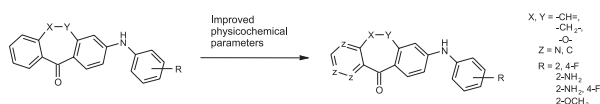
Since the binding site is highly conserved in all protein kinases, the potential selectivity for p38 vs. other kinases is an evident problem. In fact, all p38 inhibitors in clinical trials are more or less subject to this problem. Thus, there is a continuous need for structurally novel inhibitors that overcome the intrinsic problems of the common diaryl-substituted heterocyclic compounds.

The small gatekeeper of the p38 can be used to get more selective compounds. Recently several p38 inhibitors have been described^{1,2,3,4,5}, connecting the two

essential rings of the inhibitors more directly and they are therefore called "linear binders"⁵. Compounds with the linear binding mode make good use of the small gatekeeper pocket by forming a very tight, complementary surface to the linker strand. An additional peptide flip (Gly110) enhances the selectivity of these compounds⁶.

Recently we have described a structural novel class of p38 inhibitors with dibenzepinone-scaffolds⁷. This structural class is very rigid and thereby expectedly very selective. Although showing good activity in the p38 enzyme assay many substituted dibenzepinones are suffering from high lipophilicity and low solubility.

In order to optimize these physicochemical parameters, we synthesized different substituted aza-analogous dibenzepinones. Here we describe the synthesis and biological data of these novel compounds.



References

- [1] Ottosen et al., J. Med. Chem., 46, 5651 (2003)
- [2] Pearlman et al., J. Med. Chem., 44, 3417 (2001)
- [3] Natarajan et al., Bioorg. Med. Chem. Lett., 13, 273 (2003)
- [4] Revesz et al., Bioorg. Med. Chem. Lett., 14, 3601 (2004)
- [5] Lee et al., Curr. Med. Chem., 12, 2979 (2005)
- [6] Fitzgerald et al., Nat. Struct. Biol., 20, 764 (2003)
- [7] Laufer et al., J. Med. Chem., 49, 7912 (2006)

P243

NEW HETEROCYCLIC ARYLIDENE DERIVATIVES WITH ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITY

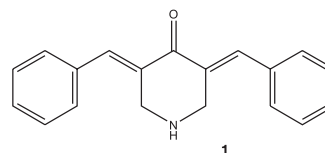
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Inflammation is a complex phenomenon involving interrelationships humoral and cellular reactions through a number of inflammatory mediators. It is a usual symptom covering different pathologies and there are still many questions to be answered in order to understand the inflammatory process as well as a need for better-tolerated and more efficient non-steroidal anti-inflammatory drugs. In the pathways of the inflammatory process, the implication of free radicals is particularly important. It has been also reported that anti-inflammatory drugs may be effective in the prevention of free radical mediated damage. The functional relationship between inflammation and carcinogenesis has been extensively examined in numerous molecular studies, revealing potential novel

targets for the treatment and chemoprevention of a number of different cancers.[1]

A number of α , β -unsaturated ketones display cytotoxic and anticancer properties.[2,3] The rationale for their synthesis and antineoplastic evaluation is based in part on their predicted mode of action, i.e., interaction with cellular thiols with little or no affinity for hydroxy and amino groups found in nucleic acids.[4,5] Conversion of certain conjugated enones into the corresponding Mannich bases led to significant increases in both the rates of thiol alkylation and cytotoxicity. The increased rate of thiolation may be due to the protonated Mannich bases stabilizing the reaction intermediates. A number of tumors have lower pH than corresponding normal cells. Hence Mannich bases may display selective toxicity to such neoplasms since there will be a higher percentage of the molecules in the ionized form in the tumors) than is the case with the corresponding α , β -unsaturated ketones. According to this compound 1 was synthesized and showed high activity toward the murine lymphocytic leukemia P388/MRI cell line and did not bind to a synthetic DNA, poly [d(AT)].[6]



Based on the fact that free radicals and lipoxygenase (LOX) are implicated in cancer, we present here the antioxidant, the LOX inhibitory activity and the *in vivo* anti-inflammatory activity of two analogues.

The compounds have been identified using IR, ¹H-NMR, ¹³C-NMR and elemental analyses. Lipophilicity as R_m values was determined using RPTLC.

The derivatives were screened for their biological activity *in vitro* on: a) soybean lipoxygenase inhibition, b) interaction with 1,1-diphenyl-2-picryl-hydrazyl (DPPH) stable free radical, c) the HO $\cdot$ mediated oxidation of DMSO, d) antiradical activity as loss of ABTS $\cdot$ radical, e) inhibition of lipid peroxidation using the AAPH protocol, f) scavenging of superoxide anion radicals, g) *in vivo* for the inhibition of carrageenin induced rat paw edema.

The compounds have shown potent antioxidant activity, good anti-inflammatory activity and high inhibition of soybean lipoxygenase as a result of their physicochemical features. The results are discussed in terms of the structural and physicochemical characteristics of the compounds.

Acknowledgements

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References

- [1] Coussens, L.M., Werb, Z. *Nature*, 420, 860-867, 2002
- [2] Dimmock, J.R., Ellas D.W., Beazely, M.A., Kandepu, N.M. *Curr.Med.Chem.* 6, 1125-1149, 1999
- [3] Dimmock, J.R. *et al*, *J.Med.Chem.*42, 1358-1366, 1999
- [4] Mutus B. *Et al*, *Anal. Biochem.* 177, 237-243, 1989
- [5] Batuja, G. *et al*, *Chem. Ind.* 2053-2054, 1964; 6. Dimmock, J.R. *et al*, *J.Med.Chem.*44, 586-593, 2001

P244

PHARMACOLOGICAL PROFILES OF NEW CARBOTHIOAMIDES

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The goal of this study was to investigate the efficacy and the pharmacodynamics of newly synthesized *N*-substituted *carbothioamides* focusing on their vasodilating and spasmolytic action. A range of compounds with different substituents on the phenyl moiety was synthesized. The effects of two compounds (Fig. 1) on vascular smooth muscles and terminal ilea are presented.

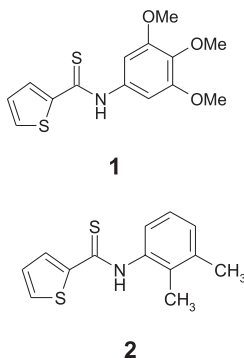


Fig.1

Force of contraction (f_c) on aortic-rings, arteria pulmonalis-rings (precontracted with 90 mM KCl) and terminal ilea (precontracted with 60 mM KCl) of guinea pigs was measured, using the method described by Reiter¹. Compound **1** significantly reduced f_c in terminal ilea with an IC_{50} of 2.05 μ M ($n=5$, $P<0.001$), in aortic rings with an IC_{50} of 40.6 μ M ($n=9$, $P<0.001$) and in arteria pulmonalis rings with an IC_{50} of 47.4 μ M ($n=5$, $P<0.001$). The effect of compound **2** on the isolated smooth muscle preparations was less pronounced with an IC_{50} of 4.7 μ M ($n=6$, $P<0.001$) in terminal ilea, an IC_{50} higher than 100 μ M in aortic rings and with an IC_{50} of 24.5 μ M ($n=5$, $P<0.001$) in arteria pulmonalis rings.

To elucidate the possible mode of action compound **1** was studied in presence of *glibenclamide*, a K_{ATP} -channel inhibitor, *nitro-L-arginine*, an inhibitor of the endothelial

nitric oxide (NO) synthase, *acetylcholine chloride*, *histamine dihydrochloride* and *phenylephrine hydrochloride* in terminal ilea and aortic rings.

Our results suggest that compound **1** induces relaxation in guinea pig smooth muscle preparations by α -adrenoceptor inhibition and an endothelium-dependent pathway, involving NO-cGMP. Calcium antagonistic effects may play a role as well.

Therefore this new compound **1** with its potent spasmolytic activity could be beneficial for treatment of gastrointestinal disorders.

Reference

- [1] Reiter M (1967) Evaluation of inotropically active drugs on isolated papillary muscle. *Drug Res* 17:1249-1253

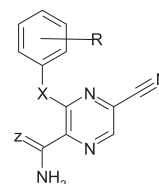
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SYNTHESIS AND ANTIFUNGAL PROPERTIES OF PYRAZINAMIDE ANALOGUES

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Mycoses are on the rise. The high incidence of invasive fungal infections has correlated with a concomitant increase of immunocompromised population¹. According to the US study², candidemia belongs to the leading nosocomial bloodstream infections with a mortality rate of approximately 40%. The dramatic development of resistant fungal strains remains a serious challenge for more intensive research on antimycotics³. Some pyrazinamide analogues, 3-arylaminopyrazine-2,5-dicarbonitriles, have already demonstrated a high antimycobacterial and/or antifungal activity in a previous study⁴. Accordingly with the observed structure-activity relationships among the formerly described pyrazine derivatives, this study has focused on the synthesis of novel *N*-substituted 3-amino-5-cyanopyrazine-2-carboxamides and corresponding carbothioamides. General structure of the compounds is displayed on the figure below. The antifungal activity was evaluated *in vitro* on the following fungal pathogens: *Candida albicans*, *Candida glabrata*, *Trichosporon asahii*, *Trichophyton mentagrophytes*, *Aspergillus fumigatus*, and *Absidia corymbifera*. Fluconazole was used as a reference drug.



X = NH, NH-NH Z = O, S R = Cl, Br, NO₂, OCF₃, CF₃

This study was supported by the Ministry of Education of the Czech Republic (MSM002160822).

References

- [1] Nosanchuk, J. D.: Recent Patents on Anti-Infective Drug Discovery, 2006, 1, 75 – 84.
- [2] Edmond, M. B., Wallace, S. E., McClish, D. K., Pfaller, M. A., Jones, R. N., Wenzel, R. P.: Clinical Infectious Diseases. 1999, 29, 239 – 244.
- [3] Neely, M. N., Ghannoum, M. A.: Eur. J. Microbiol. Infect. Dis., 2000, 19, 897 – 914.
- [4] Palek, L., Dvorak, J., Svobodova, M., Buchta, V., Jampilek, J., Dolezal, M.: Arch. Pharm., 2008, 341, 61 - 65.

P246

α -ALKYL-SUBSTITUTED PIRINIXIC ACID DERIVATIVES AS DIRECT, POTENT AND SELECTIVE INHIBITORS OF THE MICROSOMAL PGE₂ SYNTHASE-1

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The beneficial effects of cyclooxygenase (COX)-2 selective drugs (coxibs) over classical non steroidal anti-inflammatory drugs (NSAIDs) in the therapy of inflammation are contrasted by an elevated myocardial infarction rate due to inhibition of physiologically beneficial prostaglandins (PG) such as PGI₂. Cloning and progress in the understanding of the COX-2-coupled microsomal PGE₂ synthase-1 (mPGES-1) now opens pharmacological possibilities to selectively inhibit the formation of pro-inflammatory PGE₂ without affecting PGI₂ biosynthesis. Up to now, only the unselective indole MK-886, and derivatives thereof (Riendeau et al., 2005) as well as phenanthrene imidazoles (Coté et al., 2007) have been described as synthetic mPGES-1 inhibitors. We have identified α -alkyl substituted pirinixic acid derivatives as novel class of mPGES-1 inhibitors. Thus, pirinixic acid derivatives inhibit the conversion of PGH₂ to PGE₂ in microsomal preparations of IL-1 β -stimulated A549 cells in a concentration-dependent manner being about equipotent with MK-886 (IC₅₀ $\approx$ 1 μ M). Importantly, inhibition of COX-1 and COX-2 in cellular systems and direct inhibition of isolated COX enzymes is significantly less pronounced (IC₅₀ > 10 μ M). Plasmon resonance spectroscopy indicates a direct interference between pirinixic acid derivatives and purified in-vitro translated mPGES-1 (K_d $\approx$ 10 μ M). Further more, pirinixic acid derivatives blocked PGE₂ formation in a cellular test system based on A549 cells using [³H]-arachidonic acid and separation of PGE₂ by RP-HPLC as well as in a whole blood assay

consisting of a novel combined RP-HPLC / EIA detection method. Together, pirinixic acid derivatives were developed as a novel class of potent and selective mPGES-1 inhibitors that efficiently suppress PGE₂ formation without significant inhibition of COX enzymes.

References

- [1] Coté et al. (2007) *Bioorg Med Chem Lett.* 17(24):6816-6820.
- [2] Riendeau et al. (2005) *Bioorg Med Chem Lett.* 15(14):3352-3355.

P247

STRUCTURAL MODIFICATIONS ON COUMARIN RING. SMALL COUMARIN MOLECULES AS TEMPLATE OF DESIGNING POTENT ANTI-INFLAMMATORY AND ANTIPLATELET AGENTS

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Thrombosis is the current most common cause of ischemic cardiovascular disease (CVD) such as myocardial infarction and stroke. The pathogenesis of atherothrombosis is a complex phenomenon where both inflammation and platelet aggregation play fundamental roles. Therefore, inflammation might play an important role in the initiation and progression of cardiovascular diseases (CVDs) [1]. It would be quite effective to develop a series of "multi target-directed-ligands", [2] where a compound could act both as an anti-inflammatory and antiplatelet agent.

Coumarins have been reported to have multiple biological activities. They have been used to treat diverse ailments as cancer and cardiovascular diseases. The coumarin molecule has been shown to possess unique antiedema and anti-inflammatory activities. Various coumarin-related derivatives are recognized as inhibitors of the lipoxygenase and cyclooxygenase pathways of arachidonate metabolism but also of neutrophil-dependent superoxide anion generation, lipid peroxidation and free radicals scavenging [3].

We have reported already a series of coumarin compounds, with potent anti-inflammatory and antioxidant activity [3-6]. A series of several substituted coumarin derivatives have been synthesized, according to Pechman synthetic procedure, as to further evaluate their anti-inflammatory, antioxidant and antiplatelet activities. These compounds are tested both *in vivo* for their anti-inflammatory activity, in carrageenin rat paw edema, and *in vitro*, for their antioxidant activity (searching their possible role as free radical scavengers) and for their

antiplatelet and clot destabilizing activity. Since lipophilicity is an important physicochemical property, we have determined log P values both experimentally and theoretically. [3] The results are discussed in terms of structural activity relationships considering further design of new potent coumarin derivatives with both anti-inflammatory and antiplatelet activity.

References

- [1] Rauch, U. et al. Ann. Intern. Med., 2001, 134, 224-238.
- [2] Cavali, A., Bolognesi, M.L., Minarini, A., Rosini, M., Tumiatti, V., Recanatini, M., Melchiorre, C., J. Med. Chem., 2008, 51, 347-372.
- [3] Kontogiorgis, C., Hadjipavlou-Litina D. J. Med. Chem., 2005, 48, 6400-6408.
- [4] Kontogiorgis, C.A., Xu, Y., Hadjipavlou – Litina, D., Luo, D. Free Radic Res, 2007, 41, 1168-1180.
- [5] Kontogiorgis, C.A., Savvoglou, K., Hadjipavlou-Litina, D.J. J. Enz. Inhib. Med. Chem., 21, 2006, 21-29.
- [6] Kontogiorgis, C., Hadjipavlou-Litina, D. Bioorg. Med. Chem. Lett., 2004, 14, 611-614.

P248

CHEMETICS™ - DRUG DISCOVERY IN ONE TUBE

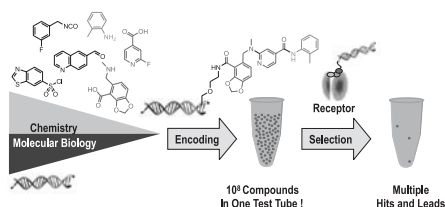
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Nuevolution's Chemetics™ technologies currently allow rapid synthesis and screening of 10e8 drug-like small molecules. Each small molecule in a Chemetics library is tagged with a DNA molecule encoding its structure. The DNA-tag allows stringent affinity selection to be performed on virtually any target.

Our methods for generating libraries include DNA-templated chemical synthesis, and split-and-mix based DNA-tagging synthetic approaches. The library of DNA-encoded small molecules is generated in one tube, through the combination of drug fragments. The fragments (pre-synthesized by standard organic chemistry) are linked together with a variety of different attachment chemistries. Therefore, any relevant chemical motif may be represented in the library, ensuring high diversity.

The power of the Chemetics approach has been demonstrated by identification of high affinity small molecule ligands to several receptors from 10e8-membered libraries.



P249

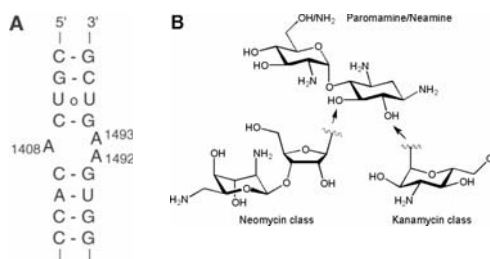
CHEMICAL BIOLOGY OF SMALL MOLECULES INTERFERING WITH PROTEIN- SYNTHESIS

Kythreoti Georgia, Zografos Alexandros L., Katsoulis Ioannis, Athanasios Papakyriakou, Anastasopoulou Panoula, Mavridis Ioannis, Vourloumis Dionisios*

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The bacterial ribosome is a key target for many natural and semi-synthetic antibiotics, such as aminoglycosides, tetracyclins, and macrolides, which interact preferentially with its RNA components (rRNA). The decoding, or A-site, an internal loop within the 16S rRNA, is the molecular target for natural aminoglycoside antibiotics (Scheme) which interfere with the conformational flexibility of two adenines involved in mRNA decoding, thereby inducing an increased error rate in protein synthesis and ultimately leading to bacterial cell death.

Recent crystallographic advances in the field of RNA along with our molecular modeling studies, assay development and synthetic chemistry efforts, will be presented. Our studies aim at improving the pharmacological and resistance profiles of natural aminoglycoside antibiotics which are used as starting points and thus provide the necessary knowledge, for specifically screening newly developed small molecules against well defined and characterized RNA targets.



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CONSISTENT HOMOLOGY MODELLING AND COMPARATIVE ANALYSIS OF THE ANTIPSYCHOTIC DRUGS RECEPTOROME

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It is widely recognized that some of the most clinically useful antipsychotic drugs have a complex pharmacological profile, being able to interact with many different receptors¹ with different affinity. Therefore, for these drugs (and probably for many others in different therapeutic areas), the concept of single target receptor should be replaced by a target receptor set or "receptorome".

One of the implications of such a multireceptorial approach is the need to obtain structural models of the receptorome binding sites, suitable for being used in comparative analysis. It is critically important to obtain the structures by modelling methods that provide consistent solutions that allow a relevant exploitation of structural differences.

In this study we present 3D models built for a set of 14 GPCR potentially involved in the pharmacological behaviour of antipsychotic drugs. All these models are based on the recently published structure of the human β_2 adrenergic receptor² and obtained following an automated protocol, which provides consistent and comparable structures.

Together with the modelled structures, we present the results of diverse comparative analysis of the GPCR binding sites, carried out with different methods that allow the description of the molecular diversity of the structures under study (sequence analysis, residues principal properties, molecular interaction fields, etc.). The results unveil some interesting receptor clustering which could be put in relationship with observed pharmacological properties of the antipsychotic drugs.

References

- [1] Roth BL, Sheffler DJ, Kroeze WK. Magic shotguns versus magic bullets: selectively non-selective drugs for mood disorders and schizophrenia. *Nat Rev Drug Discov* 2004;3(4):353-9.
- [2] Cherezov V, Rosenbaum DM, Hanson MA, et al. High-resolution crystal structure of an engineered human β_2 -adrenergic G protein-coupled receptor. *Science* 2007;318(5854):1258-65.

P251

REGIOSELECTIVE SYNTHESIS & BIOLOGICAL EVALUATION OF NOVEL HIGHLY POTENT AND SPECIFIC 2-THIOIMIDAZOLE-BASED P38 MAPK INHIBITORS WITH LOW CYP450 INTERACTION POTENTIAL

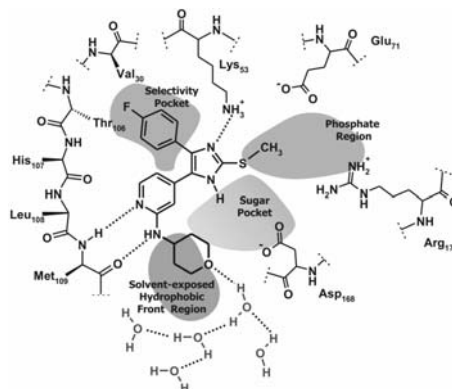
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Targeting disease-related protein kinases has a huge therapeutic potential, but discovering small molecule drugs that can disrupt the intracellular phosphorylation cascade is an enormous challenge, as all Ser/Thr/Tyr kinases encoded in the human genome have very similar structures and use ATP as their native co-substrate. Several recent success stories, however, indicate that kinase targets might be more controllable than has been originally thought.[1] Imidazoles represent important bioactive scaffolds in medicinal chemistry exhibiting a broad range of pharmacodynamic effects.[2] More than 2.500 structures are listed in drug discovery databases and over 3.000 patents have been claimed for imidazole-

based structures. Recent imidazole pharmacophores have targeted various mitogen-activated protein (MAP) kinases. The serine/threonine MAPK p38 plays a central role in the signalling network responsible for the upregulation of proinflammatory cytokines like $\text{TNF}\alpha$ and $\text{IL-1}\beta$ driving the pathogenesis of several (inflammatory) autoimmune diseases and offers, therefore, a valid target for orally available small molecule anti-inflammatory drugs. Here, 2-sulfanylimidazole derivatives have some advantages over prototype inhibitors (SB203580), e.g. lower cytochrome P450 (CYP450) interactions and better kinetic and metabolic properties.[3] The regioselective synthesis, biological testing, and SAR of novel 2,4,5- and 1,2,4,5-substituted 2-thioimidazoles are described.[1, 4] Amino-, oxy- or thioxy substituents at the 2-position of the pyridinyl moiety were evaluated for their contributions to inhibitor potency and selectivity against p38 MAPK as well as for the ability to minimize CYP450 inhibition. Incorporation of functionalized (cyclo)aliphatic amino substituents (e.g. tetrahydropyranilamino; see Figure), which positively interacted with the solvent-exposed front region of the enzyme, led to the identification of extremely potent p38 inhibitors with p38-IC_{50} values in the low single-digit nanomolar range. Approximately 90 pyridinylimidazole-based compounds with a range of potencies against p38a MAPK were further investigated for their ability to inhibit the release of tumor necrosis factor- α ($\text{TNF}\alpha$) and/or interleukin- 1β ($\text{IL-1}\beta$) from human whole blood. Some of the most promising drug candidates underwent selectivity profiling against a panel of 17 different kinases besides p38 α and/or were tested for their interaction potential toward a number of metabolically relevant CYP450 isozymes. As a result, a variety of selective and highly potent p38 inhibitors were selected for further preclinical development.



References

- [1] Laufer, S.; Hauser, D.; Domeyer, D.; Kinkel, K.; Liedtke, A.; J. Med. Chem. 2008, in press; [2] Bellina F.; Cauteruccio, S.; Rossi, R.; *Tetrahedron* 2007, 63, 4571-4624; [3] Laufer, S.; Albrecht, W.; *Inflamm. Res.*, 2004, 53, Suppl. 3 [S215]; [4] Laufer, S.; Hauser, D.; Liedtke, A.; *Synthesis* 2008, 2, 0253-0266.

P252

DEVELOPMENT OF TWO MICROPLATE OR MICROBEAD BASED ASSAYS TO SCREEN P38 δ MAPK INHIBITORS

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P38 mitogen-activated protein kinases (MAPK) are a family that are activated by cellular stress and play an important role in several processes such as cell transformation, proliferation, differentiation and apoptosis. At present, four p38 isoforms (α , β , γ and δ) have been identified. These isoforms share many similarities like upstream activation, approximately 60% amino acid homology and the dual phosphorylation site motif Thr-Gly-Tyr in subdomain VIII of the catalytic domain but differ in inhibitor sensitivity, substrate selectivity and tissue distribution.

P38 α is mainly involved in biosynthesis and release of proinflammatory cytokines as IL-1 β and TNF α . P38 δ however seems to activate MMPs independently from TNF α -release, making it an attractive target against cartilage loss in osteoarthritis (Gay et al.⁽¹⁾). Essential base for all drug discovery programs is a fast and reliable assay to screen p38 δ inhibitors.

We present the development and optimization of a 96 well plate p38 δ assay based on already known p38 α tests described by Forrer et al.⁽²⁾, Thuma et al.⁽³⁾ Peifer et al.⁽⁴⁾ and Linsenmaier et al.⁽⁵⁾.

The assay includes the immobilisation of the substrate GST-c-jun on microtiterplates, followed by addition of a mixture including the test compound, ATP as co-substrate and the enzyme and finally measurement of the dual phosphorylation of c-jun by phospho-specific antibodies.

We also describe the establishment of a faster method to measure the phosphorylation of c-jun by using Luminex technology. Therefore the GST-c-jun was immobilised on GSH-microspheres, subsequent addition of the test solution including test compound, ATP and p38 δ and quantifying of the dual phosphorylation of c-jun with aid of specific antibodies.

Advantages for the Luminex assay are that the described method is a non-wash assay, including a smaller sample volume and carrying out the assay within a shorter time compared to other methods.

References

- [1] Gay, R. et al. :Synovial fibroblasts : key players in rheumatoid arthritis. *Rheumatology* (Oxford, United Kingdom) 2006, 45(6), 669-675.
- [2] Forrer, P et al. :Enzyme-linked immunosorbent assay for measurement of JNK, ERK, and p38 kinase activities. *Biol-Chem.* 1998 Aug-Sep; 379(8-9): 1101-11

[3] Thuma, S. et al. :An immunosorbent, nonradioactive p38 MAP kinase assay comparable to standard radioactive liquid-phase assays. *Anal Biochem.* 2005 Sep; 1;344 (1):135-7

[4] Peifer, C. et al. :Development and optimization of a non-radioactive JNK3 assay. *Combinatorial Chemistry & High Throughput Screening* 2006, Sep; 9(8), 613-618

[5] Linsenmaier, S. et al. :Development of a Microsphere-Based p38 Kinase No-Wash Assay *J Biomol Screen.*2006; Nov; 528-536

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SYNTHESIS AND ANTITUMOR EVALUATION OF 1,8-NAPHTHYRIDINE-2-(1H)-ON-3-CARBOXAMIDE DERIVATIVES AS CB₂ SELECTIVE AGONISTS

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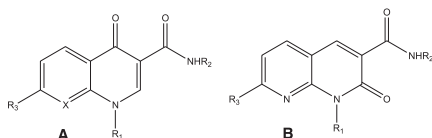
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In the last two decades, research has strongly increased the knowledge of cannabinoids biology and pharmacology. The pharmacological effects of cannabinoids are considered to be mediated through at least two G-protein-coupled seven-transmembrane receptors (GPCRs), namely CB₁ and CB₂. The CB₁ subtype is mainly located in the central nervous system. The CB₂ receptor is expressed primarily in the cells associated with the immune system, and is also found in microglia cells, and overexpressed in several human cell lines. The cannabinoids have been found to modulate several signalling pathways that are involved in the control of cell fate: they stimulate mitogen-activated protein kinases (MAPKs), such as the extracellular-signal-regulated kinase (ERK), the stress-activated kinase (JNK) and p-38 MAPK, which have prominent roles in the control of cell growth and differentiation. Furthermore they can modulate sphingolipid metabolising pathways by inducing sphingomyelin breakdown and then acutely increasing the levels of ceramide, a lipid second messenger that can induce apoptosis and cell cycle arrest. Finally they can reduce the expression of vascular endothelial growth factor (VEGF) and other pro-angiogenic cytokines. Although cannabinoids have a favorable drug safety profile, their use in the clinic is severely limited by their psychoactivity. Because the unwanted psychotropic effects are mediated largely or completely by neuronal CB₁ receptors, the most obvious alternative possibility is to target CB₂ receptors alone.

We have previously reported the binding results of a series of 1,8-naphthyridin- and quinolin-4(1H)-on-3-carboxamide derivatives (**A**). These compounds generally exhibit a remarkable CB₂ affinity, with a K_i value in the nanomolar range, which was also accompanied by a high

selectivity for the same receptor. In the present study 1,8-naphthyridine-2-(1*H*)-on-3-carboxamide derivatives (**B**) were synthesized and tested on membranes from HEK-293 cells transfected with either the human CB₁ or CB₂ receptor, to determine their affinities towards both cannabinoid receptors. Furthermore in order to obtain information about SAR of these compounds, we docked them into the three-dimensional model of CB₂ receptor.

The newly synthesized CB₂ receptor ligands, showing the better binding profiles in the series, were evaluated for their *in vitro* effect as anticancer agents. After 48h drug-exposure, an antiproliferative activity was observed on Jurkat and U87 cell lines expressing CB₂ receptors.



References

- [1] Manera, C. *et al.*, J. Med. Chem., 2006, 49, 5947-5957.
- [2] Manera, C *et al.*, Bioorg. Med. Chem., 2007, 17, 6505-1933.

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1,4-DIOXANE RING AS A PROMISING TEMPLATE OF NOVEL 5-HT_{1A} FULL AGONISTS

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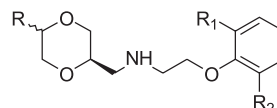
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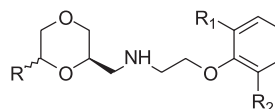
Among the large number of serotonergic receptors the 5-HT_{1A} subtype has grown into a considerable research due to its involvement in psychiatric disorders. 5-HT_{1A} Agonists may be useful antidepressant [1] and neuroprotective agents [2]. Since the limited clinical efficacy of partial agonists, such as buspirone, the first 5-HT_{1A} agent launched in the market, seems to be related to their low level of intrinsic activity [3], the discovery of 5-HT_{1A} full agonists proves to be determinant.

In the present study the α_1 -adrenoreceptor (α_1 -AR) antagonist WB 4101 was chosen as lead molecule because it is well known that this and related compounds are effective ligands also for 5-HT_{1A} serotonergic receptor and a high degree of omology exists between these two receptor families [4]. The quite planar 1,4-benzodioxane template of WB 4101 was replaced by the less conformationally constrained 1,4-dioxane ring substituted in its positions 5 or 6 with one or two pendant phenyl rings (compounds **1-12**). The use of 1,4-dioxane was encouraged by a recent study of ours in which this nucleus demonstrated its bioversatility proving to be a suitable scaffold for efficacious and selective muscarinic agonists [5].

All compounds were tested at 5-HT_{1A} receptors, expressed in HeLa cells, and human cloned α_1 -AR subtypes, expressed in Chinese Hamster Ovary (CHO) cells. Compounds **2b**, **5b**, and **10-12**, selected on the basis of their highest 5-HT_{1A} affinity values (8.4-9.2), were examined in the [³⁵S]GTPγS at the human cloned 5-HT_{1A} receptors. The most interesting compound was **12** behaving as a potent full agonist with a pD₂ value (8.28; % max 106.3) significantly higher than that of 5-hydroxytryptamine. It also showed a good selectivity for 5-HT_{1A} towards the α_{1A} -, α_{1B} - and α_{1D} -AR subtypes (binding assays: 251.2, 251.2, and 50.1; functional assays: 37.2, 109.6, and 11.5, respectively). Therefore, it represents a new lead for developing potent full 5-HT_{1A} agonists significantly selective over α_1 -ARs.



- 1a: R = C₆H₅ (trans); R₁ = R₂ = H
 1b: R = C₆H₅ (cis); R₁ = R₂ = H
 2a: R = C₆H₅ (trans); R₁ = H; R₂ = OCH₃
 2b: R = C₆H₅ (cis); R₁ = H; R₂ = OCH₃
 3a: R = C₆H₅ (trans); R₁ = R₂ = OCH₃
 3b: R = C₆H₅ (cis); R₁ = R₂ = OCH₃
 7: R = (C₆H₅)₂; R₁ = R₂ = H
 8: R = (C₆H₅)₂; R₁ = H; R₂ = OCH₃
 9: R = (C₆H₅)₂; R₁ = R₂ = OCH₃



- 4a: R = C₆H₅ (trans); R₁ = H; R₂ = H
 4b: R = C₆H₅ (cis); R₁ = H; R₂ = H
 5a: R = C₆H₅ (trans); R₁ = H; R₂ = OCH₃
 5b: R = C₆H₅ (cis); R₁ = H; R₂ = OCH₃
 6a: R = C₆H₅ (trans); R₁ = R₂ = OCH₃
 6b: R = C₆H₅ (cis); R₁ = R₂ = OCH₃
 10: R = (C₆H₅)₂; R₁ = R₂ = H
 11: R = (C₆H₅)₂; R₁ = H; R₂ = OCH₃
 12: R = (C₆H₅)₂; R₁ = R₂ = OCH₃

References

- [1] Blier, P.; Bergeron, R.; de Montigny, C. *Neuropsychopharmacology* 1997, 16, 333-338.
- [2] Torup, L.; Møller, A.; Sager, T. N.; Diemer, N. H. *Eur. J. Pharmacol.* 2000, 395, 137-141.
- [3] Glennon, R. A.; Malgorzata, D. *Serotonin* 1997, 2, 351-372.
- [4] Trumpp-Kallmeyer, S.; Hoflack, J.; Bruinvels, A.; Hibert, M. J. *Med. Chem.* 1992, 35, 3448-3462.
- [5] Piergentili, A.; Quaglia, W.; Giannella, M.; Del Bello, F.; Bruni, B.; Buccioni, M.; Carrieri, A.; Ciattini, S. *Bioorg. Med. Chem.* 2007, 15, 886-896.

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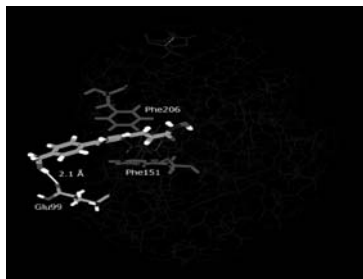
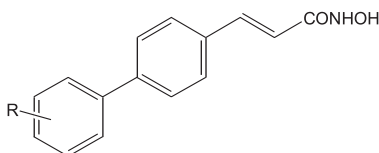
DESIGN, SYNTHESIS, AND EVALUATION OF BIPHENYL-4-YL-ACRYLOHYDROXAMIC ACID DERIVATIVES AS HISTONE DEACETYLASE (HDAC) INHIBITORS

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Inhibitors of histone deacetylase (HDAC) enzymes have recently gained prominence as an emerging class of anticancer agents.¹

Molecular modeling studies on homology model of HDAC2 were performed to design novel structurally simple HDAC inhibitors. They are characterized by a hydroxamic acid as the zinc chelating head group, attached to a cinnamic linker domain and an aromatic group as a *cap* structure.



The substituted aryl or heteroaryl derivatives were obtained by a Suzuki coupling reaction between methyl 4-bromocinnamate and the appropriate aromatic boronic acids. The compounds were tested in an *in vitro* assay with the isoenzyme HDAC2, and a good correlation of the docking energy with the inhibitory activity against HDAC2 was observed.

Most compounds showed an antiproliferative activity comparable to that of SAHA on five tumor cell lines. In human ovarian carcinoma IGROV-1 cells, selected compounds of this series were more effective than SAHA in inducing α -tubulin and p53 acetylation. At concentrations causing comparable antiproliferative effects, the tested compounds were more effective than SAHA in inducing apoptotic cell death. Compounds selected for *in vivo* evaluation on H-460, HCT-116 and IGROV-1 tumor models exhibited a significant antitumor activity at well tolerated doses, thus suggesting a good therapeutic index.

[1] Elaut, G.; Rogiers, V.; Vanhaecke, T. The pharmaceutical potential of histone deacetylase inhibitors. *Curr. Pharm. Des.* 2007, 13, 2584-2620

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PARASITICIDAL TRIFLUOROMETHANESULFONANILIDES

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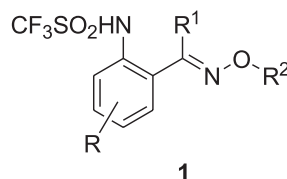
Parasites cause significant health afflictions in humans and animals.

The cat flea, *Ctenocephalides felis*, and the brown dog tick, *Rhipicephalus sanguineus* are common nuisance ectoparasite species that plague companion animals. These pests cause blood loss anaemia, allergic dermatitis, and are vectors for infectious pathogens. Although the introduction of the topical insecticides fipronil, imidacloprid and selamectin has revolutionized flea control on cats and dogs, only fipronil and selamectin are known to be efficacious as acaracides. Moreover, since resistance to current drugs is a distinct possibility, the development of new agents to control these parasites must continue to be pursued.

As part of a program to find new drugs to control important ectoparasites afflicting companion animals, we have discovered novel trifluoromethanesulfonanilide (TFMS) oxime ethers **1** with potent *in vitro* insecticidal and acaricidal activity.

Many of these TFMS oxime ethers also displayed significant activity against the endoparasite *Haemonchus contortus*, a pathogenic gastrointestinal nematode of ruminants that causes substantial economic losses to livestock production worldwide. Current control of haemonchosis primarily depends on the use of anthelmintics (e.g., ivermectin). However, since their use is being compromised by the spreading prevalence and severity of multi-drug parasite resistance, there is an urgent need for anthelmintics with new modes of action.

The synthesis, SAR and *in vitro* parasitocidal activity of TFMS oxime ethers, and other classes of TFMS, will be presented.



R = halo, CF₃

R¹ = H, alkyl, phenyl

R² = alkyl, aryl

References

- [1] Ali, A.; Altamore, T. M.; Bliese, M.; Fisara, P.; Liepa, A. J.; Meyer, A. G.; Nguyen, O.; Sargent, R. M.; Sawutz, D. G.; Winkler, D. A.; Winzenberg, K. N.; Ziebell, A. *Bioorg. Med. Chem. Lett.* 2008, 18, 252.
- [2] Meyer, A. G.; Winzenberg, K. N.; Sawutz, D. G.; Liepa, A. J. US Patent 7312248, 2007.

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STRUCTURAL MODIFICATIONS OF ESTRONE LEADING TO COMPOUNDS WITH ANTIPROLIFERATIVE ACTIVITY TOWARD CANCER CELL LINES

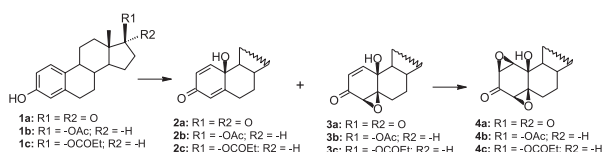
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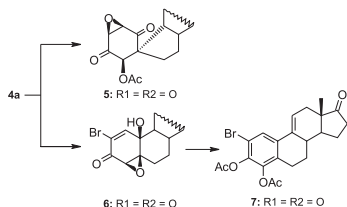
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Oxidation of steroidal phenols **1** under mild reaction conditions yields corresponding quinols **2** and epoxyquinols **3**¹, and epoxidation of epoxyquinol substructure led to diepoxides **4**². In such a way, a series of A-ring polyoxygenated compounds were obtained. Further synthetic modifications of diepoxides gave aromatic, as well as spiro compounds with antiproliferative activity³.

It is important to note that performed structural changes of steroidal phenols led to compounds with different biological activities, such as antiproliferative action on cancer cell lines. An antineoplastic activity of obtained compounds toward three human carcinoma cell lines was examined. The results showed that almost all tested compounds expressed strong activity, mostly phenotype independent.



	IC ₅₀ (nM)		
	Fem-x	HeLa	K ₅₆₂
2a	> 100	> 100	> 100
2b	> 100	> 100	> 100
2c	62	24	34.5
3a	7.10	5.73	6.22
3b	1.58	2.23	3.50
3c	1.16	1.48	1.30
4a	1.55	1.35	1.50
4b	0.51	1.11	0.84
4c	0.55	0.79	0.84
5	9.15	30.10	7.88
6	6.00	2.50	1.20
7	13.00	40.70	12.50



- [1] B. A. Šolaja, D. R. Milic, M. J. Gašić, *Tetrahedron Lett.* 1996, 37, 3765-3768.
- [2] D. Milic, T. Kop, Z. Juranic, M. J. Gašić, B. Šolaja, *Bioorg. Med. Chem. Lett.* 2001, 11, 2197-2200.
- [3] D. Milic, T. Kop, Z. Juranic, M. J. Gašić, B. Tinant, G. Pocsfalvi, B. A. Šolaja, *Steroids* 2005, 70, 922-932.

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NOVEL ACETYLCHOLINESTERASE REACTIVATORS AGAINST PARAOXON INTOXICATION – SAR STUDY

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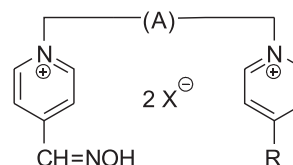
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The reactivators of acetylcholinesterase (AChE, EC 3.1.1.7) are very important components in the treatment of intoxications caused by organophosphate inhibitors such as nerve agents and pesticides [1]. These inhibitors covalently bind to active site of mentioned enzyme and irreversibly inhibit its activity. The reactivator breaks the inhibitor-enzyme covalent bond and restores its activity. Unfortunately, there is no reactivator applicable for every type of inhibitor; it means that every structural change in the molecule of inhibitor needs a specific structure of the reactivator [2].

Several series of AChE reactivators have been prepared in last 5 years in Czech Republic. The key structural features have changed during this period. Namely, the bisquaternary compounds with two hydroxyiminomethyl (oxime) groups were turned into mono-oxime compounds [3], whereas the second heteroarenium ring carried another moiety. This second non-oxime moiety highly influenced an activity and also toxicity of the reactivator. Finally, the SAR study for paraoxon-inhibited AChE was developed.



[1] Bajgar, J. *Adv. Clin. Chem.* 2004, 38, 151.

[2] Marrs, T.C.. *Pharmacol. Therapeut.* 1993, 58, 51.

[3] Musilek, K.; Jun, D.; Cabal, J.; Kassa, J.; Gunn-Moore, F.; Kuca, K. *J. Med. Chem.* 2007, 50, 5514.

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SELENOCYSTEINE IN PEPTIDE DRUG DESIGN

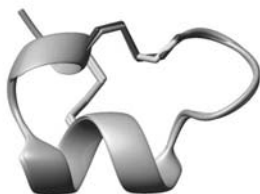
Markus Muttenthaler^a, S. T. Nevin^b, A. A. Grishin^b, S. T. Ngo^b, P. T. Choy^b, N. L. Daly^a, S-H. Hu^a, J. L. Martin^a, P. G. Noakes^b, D. J. Craik^a, D. J. Adams^b, P. F. Alewood^a

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Many biologically active peptides contain multiple cysteine links that are crucial for their activity. Selenocysteine is considered the most conservative substitution for cysteine with the advantage of the diselenide bond being more stable under reducing environment than the disulfide bond.

Our work illustrates how selenocysteine, when strategically incorporated into cysteine-rich bioactive peptides can facilitate and direct their folding. The isosteric character and the folding properties were assessed in a detailed study on potent alpha-conotoxins, which are small ligands that target the nicotinic acetylcholine receptors. Alpha-conotoxins are found in predatory cone snails, contain 8-16 residues with 4 cysteines comprising 2 disulfide bonds. Some of these alpha-conotoxins are potential drug leads for the treatment of neuropathic pain (1). Structure activity studies on several potent and selective alpha-conotoxins confirmed the isosteric character of selenocysteine bonds (CD, NMR, X-Ray) and revealed that the selenium analogues have the same or higher activity than their native analogues.

Superimposition of the crystal structures of an alpha-conotoxin with its selenium-analogue, showing the diselenide bond in red and the disulfide bond in yellow.



References

- [1] M. Vincler *et al.*, *Proc. Natl. Acad. Sci. USA* 103, 17880 (2006).

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ANTIMALARIAL ACTIVITY OF 1-ARYL-3,3-DIALKYL-TRIAZENES

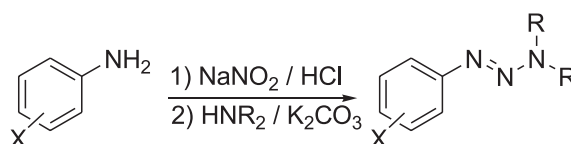
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1-Aryl-3,3-dialkyltriazenes have been used in many ways in organic syntheses and are known as anticancer drugs represented by dacarbazine. We explored the additional bioactivities of the triazenes. Until now, 1,3-diaryl-triazenes, Isometamidium and Berenil are known as chemical compounds with antimalarial activity with the triazene structure. Isometamidium is known to inhibit histamine N-methyltransferase and diamine oxidase both *in vitro* and *in vivo*, which is important for antimalarial activity. And it is also known that Berenil is 125 times more active than CQ in antimalarial activity *in vivo*. But, no report related to antimalarial activities of 1-aryl-3,3-dialkyltriazenes has appeared yet.

Now we revealed that the antimalarial activity of 1-aryl-3,3-dialkyltriazenes to *Plasmodium berghei* NK-65 in infected mice was evaluated at an intraperitoneal dose of 100 mg/kgbw. Some of these compounds were found to possess potent antimalarial activity.



The substitution of 3,3-dimethyl and 3,3-tetramethylene groups on the 3-nitrogen of the triazene group showed good effects, and *p*-trifluoromethylphenyl, *p*-chlorophenyl and mesityl groups on the 1-nitrogen of the triazene group did as well.

These results indicated that the triazenes are new candidates for antimalarial drugs.

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ARTIFICIAL NEURAL NETWORKS IN ADMET MODELING: PREDICTION OF BLOOD-BRAIN BARRIER PERMEATION AND ORAL ABSORPTION

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The new regulatory framework proposed by the European Union in 2003 for the Registration, Evaluation and Authorization of Chemicals (REACH) [1] has contributed to the increasing importance of alternative methods to animal-experimentation. According to REACH regulation, toxicity and safety data for each chemical (more than 30,000) will need to be provided. Thus, in recent years, the development of *in vitro* and *in silico*-methods for the prediction of chemical properties such as toxicity and pharmacokinetics (i.e., ADME properties) has received a great deal of attention.

Within this context, the purpose of this work is to develop *in silico* models to predict ADME properties as blood brain barrier permeation and oral absorption of any chemical substance. As theoretical tool, Artificial Neural Networks (ANNs) is a modeling methodology whose application in some areas of Medicinal Chemistry such as

QSPR, QSAR and prediction of pharmacokinetic properties has increased spectacularly in recent years. The successful application of ANNs in structure-property models requires the numerical representation of the structure of the compounds. Taking into account this idea, the program CODES[®][2], created in Instituto de Química Médica (CSIC, Spain) based on neural computing, enables the easy generation of the required numerical descriptors of the structures involved in the study. This singular way to encode chemical entities converts CODES in an excellent tool to carry out QSAR and QSPR studies [3-4]. The particularity of the present study lies in the use of the artificial neural network approach for the generation of numerical descriptors of structures by means a non-supervised neural network learning with the only knowledge of their chemical structure (SMILES code).

Two supervised artificial neural network models have been developed for the accurate prediction of the blood-brain barrier partition (in LogBB scale) and oral absorption (in HIA% scale) of chemical compounds, respectively. A structural diverse set of compounds of known experimental LogBB and HIA value were chosen for these studies. The molecules were defined by means of a non-supervised neural network using our CODES program. The models obtained averages 73% (Log BB) [5] and 72% (HIA%) of accuracy in the external prediction set. The models are able to predict correctly the behavior of a very heterogeneous series of compounds in terms of the blood-brain barrier permeation and oral absorption. The results indicate that this methodology (ANN and CODES) may represent an useful tool for the prediction of ADMET properties. CODES[®] is available free of charge for academic institutions.

References

- [1] URL: http://ec.europa.eu/enterprise/reach/index_en.htm, 2007.
- [2] M. Stud CODES[®]; v1.0 (revision 3) ed.
- [3] Gil C., Dorronsoro I, Castro A and Martinez A. *Bioorg. Med. Chem. Lett.* 2005, 15, 1919-1921.
- [4] Ochoa C., Rodriguez J., Rodriguez M., Chana A., Stud M., Alonso-Villalobos P. and. Martinez Grueiro M.M. *Med. Chem. Res.* 1997, 7, 530-545.
- [5] Guerra A., Paez J.A. and Campillo, N.E. Artificial Neural Networks in Medicinal Chemistry: Establishment of a Neural Network Model for the Prediction of Blood-Brain-Barrier Passage. QSAR & Combinatorial Science. 2007. DOI: 10.1002/qsar.200710019

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DESIGN AND SYNTHESIS OF NEW N-[(TRIFLUOROMETHYLPHENYL)-PIPERAZIN-1-YL]-ALKYL 3-ARYLPYRROLIDINE-2,5-DIONES AS POTENT ANTI-CONVULSANT AGENTS

Jolanta Obniska and Krzysztof Kaminski

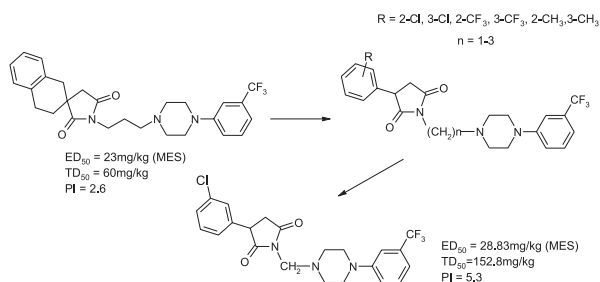
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One of the most popular fluorine-containing functional group is the trifluoromethyl moiety, which may affect the bioavailability because of increase membrane permeability as well as by enhancing metabolic stability, especially by blocking of oxidative processes. Thus compounds with trifluoromethyl groups are the focus of much interest in modern medicinal chemistry and play a significant role in development of new drugs, including anticonvulsant active molecules.¹

In view of the above, in our previous studies in a group of 1,3-substituted pyrrolidine-2,5-diones, we have synthesized library of spirosuccinimides containing at the imide nitrogen atom 1-alkyl-4-phenylpiperazines with different substituents at the phenyl ring. In this series of compounds, all derivatives with the trifluoromethyl group at the position-3 of the 4-phenylpiperazine moiety exhibited anticonvulsant activity, especially in the MES test.^{2,3} The most active was N-[2-{4-(3-trifluoromethylphenyl)-piperazin-1-yl}-propyl]-[7,8-f]benzo-2-aza-spiro[4.5]decane-1,3-dione with the ED₅₀=23mg/kg, and the TD₅₀ = 60mg/kg. Looking for the more potent compounds, in the present study we designed and synthesized new series of derivatives in which we replaced the spiro nucleus by the differently substituted phenyl rings. The results obtained have shown, that the introduction of the aromatic ring at the position-3 of pyrrolidine-2,5-dione moiety as well as the presence of N-[4-(3-trifluoromethylphenyl)-piperazin-1-yl]-alkyl fragment are essential for bioactivity.

The main modifications and structures of the most active molecules are presented below.

All the compounds were tested for their anticonvulsant activity within the Antiepileptic Drug Development (ADD) program (Epilepsy Branch, Neurological Disorders Program, National Institute of the Neurological and Communicative Disorders and Stroke (NINCDS), Bethesda, USA), by using of the procedures described elsewhere.⁴



References

- [1] H. A. Schenck, P. W. Lenkowski, J. P. Stables et al, *Bioorg. Med. Chem.* 12 (2004) 979-993.
- [2] J. Obniska, M. Kolaczowski, A. Bojarski, B. Duszynska, *Eur. J. Med. Chem.* 41 (2006) 874-881.
- [3] J. Obniska, K. Kaminski, E. Tatarczynska, *Pharmacol. Rep.* 58 (2006) 207-214.
- [4] H. J. Kupferberg; *Epilepsia*, 30 suppl. (1989) 51-56.

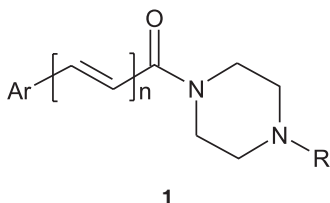
P263

DESIGN AND SYNTHESIS OF NOVEL, ORALLY ACTIVE, PIPERAZINE-DERIVED PAR1 ANTAGONISTS AS ANTI-PLATELET AGENTS

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The activation of platelets by thrombin is mediated via proteolytic activation of specific cell surface receptors known as protease-activated receptors (PARs). PAR1 is the major thrombin-activated receptor on human, monkey and guinea-pig platelets. They are activated by a unique mechanism in which a proteolytic enzyme such as thrombin binds to the receptor and cleaves its extracellular domain at Arg41-Ser42. The newly unmasked amino terminus binds intramolecularly to the proximally located transmembrane loop of the GPCR, eliciting intracellular signalling. This very rare intramolecular activation mechanism makes this target particularly difficult to address since an external antagonist ligand will have to compete with a very favoured intramolecular activation. We have recently discovered a novel series of compounds derived from piperazine (**1**) that competitively antagonize PAR1.



This series of compounds was optimised and led to products acting as antagonists of SFLLR-induced calcium efflux in CHO cells and inhibitors of SFLLR-induced aggregation of human platelets. *In vivo*, these compounds were able to delay thrombus formation in an intravenous and oral shunt model in the rat and an intravenous thrombosis model in the guinea-pig. Synthesis of these compounds and structure-activity analyses will be presented as well as the pharmacological profile of our lead compounds.

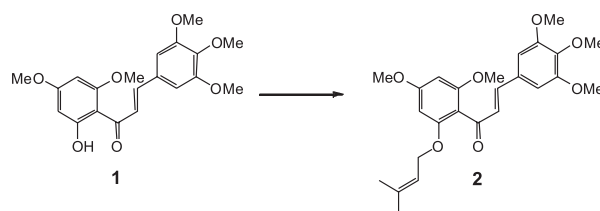
P264

SYNTHESIS AND CYTOTOXIC EFFECT OF 2'-PRENYLOXY-3,4,4',5,6'-PENTAMETHOXYCHALCONE

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Prenylflavonoids are compounds naturally occurring in plants showing a large diversity of pharmacological activities, namely antitumor [1-4]. Due to these interesting biological effects our research group has been focusing on the study of this class of heterocyclic compounds. In this work we describe the synthesis of a prenylated chalcone (**2**), by stirring at room temperature a solution of 2'-hydroxy-3,4,4',5,6'-pentamethoxychalcone (**1**) in dichloromethane and toluene with prenyl bromide in the presence of tetrabutylammonium hydroxyde [4], and its structure characterization by IR, UV, MS and NMR (¹H, ¹³C, HSQC and HMBC). The effect of compound (**2**) on the *in vitro* growth of two human tumor cell lines, MCF-7 (breast) and NCI-H460 (non-small cell lung), using sulforhodamine B method [5] is also described. Although the starting material was not found to have cytotoxic effect (GI₅₀ > 150 μM), the introduction of one *O*-prenyl group, was responsible for an improvement on this effect (GI₅₀ (MCF-7) = 68.3 μM and GI₅₀ (NCI-H460) = 73.4 μM).



References

- [1] Barron, D. et al. *Phytochemistry* 1995, 43, 921.
- [2] Botta, B. et al. *Curr. Med. Chem.* 2005, 12, 713.
- [3] Brahmachari, G., Gorai, D. *Curr. Org. Chem.* 2006, 10, 873.
- [4] Daskiewicz, J.-B. et al. *J. Med. Chem.* 2005, 48, 2790.
- [5] Shekan, P. et al. *J. Natl. Cancer Inst.* 1990, 82, 1107.

Acknowledgments:

FCT (I&D 226/2003), FEDER, POCI for financial support and for the PhD grant to Marta Neves (SFRH/BD/21770/2005).

P266

ANTIPROLIFERATIVE ACTIVITY OF SOME [1,2,5]SELENADIAZOL DERIVATIVES

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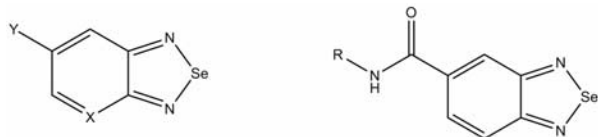
Accumulating evidence indicates that selenium compounds possess anticancer properties¹⁻³. Low blood selenium levels were found to be associated with an increased incidence and mortality from various types of cancers. Numerous studies strongly support the use of organoselenium compounds as antiproliferative agents in lung, colon, leukaemia and breast among other although its mechanism of action has been reported to be multifactorial.

In order to increase our knowledge about selenium and cancer we carried out the synthesis and biological evaluation of novel organoselenium compounds according to general structures shown below:

In recent years selenium heterocyclic compounds have shown cytotoxic activity against several cancer cell lines, as Ebselen and 2-aryl/alkyl selenazolidine-4(*R*)-carboxylic acids⁴. So, compounds synthesized by us contain selenium atom on heterocyclic system and we modify some structural features, as presence of one or more amide bonds, symmetry and different aromatic rings fused to selenodiazol system.

We evaluated cytotoxic activity of these compounds in four cancer cell lines, leukaemia (CCRF-CEM), lung (HTB-54), colon (HT-29) and breast (MCF-7) and one nontumoral breast epithelium line (CRL-8799). We also determined the apoptotic activity of all compounds in MCF-7 cell line.

We observed that MCF-7 cell line is the most sensitive to these compounds. One compound showed interesting antiproliferative values (GI_{50} and $TGI < 10 \mu M$) for MCF-7 and HTB-54. Furthermore, the number of apoptotic cells for this compound is more than obtained for camptothecin.



References

- [1] Letavayova, L.; Vickova, V.; Brozmanova, J. *Toxicology* 2006, 227, 1-14.
- [2] Smith, M.L.; Lancia, J.K.; Mercer, T.I.; Ip, C. *Anticancer Res.* 2004, 24, 1401-1408.

[3] Zaidman, B.Z.; Yassin, M.; Mahajna, J.; Wasser, S.P. *Appl. Microbiol. Biotechnol.* 2005, 67, 453-468.

[4] Franklin, M.R.; Moos, P.J.; El-Sayed, W.M.; Aboul-Fadl, T.; Roberts, J.C. *J. Biochem. Mol. Toxicol.* 2007, 168, 211-220.

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NOVEL SELANYLACETIC ACID DERIVATIVES AS ANTIPROLIFERATIVE AGENTS

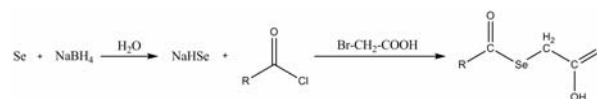
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Several studies in animal models and in humans have demonstrated the anticarcinogenetic and chemopreventive effects of some selenocompounds¹. Between the action mechanisms involved in this effects induction of apoptosis and stress oxidative are the most representative². There is increasing evidence to suggest the selenium works by inhibiting important molecular pathways of the early carcinogenesis in a variety of experimental models and that the anticancer activity is dependent on its chemical form.

Based in these findings and previous work of our research team^{3,4}, we planned the synthesis and biological evaluation of novel organoselenium compounds according to general procedure:



We evaluated cytotoxic activity of these compounds in four cancer cell lines, leukaemia (CCRF-CEM), lung (HTB-54), colon (HT-29) and breast (MCF-7) and one nontumoral breast epithelium line (CRL-8799). Results are expressed as GI_{50} (concentration that reduces by 50% the growth of treated cells with respect to untreated controls), TGI (dose that inhibits 100% of cell growth) and LD_{50} (dose that kills 50% of cells). Furthermore, we determined the apoptotic activity of one representative compound in MCF-7 and CCRF-CEM cell lines.

Our results showed that MCF-7 cell line is the most sensitive to these compounds with very interesting antiproliferative values (GI_{50} and $TGI < 10 \mu M$). HT-29 and HTB-54 have poor sensitivity to these compounds and all of them are inactives in CCRF-CEM.

References

- [1] Brinkman, M.; Reulen, R.C.; Kellen, E.; Buntinx F.; Zeegers, M.P. *Eur. J. Cancer* 2006, 42, 2463-2471.
- [2] Li, G.X.; Hu, H.B.; Jiang, C.; Schuster, T.; Lu, J.X. *International J. Cancer* 2007, 120, 2034-2043.
- [3] Plano, D.; Sanmartín, C.; Moreno, E.; Prior, C.; Calvo, A.; Palop, J.A. *Bioorg. Med. Chem. Lett.*, 2007, 17, 6853-6859.
- [4] Sanmartín, C.; Plano, D.; Palop, J.A. *Mini-reviews Med. Chem.*, In press.

P268

TOWARDS NONPEPTIDIC RADIOLIGANDS AND FLUORESCENT PROBES FOR THE NEUROPEPTIDE Y₂ RECEPTOR

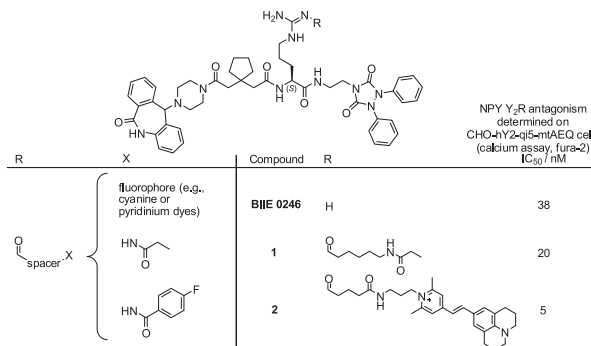
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Based on the (S)-argininamide BIIE 0246^[1], the first highly potent and selective non-peptide neuropeptide Y (NPY) Y₂ receptor (Y₂R) antagonist, we started to develop N^G-substituted analogues as potential fluorescence-, radio- and PET ligands. As shown previously, the introduction of variable electron-withdrawing groups into the N^G-position of BIIE 0246 is tolerated depending on the chemical nature of the substituent^[2]. The functionalization of BIIE 0246 with different ω-amino acyl substituents yielded precursors for the preparation of variously labeled nonpeptidic Y₂R ligands: potential radioligands (³H, ¹⁸F) (cf. **1**) as well as fluorescent probes (cf. **2**). The new substances proved to be highly potent Y₂R antagonists comparable or even superior to the parent compound BIIE 0246. In order to determine the K_i and IC₅₀ values, the synthesized compounds were investigated in a flow cytometric binding assay and in a spectrofluorimetric Ca²⁺ assay^[3]. Furthermore, the synthesized compounds were investigated for Y₂R selectivity versus Y₁, Y₄, and Y₅ receptors.

³H-labeled Y₂R ligands should be useful tools for the determination of receptor affinities in binding assays, and ¹⁸F-PET ligands are of potential value for molecular *in vivo* imaging. The high affinity fluorescent probes, in particular arginine derivatives labeled with pyrylium dyes, should be useful in fluorescence microscopy and to replace Cy5-NPY in flow cytometric equilibrium binding studies.



References

- [1] Doods, H. et al., *Eur. J. Pharmacol.* 1999, 384, R 3-5.
- [2] Brennauer, A., PhD thesis, University of Regensburg, 2006
- [3] Ziemek, R., et al., *Eur. J. Pharmacol.* 1999, 551, 10-18

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DESIGN AND SYNTHESIS OF 1-C-LINKED DIPHOSPHATE ANALOGUES OF UDP-N-AC-GLUCOSAMINE AND UDP-N-AC-MURAMIC ACID

Andrej Babic,^a Stanislav Gobec,^a Christine Gravier-Pelletier,^b Yves Le Merrer,^b and Slavko Pecar^{a,c}

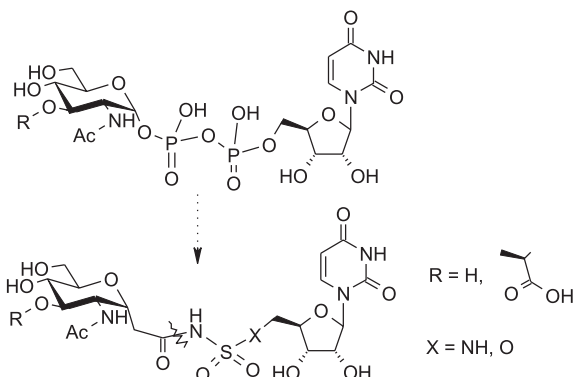
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Peptidoglycan is an essential component of the bacterial cell wall which preserves cell integrity. *N*-Acetyl-glucosamine (GlcNAc) and *N*-Acetyl-muramic acid (MurNAc) are present in almost all eubacteria as constituent units of the bacterial cell wall peptidoglycan [1]. In the biosynthetic pathways they are utilized in the form of uridinediphosphate (UDP) precursors by several bacterial enzymes of the peptidoglycan biosynthesis.

Analogues of these important peptidoglycan precursors could serve as useful starting points for designing new enzyme inhibitors. The convergent synthesis of new 1-C-linked *O*- and *N*-sulfamoylamido analogues of UDP-GlcNAc and UDP-MurNAc is presented. To render the target compounds chemically and enzymatically stable, C-linked glycosides were chosen as a synthetically feasible alternative to natural α-O-glycosides. The key compound 1-allyl-*N*-acetylglucosamine has been synthesized using the Keck radical allylation [2] under microwave-accelerated conditions and converted into 1-C-linked-MurNAc and GlcNAc carboxylic acid derivatives. Coupling with protected *N*- and *O*-sulfamoyl uridine derivatives and the ensuing deprotection reactions gave stable diphosphate analogues of UDP-GlcNAc and UDP-MurNAc.



References

- [1] Barreateau, H.; Kovac, A.; Boniface, A.; Sova, M.; Gobec, S.; Blanot, D. *FEMS Microbiol. Rev.* 2008, 32, 168-207.
- [2] Keck, G. E.; Yates, J. B. *J. Am. Chem. Soc.* 1982, 104, 5829-5831.

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SQUIRRELNVO: LEAD-FINDING OF PPAR α AGONISTS BY DE NOVO DESIGN

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Ligand-based development of novel lead structures is a typical scenario in medicinal chemistry. Computer-aided molecular design can play a significant role in this process [1]. Here, we propose a novel technique for lead-finding: de novo design by bioisosteric replacement. Our tool SQUIRRELNovo generates suggestions for query ligand parts which can be visually inspected and combined in a new lead structure by medicinal chemist's expertise. The proposed bioisosteric groups are chosen according to their steric [2] and pharmacophoric [3] properties.

In a case study we applied SQUIRRELNovo to scaffold-hopping from the potent PPAR α agonist GW590735 [4]. In the design process, two moieties were replaced which were shown to be responsible for compound potency. The new lead structure yielded submicromolar activity on PPAR α in a cell-based reporter-gene assay [5,6].

Literature:

- [1] Schneider, Baringhaus, 2008 *Molecular Design – Concepts and Applications*. Wiley-VCH: Weinheim, New York.
- [2] Proschak *et al.*, *J. Comput. Chem.* 2008, 29, 108.
- [3] Tanrikulu *et al.*, *ChemBioChem* 2007, 8, 1932.
- [4] Sierra *et al.*, *J. Med. Chem.* 2007, 50, 685.

[5] Rau *et al.*, *Planta Med.* 2006, 72, 881.

[6] Derksen *et al.*, *ChemMedChem* 2006, 1, 1346.

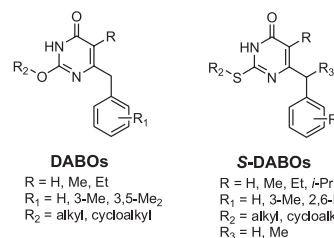
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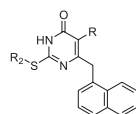
NOVEL DIHYDRO-ALKYLTHIO-BENZYL-OXOPYRIMIDINES (S-DABOs): PICOMOLAR ACTIVITY AGAINST WILD-TYPE HIV-1 AND NANOMOLAR ACTIVITY AGAINST CLINICALLY RELEVANT MUTANT STRAINS

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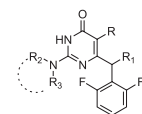
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Since their discovery by our research group in 1992, 3,4-dihydro-2-alkoxy-6-benzyl-4-oxopyrimidines (DABOs) have been subjected to many structural modifications in order to obtain better non-nucleoside reverse transcriptase inhibitors (NNRTIs) as potential candidates for the treatment of AIDS [1]. The first series of inhibitors showed an alkoxy chain at C2 position and a benzyl/3-methylbenzyl/3,5-dimethylbenzyl group at the C6 position of the pyrimidine ring (DABOs). The replacement of the C2-alkoxy with a C2-alkylthio chain (S-DABOs) and the insertion of halogen atoms (chloro, fluoro) at the 2- and 2,6-positions of the C6-benzyl moiety afforded highly potent compounds (F₂-S-DABOs), active at nanomolar concentration against wt HIV-1. By means of bioisosteric substitution of C2-alkylthio with a C2-alkylamino moiety, we disclosed a new series of compounds (Amino-DABOs), belonging to the DABO family, endowed with broad spectrum and nanomolar HIV-1 inhibitory activity [2]. Here we present two novel series of S-DABOs (**1,2**), characterized by promising substitution profiles at the C6 position (2,6-difluorobenzyl/2,6-dichlorobenzyl/2-chloro, 6-fluorobenzyl) and at the C2 position (alkylthio, cycloalkylthio or oxophenethylthio) of the pyrimidine ring. In fact, some compounds showed picomolar activity against wild type HIV-1 and nanomolar activity against various clinically relevant mutant strains. The so called oxophenethyl-S-DABOs (**1**) also showed an interesting and unexpected structure-activity-relationship profile for this family of NNRTIs [3].

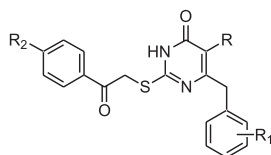




DATNOs
R = H, Me
R₂ = alkyl, cycloalkyl

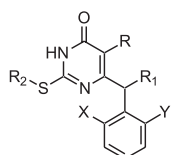


Amino-DABOs
R, R₁ = H, Me
R₂, R₃ = H, alkyl, (hetero)cycloalkyl



Oxophenethyl-S-DABOs (1)

R = H, Me, Et, *i*-Pr, *s*-Bu
R₁ = 2,6-Cl₂, 2-Cl-6-F, 2,6-F₂
R₂ = H, F, OMe



2,6-Dihalo-S-DABOs (2)

R = H, Me, Et
R₁ = H, Me, Et
R₂ = alkyl, cycloalkyl
X, Y = F, Cl

References

- [1] Artico M. Selected non-nucleoside reverse transcriptase inhibitors (NNRTIs): the DABOs family. *Drug Future* 2002;27:159-175.
- [2] Mai A, Artico M, Rotili D, Tarantino D, Clotet-Codina I, Armand-Ugón M, Ragno R, Simeoni S, Sbardella G, Nawrozki MB, Samuele A, Maga G, Esté JA. Synthesis and Biological Properties of Novel 2-Aminopyrimidin-4(3*H*)-ones Highly Potent Against HIV-1 Mutant Strains. *J Med Chem* 2007;50:5412-5424 and references cited therein.
- [3] Nawrozki MB, Rotili D, Tarantino D, Botta G, Eremychuk AS, Samuele A, Facchini M, Armand-Ugón M, Clotet-Codina I, Novakov IA, Orlinson BS, Maga G, Esté JA, Artico M, Mai A. 5-Alkyl-6-benzyl-2-(2-oxo-2-phenylethylthio)pyrimidin-4(3*H*)-ones, a New Series of Anti-HIV-1 Agents of the DABO Family with Unexpected Structure-Activity-Relationship Profile. *J Med Chem* 2008, accepted for publication.

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NOVEL SIRTUIN INHIBITORS WITH STRONG APOPTOTIC AND CYTODIFFERENTIATING EFFECTS IN HUMAN LEUKAEMIA CELLS

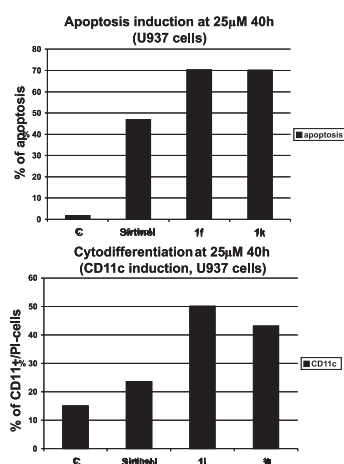
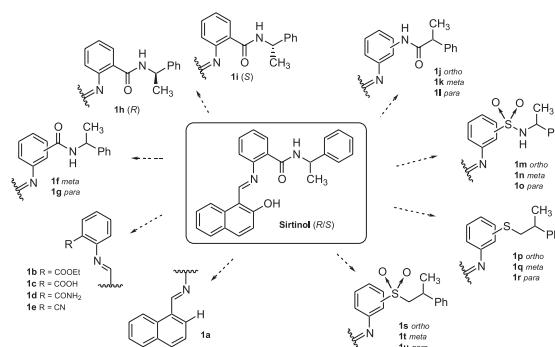
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Sirtuins are NAD⁺-dependent deacetylases and/or mono-ADP-ribosyl transferases (SIRT1-7) that can deacetylate and/or ADP-ribosylate histones and important non-histone proteins, such as the tumor suppressor p53, the BCL6 oncoprotein and the α -tubulin [1]. Since they are involved in gene silencing, DNA repair, chromosomal stability, cell cycle progression, metabolism regulation and longevity, human sirtuins (hSIRT1-7) are now pro-

posed as potential pharmacological targets to treat several diseases, first of all cancer [2].

To date, a small number of sirtuin inhibitors (SIRTi) has been described [2]. One of these, sirtinol, pushed us to synthesize a first series of sirtinol analogues and derivatives (**1a-i**) that were tested against recombinant yeast Sir2, human SIRT1 and SIRT2, and *in vivo* with a yeast phenotypic assay [3,4]. Two isomers, namely *meta*- and *para*-sirtinol were from 2- to 10-fold more potent than sirtinol against human SIRT1/2 enzymes and as potent as prototype in yeast assay [4]. On these bases and in a search for more potent SIRTi as apoptotic and/or cytodifferentiating agents, we prepared a second series of sirtinol analogues (**1j-u**) by studying the replacement of the benzamide linkage of the prototype with other bioisoster groups (such as anilide, sulfonamide, sulphonylmethyl and sulphonylmethyl moieties) with *ortho*, *meta*, and *para* regiochemistry [5]. Some of the new derivatives were more potent than sirtinol against hSIRT1 and hSIRT2, and much more selective against hSIRT2. Many of them also showed very high apoptosis induction and/or strong cytodifferentiating properties (CD11c induction) in human leukaemia U937 cells [5].



References

- [1] Michan S, Sinclair D. Sirtuins in mammals: insights into their biological function. *Biochem J* 2007;404:1-13.
- [2] Mai A. The therapeutic uses of chromatin-modifying agents. *Expert Opin Ther Targets* 2007;11:835-851.
- [3] Grozinger CM, Chao ED, Blackwell HE, Moazed D, Schreiber SL. Identification of a class of small molecule inhibitors of the sirtuin family of NAD-dependent deacetylases by phenotypic screening. *J Biol Chem* 2001;276:38837-38843.
- [4] Mai A, Massa S, Lavu S, Pezzi R, Simeoni S, Ragno R, Mariotti FR, Chiani F, Camilloni G, Sinclair DA. Design, Synthesis, and Biological Evaluation of Sirtinol Analogues as Class III Histone/Protein Deacetylase (Sirtuin) Inhibitors. *J Med Chem* 2005;48:7789-7795.
- [5] Sinclair DA, Mai A. Sirtuin-inhibiting sirtinol analogs for cancer treatment. *PCT* 2007, WO-2007084162.

P273

NOVEL HISTONE ACETYLTRANSFERASES MODULATORS: POTENCY AND SELECTIVITY

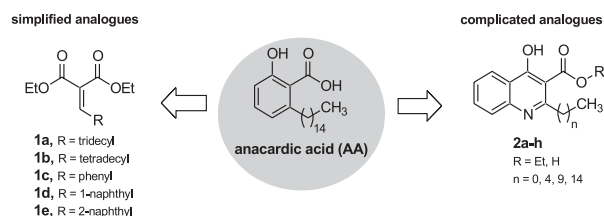
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Histone acetyltransferases (HATs) are key enzymes that acetylate the ϵ -amino groups of specific lysine residues in histones, as well as in non-histone proteins (p53, α -tubulin, etc.), thus regulating transcriptional activation, cell cycle progression, DNA repair and other genomic processes. HAT proteins have been phylogenetically divided into several classes, including the GCN5-*N*-acetyltransferase (GNAT), the MYST and the p300/CBP families, each of which exhibits distinct histone lysine specificities [1]. Overexpression, translocations, missense and deletion mutations in HAT genes (p300/CBP, etc.) and mistargeted and deregulated activities of specific enzymes (GCN5/PCAF, p300, etc.) have been reported to play an important role in a variety of solid and haematological tumours. Selective modulators of HAT enzymes might therefore exert useful therapeutic applications in cancer [1]. To date, a small number of HAT modulators has been reported [1].

Starting from the natural product anacardic acid (AA), a well known p300 and PCAF inhibitor [2], we prepared two series of molecules structurally related to AA, **1a-e** and **2a-h** (as simplified and complicated analogues, respectively) and tested them against a panel of HAT enzymes (p300, PCAF, CBP and GCN5) and on human leukaemia U937 cells for evaluating their potential anticancer properties. The pentadecylidenemalonate **1b**, for its ability to potentiate PCAF (GNAT family) activity while

inhibiting that of CBP (p300/CBP family) was identified as the first mixed activator/inhibitor of HATs and also showed powerful apoptotic effects in U937 cells [3]. The quinolines **2a-h**, obtained by combining the structural features (i.e., the C2- and C3-disubstituted quinoline moiety) of the GCN5 inhibitor MC1626 (a 3-carbethoxy-2-methylquinoline previously reported by us) [4] with those typical of AA (i.e., the salicylic moiety), showed to be not active against GCN5 and PCAF, but selective and in some cases stronger than AA in inhibiting p300/CBP.



References

- [1] Mai A. The therapeutic uses of chromatin-modifying agents. *Expert Opin Ther Targets* 2007;11:835-851.
- [2] Balasubramanyam K, Swaminathan V, Ranganathan A, Kundu TK. Small molecule modulators of histone acetyltransferase p300. *J Biol Chem* 2003;278:19134-19140.
- [3] Sbardella G, Castellano S, Vicidomini C, Rotili D, Nebbioso A, Miceli M, Altucci L, Mai A. Identification of Long Chain Alkylidenemalonates as Novel Small Molecule Modulators of Histone Acetyltransferases. *Bioorg Med Chem Lett* 2008; in press.
- [4] Mai A, Rotili D, Tarantino D, Ornaghi P, Tosi F, Vicidomini C, Sbardella G, Nebbioso A, Miceli M, Altucci L, Filetici P. Small-Molecule Inhibitors of Histone Acetyltransferase Activity: Identification and Biological Properties. *J Med Chem* 2006;49:6897-6907 and references cited therein.

P274

ORGANOMETALLIC-BASED INHIBITORS OF CARBONIC ANHYDRASES TARGETING HYPOXIC TUMOURS

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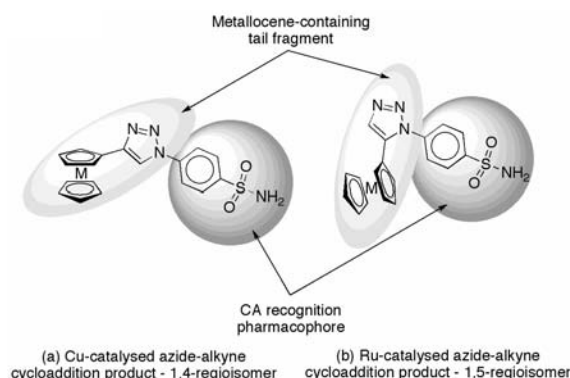
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Whilst the medicinal applications of metals dates back almost 5000 years,¹ it was the discovery of the anti-cancer agent cisplatin that has led to the development of modern day medicinal inorganic chemistry. Since this time, a large amount of research has been undertaken to further explore the positive therapeutic effects of metal containing compounds. The metallocenes (ferrocene, ruthenocene) are one such class of compounds, whose aromatic-like properties and ability to partake in redox

processes make them interesting compounds from a medicinal chemistry viewpoint.

There are currently 16 known carbonic anhydrase (CA) isozymes found in humans, possessing catalytic activity for the reversible hydration of carbon dioxide to aqueous bicarbonate. While some isozymes are widely distributed throughout the body, hCA IX and XII (h = human) have been observed to be over-expressed in hypoxic tumour cells, and are a target for potential anticancer treatments.² Our research focuses on the attachment of organometallic “tail” fragments to an arylsulfonamide (ArSO_2NH_2) CA recognition pharmacophore (figure 1). This “tail approach” strategy provides an avenue to explore CA isozyme differentiation in addition to imparting desired physicochemical properties into the inhibitor molecule. A range of synthetic techniques, including a novel ‘click-tailing’³ approach (figure 1), will be presented in addition to the CA inhibition effects of the metallocene-based compounds.



References

- [1] Zhang, C. X. and Lippard, S. J., *Curr. Opin. Chem. Biol.*, 2003, 7, 481-489.
- [2] Supuran, C. T., *Nat. Rev. Drug Disc.*, 2008, 7, 168-181
- [3] Salmon, A. J., Williams, M. L., Innocenti, A., Vullo, D., Supuran, C. T., Poulsen, S.-A., *Biorg. Med. Chem. Lett.*, 2007, 17, 5032-5035.

P275

THE QUANTIFICATION OF THE CATION- π INTERACTION IN THE S4 POCKET OF FACTOR XA

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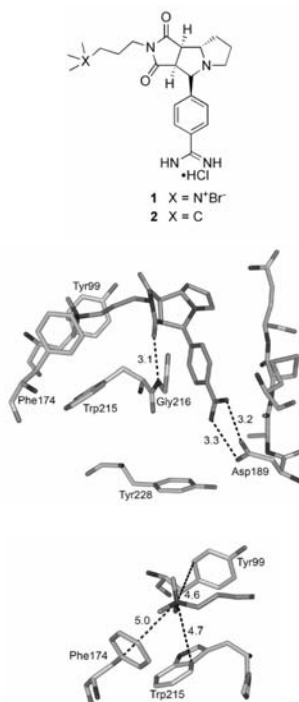
The most important interactions between a drug and its target enzyme are noncovalent. The cation- π interaction is considered an essential factor in protein-ligand

binding. The cation- π interaction is an electrostatic attraction between a cation and the negative electrostatic potential on the face of a π -system.¹

Human factor X is a vitamin K-dependent glycoprotein, synthesized in the liver and secreted into the bloodstream as a zymogen. It is a central serine protease of the blood coagulation cascade and provides a link between the extrinsic and intrinsic pathways. Factor Xa cleaves prothrombin to generate thrombin, which leads to the formation of fibrin.²

Thrombin was a target in molecular-recognition studies in the *Diederich* group. Inhibitors **1** and **2** were synthesized to investigate the cation- π interaction in the D pocket of thrombin. Surprisingly, the onium ion inhibitor **1** did not show much activity against thrombin but was found to be a highly active factor Xa inhibitor. The corresponding non-cationic inhibitor **2**, however, showed reversed activity. Presumably, the cation- π interaction between Trp215 in the D pocket of thrombin and the onium ion is not strong enough to compensate for the desolvation energy lost during binding. In the S4 pocket of factor Xa, the aromatic box formed by the three aromatic residues Phe174, Tyr99 and Trp215 is able to compensate for the loss in desolvation energy. The cation- π interaction was quantified by comparing the binding affinities of **1** and **2**. A second, different ligand series will also be reported.

Inhibitor **1** was co-crystallized with factor Xa. The X-ray co-crystal structure confirms the preferred position of the onium ion in the S4 pocket. The tricyclic linker positions the ammonium ion in the middle of the aromatic box at an almost ideal distance for cation- π interactions.³



- [1] N. Zacharias, D. A. Dougherty, *Trends Pharmacol. Sci.* 2002, 23, 281-287.
- [2] E. W. Davie, K. Fujikawa, W. Kisiel, *Biochemistry* 1991, 30, 10363-10370.
- [3] K. Schärer, M. Morgenthaler, R. Paulini, U. Obst-Sander, D. W. Banner, D. Schlatter, J. Benz, M. Stihle, F. Diederich, *Angew. Chem. Int. Ed.* 2005, 44, 4400-4404.

P276

SYNTHESIS AND EVALUATION OF CYTOTOXIC ACTIVITY OF NOVEL SYNTHETIC LUPANE DERIVATIVES

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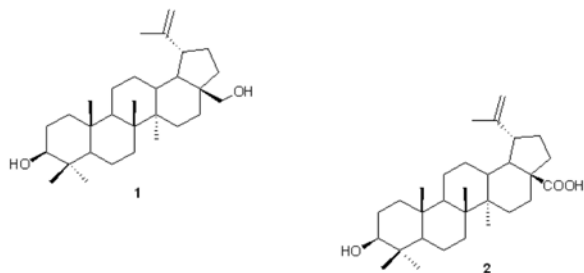
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In developing countries malignant tumors are the second most common cause of death, after cardiovascular diseases, comprising 23-25 percent of total. Prevention of cancer remains a primary need, and new chemopreventive agents must be developed for this purpose.

Triterpenes represent a varied class of bioactive natural products.^{1, 2} Thousands of structures have been reported with hundreds of new derivatives described each year.³

Natural occurring lupane triterpenoids such as betulin (1) and betulinic acid (2) have demonstrated antitumor activity against several cancer cell lines as melanoma⁴, neuroblastoma⁵, brain tumor⁶, leukaemia⁷, and others.

Using compounds 1 and 2 as starting materials a series of novel lupane derivatives have been synthesized and tested for their cytotoxic potential against several cancer cell lines.



References

- [1] Dzubak, P; Hajdich M; Vydra, D; Hustova, A; Kvasnica, M; Biedermann, D; Markova, L; Urban, M; Sarek, J. Pharmacological activities of natural triterpenoids and their therapeutic implications. *Nat. Prod. Rep.* 2006; 23(3): 394-411.
- [2] Tolstikova, TG; Sorokina, IV; Tolstikov GA; Tolstikov AG; Flekhter OB. Biological activity and pharmacological prospects of lupane terpenoids: I. Natural lupane derivatives. *Russian J. Bioorg. Chem.* 2006; 32(1):37-49.

- [3] Connolly, JD; Hill, RA. Triterpenoids. *Nat. Prod. Rep.* 2007; 24(2): 465-486.
- [4] Pisha, E. et al. Discovery of betulinic acid as a selective inhibitor of human-melanoma that functions by induction of apoptosis. *Nat. Med.* 1995; 1(10):1046-1051.
- [5] Fulda, S; Jeremias, I; Pietsch, T; Debatin, KM. Betulinic acid: a new chemotherapeutic agent in the treatment of neuroectodermal tumors. *Klin. Padiatr.* 1999; 211(4):319-322.
- [6] Fulda, S; Jeremias, I; Steiner, H; Pietsch, T; Debatin, K-M. Betulinic acid: a new cytotoxic agent against malignant brain-tumor cells. *Int. J. Cancer.* 1999; 82:435-441.
- [7] Ehrhardt, H; Fulda, S; Fuhrer, M; Debatin, KM; Jeremias, I; Betulinic acid-induced apoptosis in leukemia cells. *Leukemia*.2004; 18(8):1406-1412.

Acknowledgments

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IMPLICATIONS FOR SELECTIVITY OF 3,4-DIARYL-QUINOLINONES AS p38 α MAP KINASE INHIBITORS

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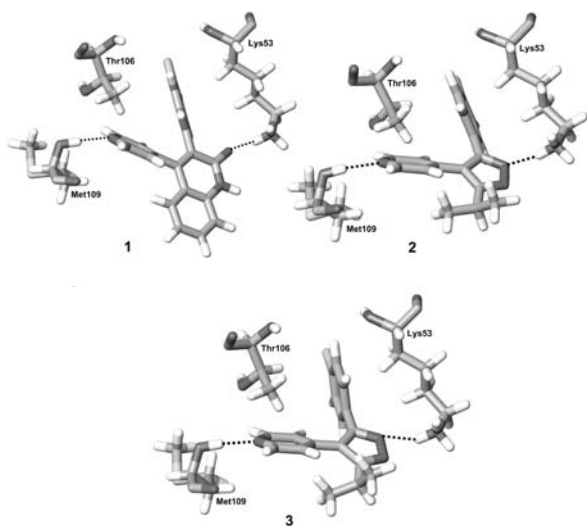
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In this study we report on the specificity profiling of the mitogen activated protein kinase (MAPK) inhibitors 1, 2 and 3 in a panel of 78 protein kinases including the MAPK isoforms p38 ($\alpha, \beta, \gamma, \delta$), JNK1/2/3 and ERK1/2/8 showing compound 1 (3-(4-fluorophenyl)-4-pyridin-4-ylquinolin-2(1H)-one) to be highly selective for p38 α MAPK. In contrast, besides p38 α the related isoxazoles 2 and 3 significantly inhibited JNK2/3 and further protein kinases beyond the MAPK family such as PKA, PKD, Lck and CK1^[1]. In order to investigate a plausible binding mode of these compounds the PDB structures of p38 α and JNK3 were used for molecular modeling studies. The rational binding modes of 1, 2 and 3 in the ATP binding pocket of p38 α (PDB: 1A9U) are shown below.

Accordingly to the typical binding mode of compounds bearing a vicinal 4-F-phenyl/pyridine pharmacophore in p38 α a hydrogen bond from Met109 (hinge region) is addressed to the pyridine nitrogen. The 4-F-phenyl moiety is situated in the hydrophobic pocket I with the vicinal 4-F-phenyl/pyridine moiety clasp around the "gate-keeper" residue Thr106 and thereby accounting for p38(α)-selectivity. Furthermore a hydrogen bond from Lys53 toward the carbonyl-oxygen (1) and to the isoxazole ring-nitrogen (2) or -oxygen (3) is formed, respectively.

However, besides a sequence alignment approach homology models were generated for MAPK isoforms for which PDB data were not published and subsequently used for extended docking studies (used programs:

GLIDE, FlexiDock). Key residues involved in ligand-protein interactions in the highly conserved ATP binding pockets were compared. Significantly the relevant amino acids Met and Lys are overall conserved but the gate-keeper residue differs within these enzymes [1]. While the small-sized Thr106 is unique to p38 α more spacious residues can be found at this position in JNK3 and further MAPK and thereby potentially determining selectivity of six-membered core compound **1** for p38 α but not for the five-membered core compounds **2** and **3** [2].



References

- [1] Christian Peifer, Robert Urich, Verena Schattel, Mohammed Abadleh, Stefan Laufer, "Implications for selectivity of 3,4-Diarylquinolinones as p38 γ MAP kinase inhibitors", *Bioorg. Med. Chem. Lett.* 2008, 4(18), 1431-1435.
- [2] Christian Peifer, Katrin Kinkel, Mohammed Abadleh, Dieter Schollmeyer, Stefan Laufer, "From Five- to Six-Membered Rings: 3,4-Diarylquinolinone as Lead for Novel p38MAP Kinase Inhibitors", *J. Med. Chem.*, 2007, 50, 1213-1221.

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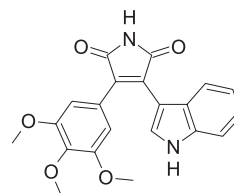
DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION OF NOVEL 3-ARYL-4-(1H-INDOLE-3YL)-1,5-DIHYDRO-2H-PYRROLE-2-ONES AS VEGF-R INHIBITORS

Schlosser J., Laufer S., Peifer C.

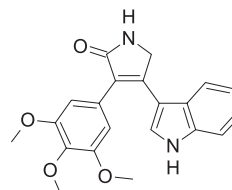
Institute of Pharmacy, Department of Pharmaceutical / Medicinal Chemistry, Eberhard-Karls Universität, Auf der Morgenstelle 8, 72076 Tübingen, Germany

In this study we report on the design, synthesis and biological evaluation of pyrrole-2-one **2** to be a highly potent VEGF-R2/3 inhibitor with IC₅₀ values of 31/37 nM. The novel 3,4-diaryl-2H-pyrrole-2-ones were designed based on the binding mode received by molecular modelling of the corresponding 1H-pyrrole-2,5-dione (maleimide) VEGF-R2/3 inhibitor **1** indicating two H-bond

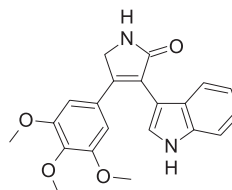
ligand-protein interactions in the ATP pocket for the amide **2** but not for the isomer **3**. Flexible synthetic routes to 3,4-diaryl-2H-pyrrole-2-ones and structure-activity relationships for the compounds in a panel of 24 therapeutically relevant protein kinases (IC₅₀ values) are presented. According to the *in vitro* data the compounds **1** and **2** were found to possess highly potent antiangiogenic activities in the cellular HLMEC (human lung-derived microvascular endothelial cells) sprouting assay and also slightly induced apoptosis in HDMECs (human dermal microvascular endothelial cells) whereas **3** was determined to be significantly less active. Hence the pyrrole-2-one moiety was dissected from the corresponding maleimide protein kinase inhibitor as a suitable key pharmacophore.[1]



Compound 1



Compound 2



Compound 3

References

- [1] Christian Peifer, Roland Selig, Katrin Kinkel, Dimitri Ott, Frank Totzke, Christoph Schächtele, Regina Heidenreich, Martin Röcken, Dieter Schollmeyer and Stefan Laufer: Design, Synthesis and Biological Evaluation of Novel 3-aryl-4-(1H-indole-3yl)-1,5-dihydro-2H-pyrrole-2-ones as VEGF-R inhibitors *J. Med. Chem.*, submitted

P279

CLASSIFICATION OF ABCB1 SUBSTRATES USING SIMILARITY BASED DESCRIPTORS

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ABC (ATP-binding cassette) transporters are membrane-bound, ATP-dependent efflux pumps whose most prominent representative is ABCB1 (P-glycoprotein). Since ABCB1 influences drug-drug interactions, oral availability and brain permeation of drugs, the current focus in the area shifted from inhibitor design to substrate prediction. Within this work we focus on a classification method using similarity based techniques in order to separate ABCB1 substrates from nonsubstrates.

Recently we could demonstrate that Similarity Based Descriptors (SIBAR) are a useful tool to establish predictive *in silico* models for drug/ABCB1 interaction. In this work we present a classification study of 240 ABCB1 substrates and non substrates. SIBAR uses a set of reference molecules in order to calculate the Euclidian distances between reference compounds and probe molecules on basis of a set of molecular descriptors. These distance-based similarity values are further used as a new set of SIBAR-descriptor for classification and PLS. Molecular descriptors used in this work are a set of ADME related descriptors as well as VSA and 3D Autocorrelation vectors. ADME descriptors are based on features important in the concept of absorption, distribution, metabolism and elimination whereas VSA descriptors focus on binned surface area contributions for logP, molar refractivity and partial charge properties. Autocorrelation vectors are topological descriptors providing information on the level of correlation between two objects in terms of their specific structural or physico-chemical properties.

For calculation of the SIBAR values an extended size and number of reference sets were defined. Each of the three sets was obtained differently out of a 660.961 compounds containing database: (i) as overall most diverse compounds, (ii) as most extreme compounds and, (iii) as 0.01% of extremes. With respect to classification algorithms we compared linear and non-linear methods using Binary QSAR and a Support Vector Machine (SVM). Results show that prediction of non substrates performs better than prediction of substrates with a cross-validated specificity of 94% and a sensitivity of nearly 78%. Cross-Validation showed best results for 3D Autocorrelation based SIBAR descriptors with an overall accuracy of 85%. This results further strengthen previous results obtained for a small set of propafenone-type ABCB1 inhibitors, which showed the advantage of shape similarities over topological similarity value at least when used in the SIBAR workflow. The contribution of real shape similarity values is currently under active investigation.

Acknowledgement

We are grateful for financial support from the Austrian Science Fund (grant L344-N17)

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A CONVERGENT ROUTE TO SCLERITODERMIN A AND ANALOGS

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Marine sponges of the order Lithistida are excellent sources of bioactive metabolites. They produce cyclic peptides with non-proteinogenic amino acids and polyketide moieties. Scleritodermin A (**1**), Figure 1, a macrocyclic compound, was isolated by Schmidt and co-workers from the sponge *Scleritoderma nodosum*.¹ It shows significant cytotoxic activity against a panel of human tumor cells lines (IC₅₀ <2 µM). The structure of Scleritodermin A incorporates a novel conjugated thiazole moiety 2-(1-amino-2-*p*-hydroxyphenylethane)-4-(4-carboxy-2,4-dimethyl-2*Z*,4*E*-propadiene)-thiazole (**ACT**), L-proline, L-serine and the unusual amino acids keto-*allo*-isoleucine and *O*-methyl-*N*-sulfo-D-serine.

During the last years, we have embarked in the synthesis of different fragments towards the total synthesis of Scleritodermin A and analogs. Recently, we described the synthesis of the **ACT** fragment and its coupling to the α,β-ketonitrile derived from L-*allo*-Ile to obtain the key intermediate C₁-N₁₅ fragment of Scleritodermin A.²

In the present work, we present a convergent strategy that involves the coupling between the **ACT** or **ACO** fragment and α,β-ketonitrile peptide (**3**), the results from the lactonization process (macrocyclization) and the biological evaluation of synthetic fragments.

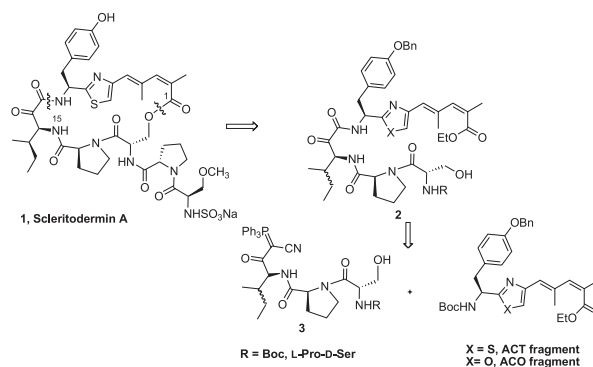


Figure 1

Acknowledgments

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References

- [1] Schmidt, E. W.; Ravento-Suarez, C.; Bifano, M.; Menendez, A. T.; Fairchild, C. R.; Faulkner, D. J. *J. Nat. Prod.* 2004, 67, 475.
- [2] Sellanes, D.; Manta, E.; Serra, G. *Tetrahedron Lett.* 2007, 48, 1827.

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TARGETING HIV-1 REVERSE TRANSCRIPTASE DIMERIZATION BY TRIPEPTIDES

Sonsoles Velázquez,¹ Carlos García-Aparicio,¹ M. Teresa Peromingo,¹ Pedro A. Sánchez-Murcia,¹ Ana San-Félix,¹ Federico Gago,² Nicolas Sluis-Cremer,³ Jan Balzarini⁴ and María-José Camarasa¹

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Reverse Transcriptase (RT) is one of the main targets in the development of chemotherapy against HIV. The active form of the enzyme is an asymmetric heterodimer formed by two subunits, named p66 and p51. Because dimerization of RT is a prerequisite for enzymatic activity, interference with the dimerization could constitute an alternative antiviral strategy to the conventional RT inhibition, particularly against resistant virus strains. We have shown that TSAO derivatives (a peculiar NNRTI family) are the first example of a small non-peptide molecule that interfere with the dimerization process of the enzyme, upon binding to the $\beta 7/\beta 8$ loop of the p51 subunit. This interface region plays a key role in RT dimerization. Examination of this dimer interface region reveals a number of hydrogen bonds between both subunits that can be important for dimerization. Two residues of this loop seem particularly important for enzyme stability (and thereby enzyme activity), the highly conserved Asn-B136 and the Glu-B138 (key interaction residue of TSAO derivatives with the enzyme).

The specific aim of this work is the search for novel molecules that may be able to interfere with the dimerization process. Thus, we propose the design, synthesis and biological evaluation of tripeptide derivatives that may mimic the key interactions of Asn-B136 and Glu-B138 with the p66 subunit. As N-terminal residue an Asn residue was chosen. As C-terminal residue an amino acid of aromatic nature (Phe, Tyr) to interact by π -stacking with the Tyr-A181 of the p66 subunit, or a Glu residue itself were used. As central residue a D-Pro or more flexible residues such as Gly or Ala have been introduced. The designed peptides were prepared with different N-/C-terminal capping groups.

The ability of the compounds to interfere with HIV-1 RT dimerization and DNA polymerase activity of RT using *in vitro* assays will be reported. Their inhibitory effect

against HIV-1 and HIV-2 replication in cell-culture will be also described.

References

- [1] N. Sluis-Cremer, G. I. Dimitrienko, J. Balzarini, M. J. Camarasa and M. A. Parriak. *Biochemistry*, 2000, 39, 1427-1433.
- [2] N. Sluis-Cremer, N. Hamamouch, A. San-Félix, S. Velázquez, J. Balzarini and M. J. Camarasa. *J. Med. Chem.* 2006, 49, 4834-4841.
- [3] F. Rodríguez Barrios, C. Pérez, E. Lobatón, S. Velázquez, C. Chamorro, A. San-Félix, M. J. Pérez-Pérez, M. J. Camarasa, H. Pelemans, J. Balzarini and F. Gago. *J. Med. Chem.*, 2001, 44, 1853-1865.

P283

NEW MRI CONTRAST AGENTS AND THEIR CELL PENETRATING PEPTIDES CONJUGATES: WHY AND HOW?

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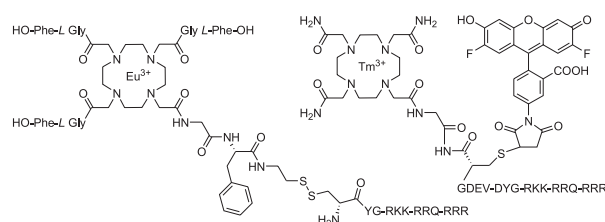
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Over the last two decades there have been dramatic developments in the use of lanthanide(III) complexes as responsive MRI contrast agents.¹ A wide variety of MRI contrast agents have been designed for the detection of various physiological parameters and enzymatic activities.

We have recently developed² a new temperature responsive PARACEST contrast agent intended to be used for the MRI diagnostics of brain tumors. The major drawback associated with this contrast agent is its inability to cross various biological barriers (e. g. blood brain barrier). To address this problem, we are attempting to conjugate it with cell penetrating peptide. Synthetic studies towards the modified chelator as well as the assembly of the whole molecule (structure on the left) will be discussed.

In addition to temperature responsive MRI contrast agents we are also interested in developing responsive MRI contrast agents capable of detecting various enzymatic activities. A DOTAM derived peptide conjugate (structure on the right) has been designed and synthesized as an MRI and fluorescence microscopy dual probe for the detection of caspase-3 enzymatic activity.



References

- [1] Yoo, B.; Pagel, M. D. *Front. Biosci.* 2008, **13**, 1733-1752.
- [2] (a) Wojciechowski, F.; Suchý, M.; Li, A. X.; Azab, H. A.; Bartha, R.; Hudson, R. H. E. *Bioconjugate Chem.* 2007, **18**, 1625-1636. (b) Li, A. X.; Wojciechowski, F.; Suchý, M.; Jones, C. K.; Hudson, R. H. E.; Menon, R.; Bartha, R. *Magn. Reson. Med.* 2008, **59**, 374-381.

P284

TRACKING DRUG-LIKENESS IN THE CHEMICAL SPACE OF PPAR- γ AGONISTS

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High-throughput screening of absorption, distribution, metabolism, elimination / toxicology (ADME/Tox) characteristics has been introduced in the early steps of Drug Design as an emergent demand to reduce attrition rate of drug candidates due to poor pharmacokinetic profiles, poor efficacy as well as toxicity side effects. In this aspect, certain physicochemical and molecular properties are considered for in silico estimation of bioavailability, while relevant cut off values or ranges have been proposed to serve as filters to distinguish drugs from non drugs [1]. Lipinski's 'rule of five' (RoF) suggests upper limits for molecular weight, lipophilicity, expressed as ClogP, hydrogen bond donor and acceptor sites and is widely used to discriminate candidates which are expected to show poor oral absorption, or to present formulation problems [2]. In the present study, evaluation of drug-likeness was performed in the case of PPAR- γ agonists, a category of chemical compounds with increasing interest as drug candidates against diabetes mellitus. For this purpose, a large data set of 1152 relevant compounds available in literature, was compiled and explored in respect to the RoF criteria as well as to additional molecular properties, such as molar refractivity (CMR), topological polar surface area (TPSA), rotatable bonds (RB) and the number of rings (NRG). Since the compounds contain ionizable groups, logD_{7.4} values, reflecting the lipophilicity at physiological conditions, were also analyzed. Descriptors were calculated by widely recognized software packages. The distribution of the molecular properties within the data set was assessed and the conformity to the established cut off values or ranges was evaluated. Violations concerning excess ClogP, MW and RB were observed for 48%, 24% and 68% of the compounds respectively, while 21% had a RoF violation score equal to 2. TPSA, CMR and NRG were found to lie in all cases on the limit of the proposed cut off values or ranges. The mean logD_{7.4} was close to the corresponding upper limit, with 59% of the compounds exceeding it. The same

analysis was repeated after division of the data set into two groups, according to activity levels, expressed as pEC₅₀ values and concerning PPAR- γ gene transactivation. Compounds with pEC₅₀ $\geq$ 7 were classified as active and those with pEC₅₀ < 7 as less active or inactive. The distribution of molecular properties was assessed within each group. A score in violation of RoF equal to 2 in regard to ClogP and MW was found for 40% of the active compounds. This percentage was considerably lower in the group of less active/inactive compounds, indicating that increase in lipophilicity and molecular size may be a safe way to achieve higher PPAR- γ activity levels. However, in a more holistic approach for future design of PPAR- γ agonists, improved molecular properties and evaluation of drug-likeness should also be taken into consideration in order to enhance the chance for success in further clinical development.

References

- [1] Muegge I. Selection criteria for drug-like compounds. *Med. Res. Rev.* 23:302-321 (2003)
- [2] Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv. Drug Deliv. Rev.*, 23: 3-25 (1997)

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SYNTHESIS AND CYTOTOXIC EVALUATION OF ARYLAMINO- AND ALKYLAMINOISOQUINOLINQUINONES

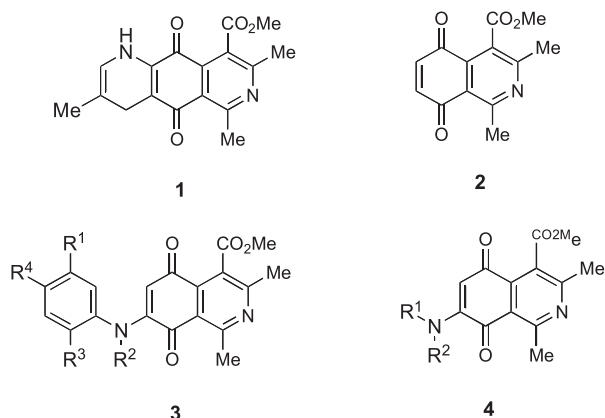
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A structural pattern consisting of an amino group attached to a quinone nucleus seems to be a critical segment in determining the antitumor activity of natural quinolinquinones such as *Streptonigrin* and *Lavendamycin*.¹ Recently, we have reported that aminoisoquinolinquinone-containing compound **1** exhibits significant cytotoxic activity against a panel of cancer cell lines at submicromolar concentrations.² Prompted by these results, we decided to study the synthesis of various arylamino- and alkylaminoisoquinolines from isoquinolinquinone **2** to evaluate the influence of the nature of the substituted amino group on the antitumor activity. Quinone **2** required for the study was prepared from acetylbenzoquinone and the respective enamines *via* a one pot procedure.³ The novel anilino-1,4-quinones (**3**, **4**) were obtained by reaction of quinone **2** with the respective amines using CeCl₃ as the catalyst. The structures of the new compounds were established by ¹H-, ¹³C NMR and 2D NMR techniques.



The evaluation of the anilinoquinones on a panel of four cancer cell lines is presented and their activity is discussed in the light of molecular descriptors such as lipophilicity, LUMO energies and half-wave potentials. In conclusion we have developed a facile and highly regioselective access to arylaminoaisoquinolin- and arylaminopyrimidazinoisoquinolinquinones endowed with potential antitumor activity.

- [1] Rao, K. V. *Cancer Chemother. Rep.* 1974, 4, 1.
- [2] Valderrama, J. A.; González, M. F.; Pessoa-Mahana, D.; Tapia, R. A.; Fillion, H.; Pautet, F.; Rodriguez, J. A.; Theoduloz, C.; Schmeda-Hirschmann, G. *Bioorg. Med. Chem.* 2006, 14, 5003-5011.
- [3] Valderrama, J. A.; González, M. F.; Pessoa-Mahana, D.M.; Colonelli, P.; Vázquez, D. *Synlett.* 2006, 2777-2780.

This work was financially supported by FONDECYT (GRANT 1060591).

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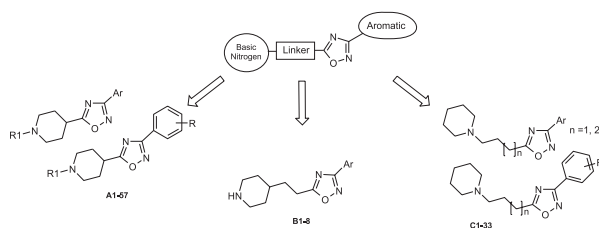
DISUBSTITUTED 1,2,4 OXADIAZOLES AS ALPHA7 NICOTINIC RECEPTOR AGONISTS

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A growing body of data strongly indicates neuronal nicotinic receptors as potential therapeutic targets in various cognitive disorders. In particular the use of pharmacological agents that selectively activate $\alpha 7$ nAChRs has been proposed for the symptomatic and disease modifying treatment of Alzheimer's disease.

In a program aimed at the identification of agonist of the $\alpha 7$ receptor, three distinct series of disubstituted 1,2,4 oxadiazoles have been efficiently synthesized by the reaction of an appropriate arylamidoxime with piperidine or alkylpiperidine carboxylic acids, under microwave irradiation conditions in good yields. Series extension has been achieved by the introduction of aryl substituents via Suzuki coupling reactions.



All compounds have been tested in parallel in a Ca^{2+} influx FLIPR assay in GH4C1 cells stably expressing rat $\alpha 7$ -nAChR and in a set of ADMET profiling assays. Single concentration and dose response experiments indicate that this class of compounds contains specific and unspecific $\alpha 7$ -nAChR agonists with the unspecific activity probably due to some effect on L-type Ca^{2+} channels. Results of the ADMET profiling did not underline major liabilities increasing the value of this series of compounds.

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SYNTHESIS OF NEW QUINOXALINE-2-CARBONITRILE-1,4-DIOXIDE DERIVATIVES AS ANTI-MYCOBACTERIUM TUBERCULOSIS AGENTS

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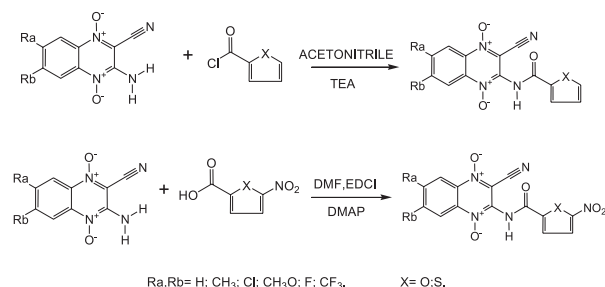
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Tuberculosis is an infectocontagious disease caused by the bacteria *Mycobacterium tuberculosis*. It is transmitted by air and is one of the principal causes of disease and death in the world. In 2006, 9.2 million cases were recorded and 1.7 million deaths were caused by TB¹.

Due to the aforementioned data and continuing with our line of research^{2,3}, 40 new compounds were synthesized with antitubercule activity.

The synthesis of these compounds was carried out following the scheme below^{4,5}:

All of these derivatives are also being tested as anti-*Mycobacterium tuberculosis* agents by the Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF) and some of them showed MIC values less than 6.25 $\mu\text{g/mL}$.



Acknowledgements

Antimycobacterial data was provided by the Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF) through a research and development contract with the U.S. National Institute of Allergy and Infectious Diseases (NIAID).

References

- [1] www.medisur.sld.cu/index.php/medisur/announcement/view/4768
- [2] Miguel Angel Ortega; Yolanda Sainz; Maria Montoya. *Arzneimittel-Forschung /Drug Research* 2002 52 (2) 113
- [3] F.J. Martinez Crespo; J.A. Palop; Y. Sainz; S. Narro. *J. Heterocyclic. Chem.*, 1996, 33, 1671-
- [4] Joseph F. Dellaria, Jr., Kevin J. Sallin. *Bioorg. Méd. Chem.* 1993, 305-308.
- [5] Rajendra P J. *Med. Chem.* 2004, 47 5276.

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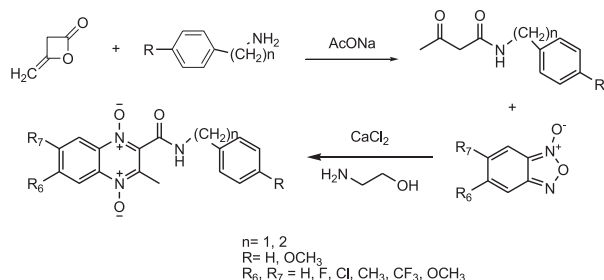
NEW AMIDE DERIVATIVES OF QUINOXALINE 1,4-DI-N-OXIDE AS ANTI-MYCOBACTERIUM TUBERCULOSIS AGENTS

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Tuberculosis (TB), an infection of *Mycobacterium tuberculosis*, is a contagious disease with high mortality worldwide. It is estimated that 1.6 million deaths resulted from TB in 2005 and there are an estimated 8 million new cases each year¹.

Several papers have been published in which both synthesis and biological activity assessments of a large number of quinoxaline and quinoxaline 1,4-di-N-oxide derivatives have been described²⁻⁴. Here we describe the synthesis and the antitubercular activity of new amide quinoxaline 1,4-di-N-oxide derivatives.



This series of derivatives was evaluated against H37Rv strain of *M. tuberculosis* and certain parameters of cytotoxicity and selectivity were established, following the TAACF program. The assayed compounds are active with MIC values ranging from 0.2 to 8.6 µg/mL. Moreover,

the tested derivatives show excellent selectivity index (SI between 10 and 500) due to a low cytotoxicity against VERO cells.

Acknowledgements

Antimycobacterial data was provided by the Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF) through research and development contracts. R. Villar was awarded a post-doctoral grant supported by the "Gobierno de Navarra".

References

- [1] World Health Organization. March 2007, posting date. [Online.]
- [2] Jaso, A.; Zarranz, B.; Aldana, I.; Monge, A. *Eur. J. Med. Chem.* 2003, 38, 791-800.
- [3] Ortega, M. A.; Montoya, M. E.; Jaso, A.; Zarranz, B.; Tirapu, I.; Aldana, I.; Monge, A. *Pharmazie*, 2001, 56, 205-207.
- [4] Ortega, M. A.; Sainz, Y.; Montoya, M. E.; Jaso, A.; Zarranz, B.; Aldana, I.; Monge, A. *Arzneim-Forsch.* 2002, 52, 113-119.

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ATOM CONTRIBUTION VISUALIZATION OF PLS-QSAR-EQUATIONS PREDICTING P-GLYCOPROTEIN INHIBITORY ACTIVITY

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As early assessment of bioavailability and toxicity becomes increasingly important, prediction of interaction with proteins like P-glycoprotein (ABCB1), a drug efflux pump belonging to the ABC-protein-family, is currently one of the challenging tasks in safety sciences. ABCB1 is not only one of the primary causes for multiple drug resistance in tumour therapy, it is also involved in intestinal uptake, biliary and renal excretion and brain uptake of drugs [1]. Although several local and global in silico models already exist, there is only very little information available which can guide the medicinal chemist in the lead optimisation process.

Hence, getting one step beyond by visualizing atom contributions might support compassing and understanding QSAR-equations and thus provide information on the next synthetic modifications to the medicinal chemist. We developed a tool using MOE's own scripting language SVL [2] to display contributions of atoms with respect to QSAR-equations derived from homogeneous series of propafenone-type ABCB1 inhibitors. The tool intends to enable the user to get a graphic impression of molecule parts contributing positively or negatively to pharmacological activity. This is visualised not only for the PLS-QSAR-equation altogether, but also for each specific descriptor used in the final QSAR equation. Thus, in con-

trast to Sybyl's HQSAR [3] we analyse atomic contributions with respect to their individual physicochemical contributions.

This method relies on descriptors being calculated on an atom-by-atom basis, as the overall molecule properties have to be broken down to its respective atoms. This is performed by recalculating the values for each atom considering the original calculation of the property. Out of the approximately 180 2D-descriptors shipped with MOE, around 100 meet this criterion including the binned VSA-descriptors [4] which give good QSAR-equations concerning ABCB1. To show the validity of the concept several in house data sets have been re-analysed and compared with the results of previous SAR studies.

References

- [1] Ecker, GF. QSAR Studies on Drug Transporters Involved in Toxicology, in: *Computational Toxicology: Risk Assessment for Pharmaceutical and Environmental Chemicals*, John Wiley & Sons, Inc., 2007
- [2] MOE (Molecular Operating Environment), Chemical Computing Group, Montreal, Canada
- [3] SYBYL 8.0, Tripos International, 1699 South Hanley Rd., St. Louis, Missouri, 63144 USA
- [4] Labute, P. A widely applicable set of descriptors, *J Mol Graph Model*. 2000;18 (4-5):464-77.

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PYRIDOISOTHIAZOLONES AS NEW INHIBITORS OF HISTONE ACETYLTRANSFERASES

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Acetylation and deacetylation of histones play an important role in the control of gene expression of the cell. Histone acetyltransferases (HAT) and deacetylases (HDAC) are responsible for these epigenetic modifications. Dysregulation of these enzymes is known to cause malignant transformation and cancer, thus making them important new drug targets.

While HDAC-inhibitors are well studied and SAHA (INN: vorinostat, Zolinza[®]) was the first deacetylase inhibitor approved by the Food and Drug Administration for the treatment of cutaneous T-cell lymphoma, few druglike inhibitors are available for HAT so far. We have reported on analogues of anacardic acid as HAT inhibitors before.¹

Based on published isothiazolone inhibitors² we performed a similarity based virtual screening in the the NCI compound repository and commercial databases. Phenyl- and pyridoisothiazolones were selected as hit

candidates and tested for in-vitro inhibition on histone peptides as a substrate and recombinant PCAF as the enzyme source. A time-resolved fluorescent assay based on an anti-acetyllysine primary antibody and an Eu-labeled secondary antibody was used to determine the inhibitory effects in vitro. IC₅₀ values for inhibition of acetylation of peptides based on histone H3 and H4 were determined in the low micromolar range in vitro. Selected inhibitors were characterized in terms of changes in acetylation of histone H3 and H4 in SK-N-SH neuroblastoma cells. Growth inhibition studies as well as quantitative imaging microscopy and western blots were performed to characterize the activity of the inhibitors in these cells

There was a clear correlation between the cytotoxic effects and the HAT inhibitory potency in vivo.

The effects on protein acetylation and HAT activity make them interesting tools for epigenetic research as well as interesting drug candidates for anti-cancer therapy. Further analogues will be synthesized to generate compounds with increased HAT inhibitory potency and optimized cellular activity.

References

- [1] Eliseeva et al. *Mol Cancer Ther* 2007;6:2391-8.
- [2] Stimson et al.; *Mol Cancer Ther* 2005;4:1521-32

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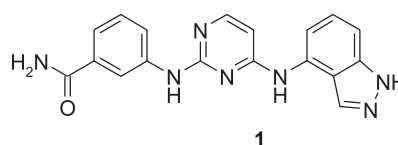
DISCOVERY OF A SERIES OF BISANILINOPYRIMIDINES AS SYK INHIBITORS FOR THE TREATMENT OF ALLERGIC RHINITIS

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Spleen tyrosine kinase (SYK) has been identified as performing a key role in the release of allergic and inflammatory mediators in mast and B-cells and hence as a target for treatment of allergic conditions including allergic rhinitis and of autoimmune diseases^(1,2,3).

This poster describes the development of a novel series of bisanilinopyrimidine SYK inhibitors, from a cross-screening lead (1), to potential intranasal clinical candidates. Crystallography of the inhibitors bound to SYK will be discussed, enzymatic and cellular SAR will be shown and *in vivo* data provided on key members of the series.



- [1] Targeting Syk as a treatment for allergic and autoimmune disorders. Wong, Brian R.; Grossbard, Elliott B.; Payan, Donald G.; Masuda, Esteban S. Expert Opinion on Investigational Drugs, 2004, 13(7), 743-762.
- [2] Manipulation of signaling to control allergic inflammation. Colgan John; Rothman Paul, Roy J. and Lucille A. Current opinion in allergy and clinical immunology (2007), 7(1), 51-6.
- [3] Syk kinase as a treatment target for therapy in autoimmune diseases. Kytitaris, Vasileios C.; Tsokos, George C. Clinical immunology (2007), 124(3), 235-7.

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THREE-DIMENSIONAL MODEL OF HUMAN GABA TRANSPORTER hGAT-1 FOR VIRTUAL SCREENING

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A three-dimensional model of the human Na⁺/Cl⁻—dependent γ -aminobutyric acid (GABA) transporters hGAT-1 was developed by homology and refined by molecular modeling using the crystal structure of a bacterial homologue Leucine transporter from *Aquifex aeolicus* (LeuT_{Aa}) as a template. Interactions of GABA and the substrate binding pocket were investigated using docking studies. Out of 13 amino acids forming the active site, 6 are different between LeuT_{Aa} and hGAT-1 and should be responsible for the different substrate selectivity. In particular the exchange of Serine 256 and of Isoleucine 359 in LeuT_{Aa} against Glycine and Threonine, respectively, in hGAT-1 is the main reason for the different substrate selectivity of the latter.

Virtual screening of our in-house database of known hGAT-1 inhibitors shows that the hGAT-1 model is very well suited for searching libraries of small compounds as potential hGAT-1 inhibitors. Especially when using the software LigandFit (Accelrys) with a consensus scoring using DOCKSCORE, LigFit_CFF_2, -PLP1, and -PLP2 as scoring functions, the activities of known inhibitors like nipecotic acid or (S)-4-amino-2-hydroxybutyric acid are very well predicted by the modelled hGAT-1 structure. However, the closed character of the active site limits this approach to molecules with a molecular mass below 250.

References

- [1] Yamashita, A.; Singh, S. K.; Kawate, T.; Jin, Y. & Gouaux, E. *Nature*, 2005, 437, 215-223

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DISCOVERY AND DEVELOPMENT OF PIPERAZINE SULFONAMIDES AS POTENT INHIBITORS OF THE 11BETA-HSD1

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Glucocorticoid hormones are important chronic regulators of metabolism. Intracellular reactivation of inactive glucocorticoids has emerged as a key mechanism for regulation and amplification of glucocorticoid action. The reactivation is catalyzed by 11 β -Hydroxysteroid Dehydrogenase type 1 (11 β -HSD1). Mice over-expressing 11 β -HSD1 in adipose or liver display a phenotype very similar to metabolic syndrome, while 11 β -HSD1 knock out mice show a marked improvement in insulin sensitivity, lipid and cholesterol profiles. These data indicate that inhibitors of 11 β -HSD1 could be novel therapeutics for patients with type 2 diabetes, obesity and metabolic syndrome. In this report, we will present the discovery of some piperazine sulfonamides as novel and potent 11 β -HSD1 inhibitors. The synthesis and SAR of these sulfonamides will be discussed.

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A NOVEL SERIES OF 2-MERCAPTOHEXANOIC ACID DERIVATIVES ADDRESSING CRUCIAL ISOLEUCIN-INTERACTIONS WITHIN THE PPAR α -LIGAND BINDING DOMAIN

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The peroxisome proliferator activated receptors (PPARs) are among today's most prominent targets for antidiabetic and antilipidemic drugs. Activation of different PPAR subtypes leads to elevated expression of apolipoproteins and cellular glucose transporters, leading to enhanced clearance of lipids and glucose from the blood. PPAR α selective fibrate-type of drugs are well established for the treatment of dyslipidemias and the PPAR γ selective glitazones have proven valuable tools for the treatment of type 2 diabetes. As dyslipidemia and insulin resistance are often associated in the metabolic syndrome, the combination of the beneficial therapeutic effects of fibrates and glitazones appears to be a desirable approach. However, as dual PPAR α/γ activating glitazars have so far all failed in clinical development, there is still a necessity for new lead structures¹. All glitazars are either equivalent activators of PPAR α and γ or PPAR γ

preferential compounds. Thus, highly active PPAR α activators with graded PPAR γ activity are one promising remaining approach. Since there are some known tools to assign subtype selectivity based on the occupation of different binding cavities, an assignment of interactions with single amino acids could offer the next step for the design of novel small molecules with predictable activity and selectivity profiles.

We discovered a PPAR α -unique amino acid interaction responsible for the high activity and selectivity of our compounds. These are 2-mercaptohexanoic acid derivatives which achieve nanomolar PPAR α -activities.

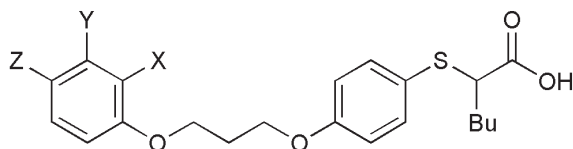


Fig.1

Based on docking experiments with the PPAR α -Ligand-Binding-Domain, SAR of the lead structure will be highlighted. We conserved α -alkyl substitution whose positive effects on PPAR activity we have shown recently². As essential for the high activities on PPAR α , we identified the interaction of the backbone of our lead structure with the amino acids Ile339 and Ile272. By means of structural variation of the concerning moiety we substantiated the benefit for PPAR α activity achievable by utilizing this interaction. Our current most potent compound activates PPAR α with an EC₅₀ value of 1.7nM and shows about 1200-fold selectivity versus PPAR γ (EC₅₀ 2.1 μ M). Additionally, in order to investigate the PPAR-activity for the distinct enantiomers of our compounds, data for preparatively HPLC-separated enantiomers were obtained.

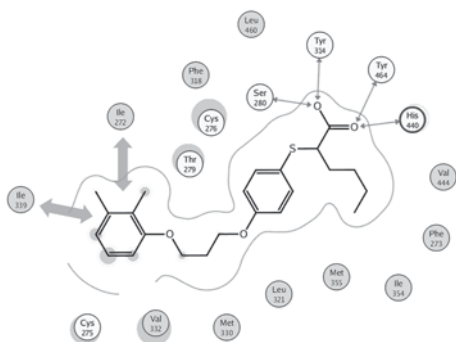


Fig.2

[1] Rau, O.; Zettl, H.; Popescu, L.; Steinhilber, D.; Schubert-Zsilavecz, M. ChemMedChem 2008, 3, (2), 206-221.

[2] Rau, O.; Syha, Y.; Zettl, H.; Kock, M.; Bock, A.; Schubert-Zsilavecz, M. Arch Pharm (Weinheim) 2008, 341, (3), 191-5.

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NEUROPROTECTIVE EFFECTS OF QUERCETIN 3-METHYL ETHER ISOLATED FROM OPUNTIA FICUS-INDICA VAR. SABOTEN

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The methanol extract [1] and the butanol fraction [2] prepared from the fruits and stems of *Opuntia ficus-indica* var. *saboten* (Cactaceae) have been reported to exert neuroprotective actions against oxidative neuronal damage induced in cortical cell cultures. Among the three flavonoids, quercetin (QUE), (+)-dihydroquercetin, and quercetin 3-methyl ether (Q3-ME), isolated from this plant, Q3-ME was found to be the most potent antioxidative neuroprotectant [3]. In the present study, we evaluated additional neuroprotective effects of Q3-ME using the primary cultured rat cortical cells and compared with that of QUE. Although both QUE and Q3-ME exhibited potent antioxidant actions in cell-free bioassays, QUE was found to induce activation of caspase 3 in the cultured cells exposed for 24 h at the concentrations of 10 μ M and above and significantly reduce the neuronal cell viability. In contrast, Q3-ME induced bcl-2 activation and maintained the cell viability. Q3-ME was also found to exert protective action against the A $_{\beta(25-35)}$ -induced neuronal damage, whereas QUE caused neurotoxicity at the same concentrations tested. The colorimetric enzyme assay and Western blotting analysis revealed that Q3-ME inhibited the A $_{\beta(25-35)}$ -induced activation of caspase 3 in a concentration-dependent fashion. In addition, QUE and Q3-ME were shown to exhibit moderate inhibition of the excitotoxicity induced by glutamate or N-methyl-D-aspartate (NMDA). Taken together, these results indicate that Q3-ME exerts antioxidant and neuroprotective actions through multiple mechanisms, suggesting that it may be beneficial for the prevention or management of neurodegenerative disorders associated with oxidative stress, A $_{\beta}$ and excitotoxicity.

References

- [1] Wie, M.-B. (2000) Yakhak Hoiji, 44: 513-519.
- [2] Cho, J. et al., (2007) J. Appl. Pharmacol. 15: 205-211.
- [3] Dok-Go et al., (2003) Brain Res. 965: 130-136.

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FACILE SYNTHESIS OF A NEW CLASS OF HETEROCYCLES, PYRAZOLO-[3,4,5-de]PHTHALAZINES, FROM BENZALPHTHALIDES AND THEIR MAO-B ACTIVITY

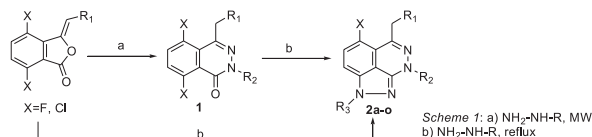
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Nitrogen-containing heterocyclic compounds are wide-spread in nature and their applications to biologically active pharmaceuticals are becoming more and more important.¹ The development of new efficient methods to synthesize *N*-heterocycles with structural diversity is one major interest of modern synthetic organic chemists.² Heterocycles containing bridgehead hydrazine: triazolophthalazines³, anthrapyrazoles⁴ and pyrroloquinolines⁵ have received considerable attention because of their pharmacological properties and clinical applications. As continuation of our study to the synthesis of heterocycles from β -haloketones and hydrazines⁶ will be described here, the design of a library of pyrazolophthalazines **2a-l** which can be synthesized in only one step from benzalpthalides and/or additionally in two step using 5,8-dihalogenophthalazinone derivatives as intermediates, affording the last procedure a wider structural variability. Last procedure was used to synthesize compounds **2m-2o** with different substitutions on R₂ and R₃ (Scheme 1). Both methodologies let to access to the desired pyrazolophthalazines in moderate to good yield (Table 1).



Product	X	R ₁	R ₂	R ₃	Yield(%)
2a	Cl	phenyl	CH ₃	CH ₃	26
2b	Cl	<i>p</i> -chlorophenyl	CH ₃	CH ₃	74
2c	Cl	<i>p</i> -fluorophenyl	CH ₃	CH ₃	25
2d	Cl	<i>p</i> -methoxyphenyl	CH ₃	CH ₃	54
2e	Cl	<i>o</i> -methoxyphenyl	CH ₃	CH ₃	21
2f	Cl	3,4,5-trimethoxyphenyl	CH ₃	CH ₃	29
2g	Cl	<i>p</i> -nitrophenyl	CH ₃	CH ₃	34
2h	Cl	α -naphthyl	CH ₃	CH ₃	17
2i	Cl	β -naphthyl	CH ₃	CH ₃	20
2j	F	<i>p</i> -chlorophenyl	CH ₃	CH ₃	45
2k	F	<i>p</i> -methoxyphenyl	CH ₃	CH ₃	15
2l	Cl	<i>p</i> -chlorophenyl	CH ₂ -CH ₂ -OH	CH ₂ -CH ₂ -OH	80
2m	Cl	Cl	CH ₃	CH ₂ -CH ₂ -OH	57
2n	Cl	Cl	CH ₂ -CH ₂ -OH	CH ₃	51
2o	Cl	Cl	H	CH ₂ -CH ₂ -OH	55

MAO inhibitory activity was tested for these compounds, finding a moderate and selective MAO-B activity for some of them.

References

- [1] Teimouri, M. B. *Tetrahedron* 2006, 62, 10849.
- [2] (a) Padwa, A.; Waterson, A. G. *Current Org. Chem.* 2000, 4, 175. (b) Orru, R. V. A.; de Greef, M. *Synthesis* 2003, 1471. (c) Kirsch, G.; Hesse, S.; Comel, A. *Curr. Org. Chem.* 2004, 1, 47.
- [3] Sorsak, L. J.; Sorsak, G.; Toplak, R.; Bevk, D.; Svete, J.; Stanovnik, B. *J. Het. Chem.* 2006, 43 (5), 1205.
- [4] Novak, R. F.; Kharasch, E. D.; Frank, P.; Runge-Morris, M. *Bioactive Molecules* 1988, 475.
- [5] Borsello, T.; Forloni, G. *Current Pharmaceutical Design*, 2007, 13 (18), 1875.
- [6] Viña, D.; del Olmo, E.; López-Perez, J. L.; San Feliciano, A. *Org. Lett.* 2007, 9, 525.

P300

DISCOVERY OF POTENTIAL ANTI-ARTHRITIS AGENTS WITH THE APPLICATION OF A NOVEL DISEASE-SPECIFIC REPORTER-GENE ASSAY

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Arthritis is a chronic degenerative arthropathy that frequently leads to chronic pain and disability. With a progressively aging population, this condition is becoming more prevalent and its treatment financially burdensome. Finding better treatments for arthritis is a major focus of medical research. It is becoming clear that inflammatory cytokines play a key role in the progression of the disease. Tumor necrosis factor alpha and interleukin-1, the two major inflammatory cytokines promote the destruction of the joints via a double mechanism. Cytokine signaling via protein kinases (MAPKs, IKKs etc.) is implicated in metalloproteinase induction and production of proinflammatory cytokines and lipid metabolites, which escalate the inflammatory process.

In order to find small molecules that block cytokine signaling an anti-arthritis screen was developed.

A recombinant plasmid was constructed, containing the firefly luciferase gene driven from multiple copies of the binding site for the NF κ B transcription factor. A plasmid containing also the neomycin resistance cassette was introduced into the SW1353 human chondrosarcoma cell line and stable transfectant clones were selected.

In the meantime we generated a kinase-focused library for screening. Based on the reported structure of kinase inhibitors relevant to the pathway we carried out a 2D fingerprint-based similarity search on AMRI's Open Access Library consisting of 230,000 compounds available in at least 1 mg quantity and in 85 % purity. Compounds having 70 % similarity expressed in Tanimoto coefficient were selected (2000 compounds),

and their number was then reduced by diversity analysis. The resulting set (272 compounds) was investigated in the anti-arthritis screen and the luciferase reporter gene activity driven by NFκB was measured. Twenty-four compounds having 70 % or lower luciferase activity were further evaluated by IC₅₀ determination. Based on the structure of the best hit (IC₅₀=7 μM) we selected a small analogue set (48) for hit validation and a closely related structure showed improved activity (IC₅₀=5 μM).

The hits were found to inhibit in the micromolar range Interleukin 1β-induced metalloproteinase gene expression in chondrosarcoma and NFκB activity in Jurkat T-cells.

P301

HIGH-THROUGHPUT CELL-BASED SCREENING OF A PHARMACOLOGICALLY ACTIVE COMPOUND LIBRARY IN REPERFUSION INJURY MODELS OF THE HEART AND KIDNEY

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Reperfusion injury plays a fundamental role in the pathogenesis of hypoxic acute tubular necrosis and myocardial infarction, and emerges as a promising target for pharmacological intervention. Drugs that reduce reperfusion induced cell death and reduce myocardial infarction size or renal nephron loss are limited and novel modalities are necessitated. We developed in vitro models of heart and kidney reperfusion injury and applied a library of pharmacologically active compounds (LOPAC) to validate the model systems.

Reduction in cell viability was initiated by treating H9c2 cardiomyocytes with hydrogen peroxide and by exposing LLC-PK renal tubular cells to transient oxygen-glucose deprivation. The cytoprotective effect of compounds was tested by consequent treatment at 3 and 30 μM final concentrations. Cell viability was evaluated after 3 and 24 hours of culture in the myocardial and at 8 and 24 hours in the renal injury model. Viability was quantitated with the methylthiazolyldiphenyl-tetrazolium bromide (MTT) method. Toxicity was also evaluated by measuring the lactate dehydrogenase activity in the cell culture supernatant.

In cardiomyocytes the most potent cytoprotective drugs included molecules that interfered with components of the DNA repair and cell cycle progression, e.g. poly(ADP-ribose) polymerase (PARP) inhibitors, topoisomerase inhibitors, cyclin dependent kinase inhibitors, or reduced energy consumption by interfering with heart myofilament function. Selected dopamine D1 receptor agonists emerged as highly potent cytoprotective drugs at

both 3 and 24 hours. These D1 agonists (chloro- and bromo-APB- and chloro-PB hydrobromide) exerted cytoprotective effect comparable to the potent PARP inhibitor PJ34 in 3 hours, and a superior effect in 24 hours as assessed by the number of viable cells. Chloro-APB hydrobromide significantly reduced peroxide induced PARP activation in H9c2 cells independent of its action on dopamine D1 receptor, but lacked direct PARP inhibitor capacity in a cell-free system. In contrast, in tubular cells inhibitors of necrosis had no significant cytoprotective effect, but inhibitors of apoptosis (caspase inhibitors Z-VAD-FMK and Ac-DEVD-CHO) and adenosine showed cytoprotective activity.

The pattern of cytoprotective drugs supports our model systems, which are applicable for high throughput screening of drug libraries. Pharmacological characterization of the models also provides a reproducible validation tool for these assay systems.

P302

SYNTHESIS AND BIOLOGICAL EVALUATION OF NEW UREA DERIVATIVES TARGETING THE MCH-R1 AND HISTAMINE H3 RECEPTORS FOR THE TREATMENT OF OBESITY

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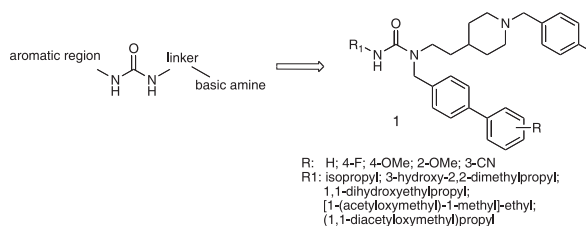
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A large percentage of people worldwide are overweight, with a body mass index (BMI) between 25 and 29.9 kg m⁻² and estimates suggest that one quarter to one third of the population is obese. Moreover, the disease incidence is increasing throughout the world [1]. This increasing prevalence of obesity is leading to increasing drug therapy based on greater understanding of novel pharmacological principles. As a result, there is a medical need for more efficacious treatment modalities for obesity [2].

Melanin-concentrating hormone is thought to be a key neuropeptide as regulator of feeding behaviour and energy balance, based on several lines of evidence [3].

The histaminergic system is also involved in the energy balance since high levels of hypothalamic histamine induces weight loss and leanness [4].

Consequently, antagonism of the aforementioned receptors could provide a viable treatment of obesity.



As a continuation of our research in this field, we have synthesized and evaluated a novel series of urea derivatives (structure **1**) with the aim of improving the MCH-R1 and the histamine H3 receptor in vitro activities maintaining hERG affinity [5].

References

- [1] Martin, L.F.; Robinson, A.; Moore, B.J.; *Pharmacoeconomics*. 2000, **18**, 335.
- [2] Clapham, J.C.; Arch, J.R.S.; Tadayyon, M.; *Pharmacol. Ther.* 2001, **89**, 81.
- [3] Della-Zuana, O.; Presse, F.; Ortola, C.; Duhault, J.; Nahon, J.L.; Levens, N.; *Int. J. Obes.* 2002, **26**, 1289.
- [4] Yoshimatsu, H.; *Peptides*. 2006, **27**, 326.
- [5] Galiano, S.; Ceras, J.; Cirauqui, N.; Pérez, S.; Juanenea, L.; Rivera, G.; Aldana, I.; Monge, A. *Bioorg. Med. Chem.* 2007, **15**, 3896.

P303

PARTIAL SYNTHESIS OF L687384: AN EASY ENTRY TO SPIROPIPERIDINE.

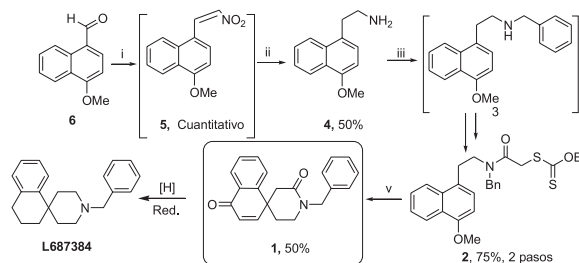
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L687384 spiroperididine (Scheme 1) is σ -receptor ligand and [1a] useful in the treatment of cocaine addiction,[1b] depression,[1c] epileptic attacks[1d] and other mental disorders. This class of compounds has been tested as neuroprotective agents,[1e] anti-inflammatories,[1f] and anti-neoplastics.[1g]

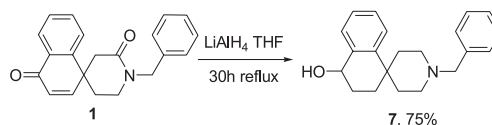
In general, there are few reports for the synthesis of **L687384** spiroperididine. Usually these synthetic routes involve several reaction steps to obtain the quaternary center (spiro).[1-7] In this work we describe a three step synthesis of spirodienone **1**, as a potential precursor of **L687384**. The key step is the oxidative radical spirolactamization to obtain spirodienone **1** through xanthate **2** (Scheme 1). Recently, we have demonstrated the efficacy of *ipso*-addition of free radicals to *p*-oxygenated aromatic rings occurs in good yield and high selectivity.[2]

Phenethylamine **4** was obtained by a reduction with LiAlH₄ of nitrostyrene **5** which was prepared from the benzaldehyde **6** through a Henry reaction in good yields.[3] The phenethylamine **4** was condensed with benzaldehyde to give in high yield the secondary amine **3** which was acylated and then transformed to xanthate **2** in 50% yield (two steps). The xanthate **2** was refluxed in 1,3-dichloroethane and 1.3 equivalents of dilauroyl peroxide (DLP) to yield Spirodienone **1**. The spiroperididine **L687384** can be obtained from spirodienone **1** with a reduction (Scheme 1).



Scheme 1. Conditions: (i) CH₃NO₂, NH₄OAc, 80 °C, 3 h. (ii) LiAlH₄, THF, reflux, 15 h. (iii) 1.C₆H₅CHO, mesh, MeOH, 14 h. 2. NaBH₄, 0 °C, 2 h. (iv) 1. ClCOCH₂Cl, Et₃N, CH₂Cl₂, 0 °C, 1 h. 2. KSCSOEt, MeOH, 2 h. (v) DLP, 1,2-dichloroethane, reflux, 8-10 h.

When the spiroperididine was treated with an excess of LiAlH₄ during 30 h it was obtained the hydroxy-spiroperididine **7** in 75% yield (Scheme 2). Although it is hard the total reduction to get the spiroperididine, the LiAlH₄ reduces carbonyls and the double bond which usually does not occur with this reducing reagent.



Scheme 2. Reduction of spirodienone 1.

The oxidative radical spirolactamization was successfully applied to obtain the spiroperididine core in three steps from commercial starting materials. Several derivatives can be prepared with this methodology.

Bibliography

- [1] (a) Maier, C.A.; Wunsch, B. *J. Med. Chem.* 2002, **45**, 438. (b) Ujike, H.; Otsuki, S. *Eur. J. Pharmacol.* 1996, **296**, 123. (c) Monographie, *Drugs Future* 1999, **24**, 133. (d) Lin, Z.; Kadaba, P.K. *Med. Res. Rev.* 1997, **21**, 537. (e) Maurice, T.; Lockhart, B.P. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry* 1997, **21**, 69. (f) Bowen, W.D. *Pharm. Acta Helv.* 2000, **74**, 211. (g) Chambers, M.; Baker; Wong, E. *J. Med. Chem.* 1992, **35**, 2033.
- [2] Ibarra-Rivera, T.R.; Gamez-Montaño, M.A.; Miranda, L.D. *Chem. Comm.* 2007, 3485.
- [3] Honda, T.; Shigehisa, H. *Org. Lett.* 2006, **8**, 657.

P304

SYNTHESIS OF LENNOXAMINE AND NEUVAMINE AZA-ANALOGS

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Lennoxamine (**1**)¹ and Neuvinamine (**2**)² are two natural products with vaso-relaxant activity, which were isolated from native plants (*Berberis darwinii* and *Berberis empetrifolia*, respectively) of South America. Several methodologies for the synthesis of these compounds have been described. However, there is few reported about the preparation of their analogs.³ The objective of this work is to develop a short synthesis to obtain the analogs **3a** and **3b** (Figure 1), using a multicomponent Ugi-type reaction as step key.⁴

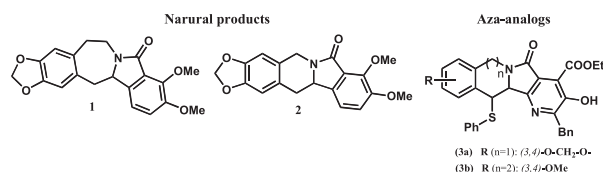
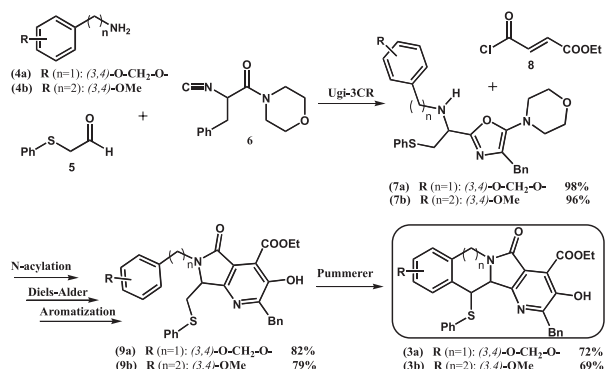


Figure 1

The synthetic strategy is shown in Scheme 1.



Scheme 1

The amine (**4**), aldehyde (**5**) and isonitrile (**6**) react in a domino process by Ugi-3CR (three components reaction) to obtain 5-amino-oxazol (**7**) in excellent yield. The pure intermediate **7** reacts with the acid chloride (**8**) involving a sequential triple process: *N*-acylation/Diels-Alder/Aromatization to obtain the compound **9** in good yield. The product **3** was obtained from the thioether (**9**) by a Pummerer cyclization.

[1] Padwa, A.; Beall, L.S.; Eidell, C.K.; Worsencorft, K.J. *J. Org. Chem.* 2001, 66, 2414-2421.

[2] Katritzki, A.R.; He, H.Y.; Jiang, R. *Tetrahedron Lett.* 2002, 43, 2831-2833.

[3] Gamez-Montañón, R.; Zhu, J. *Chem. Commun.* 2002, 20, 2448-2449.

[4] Dönnling, A.; Ugi, I. *Angew. Chem. Int. Ed.* 2000, 39, 3168-3210.

P305

NANOMOLAR INHIBITORS OF HELICOBACTER PYLORI DEHYDROQUINASE WITH POTENTIAL ANTIMICROBIAL ACTIVITY

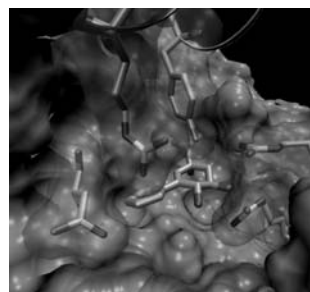
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The synergy between the AIDS epidemic and the increasing surge of multidrug-resistant isolates to antibiotics, leads to the alarming conclusion that antibiotics are losing their effectiveness. The development of new antibiotics to control these adaptable pathogens is therefore much needed. Bacteria, as well as fungi, higher plants and some apicomplexan parasites (malaria), biosynthesize the aromatic amino acids, as well as other important aromatic compounds such as folates, ubiquinone, and vitamins E and K, through a biosynthetic route known as the shikimic acid pathway.[1] The absence of the pathway in mammals, combined with its essential nature in certain microorganisms, makes the shikimic acid pathway enzymes attractive targets for the development of new antibiotics.

Our recent promising results in the development of potent competitive inhibitors against *M. tuberculosis* dehydroquinase [2,3], the third enzyme of this pathway, have encouraged us to search for inhibitors of other pathogenic bacteria such as *Helicobacter pylori*. This bacterium causes gastric and duodenal ulcers and has been classified by WHO as a class I carcinogen. *H. pylori* infects approximately two-thirds of the world's population, with the highest prevalence among adults in developing countries.

In this communication we will present our recent progress made on the design and the synthesis of *H. pylori* dehydroquinase inhibitors. [4] Molecular modelling and NMR studies to determine the bound conformation of the most potent competitive inhibitor ($K_i = 540$ nM) will be also presented.



Acknowledgements

Xunta de Galicia (PGIDT07PXIB209080PR and GRC2006/132) and the Spanish Ministry of Education and Culture (SAF2007-63533) are gratefully acknowledged. CSS and VFVP thank the Spanish Ministry of Education and Culture and Fundação para a Ciência e a Tecnologia for a FPU and FCT scholarships, respectively.

References

- [1] Abell, C. Enzymology and molecular biology of the shikimate pathway. In *Comprehensive Natural Products Chemistry*; Sankawa, U., Ed.; Elsevier Science Ltd.: Oxford, 1999. pp 573-607.
- [2] González-Bello, C.; Castedo, L. *Med. Res. Rev.* 2007, 27, 177.
- [3] (a) González-Bello, C. *et al. J. Med. Chem.* 2005, 46, 5735.
(b) González-Bello, C. *et al. ChemMedChem* 2007, 2, 194.
- [4] González-Bello, C. *et al. ChemMedChem* 2008, 3, 756.

P306

4-NITRO-N-PYRIMIDIN-2-YLBENZENESULFONAMIDE AND N²-(4-NITROPHENYL)-N¹-PROPYLGLYCINAMIDE, TWO NEW AGENTS WITH ACTIVITY AGAINST LEISHMANIA INFANTUM

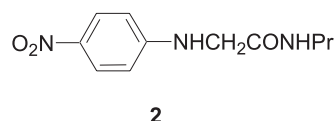
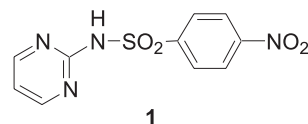
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Leishmaniasis comprises a group of diseases with distinct clinical manifestations caused by different species of the protozoan parasite *Leishmania*. Current treatment of leishmaniasis is based on chemotherapy with some attempts at immunotherapy. However, the resistance to chemotherapies and the high toxicity and cost of second-line drugs reveal the urgent need of a search for novel chemotherapeutic agents with antileishmanial activity.¹ Reports of the apparent success of nitrobenzene derivatives for the treatment of leishmaniasis have been described.²

In this communication we report on the synthesis and evaluation of the leishmanicidal activity of a series of compounds containing the nitrobenzene and sulphonamide moieties. The activity was evaluated *in vitro* versus *Leishmania infantum* 4-nitro-N-pyrimidin-2-ylbenzenesulfonamide **1** and N²-(4-nitrophenyl)-N¹-propylglycinamide **2** demonstrated a significant activity comparable with Amphotericin B. These results suggest the potential usefulness of these compounds in the ther-

apeutic control of leishmaniasis. Studies are currently in course to elucidate their mechanism of action at molecular level by studying their interaction with the DNA.



References

- [1] Croft, S. L.; Sundar, S.; Fairlamb, A. H. *Clin. Microbiol. Rev.* 2006, 19, 111.
- [2] Delfin, D.A.; Bhattacharjee, A.K.; Yakovich, A.J.; Werbovetz, K.A. *J. Med. Chem.* 2006, 49, 4196. Bhattacharya, G.; Herman, J.; Delfin, D.A.; Salem, M.; Barszcz, T.; Mollet, M.; Riccio, G.; Brun, R.; Werbovetz, K. A. *J. Med. Chem.* 2004, 47, 1823.

P307

EVALUATION OF THE TRYPANOCIDAL ACTIVITY OF A SERIES OF SULFONAMIDE DERIVATIVES

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American Trypanosomiasis (Chagas disease) is a widespread parasitic disease considered within the most relevant group of neglected tropical diseases. The World Health Organization has identified the Chagas disease as a major and increasing public health problem, particularly in Latin America. Some 16–20 million people in 17 countries are affected, out of almost 100 million who are exposed to the infection. So far, chemotherapy is the only available treatment for this disease. Two drugs are currently in use, nifurtimox (a nitrofur derivative) and benznidazole (a nitroimidazole derivative). Both compounds have limitations owing to toxicity and lack of effectiveness (active only during the acute and short-term chronic phase); moreover, production of nifurtimox has now been discontinued. Thus the development of new, efficient and safer drugs for the treatment of this disease is imperative.¹ Pharmacological interest of sulfonamides has been

established and several reports describe their antimicrobial and antiparasitic properties.²

In order to find new drugs with trypanocidal activity we have synthesized and evaluated *in vitro* 17 compounds against epimastigotes of *Trypanosoma cruzi*. Among the tested compounds, the 4-nitro-*N*-pyrimidin-2-ylbenzene-sulfonamide inhibited significantly the parasite growth. This sulfonamide derivative showed very weak cytotoxicity in macrophages and no toxicity was detected in fibroblasts, making it a promising lead compound for the development of an effective therapeutic agent.

References

- [1] Hotez, P.J.; Molyneux, D. H.; Fenwick, A.; Kumaresan, J.; Sachs, S. E.; Sachs, J. D.; Savioli, L. *New Engl. J. Med.* 2007, 357, 1018.
- [2] Everson da Silva, L.; Joussef, A. C.; Kramer Pacheco, L.; Gaspar da Silva, D.; Steindel, M.; Andrade Rebelo, R. *Bio. Med. Chem.* 2007, 15, 7553–7560. Bhattacharya, G.; Herman, J.; Delfin, D.; Salem, M. M.; Barszcz, T.; Mollet, M.; Riccio, G.; Brun, R.; Werbovets, K. A. *J. Med. Chem.* 2004, 47, 1823.

P308

ACAT INHIBITORS FROM THE FRUITS OF *PIPER LONGUM* AND *PIPER NIGRUM*

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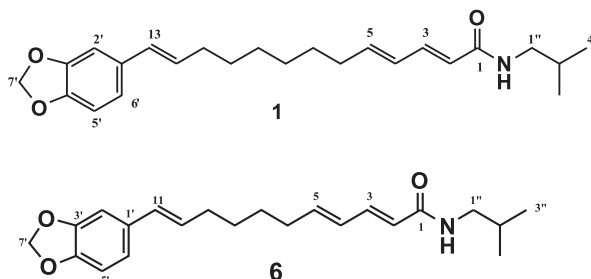
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ACAT is an intracellular enzyme that catalyzes the formation of cholesteryl esters from free cholesterol and long-chain fatty acyl coenzyme A. This enzyme plays pivotal roles in intestinal absorption of cholesterol, hepatic production of lipoproteins and accumulation of cholesteryl ester within macrophages and smooth muscle cells of the atheroma.

Bioactivity-guided fractionation of chloroform extracts of Piper species (*Piper longum* and *Piper nigrum*) led to the isolation of eleven alkamide compounds such as guineensine (1), retrofractamide C (2), (2E, 4Z, 8E)-N-[9-(3, 4-methylenedioxy phenyl)-2, 4, 8-nonatrienoyl] piperidine (3), piperonaline (4), retrofractamide A (5), piperide (6), piperolein B (7), piperchabamide D (8), pellitorin (9), dehydroretrofractamide C (10) and dehydropiperonaline (11). IC₅₀ values of 3.12 (1), 24.9 (2), 110.6 (3), 111.42 (4), 24.5 (5), 3.7 (6), 70.5 (7), 13.5 (8), 40.5 (9), 60 (10) and 90 μM (11) were obtained, according to the results of our ACAT enzyme assay system using rat liver microsomes. As a result of SAR, ACAT inhibitory activities of alkamides were positively influenced by the presence of isobutyl moiety and number of conjugated double

bond. 1 and 6 showed dose dependent inhibition of cholesteryl oleate formation in cells, the IC₅₀ values were observed at 1.87 (1) and 0.7 (6), <2.6 (1) and 5.1 μM (6), respectively. Also, they (1 and 6) showed inhibitory effects of the secretion of apolipoprotein B in HepG2 cells. 1 showed no inhibition effect on the foam cell formation and cholesterol esterification in THP-1-derived macrophages. Therefore, compounds 1 and 6 were thought to be more effect against ACAT2 than ACAT1.



P309

ANTI-INFLAMMATORY PROFILE OF P38α MAP KINASE INHIBITORS: 4-ARYL-5-HETEROARYL-2-THIO-SUBSTITUTED IMIDAZOLES

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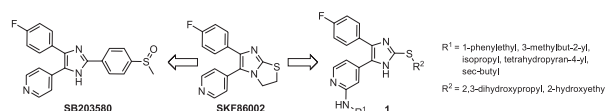
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The p38α mitogen-activated protein (MAP) kinase is required for the biosynthesis and release of pro-inflammatory cytokines like IL-1β and TNF-α. Inhibition of p38α MAP kinase could reduce the expression of these cytokines and is therefore a promising therapeutic strategy for the treatment of many inflammatory disorders such as inflammatory bowel disease, psoriasis and rheumatoid arthritis. Many tri- and tetrasubstituted pyridinyl imidazoles, derived from the early lead SKF86002, have been investigated as potent inhibitors of p38 MAP kinase, e.g. the prototype inhibitor SB203580.



However, only very few of them make use of molecular interactions with the ribose and/or phosphate binding region of the enzymes. We have tried to target these regions by substituting the imidazole C2-position with

polar thio ethers. The synthetic route to the 2-alkylsulfanyl-4-(4-fluorophenyl)-5-(2-aminopyridin-4-yl)-substituted imidazoles (**1**) as small molecule inhibitors of p38 α MAP kinase was published recently.^[1]

Potent inhibitors (IC₅₀ values down to 2 nM in the p38 α assay and down to 37 nM in TNF- α release in human whole blood) were identified by variation of substituents at the imidazole C2-thio position (R²) as well as at the 2-aminopyridinyl functionality (R¹). Substitution at the pyridinyl moiety increases both anti-inflammatory activity as well as selectivity. Compounds resulting of variation at the imidazole C2-thio ether moiety with polar groups - which may interact with the ribose and/or phosphate binding site of the enzyme - are well tolerated. Introduction of a hydroxy function at the imidazole C2-thio ether moiety (R²) results in improved inhibition of p38 α and in a substantial increase of activity in TNF- α release in human whole blood.

Selected compounds were investigated for the release of various inflammatory parameters, like interleukins (IL-2, IL-6), IFN- γ , NO production and cell proliferation in mouse spleen and peritoneal exudates cells. Most promising candidates underwent *in vivo* studies, e.g. in an acute DSS induced inflammatory bowel disease model in mice.

References

[1] Laufer, S. and Koch, P. *Org. Biomol. Chem.* 2008, 8, 437-439.

P310

STRUCTURE-ACTIVITY RELATIONSHIPS OF A SERIES OF ANTAGONISTS AT DOPAMINE D2 AND SEROTONIN 5-HT2B AND PARTIAL AGONISTS AT 5-HT1A RECEPTORS : A NEW PROFILE FOR POTENTIAL ANTIPSYCHOTICS.

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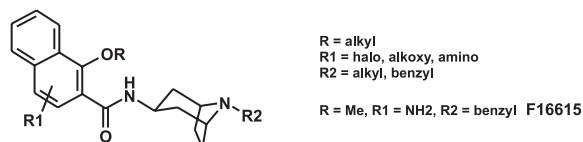
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The search for improved antipsychotics is a difficult and challenging area for human therapy. Numerous approaches have led to the identification of new compounds with various mechanism of action, but often old drugs are the last resort in case of relapse. Hence, we developed a program for the identification of effective and well-tolerated novel antipsychotics.

A new series of tropane-naphthamides was synthesized and different profile of receptor binding were obtained according to the pattern of the aromatic substituent. Modifications of the archetypic structure of tropapride (**1**) as potent dopamine D2 antagonist, led to the identification of a series with additional potent 5-HT2B antagonism. Substitution at position 4 of the naphthyl ring

determined the balance between the binding profile of 5-HT2A serotonin antagonism and 5-HT1A serotonin partial agonism.

Synthetic strategies and modifications of the tropane ring are presented. Tropane ring was mandatory to retain potency at dopamine D2 receptors while the dual presence of tropane and naphthamide conferred potent serotonin 5-HT2B receptor antagonism and partial 5HT1A agonism. Potent antagonism at D2 dopamine receptors was shown *in vivo* by antagonism of methylphenidate-induced behaviour in rats, a model for potential antipsychotic activity. Furthermore, partial agonism at 5-HT1A receptors led to a low propensity to induce catalepsy (a model of extra-pyramidal symptoms) and activity against scopolamine-induced cognitive deficit in rat. Antagonism at 5-HT2B receptors *in vivo* was demonstrated by inhibition of MDMA hyperactivity in mice (**2**). The mechanistic implications of such a profile are discussed.



This novel and promising profile of activity (combined D2 and 5-HT2B antagonism with 5-HT1A partial agonism) led to the selection of F16615 as a putative new antipsychotic for further pharmacological investigations.

References

[1] *Drug of the Future* 1984, 9, 673.

[2] Doly S. *et al. J. Neurosci.* 2008, 28, 2933.

P311

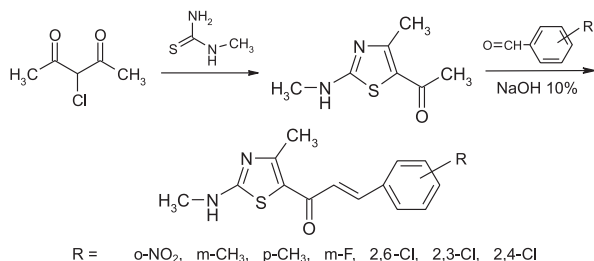
SYNTHESIS AND EVALUATION OF ANTI-INFLAMMATORY ACTIVITY OF SUBSTITUTED (E)-1-(4-METHYL-2-(METHYLAMINO)THIAZOL-5-YL)-3-PHENYLPROP-2-EN-1-ONES

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Inflammation is a natural protective reaction of human organism against tissue damage which is induced by physical injuries, chemical substances, pathogenic microorganisms and other factors. Cyclooxygenase (COX) and lipoxygenase (LOX) produce two groups of arachidonic acid metabolites, prostaglandins (COX products) and leukotrienes (LOX products) that play a key role in inflammation. Non steroidal anti-inflammatory drugs act by inhibiting COX isoenzymes. Development of agents with balanced COX-1/2 inhibitory action combined with LOX inhibition seems to be the choice of preference in an effort to minimise undesired side effects of the compounds. As a continuation of our outgoing project on the synthesis of thiazole derivatives with probable anti-inflam-

matory activity and taking into account that many chalcones have also shown anti-inflammatory activity, we synthesized a number of chalcones which are thiazole derivatives, tested their possible anti-inflammatory activity and evaluated their COX and LOX inhibitory action. The title compounds were synthesized according to Scheme 1.



Scheme 1

P312

SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF SOME 4-OXO-QUINOLIN-3-CARBOXYLIC ACID DERIVATIVES

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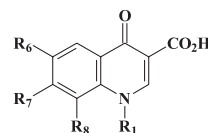
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The discovery of fluoroquinolones after 1980 represented a decisive step forward for chemical anti-infectious therapy. A large number of fluoroquinolones are used today in medical practices and some of them are deemed by leading pharmacologists to be of vital importance to anti-infectious therapy [1-5]. The research for an ideal quinolone continues worldwide, such a quinolone must be biologically active on a large spectrum of gram positive and gram negative bacteria, aerobic and anaerobic germs and mycobacteria, must have a few side effects as possible, excellent solubility in water and to be taken orally.

The goal of our research work was to obtain by synthesis new derivatives of quinolin-3-carboxylic acid with the general formula:



R₁ = ethyl, isopropyl, 2-butyl, 2-pentyl, benzyl;

R₂ = hydrogen, fluor, chlor, methyl;

R₇ = 3-methyl-piperazinyl, 4-methyl-piperazinyl, 3-methyl-piperidinyl, 4-methyl-piperidinyl, morpholinyl;

R₈ = chlor, methyl.

Figure 1

These quinolin-3-carboxylic acids have been analysed by physico-chemical techniques (elemental analysis, ¹H-NMR, ¹³C-NMR, IR spectra, thin layer chromatography). The new quinolones were evaluated for in vitro activity by determining minimum inhibitory concentration against a variety of bacteria. The results of present study indicate that substituent combination in the quinolone ring might produce powerful antibacterial agents such as compound FPQ₂₈ – MIC = 0,125 µg/ml against E. coli 25922, MIC = 0,06 µg/ml against S. aureus ATCC29213, MIC = 8 µg/ml against P. aeruginosa ATCC27813.

References

- [1] D.T.Chu, P.B.Fernandes- *Advances in drug research*, 21, 39-144(1991)
- [2] M.Neuman-“*Vade-Macum Antibiotics*”, Paris (1989)
- [3] Lednicer D.; Mitscher L.A.-, *The Organic Chemistry of Drug Synthesis*, vol.4,141-145, (1990).
- [4] Scot L.Dax-“*Antibacterial Chemotherapeutic Agents*”, Blackie Academic&Profesional, London (1997).
- [5] H. Koga, A. Itoh, S. Murayama, S. Suzue, T. Irikura, - *J. Med. Chem.*, 23, 1358-1363 (1980)

P314

NEW CYCLOHEXYLIDENEHYDRAZIDE AND 1-THIA-4-AZASPIRO[4,5]DECAN-3-ONE DERIVATIVES OF 5-iodo-3-phenyl-1H-indole

Kazan F, Ozkirimli S, Çapan G

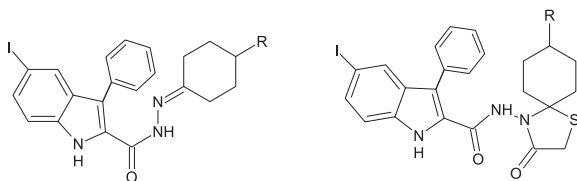
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Development of new active compounds for viral infections is a high priority goal. A number of diverse chemical structures have been shown to be potent RT-I inhibitors. Amongst them indole derivatives **Delavirdine** and **Ateviridine** are promising nonnucleoside RT inhibitors.

Their use as monotherapy may be limited because of rapid onset of resistance and hypersensitivity reactions. Therefore, there is still an existing need for new antiviral agents.

4-Thiazolidinones have been reported to exhibit antibacterial (1), antifungal (2) and antiviral (3) activities.

As a part of an ongoing project directed towards the design and synthesis of bioactive molecules, we prepared new 5-iodo-3-phenyl-N-[4-(non)substituted cyclohexylidene]-1*H*-indole-2-carbohydrazides and 5-iodo-3-phenyl-N-(3-oxo-1-thia-4-azaspiro[4,5]dec-4-yl)-1*H*-indole-2-carboxamides to investigate their antiviral potency.



R: H; 2-CH₃; 3-CH₃; 4-CH₃; 4-C₂H₅; 4-C₃H₇

References

- [1] Ur F, Cesur N, Birteksöz S, Otük G, *Arzneim.-Forsch./Drug Res* 2004; 54:125-129
- [2] Capan G, Ulusoy N, Ergenc N, Kiraz M. *Montsh Chem* 1999; 130: 1399-1407
- [3] Rao A, Balzarini J, Carbone A, Chimirri A, Clercq E, Monforte AM, Monforte P, Pannecouque C, Zappala M. *Farmaco* 2004; 59: 33-39

P315

SYNTHESIS AND ANTIMICROBIAL EVALUATION OF NOVEL BENZIMIDAZOLES

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Aromatic diamidines, such as pentamidine, hexamidine, propamidine, berenil, and related compounds are recommended for the treatment of various infectious diseases caused by bacteria, amoeba, fungi, and protozoa [1]. In particular pentamidine is a very effective drug against *Human African Trypanosomiasis*, *Leishmaniasis* and *Pneumocystis pneumonia*. However those drugs are characterized by important limitations (poor bioavailability and severe side effects) generally attributed to the high basicity of the amidine functions. In order to improve their pharmacological profile, we decided to include those amidine functions into conjugated structures and more particularly into benzimidazole ring systems.

The target compounds can be synthesized from α,ω -dibromoalkanes and hydroxybenzaldehydes, followed by a reaction with a 1,2-phenylenediamine. Selected bisbenzimidazoles were first synthesized under classical conditions. Our particular interest in the design of green protocols [2] led us to also perform the experiments under microwave irradiation. In such a way reaction times could

be reduced by a 50-fold factor. Overall yields ranged from 70 to 90 %.

Various antimicrobial tests have been realized and preliminary data indicated that amongst those derivatives some exhibited interesting properties as antileishmanial agents. It has also been demonstrated that those compounds had no bactericide effect.

Acknowledgements:

The authors are grateful to F.R.I.A. (doctoral grant, A.P.) and F.N.R.S. (J.J. VDE) for a financial support.

References:

- [1] M.N.C. Soeiro, E.M. De Souza, C.E. Stephens, D.W. Boykin, *Expert Opin. Investg. Drugs* 2005, 14, 957-972
- [2] J.J. Vanden Eynde, A. Mayence, *Intl.J. Med. Biol. Environ.* 2000, 28, 89-95

P317

GASTROPROTECTIVE EFFECT AND CYTOTOXICITY OF DITERPENES FROM THE CHILEAN LAMIACEAE SPHACELE CHAMAEDRYODES (BALBIS) BRIQ.

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The herb *Sphacele chamaedryodes* (Balbis) Briq. (Lamiaceae) is known under the common name "lahuen" and occurs in the coastal range of central Chile. Abietane diterpenes have been described as constituents of *Sphacele* species but *S. chamaedryodes* has been not previously investigated. Abietane diterpenes display several biological effects, including antiulcerogenic and antiproliferative activity in human tumoral cell lines (1, 2). Following our studies on bioactive terpenes from South American plants, we now report the isolation, gastroprotective effect and cytotoxicity of abietane diterpenes from "lahuen".

From the aerial parts of *S. chamaedryodes* five phenolic and five *p*-quinone abietanes, the sesquiterpene spathulenol and two flavonoids were isolated and characterized by spectroscopic means. The main diterpene from the plant was carnosol (7).

The cytotoxicity of the compounds was assessed on human lung fibroblasts and gastric adenocarcinoma cells (AGS). Cell viability was determined by means of the neutral red uptake assay. The compound 6,7-dehydroyleanone (1) and taxoquinone (5) showed low and selective effect towards fibroblasts and AGS cells, respectively. However, royleanone (2), 7,20-epoxyroyleanone (3), horminone (6), 7-oxo-11,12,14-trihydroxy-8,11,13-abietatrien-20-al (8) and spathulenol

proved to be highly cytotoxic with IC₅₀ values in the range of 11-67 μ M. According to our results, most of the assayed diterpenes were more cytotoxic than carnosol and the reference drug lansoprazole, presenting IC₅₀ values of 306 μ M for fibroblasts and 162 μ M for AGS cells, respectively. The selective cytotoxicity of compound **1** (IC₅₀: 61 and 366 μ M) and **5** (IC₅₀: 310 and 27 μ M) against AGS cells and fibroblasts, respectively, ameritate additional studies using different endpoints. No clear relationship between cytotoxicity and lipophilicity could be observed.

The gastroprotective effect of the compounds was studied on the HCl/EtOH-induced gastric lesions model in mice. A dose-response study of carnosol at 5, 10 and 20 mg/kg was performed to define the dose at which the naturally occurring diterpenes from the plant should be compared. Lansoprazole at 20 mg/kg reduced gastric lesions by 64.7% ($P < 0.01$) being statistically similar to carnosol at the doses of 10 and 20 mg/kg.

The eight compounds obtained in amount >3 mg were assessed at a single oral dose of 5 mg/kg. Regarding the compounds **1-3**, **5** and **6**, presenting a *p*-quinone moiety, the gastroprotective effect was 54.8, 71.0; 65.2, 36.2 and 52.9%, respectively. In this group, the best effect was elicited by royleanone (**2**), lacking functionality at C-7. In the C-7 hydroxylated derivatives, the activity was much lower for the 7 β -OH isomer. The phenolic diterpenes **7** and **8** inhibited gastric lesions by 49.8 and 53.4%, respectively being less active than lansoprazole. The most active plant constituents identified in the present work were royleanone (**2**) and its 7,20-epoxyde (**3**), reducing gastric lesions by 71 and 65.2%, respectively. Under the same experimental conditions, the effect of lansoprazole at 20 mg/kg was 64.7%. Additional studies are required to assess the action mechanism(s) of the active diterpenes from this Lamiaceae plant.

Supported by FONDECYT N° 1060841 and Programa de Productos Bioactivos, U. de Talca.

References

- [1] San Feliciano, A., Gordaliza, M., Salinero, M. A., Del Corral, J. M., 1993. Abietane acids: sources, biological activities, and therapeutic uses. *Planta Med.* 59, 485-490.
- [2] Gaspar, C., Pedro, M., Simoes, M.F.A., Nascimento, M.S.J., Pinto, M.M.M., Rodríguez, B., 2002. Effect of abietane diterpenes from *Plectranthus grandidentatus* on the growth of human cancer cell lines. *Planta Med.* 68, 839-840.

P318

SYNTHESIS AND ANTI-PNEUMOCYSTIS ACTIVITY OF NEW N-SUBSTITUTED BENZAMIDINES

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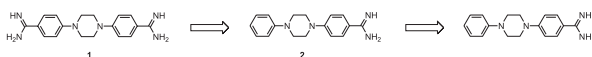
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Pneumocystis pneumonia is a fungal disease caused by a microorganism called *Pneumocystis jirovecii*. This lethal pathology affects immunosuppressed persons [1], like transplanted patients or AIDS infected people. Despite the importance of the disease, few treatments exist and resistance to classical medicines appears.

Few years ago, we showed that piperazine-1,4-bis-benzamidine **1** [2] forms a promising lead compound characterized by an anti-*Pneumocystis in vitro* activity at a micromolar concentration. Moreover, recent results obtained by our group showed that the simplification of the structure **1** to an analog bearing only one benzamidine function, does not cause a dramatic decrease of the antifungal activity. That interesting result encouraged us to prepare a library of monobenzamidines, analogs of **2**, substituted on the amidine function by alkyl or alkylaryl groups.



Particular care was taken in order to optimize the experimental conditions by performing some reactions under microwave irradiation.

All the prepared compounds were evaluated *in vitro* against *Pneumocystis carinii* rat strains at three concentrations (50 μ g/ml ; 10 μ g/ml and 0.1 μ g/ml). Interestingly, the nature of the substituent dramatically affects the antifungal activity.

Acknowledgments

The authors are grateful to F.R.I.A. (doctoral grant, D.S.) and F.N.R.S. (J.J.V.E.) for financial support.

References

- [1] B. D. Kim, *Prim Care Update Ob/Gyns*. 2002, 9, 41.
- [2] M.T. Cushion, P.D. Walzer, A. Ashbaugh, S. Rebholz, R. Brubaker, J.J. Vanden Eynde, A. Mayence, T.L. Huang, *Antimicrob. Agents Chemother.* 2006, 50, 2337.

P319

DISCOVERY OF CYTOTOXIC MACROLIDES FROM MARINE DINOFLAGELLATE

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[Purpose] Marine dinoflagellates *Amphidinium* species are known as producers of unique secondary metabolites. We have been isolated a series of cytotox-

ic macrolides from endosymbiotic dinoflagellates *Amphidinium* sp., and some of them exhibit potent cytotoxicity and antitumor activity [1]. In our continuing investigation for new anticancer drug leads from the dinoflagellate *Amphidinium* sp., we have developed the methodology for rapid screening of novel cytotoxic macrolides using genomics and metabolomics analyses. Application of the method for benthic *Amphidinium* dinoflagellates led to the discovery of the new *Amphidinium* strain HYA024, which produced unknown cytotoxic macrolides, and the isolation of a series of novel cytotoxic macrolides, designated as iriomoteolides from the strain. In this symposium, we describe the isolation and cytotoxic activities of iriomoteolides.

[Methods] Genomics analyses of 263 *Amphidinium* strains isolated from benthic sources collected off South-East Islands, Japan, were performed by using the one-cell PCR on the basis of the specific DNA primers for macrolide-producing *Amphidinium* strains. 38 strains positive in the PCR studies were cultivated in seawater medium. The extracts were further evaluated by cytotoxic screening against human B lymphoma cells and metabolomics analyses using HMQC and 2D-TLC to discover the ability producing novel cytotoxic macrolides for the *Amphidinium* strain HYA024 collected off Iriomote Island, Japan. The harvested algal cells obtained by mass-cultivation were extracted with MeOH/toluene. The toluene-soluble fractions of the extract were subjected to SiO₂ gel, C₁₈, and then NH₂-SiO₂ columns. Macrolide-containing fractions were separated by C₁₈ HPLC to afford iriomoteolides-1a, 1b, 1c, 2a, 3a, 4a, and 5a, and 7a [2–4]. Structures of iriomoteolides were elucidated on the basis of detailed analyses of 2D NMR spectra.

[Results and Discussion] Iriomoteolides-1a ~ 1c are novel 20-membered macrolides having a novel carbon skeleton with six C₁ branches. On the other hand, iriomoteolide-2a is the first 23-membered macrolide consisted of a successive carbon chain from natural sources, while iriomoteolides-3a and 7a are novel 15- and 17-membered macrolides with an allyl epoxide moiety, respectively. Iriomoteolide-4a is novel 16-membered macrolide having a unique carbon skeleton associated with five hydroxyl groups and an isoprene-like side chain, while iriomoteolide-5a is a novel 20-membered macrolide possessing 5-hydroxyl groups and three portions of vicinally locating C₁ branches. Iriomoteolides exhibited cytotoxic activity against human B lymphoma DG-75 cells. Particularly, iriomoteolides-1a and -2a showed 5 ~ 20 times potent than that of doxorubicin. Body text typed in Arial 10, with 14pt line spacing

References

- [1] Kobayashi, J.; Tsuda, M. *Nat. Prod. Rep.* 2004, 21, 77-93.
- [2] Tsuda, M.; Oguchi, K.; Iwamoto, R.; Okamoto, Y.; Kobayashi, J.; Fukushima, E.; Kawabata, J.; Ozawa, T.; Masuda, A.; Kitaya, Y.; Omasa, K. *J. Org. Chem.* 2007, 72, 4469-4474.

[3] Tsuda, M.; Oguchi, K.; Iwamoto, R.; Okamoto, Y.; Fukushima, E.; Kawabata, J.; Ozawa, T.; Masuda, A. *J. Nat. Prod.* 2007, 70, 1661-1663.

[4] Oguchi, K.; Tsuda, M.; Iwamoto, R.; Okamoto, Y.; Kobayashi, J.; Fukushima, E.; Kawabata, J.; Ozawa, T.; Masuda, A.; Kitaya, Y.; Omasa, K. *J. Org. Chem.* 2008 73, 1567-1570.

P320

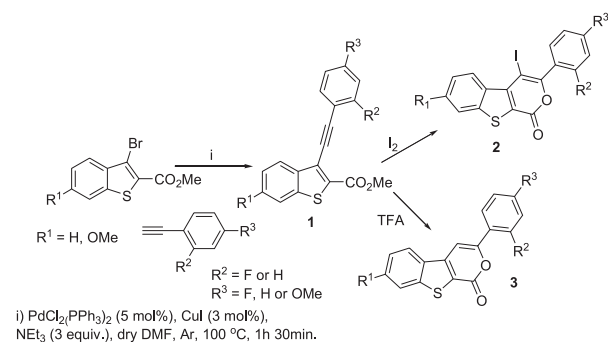
SYNTHESIS OF 3-(ARYL)BENZOTHIENO[2,3-c]-PYRAN-1-ONES FROM INTRAMOLECULAR LAC-TONIZATION OF SONOGASHIRA PRODUCTS. ANTI-TUMORAL EVALUATION AND SARs

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Several novel 3-(aryl)benzothieno[2,3-c]pyran-1-ones were prepared from Sonogashira coupling [1] products (**1**), using methyl 3-bromobenzo[*b*]thiophene-2-carboxylates and arylacetylenes. Compounds from groups **2** and **3** were obtained by regioselective intramolecular lactonization using iodine or TFA (Scheme 1).



Scheme 1

Around 20 derivatives were then investigated for their effect on the *in vitro* growth of three human tumour cell lines, MCF-7 (breast adenocarcinoma), SF-268 (central nervous system cancer), and NCI-H460 (non small cell lung cancer), using the protein-binding dye sulforhodamine B [2] assay, the method adopted by the National Cancer Institute (NCI, USA). Most of the compounds showed a high inhibitory effect on the growth of all tested cell lines, with GI₅₀ values in the μM range. It was possible to establish some structure-activity relationships for both structural groups. The best results among the Sonogashira products (**1**) were obtained for the compounds with R¹ = OMe and R² or R³ = F, while for the cyclized products **2** and **3** the most active derivatives were the ones with R¹ = OMe or H, R² = F and R³ = H, all showing GI₅₀ values around 10 μM.

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[1] Chinchilla R. and Nájera C. *Chem. Rev.* 2007, 107: 874-922.

[2] Monks A. *et al. J Natl Cancer Inst.* 1991, 83(11):757-766.

P321

THE CHARMM GENERAL FORCE FIELD (CGenFF): A CHARMM-COMPATIBLE FORCE FIELD FOR DRUG-LIKE MOLECULES¹

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The CHARMM Force Field² is widely used in computer-aided drug design for the study of target biological macromolecules. Apart from proteins, it supports nucleic acids and lipids, and has limited support for carbohydrates, with a full carbohydrate extension close to completion, allowing simulations on all commonly encountered motifs in biological systems. However, in computational medicinal chemistry, there is a high demand for simulations of biological systems interacting with drug candidates. While it is tempting to combine a biomolecular force field (like CHARMM) for a system's biological part with an organic force field (such as MMFF94) for its drug-like part, it is unlikely that this will yield properly balanced intermolecular interactions, because the nonbonded parameters are developed using different strategies for different force fields. To cure this problem, the CHARMM General Force Field (CGenFF)¹ was created. CGenFF is an organic force field explicitly aimed at simulating drug-like molecules in a biological environment represented by the CHARMM biomolecular force field.

A diverse set of more than 500 model compounds was used to parameterize CGenFF, including compounds from the bioorganic force field, general organic fragments, some cofactors, actual drugs and drug candidates. Lennard-Jones parameters were obtained from the existing CHARMM force field or optimized by reproducing experimental liquid densities and heats of vaporisation for a selection of model compounds. Partial charges for the 500+ model compounds were parameterized to reproduce water interactions and dipole moments that were determined using quantum chemical methods in the gas phase and scaled in order to be relevant for the condensed phase. For bonded parameters, high level quantum chemical equilibrium geometries, vibrational spectra

and potential energy surfaces were used as target data. Compared to other general force fields, CGenFF contains a relatively large number of atom types (more than 130) to allow for accurate parameterization over a wide chemical space. These atom types are organized hierarchically; for instance, the amide carbon type is part of the group of carbonyl carbon types, which is part of the larger group of sp² carbon types. If a parameter for a specific combination of atom types doesn't exist, this hierarchical organisation makes it easy to pick a parameter that was developed for closely related atom types. Specifically for torsions, which are ordinarily defined by 4 atoms (1-2-3-4), CGenFF also contains generic parameters which specify only the central atoms (2 and 3), and on which the force field can fall back if no exact match is found for the outer atoms (1 and 4). A similar scheme exists for improper dihedrals. It is anticipated that CGenFF will facilitate the application of CHARMM and the CHARMM biomolecular force fields to drug discovery.

References

[1] K. Vanommeslaeghe, C. Acharya and A. D. MacKerell Jr., *in preparation*

[2] A. D. MacKerell Jr., D. Bashford, M. Bellott, R. L. Dunbrack *et al.*, *J. Phys. Chem. B* 1998, 102, 3586–3616

P322

IDENTIFICATION OF BINDING SITES FOR BCL-6 INHIBITORS AND VIRTUAL SCREENING

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BCL6 is a potent transcriptional repressor with critical roles in several immunological processes, including generation and maintenance of memory T cells, chemokine gene expression in T helper cells and macrophages, and germinal center development in B cells, where its expression is downregulated upon selection for apoptosis or differentiation. BCL6 has been shown to play a central role in the pathogenesis of diffuse large B cell lymphoma, and inhibitors of BCL6-mediated repression show promise as therapeutic agents for B cell lymphomas.

The present work is part of a multidisciplinary collaboration aimed at developing novel anti-cancer drugs that

target BCL6. Our role in this collaboration is to assist in the discovery of new leads by means of *in silico* screening of a library of 943'566 commercially available compounds. Starting from X-ray structures provided by our collaborators,^{1,2} we first performed a Molecular Dynamics (MD) simulation that gave us insights into the flexibility and thermal motion of the BCL-6 protein, information used to account for protein flexibility in the docking procedure. The X-ray crystallography also led to the identification of BCL-6's protein-protein contact faces,^{1,2} on which the "binding response" algorithm³ was applied to identify "druggable" binding sites. These binding sites were subjected to a multi-step docking procedure, aimed at identifying promising BCL6 inhibitors to be obtained for experimental assay. In parallel, the same library was screened for compounds that are chemically similar to known BCL-6 inhibitors. In conclusion, a combination of ligand-based and structure-based drug design strategies was used to identify new leads in the search for anti-cancer drugs that target BCL6.

References

- [1] K. F. Ahmad, A. Melnick, S. Lax, D. Bouchard *et al.*, *Mol. Cell* 2003, 12, 1551–1564
- [2] A. F. Gethu, C. M. Corcoran, L. Cerchietti, V. J. Bardwell *et al.*, *Mol. Cell* 2008, 29, 384–391
- [3] S. Zhong and A. D. MacKerell Jr., *J. Chem. Inf. Model.* 2007, 47, 2303–2315

P323

MICROWAVE ASSISTED SYNTHESIS OF NEW 3-(1,4-DI-N-OXIDE QUINOXALIN-2-YL)-1-(4-METHOXY-PHENYL)-2-PROPEN-1-ONE DERIVATIVES

Saioa Ancizu, Raquel Villar, Beatriz Solano, Asunción Burguete, Esther Vicente, Elsa Moreno, Beatriz Ciordia, Enrique Torres, Silvia Pérez-Silanes, Ignacio Aldana and Antonio Monge

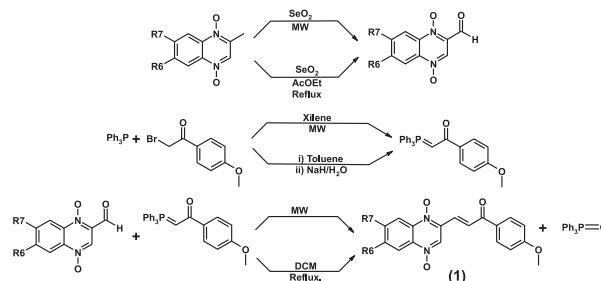
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Quinoxaline derivatives are compounds that show very interesting biological properties and their interest in medicinal chemistry is still in progress. The quinoxaline 1,4-di-N-oxide derivatives have shown promising anti-cancer activities [1, 2].

With the aim of obtaining new anticancer agents, we synthesized a novel series of 3-(1,4-di-N-oxide quinoxalin-2-yl)-1-(4-methoxyphenyl)-2-propen-1-one derivatives (**1**) through Wittig reaction [3, 4]. After a thorough study of the reaction conditions, we optimized a microwave assisted method (**scheme 1**). We carried out a comparative study with the unsubstituted compound (R6/R7=H) between the conventional method and microwave assisted method. Finally, we applied the new method in the synthesis of the other compounds. We con-

cluded that the use of the microwave radiation improves conversion ratios, decreases reaction times and minimizes quantities of solvents needed.

In addition, we tested some of the obtained derivatives for their *in vitro* anticancer activity and preliminary results have shown good cytotoxic activity.



Scheme 1. Synthesis of 1-(4-methoxyphenyl)-3-(R6,R7-1,4-di-N-oxide quinoxalin-2-yl)-2-propen-1-one derivatives

Acknowledgements

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References:

- [1] Zarranz, B.; Jaso, A.; Aldana, I.; Monge, A. *Bioorg. Med. Chem.* 2004, 12, 3711–3721.
- [2] Solano, B.; Junnotula, V.; Marín, A.; Villar, R.; Burguete, A.; Vicente, E.; Pérez-Silanes, S.; Aldana, I.; Monge, A.; Dutta, S.; Sarkar, U.; Gates, K.S. *J. Med. Chem.* 2007, 50, 5485–5492.
- [3] Westman, J. *Org. Lett.* 2001, 3, 3745–3747.
- [4] Kiddle, J.J. *Tetrahedron Lett.* 2000, 41, 1339–1341.

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ANTILEISHMANIAL ACTIVITY AND STRUCTURE-ACTIVITY RELATIONSHIP OF RING SUBSTITUTED 3-PHENYL-1-(1,4-DI-N-OXIDE QUINOXALIN-2-YL)-2-PROPEN-1-ONE DERIVATIVES

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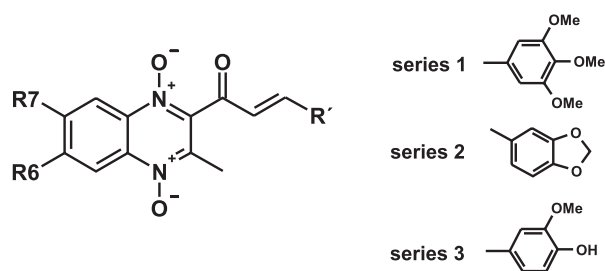
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Leishmaniasis is a tropical parasitosis caused by *Trypanosomatidae* of the *Leishmania* genus. It is estimated that 12 million people worldwide are infected. Therefore, the discovery of new active and promising compounds with antileishmanial potential remains essential for control and prevention of leishmaniasis. Quinoxaline derivatives have been reported to have antiprotozoal activity against malaria parasite and also against promastigotes forms of *Leishmania amazonensis* and *Leishmania infantum* strains [1].

As a continuation of our research in quinoxaline 1,4-di-*N*-oxide and with the aim of identifying new antileishmanial drug candidates, new series of ring substituted 3-phenyl-1-(1,4-di-*N*-oxide quinoxalin-2-yl)-2-propen-1-one derivatives have been synthesized [2] and tested for *in vitro* leishmanicidal activity against amastigotes of *Leishmania amazonensis* in axenical cultures and murine infected macrophages.

Compounds in series 1 and 3 present the best antileishmanial activities, showing IC₅₀ from 2 to 7.8 µM. Therefore, the radical methoxy at positions R₃, R₄ and R₅ seems to play an important role in the leishmanicidal activity against axenic amastigotes. (2*E*)-1-(7-Fluoro-3-methyl-1,4-dioxy-quinoxalin-2-yl)-3-(3,4,5-trimethoxyphenyl)-propenone is the most active compound and it also shows low toxicity. Studies of the impact of this product against macrophage respiratory burst reveal that its activity is not due to an enhancement of the respiratory burst.



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References:

- [1] Guillon, J.; Forfar, I.; Mamani-Matsuda, M.; Desplat, V.; Saliege, M.; Thiolat, D.; Massip, S.; Tabourier, A.; Leger, J.M.; Dufaure, B.; Haumont, G.; Jarry, C.; Mossalayi, D. *Bioorg. Med. Chem.* 2007, 15, 194-210.
- [2] Burguete, A.; Pontiki, E.; Hadjipavloy-Litina, D.; Villar, R.; Vicente, E.; Solano, B.; Ancizu, S.; Pérez-Silanes, S.; Aldana, I.; Monge, A. *Bioorg. Med. Chem. Lett.* 2007, 17, 6439-6443.

P325

NEW HETEROCYCLIC COMPOUNDS FROM 1,2,4-TRIAZOLE CLASS WITH POTENTIAL BIOLOGICAL ACTIVITY

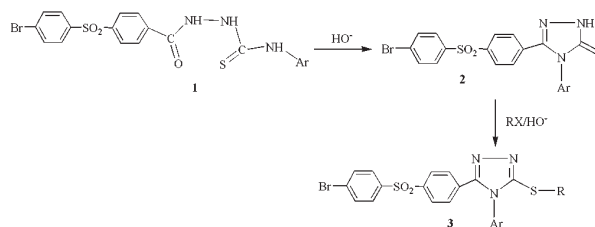
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Keywords: 1,2,4-triazole-3-thiones, alkylation, antimicrobial activity

1,2,4-Triazole and their derivatives are found to be associated with various biological activities such as: antibacterial, antifungal, anticonvulsant, anti-inflammatory, anticancer activity and anhydride carbonic inhibitors [1-8]. Moreover, several compounds containing 1,2,4-triazole ring are well known as drugs. For example, fluconazole, itraconazole and voriconazole are used as antimicrobial drugs [9], while vorozole, letrozole and anastrozole are non-steroidal drugs used for the treatment of cancer [10]. Based on these observations we have prepared a number of new compounds which contains 1,2,4-triazole moiety. The synthesis starting from 5-[4-(4-bromophenylsulfonyl)phenyl]-4-Ar-2,4,-dihydro-3*H*-1,2,4-triazole-3-thiones **2** obtained from acyltio-semicarbazides **1**, in basic media [11,12]. By treatment of 1,2,4-triazoles **2** with different alkylation agents, in basic media, new S-alkylated **3** compounds were obtained (scheme 1).



The structural elucidation of the new synthesized compounds has been performed by IR, ¹H-NMR, ¹³C-NMR, MS spectra and elemental analysis. Some newly synthesized compounds were tested for their antimicrobial effects.

References

- [1] Siddiqui A.A., Arora A., Siddiqui N., Misra A., Synthesis of some 1,2,4-triazoles as potential agents, *Indian J. Chem. Sect.B*, 2005, 44, 838-841
- [2] Abd El-Salam O.I., Mohamed S.F., Synthesis and preliminary anti-bacterial activity of some 4-substituted-N¹-2-pyridylsul-fanilamide derivatives, *J. Chin. Chem. Soc.*, 2005, 52, 1157-1164

- [3] Duran A., Dogan H.N., Rollas S., Synthesis and preliminary anticancer activity of new 1,4-dihydro-3-(3-hydroxy-2-naphthyl)-4-substituted-5H-1,2,4-triazoline-5-thiones, *II Farmaco*, 2002, 57 (7), 559-564
- [4] Al-Deeb O.A., Al-Omar M.A., El-Brollosy N.R., Habib E.E., Ibrahim T.M., Synthesis, antimicrobial, and antiinflammatory activities of novel 2-[3-(1-adamantyl)-4-substituted-5-tioxo-1,2,4-triazolin-1-yl]acetic acids, 2-[3-(1-adamantyl)-4-substituted-5-tioxo-1,2,4-triazolin-1-yl] propionic acids and related derivatives, *Arzneim.-Forsch./Drug Res.*, 2006, 56 (1), 40-47
- [5] Baluja S., Chanda S., Chabhadiya R., Kachhadia N., Nair R., Solanki A., A facile synthesis and antimicrobial activity of some 4-aryltriazoles, *J. Serb. Chem. Soc.*, 2007, 72 (6), 539-544
- [6] Kane J.M., Staeger M.A., Dalton C.R., Miller F. P., Dudley M.W., Ogden A.M.L., Kehne J.H., Ketteler H.J., McCloskey T.C., Senyah Y., Chmielewski P.A., Miller J.A., 5-Aryl-3-(alkylthio)-4H-1,2,4-triazoles as selective antagonists of strychnine-induced convulsions and potential antispastic agents, *J. Med. Chem.*, 1994, 37, 125-132
- [7] Gokce M., Cakir B., Erol K., Sahin M.F., Synthesis and antinociceptive activity of [(2-oxobenzothiazolin-3-yl)methyl-4-alkyl/aryl-1,2,4-triazoline-5-thiones, *Arch. Pharm.*, 2001, 334 (8-9), 279-283
- [126] Dogan H.N., Duran A., Rollas S., Synthesis and preliminary anticancer activity of new 1H-4,5-dihydro-3-(3-hydroxy-2-naphthyl)-4-substituted-1,2,4-triazoline-5-thione. Part. II, *Indian J. Chem. Sect. B.*, 2005, 44, 2301-2307
- [8] Almajan G. L., Innocenti A., Puccetti L., Manole G., Barbuceanu S., Saramet I., Scozzafava A. and Supuran C. T., Carbonic anhydrase inhibitors. Inhibition of the cytosolic and tumor-associated carbonic anhydrase isozymes I, II, and IX with a series of 1,3,4-thiadiazole- and 1,2,4-triazole-thiols, *Bioorg. Med. Chem. Lett.*, 2005, 15 (9), 2347-2352
- [9] Fromtling R.A., Overview of medically important antifungal azole derivatives, *Clin. Microbiol. Rev.*, 1988, 187-217
- [10] Clemons M., Coleman R.E., Verma S., Aromatase inhibitors in the adjuvant setting: bringing the gold to a standard?, *Cancer Treat Rev.*, 2004, 30 (4), 325-332
- [11] Saramet I., Almajan G.L., Barbuceanu S., Draghici C., Banciu M.D., Synthesis of some substituted aroylthiosemicarbazides, -mercaptotriazoles and -aminothiadiazoles, *Rev. Roum. Chim.*, 2005, 50 (1), 19-27
- [12] Barbuceanu S.-F., Almajan G. L., Saramet I., Draghici C., Noi compusi heterociclici din clasa 1,2,4-triazolilor si a 1,3,4-tiadiazolilor cu potentiala activitate biologica, *Rev. Chim. (Bucuresti)*, 2006, 57 (4), 355-360

P326

SCAFFOLD HOPPING BETWEEN IMIDAZOLE AND PYRIDINE – SYNTHESIS AND BIOLOGICAL EVALUATION OF NOVEL p38 MAP KINASE INHIBITORS

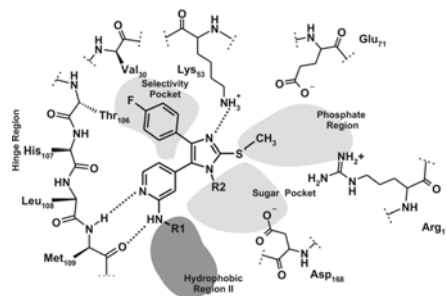
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Imidazoles represent important bioactive scaffolds in medicinal chemistry. 1,4,5-trisubstituted 2-sulfanylimidazoles target p38 mitogen-activated protein (MAP) kinases. p38 MAP kinases play a central role in the signaling network responsible for the upregulation of proinflammatory cytokines like IL-1 β and TNF- α . Nonphysiological increased cytokine levels are part of the pathogenesis of chronic inflammatory diseases like rheumatoid arthritis, osteoarthritis or Crohn's disease. Hence p38 MAP kinase is a valid target for small molecule anti-inflammatory drugs. 2-Sulfanylimidazole derivatives offer advantages over prototype inhibitors (SB203580), e.g. lower cytochrom P450 interactions and better kinetic properties [1, 2].

Essential interactions of the pyridinylimidazole inhibitors with the ATP-binding cleft are accomplished by hydrogen bond donor or acceptor functions of the 2-amino-pyridyl residues within the hinge region and space-filling lipophilic aryl residues like the 4-fluoro-phenyl residue, binding to the selectivity pocket [2].



In our recent studies we have extensively varied the R1 and R2 substituents at pyridine C-2 and imidazole N-1 positions by scaffold hopping of identical residues between the imidazole N-1 and the pyridine C-2 positions. Synthesis largely followed our previously published procedures [3]. Different residues at imidazole N-1 were introduced by use of the respective substituted triazinanes. These were synthesised by adding paraformaldehyde to a stirred solution of the amines in absolute ethanol. The same residues were introduced at the pyridine C-2 position by nucleophilic substitution of the respective 2-fluoropyridine precursor. Residues R1 and R2 may partly compete for occupying the same space. On the other hand characteristic interactions with the enzyme's sugar pocket or hydrophobic region II may be

expected depending on the substitution on R1 or R2 position. The 2-amino-pyridyl-residue and the 4-fluoro-phenyl residue are necessary mainly to gain selectivity. R1 or R2 positions are preferred positions to modify physicochemical properties and to enhance binding affinity [2]. With our study we have been able to evaluate the optimal substitution patterns for the tested residues in order to improve both p38 inhibition and physicochemical properties.

References

- [1] Laufer, S.; Hauser D.; Liedtke, A; *Synthesis* 2008, No. 2, 253–266
- [2] Laufer, S.; Hauser D.; Liedtke, A; *Journal of Medicinal Chemistry* 2008, in press
- [3] Laufer, S.; Wagner, G.; Kotschenreuther, D.; Albrecht, W.; *Journal of Medicinal Chemistry* 2003, No. 46, 3230-3244

P327

DISCOVERY OF PYRIMIDO[5,4-E][1,2,4]TRIAZINE-5,7(1H, 6H)-DIONES WITH COMBINED INHIBITORY ACTIVITY ON AKT3, CDK4 AND CDC25B

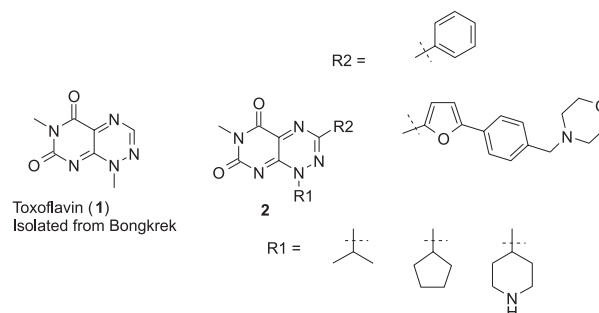
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Toxoflavin (1), which belongs to the naturally occurring pyrimido[5,4-e][1,2,4]triazines was first isolated from a coconut-based dish known in Indonesia as “bongkre” by Mertens and van Veen as early as 1931. Due to their versatile biological activity *viz.* antiviral, anti-tumour and antibiotic activity, pyrimido[5,4-e][1,2,4]triazines have attracted much attention from organic chemists and biologists. A series of toxoflavin derivatives (pyrimido[5,4-e][1,2,4]triazine-5,7(1H, 6H)-diones) was identified as potent inhibitors of Akt3, CyclinD1-Cdk4 and the phosphatase Cdc25b on the basis of their *in vitro* enzymatic activity. In cells they were shown to inhibit AKT3 and growth ($pIC_{50} > 7$). Several drawbacks of the initial series of compounds were linked to the solubility and metabolic instability. The latter was related to the presence of primary alkyl functions at the N1 position of the bicyclic scaffold. A chemistry research program, aimed at improving the physicochemical characteristics of the compounds, was focused on (1) introduction of a series of secondary alkyl functions (R1) at N1, (2) incorporation a variety of water enhancing moieties predominantly at R2.

A small number of compounds have been selected for *in vivo* evaluation in ovarian carcinoma (A2780) bearing nude mice. For each compound, the maximum tolerable dose (MTD) was determined upon a 10-day treatment

(oral administration, daily) and PK profile was obtained after single oral administration at 10 mg/kg. The compounds have been evaluated for anti-tumor efficacy using oral daily dosings at the MTD, 1/2 MTD and 1/4 MTD for 28 days. No significant anti-tumor effect of the compounds could be shown.



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UR-PI376: A NEW POTENT AND SELECTIVE HISTAMINE H₄ RECEPTOR AGONIST

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The human histamine H₄ receptor (hH₄R) belongs to the superfamily of G-protein coupled receptors and was identified by various research groups in the year 2000 based on its high homology with the hH₃R. The results of several investigations suggest that the hH₄R plays a crucial role in immunological and inflammatory processes [1]. Selective ligands, particularly agonists, are needed to pharmacologically characterize and further explore the (patho)physiological role of the hH₄R.

In our department structural modifications of the potent H₂R agonist arpromidine [2] were performed, resulting in *N*⁹-acylated imidazolypropylguanidines, a new class of guanidine-type H₂R agonists with reduced basicity [3-6]. Surprisingly, more detailed pharmacological investigations showed that several of these compounds are also (partial) agonists with potencies in the nanomolar range at the hH₃R and hH₄R.

This unexpected result prompted us to synthesize related compounds with the aim to get access to HR subtype selective compounds, in particular hH₄R agonists, and to develop such compounds as pharmacological tools.

Exchange of the acyl-guanidine moiety with a non-basic cyanoguanidine group (Fig. 1) yielded a series of potent hH₄R agonists devoid of agonistic activities at other HR subtypes. Potencies and intrinsic activities of these compounds were determined in steady-state GTPase assays using Sf9 insect cells expressing the respective hHR subtypes.

UR-PI376 turned out to be the most potent and selective hH₄R agonist in this series (Fig. 2), displaying a more

than 25-fold selectivity relative to the hH₃R and negligible activities at hH₁R and hH₂R. In contrast to other selective hH₄R agonists such as 5-methylhistamine [7] or the chiral tetrahydrofuran derivative OUP-16 [8], UR-PI376 is devoid of noteworthy agonistic activity at other HR subtypes. Due to this distinct pharmacological profile UR-PI376 will be a valuable pharmacological tool for the functional characterization of the H₄ receptor.

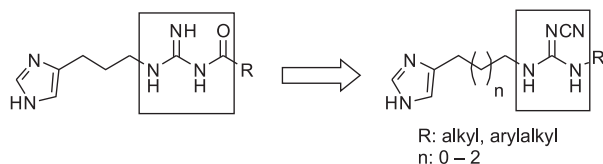


Fig. 1: Replacement of the basic acylguanidine moiety with a non- basic cyanoguanidine group

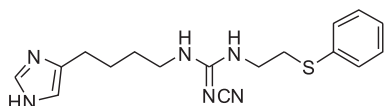


Fig. 2: The potent and selective hH₄R agonist UR-PI376 (EC₅₀ = 34 nM, E_{max} = 0.93)

References

- [1] de Esch I. et al., *Trends Pharmacol. Sci.* 2005, 26, 462 – 469.
- [2] Buschauer A., *J. Med. Chem.* 1989, 32, 1963 – 1970.
- [3] Ghorai P., *PhD thesis*, University of Regensburg, 2005.
- [4] Kraus A., *PhD thesis*, University of Regensburg, 2007.
- [5] Xie S.- X. et al., *J. Pharmacol. Exp. Ther.* 2006, 317, 139 – 146.
- [6] Xie S.- X. et al., *J. Pharmacol. Exp. Ther.*, 2006, 317, 1262 – 1268.
- [7] Lim H. D. et al., *J. Pharmacol. Exp. Ther.* 2005, 314, 1310-1321.
- [8] Hashimoto T. et al., *J. Med. Chem.*, 2003, 46, 3162 – 3165.

P330

A NEW CHALCONOID LIBRARY OF COMPOUNDS ACTIVE AGAINST MDR MYCOBACTERIUM TUBERCULOSIS

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Tuberculosis (TB) is one of the most devastating infectious diseases in the world, responsible for 2-3 million deaths annually, approximately 5000 people per day. There are estimated 8 million of new cases each year, 95% of which occur in developing countries^{1,2}. Drugs such as isoniazid (INH), Rifampicin (RMP) and ethambutol (EMB) and their combinations have historically been successful in the treatment of TB infections. In the recent past, however, poor compliance with the prolonged and complicated dosage regimens currently used to treat the disease, in conjunction with the advent of the AIDS epidemic and the increased mobility of populations, has led to the emergence of numerous multidrug-resistant (MDR) and even extremely-resistant (XDR) strains of *Mycobacterium tuberculosis*³. Resistance to the frontline agents, mainly INH and RMP, results in the treatment of patients with second-line agents. Among these second-tier drugs, thiacetazone, being used in Africa and South America, has shown to be a very effective when administered in combination with INH.⁴

The number of compounds reported in the literature based on natural products and tested on *Mycobacterium tuberculosis* experiments are huge, one of those are the chalcone derivatives.⁵ Our research group has obtained fifty-five chalconoids derived from benzylated vanillin,⁶ 2-naphthyl-acetophenone and 3,4-methylenedioxyacetophenone. The compounds were obtained by aldolic condensation between acetophenones with the corresponding aldehydes, in basic conditions (KOH 50% v/v), at room temperature for 24 hours.⁷ Compounds of type 1 with varied substituents R¹ and R² were tested *in vitro* on *M. tuberculosis* H37Rv and MDR-TB strains. The most potent compounds showed MIC values of 3.12-1.56 µM.

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References

- [1] World Health Organization. *WHO report* 2006.
- [2] Meya DB, McAdam KP. *J. Intern. Med.* 2007, 261, 309-29.
- [3] Mitchison DA. *Eur. Respir. J.* 2005, 376-9.
- [4] Houston, S.; Fanning, A. *Drugs*, 1994, 48, 689.
- [5] Lin Y, Zhou Y, Flavin MT, Zhou L, Nie W, Chen F. *Bioorg Med. Chem* 2002, 10, 2795-2802. Lall N, Hussein AA, Meyer JJ. *Fitoterapia* 2006, 77, 230-2.
- [6] Tsai S, Klinman JP. *Bioorg Chem* 2003, 31, 172-190.
- [7] Vogel AI, *Vogel's Textbook of Practical Organic Chemistry*, 5 ed. New York: John Wiley & Sons, 1989. p.1017.

P331

ENANTIOSELECTIVE SYNTHESIS OF PIRINIXIC ACID BASED DUAL PPAR α/γ AGONISTS

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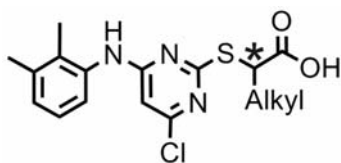
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The peroxisome proliferator activated receptor (PPAR) is a ligand dependent transcription factor playing a crucial role in the regulation of lipid and glucose homeostasis. There are three PPAR subtypes: α , δ and γ . Currently PPAR α selective fibrate and PPAR γ selective glitazone type of drugs are in clinical use for the treatment of dyslipidemia and type 2 diabetes, respectively. Dual agonists of both subtypes are in clinical development with the intention to combine both therapeutical benefits.

Previous SAR-studies in our groups indicated that the substituent in α -position of the carboxyl group plays an important role for the potency of PPAR α/γ transactivation^[1]. An enhancement of the activity of the moderately dual PPAR α/γ agonist pirinixic acid was achieved by aliphatic substitution of the α -position. The most potent compounds were the racemates of those at a chain length of four and six carbons.

For other dual PPAR α/γ -agonists distinct enantiomeric activity is shown. For example, there are significant differences of the enantiomers of Ragaglitazar concerning PPAR-activity^[2]. PPAR α and PPAR γ docking analyses of our compounds suggested the (*R*)-enantiomers to be the eutomers, showing no reasonable docking solutions for the (*S*)-enantiomers. To investigate this issue, we employed an enantioselective synthesis using chiral auxiliaries to prepare α -alkylated pirinixic acid derivatives. We were able to obtain an enantiomeric excess of up to 90 percent; determined by enantioselective HPLC with a Chiralpak QD-AX column. For PPAR γ we observed only minor differences between the distinct enantiomers, while for PPAR α the *R*-configured derivative is the more active compound substantiating the docking studies.



[1] O.Rau, Y. Syha, H. Zettl, M. Kock, A. Bock, M. Schubert-Zsilavecz; *Archiv der Pharmazie*, 2008, 341, 191-195

[2] S. Ebdrup et al.; *J. Med. Chem.*, 2003, 46, 1306-1317

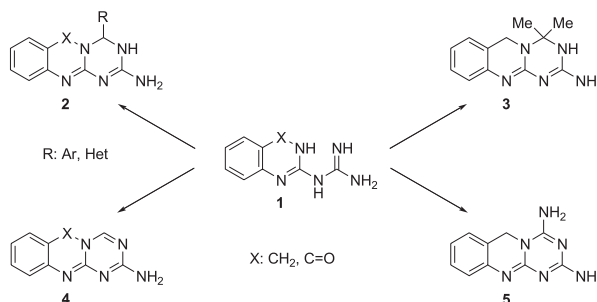
P332

QUINAZOLYLGUANIDINES IN THE SYNTHESIS OF NEW POTENTIAL ANTICANCER AGENTS

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One of the strategies that we have used in our program on the development of the synthesis of new biologically active fused 1,3,5-triazines involves reactions of azaheterylguanidines with one-carbon inserting reagents [1-8]. Recently, we have developed a new method for the preparation of 2-amino-4-(het)aryl-4,6-dihydro-1(3)(11)*H*-[1,3,5]triazino[2,1-*b*]quinazolin-6-ones (**2**, X = O) via regioselective 1,3,5-ring annulation to quinazoline nucleus [7]. Herein we report synthesis of their methylene analogues (**2**, X = CH₂). The synthetic potential of quina-zoliny-2-guanidines **1** was further extended to the preparation of other 1,3,5-triazino[2,1-*b*]quinazolines (e.g. **3-5**), including *gem*-dimethyl analogue **3** of previously reported antifolate 1,3,5-triazino[1,2-*a*]benzimidazole [2, 6]. The structural aspects, including tautomerism of the compounds obtained were investigated. The antiproliferative activity of the compounds against lung, breast and colorectal cancer cell lines was evaluated using MTT assay. The compounds **2** possessed antiproliferative activity in micromolar range. A variety of this type of compounds was prepared and structure-activity relationship was explored. The mechanism of the anticancer effect of the compounds was not associated with dihydrofolate reductase inhibition; further investigations for the identification of the targets are ongoing.



References

- [1] Dolzhenko, A.V.; Chui, W.K.; Dolzhenko, A.V.; Chan, L.W. *J. Fluorine Chem.* 2005, 126, 759-763.
- [2] Dolzhenko, A.V.; Chui, W.K. *J. Heterocycl. Chem.* 2006, 43, 95-100.
- [3] Dolzhenko, A.V.; Chui, W.K.; Dolzhenko, A.V. *Synthesis* 2006, 597-602.
- [4] Dolzhenko, A.V.; Chui, W.K.; Dolzhenko, A.V. *J. Heterocycl. Chem.* 2006, 43, 1513-1521.

- [5] Dolzhenko, A.V.; Dolzhenko, A.V.; Chui, W.K. *Tetrahedron* 2007, 63, 12888-12895.
- [6] Dolzhenko, A.V.; Chui, W.K. *Pharm. Chem. J.* 2007, 41, 470-473.
- [7] Dolzhenko, A.V.; Dolzhenko, A.V.; Chui, W.K. *J. Heterocycl. Chem.* 2008, 45, 173-176.
- [8] Dolzhenko, A.V.; Tan, B.J.; Dolzhenko, A.V.; Chiu, G.N.C.; Chui, W.K. *J. Fluorine Chem.* 2008, 129, 429-434.

P333

TOTAL SYNTHESIS OF α -(-)-CONHYDRINE VIA REGIOSELECTIVE AND DIASTEREOSELECTIVE AMINATION WITH CHLOROSULFONYL ISOCYANATE
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Naturally occurring polyhydroxylated indolizidine, pyrrolizidine, pyrrolidine, piperidine and nortophane has attracted a lot of attention from the synthetic community because of their interesting structures and glycosidase inhibitory activity. Glycosidase inhibitors are known to be used for treating diabetes, viral diseases (HIV and influenza), bacterial infections, cancer and immunological disorders.

Chiral oxazolidin-2-ones are a very interesting intermediate in the synthesis of 1,2-amino alcohols as well as a potential protecting group for vicinal 1,2-amino alcohols, and ideally functionalized for elaboration into polyhydroxylated indolizidine alkaloid, such as swainsonine, and castanospermine.

Recently, we reported a novel synthetic strategy for the synthesis of allylic 1,2-*anti*-amino alcohol with use of chlorosulfonyl isocyanate(CSI) and its application to the synthesis of various polyhydroxylated alkaloids including DAB1, (-)-lentiginosine, *trans*-3-hydroxy-pipecolic acid, and etc.

As a program part of our research aimed at developing enantioselective syntheses of polyhydroxylated alkaloids, we became interested in developing an efficient route for synthesizing (+)-conhydrine, which has been isolated from seeds and leaves of the poisonous plant *Conium maculatum* L. in 1856.

Because of their potent bioactivities and relatively simple structures, numerous ways of synthesizing (+)-conhydrine and their isomers have recently been reported.

In this presentation, we demonstrate a new synthetic approach to chiral oxazolin-2-one, a versatile intermediate in the synthesis of 1,2-amino alcohols, from allylic oxirane as an extension of the CSI reaction. Furthermore, we describe a novel enantioselective synthesis of α -(-)-conhydrine via the regioselective and diastereoselective amination of allylic *cis*-oxirane using CSI and ring-closing metathesis.

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THE ROLE OF STEROLS IN CANCER. SYNTHESIS AND CELL VIABILITY OF OXYSTEROLS IN TUMOR AND NORMAL CELL LINES

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Sterols are a chemical group of compounds characterized by the presence of an unsaturated tetracyclic hydrocarbon structure, with a 3- β hydroxyl group and an aliphatic side-chain at the 17- β position. These chemical features render these compounds amphiphilic and are of utmost importance for its incorporation into the lipid bilayers of the cytoplasmatic membrane. In fact, sterols are present in the membranes of all eukaryotic forms of life, being essential for their formation, maintenance and vital fluid characteristics. Without sterols, cells would need a cell wall and though superior life forms wouldn't exist.

Previous studies have shown that oxygenated sterols are cytotoxic and inhibit the growth of various cell types (1), however the mechanism by which they exert cytotoxicity remains largely unknown.

Cancer is one of the leading causes of death in the world. Can oxygenated sterols be an interesting therapeutic approach in the battle against cancer?

Frequently patients with malignant tumors exhibit cancer cachexia, a state of metabolic dysfunction, resulting from a metabolic demand of cancer cells (2). It is known that tumor cells have increased requirements in cholesterol (3) and glucose (4) relatively to normal cells, which replicate in a slower rate. Therefore cytotoxic sterols and glycosyl derivatives can be an efficient and wise solution to target cancer cells.

The purpose of this work was to synthesize oxygenated cholesterol compounds, their glycosyl derivatives and sitosterol oxygenated counterparts and to evaluate their cytotoxic potential in tumor and normal cell lines.

Biological results point to a significant cytotoxic effect of some sterols and structure-activity correlations have been set up.

Since some of the tested sterols can be absorbed from nutritional sources or produced by the human body, mainly as precursors of bile acids, a question can be raised. Do these oxysterols are beneficial or deleterious or even mere "passengers" in the human body?

References

- [1] Wielkoszynski, T. *et al BioEssays* (2006) 28: 387–398; Bradford P. G. and Awad A. B., *Mol. Nutr. Food Res.* (2007) 51: 161 – 170.
- [2] Tisdale M. J., *Nutrition* (2000) 16: 1013-1014.
- [3] Batetta B. and Sanna F., *Eur. J. Lipid Sci. Technol.* (2006) 108: 687–69.
- [4] Shaw S. Reuben, *Current Opinion in Cell Biology* (2006) 18: 598–608.

P335

A SERIES OF 3-AMINOPYRAZOLES AS POSITIVE ALLOSTERIC MODULATORS OF THE ALPHA 7 NICOTINIC ACETYLCHOLINE RECEPTOR

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The $\alpha 7$ nAChR is a pentameric, calcium permeable, ligand-gated ion channel that modulates fast synaptic transmission in the hippocampus, thalamus and cerebral cortex, three of the key regions of the CNS involved in learning and memory. The $\alpha 7$ nAChR also displays rapid desensitization, and so enhancing the receptor function via a positive allosteric modulator (PAM) may prove advantageous compared to a direct agonist. Using high-throughput screening of our compound file we have discovered a novel 3-aminopyrazole series of $\alpha 7$ nAChR PAMs and the preliminary SAR of this series will be discussed. A representative analog was shown to activate $\alpha 7$ -containing nAChRs of rat hippocampal neurons and attenuate d-amphetamine-evoked dopamine release in the rat striatum.

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CONFORMATIONALLY CONSTRAINED AZABUTYROPHENONES AS POTENTIAL ATYPICAL ANTIPSYCHOTICS

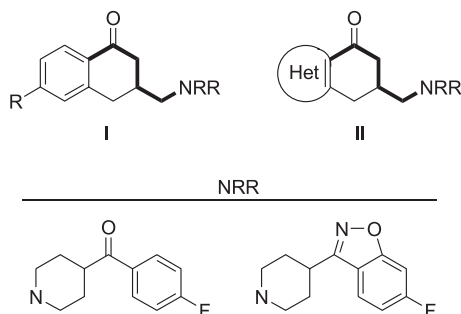
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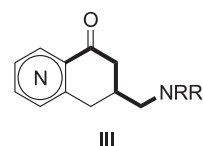
Atypical antipsychotic agents offer improved treatment of schizophrenia by combining efficacy with less propensity to cause harmful CNS side effects. Traditional attention in this field has been focused on the development of compounds that act on both dopamine and serotonin receptors. Atypical antipsychotics possess higher antagonist affinity at the serotonin 5-HT_{2A} receptor as compared with the dopamine D₂ receptor. Potential atypicality has been correlated with the ratio 5-HT_{2A}/D₂ receptor binding affinity; atypical antipsychotics show a ratio > 1.12 whereas typical neuroleptics show a ratio < 1.09.¹ It

has been shown that blockade of 5-HT_{2C} receptors and/or inverse agonist efficacy is another important feature for discriminating classical from atypical antipsychotics. It has been suggested that modulation of 5HT_{2C} receptor signaling improved the clinical response to D₂ receptor blockade by antipsychotics.^{2,3}

Previous publications of these Laboratories^{4,5} have described the synthesis of a series of atypical antipsychotic agents based on the butyrophenone structure where the aminobutyl side chain is partially incorporated in a conformationally restricted benzo- or heterocycloalkanone framework (I, II). These compounds showed good affinity at D₂ and 5-HT_{2A} with ratio values of pK_i in the range 1.08-1.20, and are devoid of cataleptogenic activity.



In this communication, we will describe our recent efforts to discover novel templates in the area of selective dual 5-HT_{2A}/D₂ antagonists for potential use as treatments for schizophrenia. A typical bioisosteric replacement of the benzene in the aminobutyrophenone pharmacophore by N-containing heterocycles such as pyridine, pyrimidine or pyridazine, has been applied to afford aza-analogues (III) with the quinoline, quinoxaline or cinnoline cores, respectively.



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References:

- [1] Meltzer, H. Y.; Matsubara, S.; Lee, J. C. *J. Pharmacol. Exp. Ther.* 1989, 25, 238-246.
- [2] Reavill, C.; Kettle, A.; Holland, V.; Riley, G.; Blackburn, T. P. *Br. J. Pharmacol.* 1999, 126, 572-574.
- [3] Richtand, N. M.; Welge, J. A.; Logue, A. D.; Keck, P. E., Jr.; Strakowski, S. M.; McNamara, R. K. *Neuropsychopharmacology* 2007, 32, 1715–1726.

[4] Raviña, E.; Masaguer, C. F. *Current Medicinal Chemistry: Central Nervous System Agents* 2001, 1, 43-62.

[5] Dezi, C.; Brea, J.; Alvarado, M.; Raviña, E.; Masaguer, C. F.; Loza, M. I.; Sanz, F.; Pastor, M. J. *Med. Chem.* 2007, 50, 3242-3255, and references cited therein.

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PHOSPHOINOSITIDE-3-KINASES (PI3KS): A MOLECULAR MODELLING APPROACH TO DRUG DISCOVERY

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Phosphoinositide 3-kinases (PI3Ks) constitute a class of enzymes that catalyze phosphorylation of the 3-hydroxyl position of phosphoinositides (PIs). The resulting second messengers, phosphatidylinositol 3,4-bisphosphate (PIP2) and phosphatidylinositol 3,4,5-trisphosphate (PIP3), can regulate a remarkably diverse array of physiological processes, including glucose homeostasis, cell growth, differentiation and motility. Eight related PI3Ks, possessing unique substrate specificity, localization and mode of regulation, have been identified in vertebrates. These include the class IA PI3Ks (p110 α , p110 β , p110 δ), which are activated by receptor tyrosine kinases, and the class IB PI3K (p110 γ), which is activated by heterotrimeric G-proteins. The exact roles of the individual isoforms are beginning to emerge.^{1,2}

The recent discoveries that the p110 α isoform undergoes gene amplification and is frequently mutated in primary tumors, together with evidence that PTEN, a lipid phosphatase which acts as a negative regulator, is a commonly inactivated tumor suppressor, have genetically validated p110 α as an attractive target for cancer therapy. This has fueled drug development, with intensive efforts underway to develop selective inhibitors of the ATP-binding site of p110 α as anticancer agents. The selective inhibition of p110 γ/δ and p110 β has also been proven valuable in the fields of inflammation, allergy, and thrombosis, respectively.



Fig. 1 Interaction model for the binding of PIK75 within p110 α

In the present communication, an integrated interaction model derived from the combined use of target-based (homology modeling and docking) and ligand-

based (3D-QSAR) approaches and capable of correlating the chemical structures of p110 α selective inhibitors (PIK75) with their biological activities (Fig. 1) is reported.³ A virtual screen that led to the discovery of new PI3K inhibitors will also be introduced.⁴

References

- [1] Knight, Z. A.; Gonzalez, B.; Feldman, M. E.; Zunder, E. R.; Goldenberg, D. D.; Williams, O.; Loewith, R.; Stokoe, D.; Balla, A.; Toth, B.; Balla, T.; Weiss, W. A.; Williams, R. L.; Shokat, K. M. *Cell* 2006, 125, 733.
- [2] Chaussade, C.; Rewcastle, G. W.; Kendall, J. D.; Denny, W. A.; Cho, K.; Grønning, L. M.; Chong, M. L.; Anagnostou, S. H.; Jackson, S. P.; Daniele, N.; Shepherd, P. R. *Biochem. J.* 2007, 404, 449
- [3] Frédéric, R.; Denny, W.A. *J Chem Inf Mod* 2008, 48(3), 629-638
- [4] Frédéric, R.; Mawson, C.; Chaussade, C.; Kendall, J. D.; Shepherd, P. R.; Denny, W. A. to be published

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DESIGN AND SYNTHESIS OF NOVEL ARYLSULFONOHYDRAZIDE INHIBITORS OF MURC

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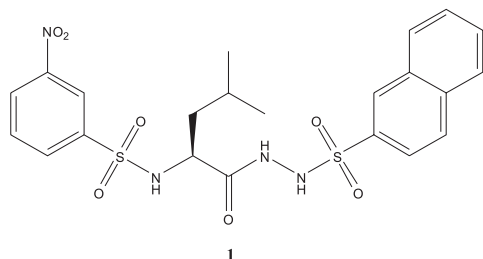
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UDP-*N*-acetylmuramyl-L-alanine ligase (MurC), is an essential cytoplasmic peptidoglycan biosynthetic enzyme which catalyzes the ATP-dependent ligation of L-alanine (Ala) and UDP-*N*-acetylmuramic acid (UNAM) to form UDP-*N*-acetylmuramyl-L-alanine (UNAM-Ala). (1) Mur ligases are highly specific and they are present only in eubacteria, thus making them attractive as targets for development of new therapeutic agents against bacterial infection (2).

In our research, a series of structurally different MurC inhibitors were designed using rational approach to the synthesis of new sulfonohydrazide analogues. Using X-ray structure of MurC co-crystallized with UNAM-Ala and Sprout (3) as a software tool for *de-novo* ligand design, a different sets of potential inhibitors were selected and redocked inside protein using Ehits[®] (4) and AutoDock (5). In our preliminary computer aided studies sulfonohydrazide moiety corresponded to one of the phosphates in the diphosphate moiety of UNAM-Ala while the leucine corresponded to the sugar moiety in the UNAM-Ala. The most promising hits were synthesized and tested for the inhibitory activity. Compound 1 was found active against *E.coli* MurC and was found to possess antibacterial activity against MRSA with MIC of 128 μ g/mL.

This work was supported by the European Union FP6 Integrated Project EUR-INTAFAR (Project No. LSHM-CT-2004-512138) under the thematic priority of Life Sciences, Genomics and Biotechnology for Health. The support from the Ministry of Higher Education, Science and Technology of the Republic of Slovenia and the Slovenian Research Agency is also acknowledged.



1
MIC (MRSA) = 128 µg/mL
RA (245 µmol/L) = 49%

References

- [1] Mengin-Lecreux, D.; Flouret, B.; van Heijenoort, J. Cytoplasmic steps of peptidoglycan synthesis in *Escherichia coli*. *J. Bacteriol.* 1982, **151**, 1109-1117.
- [2] El Zoeiby, A.; Sanschagrin, F.; Levesque, R.C. Structure and function of the Mur enzymes: development of novel inhibitors. *Mol. Microbiol.* 2003, **47**, 1-12.
- [3] Gillet, VJ; Johnson, AP; Mata, P; Sike S; Williams, P. "SPROUT - a program for structure generation" *J. Comput.-Aided Mol. Des.* 1993, **7**, 127- 153.
- [4] Zsoldos, Z.; Reida, D.; Simona, A.; Sadjada, SB; Johnson, AP. eHITS: A new fast, exhaustive flexible ligand docking system *J. Mol. Graphics Modelling.* 2007, **26**, 198-212.
- [5] Morris, GM; Goodsell, DS; Halliday, RS; Huey, R; Hart, WE; Belew, RK; Olson, AJ. Automated Docking Using a Lamarckian Genetic Algorithm and an Empirical Binding Free Energy Function *J. Comp. Chem.* 1998, **19**, 1639-1662.

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GALACTOSTATIN ANALOGUES FEATURING DOUBLE HEADED 5-N-SUBSTITUENTS: POTENTIAL CHEMICAL CHAPERONES FOR GALACTOSIDASE-DEFICIENCY BASED LYSOSOMAL STORAGE DISEASES?

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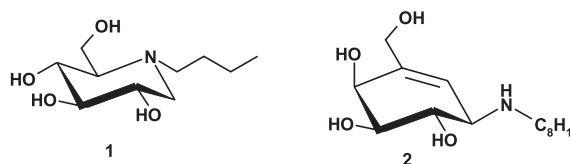
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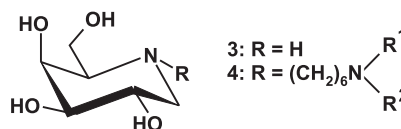
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Chaperone mediated therapy has recently emerged as a promising therapeutic principle for the improvement of conditions caused by various types of hereditary lysosomal storage diseases. For example, *N*-butyl-1-deoxynojirimycin **1** [1] as well as the 4-epi derivative of *N*-octylvalienamine **2** [2] have been shown to exert positive effects on the correct folding of mutant enzymes, thus restoring glycosidase activities to extents useful for therapeutic application.



Consequently, in context with β -galactosidase deficiencies GM1-gangliosidosis as well as Morquio B, suitable derivatives of galactostatin (**3**, 1-deoxygalactonojirimycin, 1,5-dideoxy-1,5-imino-D-galactitol) have become highly attractive targets.



We have synthesised a range of lipophilic analogues **4** of compound **3** featuring branched *N*-substituents which can be modified at will to therapeutic or diagnostic needs. For example, the attachment of large terminal groups as well as the introduction of ionisable functionalities resulted in interesting compound behaviour, excellent enzyme inhibition profiles as well as added-value analytical properties, for example for fluorescence-based detection protocols.

Details of the approach, enzyme inhibitory activities as well as preliminary results in GM1-gangliosidosis fibroblasts will be presented.

- [1] Legler, G.; Liedtke, H. *Biol. Chem. Hoppe-Seyler* 1985, **366**, 1113-1122; Osiecki-Newman, K.; Fabbro, D.; Legler, G.; Desnick, R. J.; Grabowsky G. A. *Biochim. Biophys. Acta, Prot. Struct. Mol. Enzymol.* 1987, **915**, 87-100; Karpas, A.; Fleet, G. W. J.; Dwek, R. A.; Petrusson, S.; Namgoong, S. K.; Ramsden, N. G.; Jacob, G. S.; Rademacher, T. W. *Proc. Natl. Acad. Sci. USA* 1988, **85**, 9229-9233.

- [2] Suzuki, Y. *J. Inherit. Metab. Dis.* 2006, **29**, 471-476.

P343

SYNTHESIS AND BIOLOGICAL EVALUATION OF 2-ACYLAMINO-PYRIDIN-4-YL IMIDAZOLES AS p38 MAP KINASE INHIBITORS

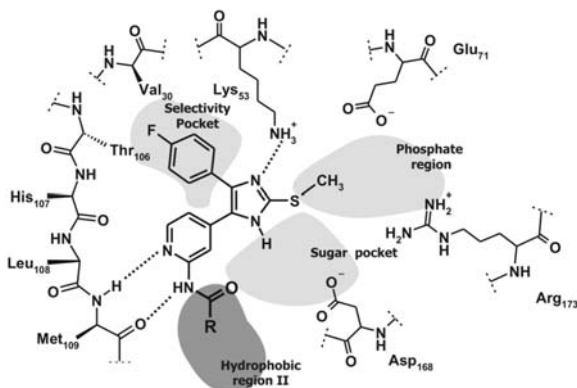
Hauser, D.^{1,2}; Unger, A.²; Stegmüller, T.¹; Ziegler K.¹; Bracht, C.¹; Bühler, S.¹; Selig, R.¹; Albrecht, W.² and Laufer, S.¹

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Inhibition of p38 MAP kinase is a valid approach for the treatment of inflammatory and autoimmune diseases. p38 was first identified as a lipopolysaccharide-activated kinase and was subsequently shown to be an important key enzyme in the regulation of the expression of both cytokines IL-1 β and TNF- α . These cytokines are important mediators of inflammation and transmit signals from one cell to another interactions with the backbone of the protein in the kinase's ATP binding cleft. We have recently published a series of imidazoles bearing 2-alkylaminopyridyl and 2-arylaminopyridinyl moieties [1, 2]. These moieties enable bidentate hydrogen bonding and interactions with hydrophobic region II and are thereby increasing binding affinity and potency of the inhibitors.

In our current study we have replaced the 2-alkylamino and 2-arylamino groups with acyl chains. The evaluated residues vary in chain length and some of the sidechains are rigidized or possess heteroatoms. Most examples have differently substituted terminal aryl moieties that allow optimal interaction with hydrophobic region II. These substitution patterns allowed us to create selective and potent new p38 MAP kinase inhibitors with high affinity to the enzyme and improved metabolic stability. We will present the synthesis and biological evaluation of promising new inhibitors that inhibit their target in the low to single digit nanomolar level.



References

[1] Laufer, S.; Hauser D.; Liedtke, A; *Synthesis* 2008, No. 2, 253–266

[2] Laufer, S.; Hauser D.; Liedtke, A; *Journal of Medicinal Chemistry* 2008, in press

P344

DESIGNED MULTIPLE LIGANDS AS NEW DRUG CANDIDATES AGAINST PARKINSON'S DISEASE

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Due to a better understanding of the pathogenesis of diseases in the recent decades, drug discovery has gradually shifted from a phenotype-based endeavor to today's single-molecular-target centered approach.¹ As a result, it has become and still is aimed mainly at the discovery of small molecules able to aim a single protein target, which is thought to be responsible for a certain disease.² This one-molecule, one-target approach has resulted in the discovery of a large number of successful drugs. Nevertheless, a highly selective ligand for a specific target does not always result in a highly efficient drug. This might be due to, for example, a complex mechanism of the disease in which multiple pharmacological targets play a role. One approach to overcome this problem is the use of Designed Multiple Ligands (DMLs).^{3,4,5}

DMLs drugs are single molecules in which two drugs have been combined. Consequently, this molecule can interact specifically on multiple targets of relevance to a disease (targeted polypharmacology).

Furthermore, G-protein coupled receptors (GPCRs) constitute a superfamily of transmembrane proteins that convert information from the extracellular environment into intracellular responses. The traditional concept of one receptor protein as a functional unit has been challenged by the recent accumulation of evidence that most monomeric GPCRs either from the same or different GPCR families are in fact 'building blocks' that form dimers or even oligomers.^{6,7,8,9} For example, in the stratum the interactions between D₂ Dopamine Receptor (D₂R) and A_{2a} Adenosine Receptor (A_{2a}R) are antagonis-

tic and have shown implications in neurological illnesses such as Parkinson's Disease (PD) and its potential treatment.^{10,11,12}

The pharmacological treatment of PD has been dominated by the use of Dopamine (D₂)-agonists such as levodopa (L-DOPA). However, long-term disability in PD is related to the progress of the underlying disease and complications associated with L-DOPA therapy. Therefore, there is a need for new treatments, which can slow the progression of this degenerative disease, reduce PD symptoms at an early stage of the disease and avoid side-effects such as dyskinesia.

Our research group has explored the strategy of designing and synthesizing libraries of DMLs as potential drug candidates against PD using combinatorial techniques. The synthesized DMLs contain a selected A_{2a}R-antagonist and D₂R-agonist which are connected by a spacer. The length and the nature of the spacer have been investigated to obtain the best interaction with GPCR dimers. In this poster we present the results obtained in the synthesis of the DMLs and the subsequent pharmacological characterization.

[1] P. T. Lansbury; Nat. Rev. Neurosci. 2004, 5 (Suppl.), S51-S53.

[2] S. Frantz; Nature 2005, 437, 942-943.

[3] R. Morphy et al.; J. Med. Chem. 2005, 48(21), 6523-6543.

[4] R. Morphy et al.; Drug Disc. Today 2004, 9(15), 641-651.

[5] A. Cavalli et al.; J. Med. Chem. 2008, 51(3), 347-372.

[6] M. Bouvier; Nat. Rev. Neurosci. 2001, 2(4), 274-286.

[7] S. Terrillon et al.; EMBO Reports 2004, 5, 30-34.

[8] S. R. George et al.; Nat. Rev. Drug Disc. 2002, 1, 808-820.

[9] S. Bulenger et al.; TRENDS Pharm. Sci. 2005, 26, 131-137.

[10] K. Fuxe et al.; Neurology 2003, 61, S19-S23.

[11] P. Svenningsson et al.; Neurology 2003, 61, S5-S9.

[12] M. Deogaonkar et al.; Brain Res. Rev. 2005, 50, 156-168.

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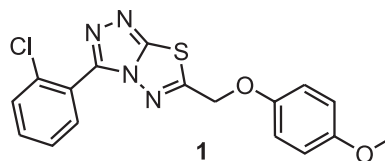
3,6-DISUBSTITUTED-[1,2,4,]TRIAZOLO[3,4-b]-[1,3,4]THIADIAZOLES: NOVEL LEAD STRUCTURES FOR p38 MAP KINASE

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The complex process of inflammation is the cause of many diseases. Prominent examples include rheumatoid arthritis, psoriasis, multiple sclerosis and inflammatory bowel diseases. In general, control of the cells of the immune defense and communication between each other runs above cytokines basically. A central role during the

regularization of the production of pro-inflammatory cytokines as interleukin 1- β (IL-1 β) and tumor necrosis factor α (TNF- α) plays the p38 mitogen activated protein kinase (p38 MAP kinase) making it a prominent target for anti-inflammatory drug discovery. New lead structures of p38 MAP kinase inhibitors were discovered by a virtual screening approach based on the Cresset's eXtended Electron Distribution (XED) model. This method allows the accurate description of molecules out of databases in terms of their surface properties for comparison with created surface properties of known inhibitors.[1] Compound 3-(2-chlorophenyl)-6-((4-methoxyphenoxy)methyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (**1**) was identified as potential hit and therefore chosen as starting point for a medicinal chemistry program. A series of 3,6-disubstituted-triazolo[3,4-b][1,3,4]thiadiazoles have been synthesized by cyclization of 5-substituted-4-amino-4H-1,2,4-triazole-3-thiols with a variety of carboxylic acids in the presence of phosphorus oxychloride.[2] Most of the key intermediate triazoles were obtained via a fast and simple method, which will be presented next to the known synthesis of Reid and Heindel.[3] The synthetic route allows variation at 3- and 6-position. From the results of p38 MAP kinase enzyme assays, we concluded several SARs.



References

[1] Cheeseright, T.; Machey, M.; Rose, S.; Vinter, A. J. Chem. Inf. Model., 2006, 46, 665-676.

[2] Malhotra, S.; Manher, V.; Chadha, V. K. Indian J. Heterocycl. Ch. 2003, 12, 257-262.

[3] Reid, J. R.; Heindel, N. D. J. Heterocyclic Chem. 1976, 13, 925-926.

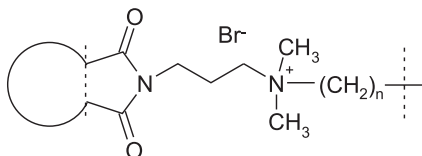
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FROM ALLOSTERIC MODULATION TO SUBTYPE-SELECTIVE AGONISTS AT MUSCARINIC ACETYLCHOLINE RECEPTORS

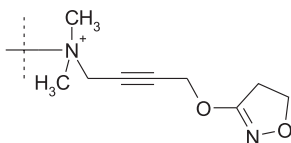
Holzgrabe, U.,* De Amici, M.,[#] Barocelli, E.,[§] Christopoulos, A.,[§] Tränkle, C.,⁺ Mohr, K.⁺

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Subtype-selective activation of G protein-coupled receptors is therapeutically desirable but difficult to achieve for many targets. Here we present a novel strategy towards this goal using muscarinic acetylcholine receptors as a model system. The five subtypes of muscarinic receptors bind their physiological messenger molecule via a highly conserved orthosteric site located within the receptors' transmembrane domains. However, there is an additional allosteric site at the extracellular entrance of this binding pocket comprised of amino acids that are less conserved among the five receptor subtypes. In order to achieve both subtype-selective binding and high affinity receptor activation, we applied the message-address concept utilizing an allosteric subtype-selective "address" linked to a highly active orthosteric agonist "message". Thus, we designed allosteric/orthosteric hybrid compounds: for the allosteric component, we used appropriate fragments of phthalimido substituted bis(ammonio)alkane compounds having pronounced M₂ subtype selectivity and no intrinsic efficacy. For the orthosteric component, we employed the non-selective high affinity agonist, iperexo, which was found to have a higher activity than the endogenous agonist, acetylcholine.



Allosteric modulator fragment



Orthosteric agonist fragment iperexo

Binding studies using wild-type and mutant receptors as well as activation studies carried out at the G protein level and in native tissue preparations revealed true bitopic allosteric/orthosteric receptor binding coupled to an M₂ selective agonism.

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THE DEVELOPMENT OF THIENO[2,3-B]PYRIDONES AS NOVEL INHIBITORS OF P38 MAP KINASE

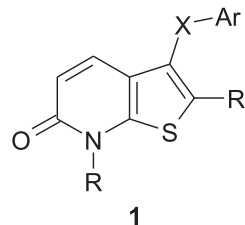
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p38 MAP kinase is a serine/threonine kinase which plays a pivotal role in inflammatory cell signalling. Importantly, p38 mediates both the expression and signalling of several key pro-inflammatory cytokines including TNF α , IL-8 and IL-1 β . Selective inhibition of p38

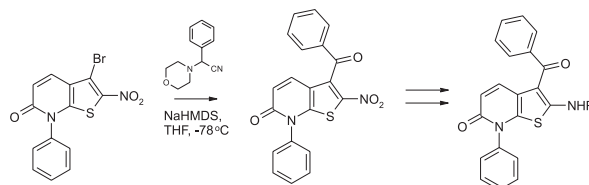
could therefore provide an effective treatment of inflammatory diseases such as rheumatoid arthritis.

We have designed and synthesised several classes of novel p38 inhibitors. Thieno[2,3-*b*]pyridones of type **1** showed good in-vitro activity, and the SAR around these inhibitors was investigated.



1

We will present our optimised synthetic route to 2-amino-3-carbonyl derivatives of structure **1**, which utilises a masked acyl-anion equivalent. We will also show how we improved the physicochemical & DMPK properties of the hit molecule to give optimised compounds that were nominated for pre-clinical evaluation. The profile of one of our pre-clinical candidates UCB1277763 will be presented, along with *in-vivo* data in the Collagen Induced Arthritis model in rat.



P349

DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION OF NOVEL TRPV1 ANTAGONISTS

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The transient receptor potential vanilloid 1 (TRPV1) is a member of ion channels and is localized on primary afferent neurons as sensory neurons innervating the bladder and gut. Activation of TRPV1 on sensory neurons by chemical stimulants including capsaicin and resiniferatoxin, as well as low pH (<6) and heat (>42°) leads to an influx of Ca²⁺ and Na⁺ ions through the channel, causing depolarization of the cell and transmission of painful stimuli. Therefore considerable efforts toward the development of a novel analgesic targeted for TRPV1 have been continued. However, the side effects, such as hypothermia and low oral bioavailability of capsaicin as TRPV1 agonist limited therapeutic uses. On the basis of the previous studies on TRPV1 agonists and antagonists, we have looked for the non-vanilloid TRPV1 antagonists by developing the ideal vanilloid equivalents, which might provide the

perfect analgesic effects without the side effects caused by TRPV1 agonists. In our efforts toward the discovery of new analgesic agents, we identified SC-0030 as potent and selective TRPV1 antagonists. In addition, we have made every possible effort to develop next generation TRPV1 antagonists on the basis of 3D-QSAR and bioisosterism. Recently, we have developed a series of novel non-vanilloid TRPV1 antagonists, resulting from focused modification on the lipophilic region and multiple H-bonding region.

P350

STRUCTURAL AND BIOLOGICAL STUDIES ON NOVEL NATURAL ANTI-MRSA AGENT, MANSONONE F

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Methicillin-Resistant Staphylococcus Aureus (MRSA) has achieved the status of “superbugs”, in that there are few antibiotics available for therapy against this pathogens. Recently, we have reported isolation, synthesis, and preliminary structure-activity relationship (SAR) study of mansonone F, which is structurally unique and highly potent in anti-MRSA activity. The structural features of mansonone F may impose that the mode of antibiotic action can be different from that of the antibiotics currently used for anti-MRSA treatments. The new mode of action has been expected to provide a solution to overcome the resistance problem of the antibacterial agents in use. We herein report anti-MRSA activities of the novel mansonone F analogues for the systemic structure-activity relationship of mansonone F. In particular, the mansonone F O1 analogues were prepared through introduction of heteroatoms using Palladium-catalyzed reaction, synthesis of phenalene skeleton by Friedel-Crafts acylation or Heck reaction, and of mansonone F, although the large substituent reduced the anti-MRSA activity. We also proposed the antibiotic mode of action of mansonone F through the investigation of the reactions of mansonone F with a variety of nucleophiles and the isolation of mansonone F binding protein.

P351

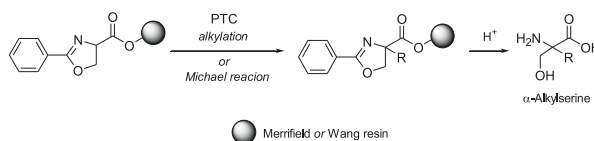
UNNATURAL α -ALKYLSERINES AS BUILDING BLOCKS FOR POTENTIAL PEPTIDE BASED DRUGS: PHASE-TRANSFER CATALYTIC SOLID-PHASE SYNTHESIS OF α -ALKYLSERINE

Jihye Lee, Mi-hyun Kim, Myoung-mo Lee, Hee-jin Lee, Sukhoon Kang, Sukchang Hong, Sang-sup Jew and Hyeung-geun Park*

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Since Merrifield has reported his pioneering work in 1963, the field of solid-phase organic synthesis has been rapidly expanding due to the speed and simplicity of the technique. Recently, we have developed the highly effective solid-phase synthesis of non-natural α -amino acid using diphenylmethyleneglycine *tert*-butyl ester under phase-transfer catalytic condition. And also, we have already reported the enantioselective phase-transfer catalytic alkylation for α -alkylserine using 2-phenyl-2-oxazoline-4-carboxylic acid *tert*-butyl ester. On the basis of these results, we attempted the solid-phase synthesis of α -alkylserine via phase-transfer catalysis.

Merrifield resin-bound substrate using ester-linker was readily prepared from 2-phenyl-2-oxazoline-4-carboxylic acid and solid resin with KF, applied for both alkylation and Michael reaction with BTTP (2.0 equiv) as a base and *n*-Bu₄N⁺Br (5 mol%) as a catalyst. After the trans-esterification using NaOMe, the resultant products were obtained in 85-93% yields.



Enantioselective solid-phase alkylation was performed using Wang resin-bound substrate using ester linker in the presence of (*S,S*)-3',4',5'-trifluorophenyl-NAS bromide (1.0 equiv) and BTTP (2.0 equiv) at 0 °C, furnishing the alkylated product in 90% chemical yield (*S*:*R*=4:1).

P352

SYNTHESIS AND PHARMACOLOGICAL EVALUATION OF MRJF4 AS NEW POTENTIAL ANTICANCER TOOL

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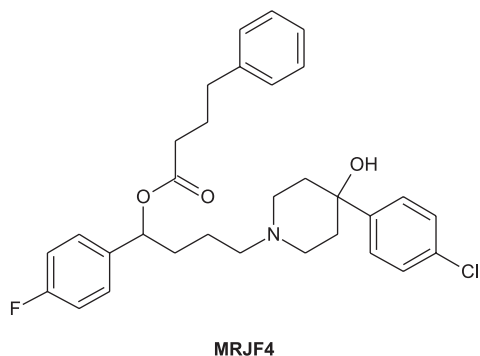
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Prostate cancer is a heterogeneous tumor composed by different groups of cells that respond to endocrine therapy in different ways. Conversion from the androgen-sensitive into the androgen-refractory type, with consequent high risk of metastatic evolution, remains a relevant clinical issue. The antiandrogenic drugs, in fact, induce cell death in androgen-sensitive cells, but are ineffective on androgen-insensitive cells.

The development of several human cancers are linked to the alteration of histone deacetylase (HDAC) activity that lead aberrant transcription of key genes regulating important cellular functions such as cell proliferation, cell-cycle regulation and apoptosis. Actually, new therapeutic strategies employ HDAC inhibitors (HDACi) alone or in combination with other drugs.

Several studies showed an overexpression of sigma receptors in various tumor cell lines, such as melanoma, small cell lung carcinoma, glioblastoma, neuroblastoma and prostate cancer, providing an involvement of these receptors in cancer process. Moreover, sigma ligands revealed high affinity for cells that overexpress the sigma receptors, suggesting their use in diagnosis for non-invasive imaging of tumor such as PET and SPECT and targeted therapy of a variety of tumors including prostate cancer.

In this study we report, for the first time, the evaluation of synergic proapoptotic and cytotoxic effects of MRJF4, an ester between sigma ligand reduced haloperidol and HDACi 4-phenylbutyric acid (4-PB) in prostate cancer LNCaP and PC3 cell lines.



4-[4-(4-chlorophenyl)-4-hydroxypiperidin-1-yl]-1-(4-fluorophenyl)butyl 4-phenylbutanoate

P353

2-PYRIDYLETHYLTHIOCARBAMATES AS NOVEL INHIBITORS OF NITRIC OXIDE PRODUCTION

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Nitric Oxide generated by iNOS is concerned in causing osteoarthritis, diabetic, Parkinsonism, and various circulatory ailments. In order to prevent NO being generated excessively, many kinds of chemical compounds have been laid out and synthesized as inhibitor. Especially, various kinds of the derivatives of L-arginine known as an endogenous substrate of NOS had been experimented about inflammatory disease. But most of these was not achieved the expected results because of low selectivity of iNOS in comparison with nNOS. Through the advance study,¹ we have established the connection between

structure and activity about NOS inhibition with synthesized yakuchinone derivatives that are similar with curcumin, could develop strong inhibitors having IC₅₀ value up to 0.1 μM. Based on this advance study, we could design 2-pyridylethylthiocarbamate analogues as a novel scaffold for the inhibition of NO production. A series of 2-pyridylethylthiocarbamate analogues were designed, synthesized, and evaluated their inhibitory activity of NO production in lipopolysaccharide (LPS)-activated macrophages.

Reference

- [1] Hwa Jin Lee, Ji Sun Kim, Joung Wha Yoon, Hee-Doo Kim, and Jae-Ha Ryu, Suppression of Inducible Nitric Oxide Synthase Expression by Yakuchinones and Their Analogues, *Chem. Pharm. Bull.* 54(3) 377-379 (2006).

P355

STRESS AND ACTIVE OXYGEN SPECIES. EFFECT OF α-TOCOPHEROL AND A NOVEL ANTIOXIDANT WITH ANXIOLYTIC ACTIVITY, DESIGNED AS AN ANTISTRESS AGENT.

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Stress, the non specific response of the body to any demand made upon it, is implicated in the pathogenesis of numerous disorders, such as cardiovascular or neurodegenerative conditions. The extensive overlap between diseases attributed to stress and oxidative damage is indicative of their potential relationship. Furthermore, there is an increasing interest nowadays in the discovery of new pharmacomolecules, which would reduce the consequences of biologic and oxidative stress upon the human body. At the same time, we find it of interest to investigate the interrelationship of the two defensive phenomena, biologic and oxidative stress, which has not been completely and clearly clarified yet.

Thereby, we studied the influence of α-tocopherol and a novel, polyfunctional compound **1** on the development of stress biomarkers, morphological and biochemical, on specific indices of radical insult, lipid peroxidation, oxidised proteins, glutathione content in brain and liver, as well as on drug metabolism. We also examined whether oxidative stress accompanies the onset of biological stress and vice versa, using α-tocopherol as a tool. Moreover, we designed and synthesised compound **1**, aiming at developing novel structures with combined actions against biologic and oxidative stress. Compound **1** integrates in one molecule an anxiolytic drug, lorazepam, esterified with GABA, an inhibitory neurotransmitter, further amidated with trolox, the antioxidant,

water soluble analogue of vitamin E. We calculated physicochemical parameters such as lipophilicity, which play an important role in pharmacodynamics and kinetics of molecules. Since biologic stress affects hepatic drug metabolic activity, we studied the effect of biologic stress and the treatment with compound **1** and α -tocopherol on this defensive activity of the liver, in which oxidative stress is also implicated.

In this research, rats were stressed by two different sets of stressors: cold plus food and water deprivation, or restrain plus food and water deprivation. The application of stress was intermittent (4x10h stress with 3x2h rest). During stress, α -tocopherol (0.42 mM/kg, 24 days) or compound **1** (0.05 mM/kg, 4 days) were administered. The appearance of biologic stress was evaluated from the decrease of thymus and spleen weight, increase of adrenal weight, as well as the concentrations of corticosterone and uropepsinogen. Oxidative stress was evaluated by determining lipid peroxidation and glutathione depletion in liver and brain, as well as oxidised proteins in the brain. The *in vitro* antioxidant activity of **1** was also determined.

It was found that the two different sets of stressors produced strong, well expressed biologic stress. The developed biologic stress was accompanied by potent oxidative stress. In this stress condition, cytochrome P450 was induced and this was expressed as increased erythromycin N-demethylation and 4-nitrophenol aromatic hydroxylation. Treatment of the stressed animals with α -tocopherol or compound **1** reduced greatly the biologic stress, restored oxidative stress completely and modified the hepatic drug metabolic activity in the stressed animals towards normal values, without, however, completely restoring them. Compound **1** was also found to be a potent inhibitor of lipid peroxidation *in vitro*, with IC_{50} 1.1 μ M, while trolox had IC_{50} 42.8 μ M. It interacted with the stable free radical DDPH, at equimolar concentration, by 80%. Trolox interacted with DPPH by 92% under the same experimental conditions.

In conclusion, compound **1** was found to be as potent as α -tocopherol in reducing biologic and oxidative stress at lower doses and shorter treatment, compared with vitamin E. Thus, it appears to be a promising lead structure for the development of agents against pathologic conditions where biologic stress, oxidative stress or both are implicated.

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PHARMACOLOGICAL CHARACTERIZATION OF MOGUNTINONES - A NOVEL CLASS OF DUAL VEGFR-2/3 AND FLT3 KINASE INHIBITORS FOR CANCER THERAPY

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Recently, it has been recognized that tumor growth and metastasis are highly associated with the overexpression of protein kinases (PK). The signalling pathway through vascular endothelial growth factor (VEGF) and its receptors (VEGFR) play an important role in the processes of angiogenesis by initiating proliferation and migration of endothelial cells, a requirement for tumor growth and metastasis. In many cases of acute myeloid leukemia (AML) several different mutations, such as FLT3-ITD (internal tandem duplications) mutations, of the receptor tyrosine kinase Flt3 lead to constitutive activation of the kinase and are associated with a poor clinical outcome using conventional chemotherapy. Therefore, we aim at characterizing the novel class of small-molecule dual VEGFR-2/3 and Flt3 kinase inhibitors, called Moguntinones, as selective tumor targeting compounds:

The compounds DHF125 and DHF150 significantly reduced angiogenesis in our *in vivo* chick embryo assay¹. In order to identify the biological target the substances were screened for inhibitory activity in a panel of 20 kinases. High affinities of the two Moguntinones to the VEGFR-2 and to Flt3 in a nanomolar range as well as a very modest selectivity against other kinases were found². Furthermore, moguntinones were evaluated in a VEGF-stimulated cell proliferation assay using human umbilical vein endothelial cells (HUVECs). We observed a high inhibitory potency of the compounds against HUVECs.

In order to demonstrate the benefit of Moguntinones against hematological malignancies the efficacy in Flt3-ITD transfected 32D cells and in primary ITD-positive AML blasts as well as in combination with chemotherapy was determined. Both compounds induced apoptosis in Flt3-ITD cells with an IC_{50} of 350nM (DHF125) and 800nM (DHF150), respectively as detected by sub G1-fraction in cell cycle analysis. These results correlated with the above mentioned high activity of our compounds against FLT3 within a nanomolar range. Using colony assays we defined a possible therapeutic window of these inhibitors: Bone-marrow cells of healthy donors were incubated with increasing concentrations of both inhibitors revealing no significant inhibition of colony formation up to a dose of 5 μ M. There is evidence to suggest that Moguntinones show no significant toxicity even at high concentrations. As clinical studies in AML will evaluate FLT3 inhibitors in combination with chemotherapy, we tested both DHF compounds *in vitro* in combina-

tion with cytosine arabinosid (Ara-C) and daunorubicine (DNR). Within a range of 1-50nM DNR and 0.3-10 μ M Ara-C, both DHFs showed synergistic effects in combination with chemotherapy in 32D-FLT3-ITD cells as revealed by apoptosis assays. Furthermore, primary AML blasts harboring the FLT3-ITD length mutation were investigated to determine the efficacy of Moguntinones in patient material. These experiments revealed apoptosis rates of 10-30% upon incubation for 72 hours.

In conclusion, Moguntinones as high potent dual VEGFR-2/3 and Flt3 inhibitors may have potential for clinical development as therapeutics in the treatment of hematological malignancies as well as solid tumors. Further investigations and the clinical profiling are ongoing.

References

- [1] Peifer, C.; Dannhardt, G. *Anticancer Res.* 2004, 24, 1545-1551.
- [2] Peifer, C.; Stoiber, T.; Unger, E.; Totzke, F.; Schachtele, C.; Marme, D.; Brenk, R.; Klebe, G.; Schollmeyer, D.; Dannhardt, G. *J. Med. Chem.* 2006, 49, 1271-1281

P357

USE OF GENE EXPRESSION SIGNATURE TECHNOLOGY TO IDENTIFY VVP808, A NOVEL INSULIN SENSITISER WITH EFFICACY IN VITRO AND IN VIVO

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Background and Aims: The aims of the current study were to develop a new small molecule library screening strategy based on a gene expression signature (GES) strategy, and to utilise this screen to identify new insulin sensitising agents.

Material and Methods: Differentiated 3T3-L1 adipocytes were rendered insulin-resistant using TNF α (3 ng/ml for 72h), and insulin sensitivity was restored by adding cocktail of known antidiabetic agents for the last 24h. Whole genome microarrays were used to profile gene expression in the insulin resistant cells, and the cells with insulin sensitivity restored. Bayesian linear discriminant data analysis was performed to find the optimal set of 11 genes that best defined the difference between the two cell states, and these genes constituted the GES. Insulin resistant 3T3-L1 cells were then treated with a library of ~1500 compounds, and analysed for similarity of expression of the 11 GES genes. Hit compounds were selected for further testing *in vitro* and *in vivo*.

Results: Using this GES strategy, we identified a family of compounds that dose-dependently improved insulin stimulated glucose transport and incorporation into lipid in 3T3-L1 adipocytes ($p=9 \times 10^{-7}$). The lead compound, designated VVP808, was then tested in animal models of insulin resistance and diabetes. Treatment of *db/db* mice with VVP808 for 7 days resulted in a dose-dependent reduction in fasting blood glucose concentration (up to 65% reduction at 50 mg/kg/d, Day 0: 24.7 ± 1.8 vs Day 7: 9.5 ± 1.0 mmol/L, $p=0.00004$). The improvement in glycemia was associated with a 7% reduction in body weight ($p=0.002$), however a subsequent pair-feeding study showed that the reduction in blood glucose concentration was independent of body weight loss. Treatment of diet-induced obese (DIO) C57Bl/6 mice with VVP808 for 21 days resulted in a dose-dependent improvement in glucose tolerance and reduction in fasting blood glucose concentration. The area under the blood glucose curve during an ipGTT was reduced by up to 28% ($p=0.037$), while fasting blood glucose was significantly reduced at doses from 20-100 mg/kg/d ($p<0.05$). These effects were accompanied by a dose-dependent drop in body weight of up to 11% at the highest dose (100 mg/kg/d, $p=0.002$).

Conclusion: The GES screening strategy successfully identified a family of compounds with insulin sensitising and antidiabetic effects *in vitro* and *in vivo*. VVP808 is a novel insulin sensitiser that also causes moderate loss of body weight, and therefore has excellent potential for development as an antidiabetic agent.

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ASYMMETRIC SYNTHESIS OF AMINOCYCLITOLS AS NEW GLYCOSIDASE INHIBITORS VIA CSI-MEDIATED AMINATION

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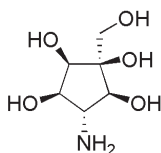
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Glycosidases are involved in a wide range of important biological processes, such as intestinal digestion, the post-translational processing of glycoproteins, and the lysosomal catabolism of glycoconjugates. Inhibitors of these enzymes have received much attention and are currently being used for treating diabetes, viral infections (e.g., HIV and influenza), malaria, and cancer. The majority of these inhibitors are iminosugars, in which an oxygen atom in a monosaccharide is replaced by a nitrogen atom, and have been often found in natural plants and microorganisms. While these iminosugars and their derivatives have been extensively studied, the development of aminocyclitol analogues as glycosidase inhibitors has received little attention.

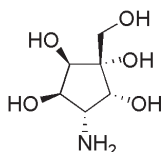
The aminocyclitols have been found in a number of natural products, e.g. bacteriohopanetetrol ether **1a** and **1b**. Bacteriohopanetetrol ether **1a** has been found in *Methylobacterium organophilum*, *Rhodopseudomonas acidophila* and *Zymomonas mobilis*, while the

diastereoisomeric bacteriohopanetetrol ether **1b** has been isolated only from cyanobacterium "*Anacystis montana*". Recent biosynthetic studies in *Z. mobilis* have shown that *N*-acetyl-D-glucosamine is the precursor of the cyclopentitol moiety in **1a**. Intrigued by the structural similarity between the cyclopentitol units in bacterial hopanoids and that found in the α,α -trehalase inhibitor of microbial origin trehalosin, we decided to undertake the synthesis of the aminocyclitols, diastereomers of **1a** and **1b**, and study their possible biological activity as glycosidase inhibitors.

In this presentation, we described an efficient stereoselective route for the preparation of aminocyclitols, which was accomplished from the readily available D-sugars via the regio- and diastereoselective amination of polybenzyl ethers using chlorosulfonyl isocyanate, intramolecular olefin metathesis leading to carbocyclic ring skeleton formation, and diastereoselective dihydroxylation.



(1S,2S,3R,4R,5R)-5-amino-2-(hydroxymethyl)cyclopentane-1,2,3,4-tetraol



(1R,2R,3R,4R,5R)-5-amino-2-(hydroxymethyl)cyclopentane-1,2,3,4-tetraol

P360

ARTEMISININ-VINYLSULFONE DOUBLE DRUGS AS ANTIMALARIALS

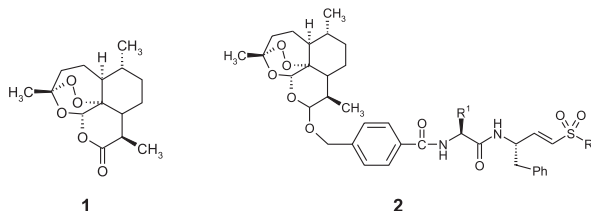
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Malaria is a major infectious disease in tropical and subtropical regions, caused by parasites of *Plasmodium* species. The major obstacle to malaria control is the emergence of drug resistance strains of *P. falciparum*, the most lethal of the malaria parasites. Artemisinin (ART), **1**, and its derivatives are the most potent anti-malarial drugs with no clinical resistance described ¹. To avoid the emergence of resistance to ART, the WHO recommended the use of ART derivatives in combination with other antimalarials ². When used in combination with other effective antimalarials, the ART derivatives have

achieved very high cure rates even against multidrug resistant strains. The double drug approach combines two different scaffolds into a single chemical entity by a linker, with the aim of improving biological activity ³. We describe the synthesis of compounds, **2**, that incorporate the ART moiety as well as a vinylsulfone scaffold. Vinylsulfones are well known inhibitors of falcipain-2 ^{4,5}, a cysteine protease involved in host haemoglobin degradation in the parasite food vacuole. The antimalarial activity of these new compounds will be presented and discussed.



[1] Schlitzer, M. *ChemMedChem*. 2007, 2, 944-986.

[2] WHO News Release 19-01-2006

[3] O'Neill, P. M.; Posner, G. H. *J. Med. Chem.* 2004, 47, 12, 2945-2964.

[4] Hogg, T. *et al. J. Biol. Chem.* 2006, 281, 35, 25425-25437.

[5] Shenai, B. R. *et al. Antimicrob. Agents Chemother.* 2003, 47, 1, 154-160.

P361

VIRTUAL SCREENING AGAINST COBRA VENOM

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α -Cobratoxin, the long chain neurotoxin from *Naja kaouthia* acts on the postsynaptic membrane of nicotinic acetylcholine receptors. It causes paralysis by preventing acetylcholine (ACh) binding to the nicotinic acetylcholine receptor. α -Cobratoxin has been proposed as a potential target for anticobratoxin drug design. The α -cobratoxin/nicotinic acetylcholine binding protein complex (1Y15) was selected to prepare α -cobratoxin active binding site for docking. The interacted amino acid residues (Ser182 – Tyr192) of nicotinic acetylcholine receptor, the binding site of α -cobratoxin, was used as the positive control to validate the prepared α -cobratoxin active binding site (RMSD < 1.2 Å). Virtual screening of the α -cobratoxin active site by use of AutoDock against the NCI diversity

set, a library of 1990 compounds with nonredundant pharmacophore profiles, has revealed 39 potential inhibitor candidates. *In vitro* radioligand (³H-epibatidine and ¹²⁵I-bungarotoxin) competition assay against nicotinic ACh binding protein (AChBP) from this list revealed that four compounds had micromolar to nanomolar inhibition. In particular, one compound, NSC121865, had 16.26 nM inhibition against AChBP from the marine species, *Aplysia californica* and had 111 and 415 nM inhibition against AChBP from fresh water snail, *Lymnaea stagnalis* and *Bolinus truncatus*, respectively. These inhibitors serve as novel templates/scaffolds for development of more potent and specific anticobratoxin.

References

- [1] Walkinshaw, M.D.; Saenger, W.; Maelicke, A. *Proc. Natl. Acad. Sci. USA*. 1980, 77, 2400.
- [2] Bourne, Y.; Talley, T.T.; Hansen, S.B.; Taylor, P.; Marchot, P. *EMBO. J.* 2005, 24, 1512.
- [3] Morris, G.M.; Goodsell, D.S.; Haliday, R.S.; Huey, R.; Hart, W.E.; Belew, R.K.; Olson, A.J. *J. Comput. Chem.* 1998, 19, 1639.

P362

SYNTHESIS AND BIOLOGICAL EVALUATION OF NEW 2-PHENYL-2,3-DIHYDRO-1,3,4-THIADIAZOLE DERIVATIVES AS NITRIC OXIDE SYNTHASE INHIBITORS.

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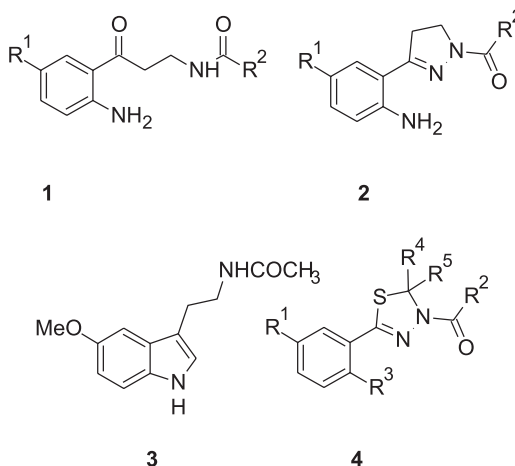
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Nitric Oxide (NO) is an important bioregulator and ubiquitous biomessenger involved in several physiological and pathological processes.¹ In mammals, NO is synthesized from *L*-arginine in various cell types by a family of nitric oxide synthases (NOS) isoenzymes.² NOSs represent a therapeutic target, since selective inhibition of one of these enzymes can help in the treatment of several disorders.

In previous papers, we have described the synthesis of several kinurenamine derivatives **1**³ and 4,5-dihydro-1*H*-pyrazole derivatives of general formula **2**,⁴ as a new type of neural nitric oxide synthase (nNOS) inhibitors.¹ Such compounds behave like a rigid analogue of the main brain metabolite of melatonin **3**, the hormone secreted by the pineal gland and a known NOS inhibitor.⁵

Herein we report the synthesis and biological evaluation of a series of new 2,3-dihydro-1,3,4-thiadiazole derivatives represented by the general formula **4**. In these molecules, the presence of the sulphur atom increases the lipophilicity of the molecule and additionally restricts the conformational freedom. A higher inhibition is expected.



References

- [1] a) Sessa, W. C.; Pritchard, K.; Seyedi, N.; Wang, J.; Hintze, T. H. *Circ. Res.* 1994, 74, 349; b) Awolesi, M. A.; Sessa, W. C.; Sumpio, B. E. *J. Clin. Invest.* 1995, 96, 1449.
- [2] a) Knowles, R. G.; Palacios, M.; Palmer, M. R. J.; Moncada, S. *Proc. Natl. Acad. Sci. USA* 1989, 86, 5159; b) McCall, T. B.; Boughton-Smith, N. K.; Palmer, R. M. J.; Whittle, B. J. R.; Moncada, S. *Biochem. J.* 1989, 261, 293.
- [3] Entrena, A.; Camacho, M. E.; Carrión, M. D.; López-Cara, L. C.; Velasco, G.; León, J.; Escames, G.; Acuña-Castroviejo, D.; Tapias, V.; Gallo, M. A.; Vivó, A.; Espinosa, A. *J. Med. Chem.* 2005, 48, 8174.
- [4] Camacho, M. E.; León, J.; Entrena, A.; Velasco, G.; Carrión, M. D.; Escames, G.; Vivó, A.; Acuña, D.; Gallo, M. A.; Espinosa, A. *J. Med. Chem.* 2004, 47, 5641.
- [5] a) Bettahi, I.; Pozo, D.; Osuna, C.; Reiter, R. J.; Acuña-Castroviejo, D.; Guerrero, J. J. *Pineal Res.* 1996, 20, 205; b) Leon, J.; Vives, F.; Crespo, E.; Camacho, E.; Espinosa, A.; Gallo, M. A.; Escames, G.; Acuña-Castroviejo, D. *J. Neuroendocrinol.* 1998, 10, 297.

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SYNTHESIS AND BIOLOGICAL EVALUATION OF PHENYL-PYRROLE DERIVATIVES AS NITRIC OXIDE SYNTHASE INHIBITORS

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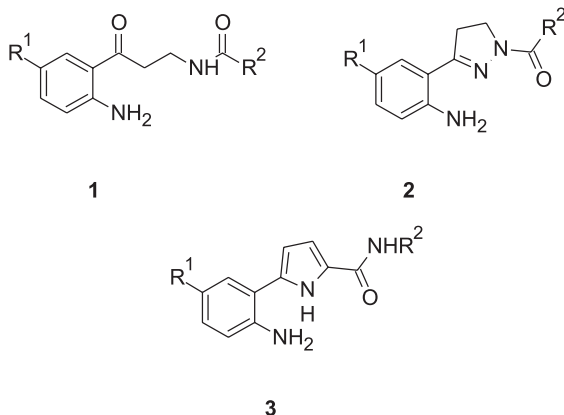
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Nitric Oxide (NO) is an important bioregulator and ubiquitous biomessenger involved in several physiological and pathological processes.¹ In mammals, NO is synthesized from *L*-arginine in various cell types by a family of nitric oxide synthases (NOS) isoenzymes.²

NOSs have been structurally classified as constitutive NOS (cNOS)³ and inducible NOS (iNOS),⁴ and cNOS has been subdivided into endothelial (eNOS) and neuronal (nNOS) attending to its localization. More recently, a mitochondrial-localized NOS isoform situated in the internal membrane of the mitochondria (mtNOS) was discovered,⁵ and in a recent paper the existence of both constitutive and inducible mitochondrial NOS (c-mtNOS and i-mtNOS, respectively) has been proven.⁶

In previous papers, we have described the synthesis of several kinurenamine derivatives **1**⁷ and 4,5-dihydro-1*H*-pyrazole derivatives of general formula **2**,⁸ as a new type of neural nitric oxide synthase (nNOS) inhibitors. Herein we report the synthesis and biological evaluation of a series of new phenyl-pyrrole derivatives represented by the general formula **3**. Some of these molecules behave as weak nNOS and iNOS *in vitro* inhibitors showing a discrete iNOS selectivity. Nevertheless, some of them significantly prevent both iNOS and i-mtNOS *in vivo* activities induced after the toxin administration in the MPTP Parkinson model.



References

- [1] (a) Sessa, W. C.; Pritchard, K.; Seyedi, N.; Wang, J.; Hintze, T. H. *Circ. Res.* 1994, **74**, 349; (b) Awolesi, M. A.; Sessa, W. C.; Sumpio, B. E. *J. Clin. Invest.* 1995, **96**, 1449.
- [2] (a) Knowles, R. G.; Palacios, M.; Palmer, M. R. J.; Moncada, S. *Proc. Natl. Acad. Sci. USA* 1989, **86**, 5159; (b) McCall, T. B.; Boughton-Smith, N. K.; Palmer, R. M. J.; Whittle, B. J. R.; Moncada, S. *Biochem. J.* 1989, **261**, 293.
- [3] (a) Schini, V.; Vanhoutte, P. J. *Pharmacol. Exp. Ther.* 1992, **261**, 553; (b) Forstermann, U.; Pollock, J.; Schmidt, H.; Heller, M.; Murad, F. *Proc. Natl. Acad. Sci. USA* 1991, **88**, 1788.
- [4] Cho, H. J.; Xie, Q. W.; Calaycay, J.; Mumford, R. A.; Swiderek, K. M.; Lee, T. D.; Natham, C. *J. Exp. Med.* 1992, **176**, 599.
- [5] Frandsen, U.; Lopez-Figueroa, M.; Hellsten, Y. *Biochem. Biophys. Res. Commun.* 1996, **227**, 88.
- [6] López, L. C.; Escames, D.; Tapias, V.; Utrilla, P.; León, J.; Acuña-Castroviejo, D. *Int. J. Biochem. Cell B.* 2006, **38**, 267.
- [7] Entrena, A.; Camacho, M. E.; Carrión, M. D.; López-Cara, L. C.; Velasco, G.; León, J.; Escames, G.; Acuña-Castroviejo, D.; Tapias, V.; Gallo, M. A.; Vivó, A.; Espinosa, A. *J. Med. Chem.* 2005, **48**, 8174.
- [8] Camacho, M. E.; León, J.; Entrena, A.; Velasco, G.; Carrión, M. D.; Escames, G.; Vivó, A.; Acuña, D.; Gallo, M. A.; Espinosa, A. *J. Med. Chem.* 2004, **47**, 5641.

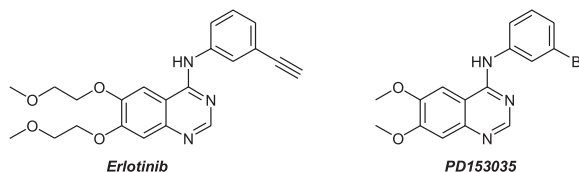
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SYNTHESIS AND EVALUATION OF ERLOTINIB ANALOGUES: EXPLOITING RING CLOSURE

Giovanni Marzaro, Luca Urbani, Adriano Guiotto, Maria Teresa Conconi, Adriana Chilin

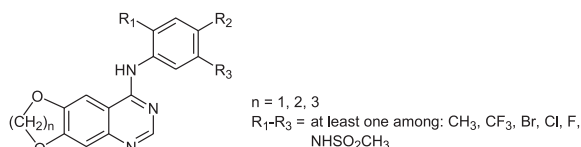
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Identification of novel targets critical for cancer growth and progression, and development of inhibitory strategies to hit these targets are fundamental to reach significant advances in cancer treatment. To date, scientific community efforts directed to small organic compound development for anticancer therapy have allowed to identify several biologically interesting classes of compounds. In the last few years, particular attention was given to 4-anilinoquinazoline derivatives, such as Erlotinib or PD153035 for their highly specific and potent antitumoral activity as tyrosine kinase inhibitor [1,2].



These compounds act as ATP-competitive antagonist towards several receptor enzyme, such as EGFR, blocking the proliferative cascade, and so inducing apoptosis. The therapeutic potential of these quinazoline derivatives is actually under extensive investigation in several clinical trials. Synthesis of these derivatives is often limited by the lack of commercially available starting products. To going over this drawback of the already known synthetic routes, our research group has developed a novel synthetic strategy, which have also been improved by microwave-assisted technique [3,4,5].

Starting from QSAR, docking and crystallographic literature data, we have decided to investigate how a condensed dioxxygenated ring might influence the biological activity of several 4-anilinoquinazoline derivatives. Structures of synthesized compounds are summarized in the figure below.



The effects on cell growth induced by all the synthesized compounds were evaluated in both human squamous carcinoma cells (A431) and mouse embryonic fibroblasts (NIH3T3) expressing EGFR at various levels. EGFR tyrosine kinase activity was also assessed by measuring the phosphorylation of a synthetic peptide substrate after treatment of A431 cells. Biological data were compared with those obtained using PD153035.

References

- [1] Dowell J. et al., *Nature reviews – drug discovery*, **4**, S14 - S15 (2005).
- [2] Bos M. et al., *Clin. Cancer Res.*, **3**(11), 2099 - 2106 (1997).
- [3] Marzaro, G.; Chilin, A.; Pastorini, G.; Guiotto, A. *Org. Lett.*, **8**, 255 - 256 (2006)
- [4] Chilin, A.; Marzaro, G.; Zanatta, S.; Barbieri, V.; Pastorini, G.; Manzini, P.; Guiotto, A. *Tetrahedron*, **62**, 12351-12356 (2006).
- [5] Chilin, A.; Marzaro, G.; Zanatta, S.; Guiotto, A.; *Tetrahedron Lett.*, **48**, 3123 - 3133 (2007).

P365

SYNTHESIS OF CONFORMATIONALLY CONSTRAINED AZA-PEPTIDES

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The introduction of conformational constraints in natural or synthetic peptides is a classic technique to modify their properties. For instance rigidification of a peptide can stabilize an active conformation or destabilize conformations which are recognized by metabolic enzymes.

Aza-peptides possess one or more amino acids in which the α -carbon is replaced by nitrogen (Figure 1).¹ Computational calculations, spectroscopic data, and crystallographic studies show that aza-amino acid residues induce β -turn geometries in peptides.² This fact can be explained by considering the extended p-system that constitutes the acylhydrazide. In addition, the hydrazine motif seems to enhance the metabolic stability of pseudopeptides.

In the last few years we have studied the chemical behaviour of 2,3-diazanorbornenes.³ In connection to our work on β -turn mimetics,⁴ we have now used this bicyclic scaffold to produce conformationally constrained tripeptide surrogates,⁵ which we use to obtain a library of ψ -melanotans⁶ with improved biological properties.⁷

We report here the synthesis of the tripeptide Trp-azaArg-Phe.

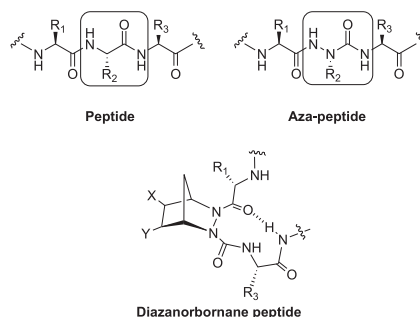


Figure 1

- [1] Gante, J. *Synthesis* 1989, 405.
- [2] Meléndez, R.E.; Lubell, W.D. *J. Am. Chem. Soc.*, 2004, **126**, 6759, and references cited therein.
- [3] a) Pérez Luna, A.; Ceschi, M.-A.; Bonin, M.; Micouin, L.; Husson, H.-P.; Gougeon, S.; Estenne Bouhtou, G.; Marabout, B.; Sevrin, M.; George, P. *J. Org. Chem.* 2002, **67**, 3522. b) Bournaud, C.; Bonin, M.; Micouin, L. *Org. Lett.*, 2006, **8**, 3041. c) Bournaud, C.; Falcioia, C.; Lecourt, T.; Rosset, S.; Alexakis A.; Micouin L. *Org. Lett.* 2006, **8**, 3581, and references cited therein.
- [4] a) Estiarte, M.A.; Rubiralta, M.; Diez, A.; Thormann, M.; Giral, E. *J. Org. Chem.* 2000, **65**, 6992. b) Piró, J.; Forns, P.; Blanchet, J.; Bonin, M.; Micouin, L.; Diez, A. *Tetrahedron: Asymm.*, 2002, **13**, 995. c) Ecija, M.; Diez, A.; Rubiralta, M.; Casamitjana, N.; Kogan, M.J.; Giral, E. *J. Org. Chem.* 2003, **68**, 9541. d) Forns, P.; Piró, J.; Cuevas, C.; García, M.; Rubiralta, M.; Giral, E.; Diez, A. *J. Med. Chem.* 2003, **46**, 5825.
- [5] For a precedent using non-functionalized diazanorbornane, see: Chakraborty, T.K.; Ghosh, A.; Ravi Sankar, A.; Kunwar, A.C. *Tetrahedron Lett.*, 2002, **43**, 5551.
- [6] A preliminary communication was presented at the "XV Congreso de la SEQT", El Escorial, Sept. 2007.
- [7] The model peptide is Melanotan 2: Hadley, M.E.; Hruby, V.J.; Blanchard, J.; Dorr, R. T.; Levine, N.; Dawson, B.V.; Al-Obeidi, F.; Sawyer, T.K. *Pharm. & Biotech.*, 1998, **11**, 575.

P366

GLYCO- AND NEOGLYCOPEPTIDES AS COMPONENTS OF VACCINES TO TARGET HUMAN ANTIGEN-PRESENTING CELLS

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Antigen presenting cells (APCs) are cells of the immune system that take up invading infectious agents and carry them to lymphoid tissue to stimulate T cells. A population of APCs in human dermis has been identified that has the potential to be very good targets for cancer vaccines.¹ An APC receptor which has a strong preference for binding small peptides bearing *N*-acetyl-galac-

tosamine (GalNac) *O*-linked to threonines was identified as a potential target molecule for new peptide-based vaccines.²

In order to target this APC receptor, a series of glycopeptides containing GalNac *O*-linked to threonines have been synthesised *via* Fmoc-SPPS. In recent years "click chemistry" approach proved to be a versatile and efficient strategy for the synthesis of neoglycopeptides.^{3,4} Synthesis of a variety of "click" analogues of the corresponding neoglycopeptides is focus of our ongoing work.

Synthesis and screening of these libraries of glyco- and neoglycopeptides bearing GalNac at different positions will allow identification of ligands capable of efficiently binding to the APC receptor. These molecules will not only provide important laboratory tools for further investigation of stimulating T cells, but will also be candidates for rapid development toward clinical trial, since related compounds have proven easy to manufacture to clinical grade and safe to administer to humans.

[1] Angel, C. E.; Georg, E.; Brooks, A. E.; Ostrovsky, L. L.; Dunbar, P. R.; *J. Immunol.* 2006, 176, 5730.

[2] Iida, S.; Yamamoto, K.; Irimura, T.; *J. Bio.Chem.* 1999, 274, 10697.

[3] van Kasteren, I. S.; Kramer, H. B.; Jensen, H. H.; Campell, S. J.; Kirkpatrick, J.; Oldham, N. J.; Anthony, D. C.; *Nature* 2007, 446, 1105.

[4] Review: Pieters, R. J.; Rijkers, D. T. S.; Liskamp, R. M. J.; *QSAR Comb. Sci.* 2007, 26, 1181.

P367

THE LEAD-GENERATION OF INHIBITORS OF GSK-3 β WITH ANTI-TUMOR ACTIVITY

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Glycogen synthase kinase 3 (GSK-3) is originally identified as a protein kinase involved in the regulation of glycogen metabolism. There are two isoforms designated GSK-3 α and GSK-3 β according to the structural differences N-terminal regions. GSK-3 β play has a pivotal role in Wnt signaling pathway, which is most closely associated with human cancer and neuronal apoptosis. Therefore, GSK-3 β has emerged as a good target for several therapeutic uses including the treatment of neurodegenerative diseases, type II diabetes, bipolar disorders, stroke, and cancer. Among them, to development of small molecule inhibitors for target-directed therapies of cancer, we focused on the treatment of cancer, especially colorectal and colon cancer as therapeutic target. Consequently, we designed pyrazolopyrimidine derivatives as potential GSK-3 β based on *in-silico* pharma-

cophore model, constructed pyrazolopyrimidine focused library and, founded the several lead compounds having anticancer activity. The detailed design, synthesis and biological evaluation of the pyrazolopyrimidinone derivatives will be presented.

P368

SYNTHESIS AND 5-LIPOXYGENASE INHIBITORY ACTIVITY OF BENZOXAZOLE AMIDES

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Leukotrienes constitute a class of potent biological lipid mediator derived from arachidonic acid, which are implicated in a range of inflammatory diseases. The initiating enzyme in leukotriene synthesis is the 5-lipoxygenase (5-LOX). And the enzyme 5-LOX is a key target in the effort to discover drugs which inhibit the pathophysiology associated with the formation of leukotrienes, such as asthma and rheumatoid arthritis. Zileuton was the first 5-LOX inhibitor brought to market for the treatment of bronchial asthma.

In this study, benzoxazole amide derivatives were designed, synthesized, and evaluated as 5-LOX inhibitors. Benzoxazole amides were prepared from the commercial amides in 2 steps; the various amides were reacted with carbon disulfide, iodomethane and sodium hydride to yield the dimethyl carbonimidodithioate, which was cyclized to benzoxazoles through refluxing in DMF. The synthesized compounds were evaluated in LTC₄ formation inhibition test. Most of the benzoxazole amide derivatives showed good biological activities at 10 μ M.

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SYNTHESIS, CYTOTOXICITY, DNA TOPOISOMERASE II AND PEROXISOME PROLIFERATION INHIBITORY ACTIVITY OF BENZONAPHTHOFURANDIONES

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Compounds contain two to four coplanar fused aromatic rings act as DNA intercalator, which causes enzyme blockade and reading errors during the replication process. A tetracyclic structural pattern consisting of a benzene ring attached to the 2-position of a naphthalene nucleus or heterocyclic ring moiety was observed among these compounds. These benzonaphthofurandiones were prepared by condensation of 2,3-dichloronaphthalene-1,4-dione and phloroglucinol or resorcinol or

4-chlororesorcinol with base catalysts in alcohol. Treatment of the synthesized compounds with dialkylaminoalkyl chloride in base and phase transfer catalysis gave the corresponding alkylaminoalkoxy derivatives. The *in vitro* cytotoxicity of the final products was evaluated by SRB method with human tumor cell lines; lung, stomach, colon, fibro sarcoma, myeloid leukemia. The IC₅₀ values of active compounds showed below 1 μ M. Also, the topoisomerase I and II inhibition was assessed and most of compounds showed high activity on topoisomerase II at a concentration of 5 μ M. When the inhibitory effect of prepared compounds on proliferation was investigated in cultured rat aortic smooth muscle cell (SMC) line A7r5, some of the tested compounds inhibited the cell growth in a dose-dependent manner.

References

- [1] Rhee, H.-K.; Park, H. J.; Lee, S. K.; Lee, C.-O.; Choo, H.-Y.P., *Bioorg. Med. Chem.* 2007, 15(14), 1651.
- [2] Giles, G. I.; Sharma, R. P. *Med. Chem.* 2005, 1, 383.
- [3] Chung, H.-J.; Jung, O.-J.; Chae, M. J.; Hong, S.-Y.; Chung, K.-H.; Lee, S. K.; Ryu, C.-K., *Bioorg. Med. Chem.* 2005, 15, 3380.

P370

SYNTHESIS AND BIOLOGICAL EVALUATION OF NEW 1-ARYL-3-ARYLAMIN-PROPAN-1-OL DERIVATIVES AS ANTIMALARIAL AGENTS

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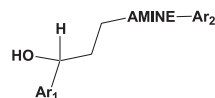
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Malaria is one of the most dangerous diseases affecting primarily poor people of tropical and subtropical regions. The search for novel drugs against specific parasites is an important goal for antimalarial drug discovery.

The increasing resistance of the malaria parasite *Plasmodium falciparum* to currently available drugs and specially to chloroquine demands a continuous effort to develop new effective therapeutic options. Identification of new molecular scaffolds structurally unrelated to existing antimalarial agents represents a valuable strategy to bypass resistance phenomena.

Accordingly, a serie of new derivatives recently published as antidepressants¹, was evaluated *in vitro* against a chloroquine-sensitive strain (3D7) of *Plasmodium falciparum*² in spectrofluorometric microassay (SYBRGreen[®]). The most active compounds are being evaluated *in vivo* against ANKA strain of *Plasmodium Berghe*³ and Ferriprotoporphyrin IX biomineralisation inhibition test

(FIBT)⁴. The obtained results confirm the potential antimalarial activity of the compounds.



Ar₁ = 3-Benzo[b]thiophenyl; 2-naftyl; phenyl; biphenyl

Amine = piperazine; 4-aminopiperidine; 1,4-diazepane; 3-aminopirrolidine

Ar₂ = Variable

References

- [1] Orus L., Pérez-Silanes S., Oficialdegui A., Martínez J., Del Castillo J.C., Mourelle M. (2002). Synthesis and molecular modeling of new 1-aryl-3-[4-arylpiperazin-1-yl]-1-propane derivatives with high affinity at the serotonin transporter and at 5-HT_{1A} receptors. *J. Med. Chem.* 45 (19): 4128-4139.
- [2] Jensen J. B., Trager W. and Doherty J. (1979). Plasmodium falciparum - Continuous Cultivation in a Semi-Automated Apparatus. *Exp. Parasitol.* 48:36-41.
- [3] Peters, W. and Robinson B.L. (1992). The chemotherapy of rodent malaria. XLVII. Studies on pyronaridine and other Mannich base antimalarials. *Ann. Trop. Med. Parasitol.* 86:455-465.
- [4] Deharo E., García R. N., Oporto P., Gimenez A., Sauvain M., Jullian V., Ginsburg, H. (2002). A non-radiolabeled ferriprotoporphyrin IX biomineralization inhibition test (FBIT) for the high throughput screening of ant malarial compounds. *Exp. Parasitol.* 100: 252-256.

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ANTICANCER, ANTI-INFLAMMATORY AND ANTIOXIDANT ACTIVITIES OF NEW QUINOXALINE AND QUINOXALINE DI-N-OXIDE DERIVATIVES

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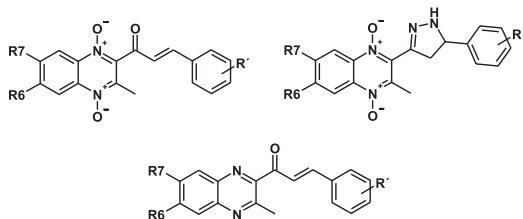
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Quinoxaline derivatives display a broad spectrum of biological properties (antifungal, anticancer, antibacterial, antihelminthic, and antiviral). Oxidation of both nitrogens of the quinoxaline ring greatly increases some of these activities, such as hypoxia-selective anticancer activity¹.

In our continuing efforts to identify new anticancer agents which can improve the current chemotherapeutic treatments, new series of quinoxaline di-N-oxide derivatives and some of their reduced analogues have been

synthesized² and tested for their *in vitro* anticancer activity. These compounds have also been tested in order to study their antioxidant activities, their role in inflammation, and their inhibition on lipoxygenase (LOX) since LOX inhibitors are able to induce the anti-carcinogenic enzymes and/or inhibit the pro-carcinogenic enzymes responsible for polyunsaturated fatty acid metabolism.



The tested compounds exhibit important scavenging activities. Two of them present higher *in vivo* anti-inflammatory activity than the reference drug, indomethacin. Furthermore, one of these compounds shows potent *in vitro* inhibition of LOX ($IC_{50} < 1 \mu M$)².

The *in vitro* anticancer activity of the compounds was evaluated against three cancer cell lines. Surprisingly, two of the reduced analogues are the most cytotoxic compounds in the colon cancer cell line HT-29 ($IC_{50} = 19 \mu M$ – $24 \mu M$).

Acknowledgements:

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References

- [1] Ganley, B.; Chowdhury, G.; Bhansali, J.; Daniels, J. S.; Gates, K. S. *Bioorg. Med. Chem.* 2001, 9, 2395–2401.
- [2] Burguete, A.; Pontiki, E.; Hadjipavlou-Litina, D.; Villar, R.; Vicente, E.; Solano, B.; Ancizu, S.; Pérez-Silanes, S.; Aldana, I.; Monge, A. *Bioorg. Med. Chem. Lett.* 2007, 17, 6439–6443

P372

FATTY ACID AMIDE DERIVATIVES AS ANTI-OBESITY DESIGNED MULTIPLE LIGANDS

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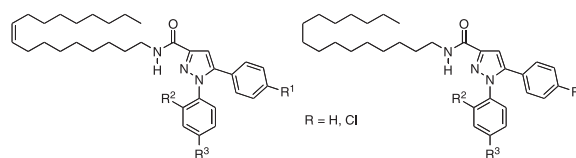
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Designed multiple ligands, DMLs, are compounds envisaged to address multiple targets with the aim of improving efficacy and safety in comparison with tradi-

tional single target pharmacology. This approach is of particular relevance in complex diseases such as metabolic disorders. Within this context, we decided to explore the idea of synthesizing a dual cannabinoid/PPAR α ligand taking into account the convergent mechanisms of peroxisome proliferator activated receptors alpha and cannabinoid receptor antagonists as modulators of appetite, lipid metabolism and carbohydrate management by the liver and adipose tissue (Pat. ES. P200702691, 2007).

The rationale was to link in the same molecule a CB1 cannabinoid antagonist motif with a group capable of activating PPAR α receptors. The cannabinoid part was the 1,5-diarylpyrazole and related five-membered structures present in Rimonabant and other well established cannabinoid ligands^[1] and the amide chosen was oleylethanolamide, OEA, a lipid mediator that regulates feeding and lipid metabolism by activating PPAR α receptors. This was further supported by a recent finding in our group in which we had proved that Rimonabant enhances the metabolic benefits of long term treatment with OEA in Zucker rats^[2].

Thus, a new series of fatty acid amide derivatives of 1,5-diarylpyrazole and other heterocycles have been synthesized in a one step procedure^[3] by treatment of the corresponding carboxylate with an aluminium complex prepared *in situ* reacting trimethylaluminium with the corresponding *N*-oleyl and *N*-hexadecylamine. This represents an improvement of the traditional preparation of amides through the acid derivative.



The compounds have been evaluated *in vivo* and *in vitro* as PPAR α activators. Cannabinoid agonist/antagonist activities have been evaluated in two of the mouse tetrad test (temperature and locomotor activity). Additionally, the anorexic effects of the new compounds have been studied *in vivo* in food deprived rats. Some compounds have shown PPAR α agonist properties and induced feeding inhibition.

- [1] Jagerovic, N.; Fernández-Fernández C.; Goya, P.; Curr. Top. Med. Chem. 2008, 8, 205.
- [2] Serrano, A.; Del Arco, I.; Pavón, F. J.; Macías, M.; Pérez-Valero, V.; Rodríguez de Fonseca, F.; Neuropharmacology 2008, 54, 226.
- [3] Jagerovic, N.; Hernandez-Folgado, L.; Alkorta, I.; Goya, P.; Martín, M. I.; Dannert, M. T.; Alsasua, A.; Frigola, J.; Cuberes, M. R.; Dordal, A.; Holenz, J.; Eur. J. Med. Chem. 2006, 41, 114.

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NEW RHODANINE – BASED INHIBITORS OF MUR LIGASES

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Bacterial resistance to currently available antibiotics has created an urgent need for discovery of novel effective antibacterial agents directed towards previously unexploited targets. One of the best known and most validated targets for antibacterial therapy is the enzymatic system responsible for the peptidoglycan biosynthesis. A large number of antibiotics currently used in therapy act by inhibiting the later extracellular steps in biosynthesis of the peptidoglycan. On the contrary, the early intracellular steps of biosynthesis of cytoplasmic peptidoglycan precursor have been less exploited to date.^[1]

The biosynthesis of peptidoglycan is a complex two-stage process involving first stage intracellular assembly of the UDP-MurNAc pentapeptide which is subsequently translocated through the cytoplasmic membrane to its outer side, where the second stage polymerization reactions take place. Enzymes of the Mur ligase family, MurC-MurF, catalyse a series of reactions leading to UDP-MurNAc pentapeptide by stepwise addition of L-Ala (MurC), D-Glu (MurD), *meso*-diaminopimelic acid or L-Lys (MurE) and D-Ala-D-Ala (MurF) to the starting MurC substrate UDP-MurNA.^[1]

Considering the facts that all four Mur ligases (MurC-MurF) are ATP-dependent enzymes and that the ATP binding site is highly conserved in Mur ligase family our hypothesis is that it might be possible to inhibit different Mur ligases with a single molecular entity through inhibition at the ATP binding site. This concept would give efficient antibacterial chemotherapeutics. Since ATP is involved in functioning of several other enzymes, e.g. kinases, the selectivity issue is very critical and should be considered in design of these multiple target chemotherapeutics. Based on this hypothesis, compounds with low micromolar inhibitory activities against several Mur enzymes were identified and will be presented.

[1] H. Barreateau, A. Kovac, A. Boniface, M. Sova, S. Gobec, D. Blanot, *FEMS Microbiol. Rev.* 2008, 32, 168-207.

P374

NEW BENZOLACTAM DERIVATIVES AS DOPAMINE D₃ RECEPTOR LIGANDS. SYNTHESIS, BINDING AFFINITY AND 3D-QSAR

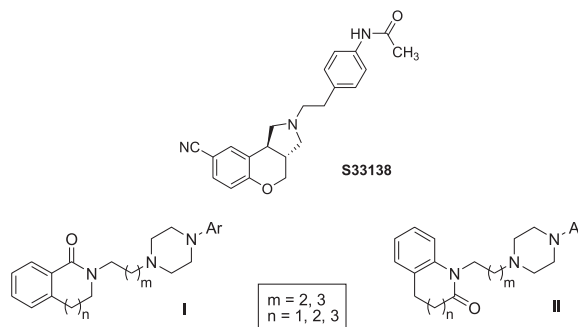
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Schizophrenia is a chronic, severe, and disabling neurodevelopmental disorder that affects about 1% of the world population. Recent studies¹ have suggested that the dopamine D₃ receptor subtype, since it is selectively located in the limbic brain areas known to be associated with cognitive and emotional functions, might be a promising rational target for development of new drugs for the treatment of this pathology.

Major progress in D₃ receptor drug development would allow us to a better understanding of this illness and to improve its treatment, as compounds with dopamine D₃ antagonist profile may give rise to beneficial antipsychotic activity avoiding extra-pyramidal side effects of the actual therapy.² Thus, compound S33138, a selective D₃ antagonist, is now in Phase II trials for the treatment of schizophrenia.³

In this communication we will report the synthesis and binding affinity of two new series D₃ antagonists (**I** and **II**) based on a benzolactam scaffold, which maintain three characteristic elements of many dopamine D₃ receptor antagonist: 1) an amine moiety, 2) a spacer, usually a linear alkyl chain, and 3) a hydrophobic residue, often connected through an amide bond.⁴ These new compounds will allow us to evaluate some of the structural requirements for high affinity and selectivity binding at the D₃ receptor. These new compounds were also used to carry out docking simulation studies and 3D QSAR analysis in order to obtain further insight into the structural requirements for high affinity and selectivity binding at the D₃ receptor.



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References:

- [1] a) Joyce, J. N.; Miador-Woodruff, J. H. *Neuropsychopharmacology* 1997, 16, 375; b) Joyce, J. N. *Pharmacol. Ther.* 2001, 90, 231.

[2] Joyce, J. N.; Millan, M. J. *Drug Discovery Today* 2005, 10, 917.

[3] Dubuffet, T.; Newman-Tancredi, A.; Cussac, D.; Audinot, V.; Loutz, A.; Millan, M. J.; Lavielle, G. *Bioorg. Med. Chem. Lett.* 1999, 9, 2059.

[4] Haebling, A. E., Stark, H. *ChemBioChem* 2002, 3, 946.

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STRUCTURE-ACTIVITY RELATIONSHIP IN A SERIES OF PPAR δ AGONISTS FOLLOWING A FIBRATE-BASED DESIGN

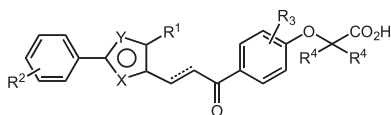
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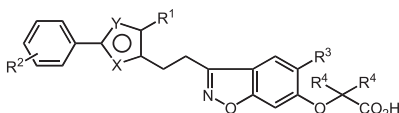
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Derivatives with general structures **I** and **II** have been synthesized and evaluated for their potential to favorably alter lipid disorders, including metabolic syndrome. Compounds were assessed for their transcriptional activation effects in cell-based transactivation assays using GAL4-PPAR chimeras and full-length receptors, as well as for their effects on lipid, glycemic and body weight variables in female obese Zucker fatty rats and in db/db mice, following one and two weeks of oral administration.

In both series were found NCEs with high in vitro selectivity for the PPAR δ receptor (nM range), as well as α/δ -, α/γ - and pan-agonists. Some compounds tested in female obese Zucker fatty rats showed an increase of HDL -cholesterol and apolipoprotein A-I, respectively. The compounds described herein are among the highest PPAR δ specific agonists described today.



Derivatives of type I



Derivatives of type II

References

- [1] S. Sakuma et al. US 6787552, 2004: PPAR Delta Activators
- [2] S. Sakuma et al. US 7265137, 2006: Activator for Peroxisome Proliferator-Activated Receptor
- [3] S. Sakuma et al. US 7119104, 2005: Activator for Peroxisome Proliferator-Activated Receptor Delta

P376

SYNTHESIS AND BIOLOGICAL EVALUATION OF A PRO-APOPTOTIC BENZODIAZEPINE (RO5-4864) PRODRUG CONJUGATED TO SHIGA TOXIN B-SUB-UNIT

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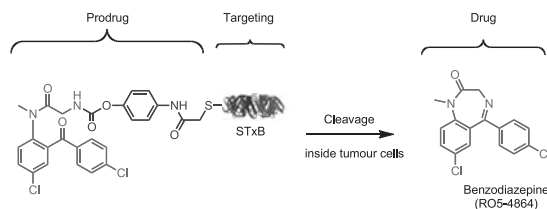
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Peripheral benzodiazepine receptors are potential targets for cancer therapeutics, using specific ligands such as the pro-apoptotic benzodiazepine RO5-4864. However, the poor water solubility of this compound has been a limitation to its use in vivo. Moreover, a promising approach to overcome side effects in cancer chemotherapy and to achieve a more tumour selective treatment is a targeted prodrug therapy. For these reasons, we propose an approach for the targeting of a prodrug form of the benzodiazepine. The tumour targeting element, Shiga toxin B-subunit, is non-toxic by itself, but selectively accumulates in different human cancer cells.

We describe an efficient synthesis scheme for the conjugation, via a cleavable linker arm, of RO5-4864 to the tumour delivery tool, the B-subunit of Shiga toxin (STxB).

The conjugate is water-soluble and specifically targets cancer cells that overexpress the glycolipid Gb3. After internalization via the retrograde transport route, the prodrug is cleaved inside cells and releases the free benzodiazepine. In summary, delivery by STxB increases the cytotoxic activity of RO5-4864 and its tumour specificity.



References

For another example of drug targeting with Shigatoxin, see:

- [1] A. El Alaoui, F. Schmidt, M. Amessou, M. Sarr, J.-C. Florent, L. Johannes, Shiga Toxin-mediated Retrograde Delivery of a Topoisomerase I Inhibitor Prodrug, *Angew. Chem. Int. Ed.*, 2007, 46, 6469-6472.

P377

SYNTHETIC APPROACHES TO TETRASUBSTITUTED IMIDAZOLES

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In 1988 the inhibition of IL-1 β production by the anti-inflammatory compound SKF86002 was reported^[1], but it took until 1994 to discover that the molecular target of the pyridinyl imidazole class of compounds indeed proved to be p38 MAPK, previously known as CSBP 32. Originally developed from the early lead SKF86002^[2], the 1st generation p38 MAPK inhibitors were structurally related to the prototypical pyridinyl imidazole SB203580. The central pharmacophore consists of a vicinal pyridine/fluorophenyl system, and to date, several inhibitors fulfilling this concept have been advanced into clinical trials^[3]. Crystallographic data demonstrate that SB203580 binds in the ATP binding site of p38 α ^[4]. In detail, the pyridine ring nitrogen is accepting a hydrogen bond from the backbone NH of Met109 in the hinge region. Vicinal to this interaction site, the 4-fluorophenyl moiety occupies the "hydrophobic pocket I" causing selectivity of vicinal pyridine/fluorophenyl inhibitors for MAPK over other PKs. First generation imidazole based inhibitors like SB202190 or SB203580 suffer from none mechanistic side effects making more structural modification necessary. Our 2-thioimidazoles proved to have decisive advantages over prototype SB203580-like 2-arylimidazoles and exhibited markedly reduced cytochrome P450 interaction, improved pharmacokinetic and metabolic properties^[5].

We tried to synthesize SKF- and SB-like inhibitors via different synthetic strategies. The initial synthetic approaches were coupling reactions such as Hiyama, Sonogashira, Suzuki-Miyaura, Negishi, Kumada-Corriu and Stille-coupling of the 4-fluorophenyl-thiazolines/dihydrothiazolines and 2-methylsulfanyl-imidazoles at the C5-position with the corresponding halogenides. The metallo-organic precursors like Zn, Mg and Li-thiazolines/dihydrothiazolines and 2-methylsulfanyl-imidazoles are readily available. The boronic acid of the aimed imidazole derivatives and the precursors of the Stille-coupling, the stannylimidazoles could not be generated. The precursor of the Hiyama-coupling, the corresponding silylimidazole, was obtained but not for all of the three aspired synthetic platforms. In summary, there are four possible coupling centers available, Mg, Zn, Li and the silylimidazole. In further coupling reactions, no coupling products could be observed. A possible explanation concerning the problem of the Kumada-coupling could be due to the presence of the nitrogen atoms which are potential metal-chelators. For explaining the challenges in building up the coupling-centers of Suzuki- and Stille-couplings, we investigated

the inverse Suzuki-coupling of the imidazole-halogenides with an aryl boronic acid^[6]. In combination with the obtainable silylated imidazoles, a possible explanation concerning the build-up of the envisaged stannylimidazoles or boronic acids could be seen as an electron density problem. Hence, we synthesized a number of SKF-like inhibitors by using alternative synthetic routes. We compared the activity data of our novel inhibitors with representative compounds of our diaryl-2-alkylsulfanylimidazoles.

References

- [1] Lee J.C., Griswold D.E., Votta B., Hanna N.; *Int. J. Immunopharmacol.*, 1988, 10, 835-83
- [2] Lantos I., Bender P. E., Razgaitis K. A., Sutton B. M., DiMartino M. J., Griswold D. E., Walz, D. T.; *J. Med. Chem.* 1984, 27, 72-75
- [3] Goldstein D. M., Gabriel T.; *Curr. Top. Med. Chem.* 2005, 5, 1017-1029.
- [4] Wang Z., Canagarajah B. J., Boehm J. C., Kassisa S., Cobb M. H., Young P. R., Abdel-Meguid S., Adams J. L., Goldsmith E. J.; *Structure* 1998, 6, 1117-1128.
- [5] Laufer, Stefan A.; Wagner, Gerd K.; Kotschenreuther, Dunja A.; Albrecht, W.; *J. Med. Chem.*, 2003, 46, 3230-3244
- [6] Laufer S., Hauser D., Liedtke, A; *Synthesis* 2008, No. 2, 253-266

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DIPEPTIDYLPEPTIDASE IV INHIBITORS FROM STRUCTURE-BASED DESIGN USING SCAFFOLD DERIVED FROM HERBAL ACTIVE COMPONENTS

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In this study, dipeptidyl peptidase IV (DPP IV) was selected as a drug target in searching for new antidiabetic drugs. Novel potent DPP IV inhibitors were developed based on the information of medicinal herbs and structured based drug design. The DPP IV template were prepared from human DPP IV crystal structure bound with 1-[2-(S)-amino-3-biphenyl-4-yl-propionyl]-pyrrolidine-2-(S)-carbonitrile (PDB: 2AJL) and validated (RMSD < 2.0 Å). The compounds with the combined structure between the scaffold of main components in herbal and the variation of side chains were docked with prepared DPP IV template by use of Autodock program suit. Hits containing SMCS function (series A) and chromone function (series B) with docking energy between -12 to -11 Kcal/mol were synthesized and found to have inhibitory action against DPP IV.

References

- [1] Lu, L.; et al. *Eur. J. Med. Chem.* 2007, 20, 1.
- [2] Qiao, L.; Baumann, C.A.; et al. *Bioorg. Med. Chem. Lett.* 2006, 16, 123.
- [3] Madar, D.J.; Kopecka, H.; et al. *J. Med. Chem.* 2006, 49, 6416.
- [4] Kim, D.; Wang, L.; et al. *J. Med. Chem.* 2005, 48, 141.

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IN SILICO PREDICTION OF THE CARDIOTOXICITY OF DRUG CANDIDATES

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The hERG (human ether-go-a-go) channel encodes a tetrameric delayed rectifier K⁺ channel, essential for normal electrical activity in the heart. Inherited mutations in the hERG gene can cause long QT syndrome, an abnormality of cardiac muscle repolarization that predisposes individuals to life-threatening arrhythmias (1). Arrhythmia can also be induced by blockade of hERG channels by various drugs, which has led to withdrawal of a number of widely prescribed drugs such as cisapride, terfenadine, sertiindole (2). Understanding the structural requirements of molecules that block hERG is necessary to avoid potential toxicity. In the absence of 3-dimensional hERG structures, homology modeling methods, using recently determined crystal structures of potassium channels (KvAP, Kv1.2, KcsA) are applied. The models are validated and refined making use of experimental data, conservation analysis and molecular dynamics simulations. Using the 3-dimensional models, a series of blockers with known affinities are docked and free binding energies are calculated. To validate our approach, a comparison with other potassium channels, with known structure and drug interactions is performed. Based on the 3-dimensional structure models, we aim at identifying important structural features underlying hERG inhibition and answering the question of multiple putative binding sites. Understanding of structure-activity relationships between hERG and drug molecules will enable to assess the risk of hERG inhibition by potential drug-candidates.

- [1] Splawski I, Shen J, Timothy KW, Lehmann MH, Priori SG, Robinson JL, Moss AJ, Schwartz PJ, Towbin JA, Vincent GM, Keating MT. Spectrum of mutations in long-QT syndrome genes KvLQT1, hERG, SCN5A, KCNE1, and KCNE3. *Circulation* 2000; 102:1178-1185.
- [2] Sanguinetti MC, Tristani-Firouzi M. hERG potassium channels and cardiac arrhythmia. *Nature* 2006; 440:463-469.

P381

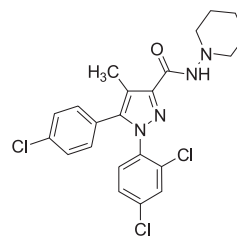
NEW CB1 RECEPTOR ANTAGONISTS: SYNTHESIS AND PHARMACOLOGICAL EVALUATION

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Rimonabant, 5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-N-(piperidin-1-yl)-1H-pyrazole-3-carboxamide, SR141716 is a potent and selective antagonist for the CB1 receptor¹. It was launched in 2006 by Sanofi-Aventis for the treatment of obesity and associated risk factors.

Our interest in this field prompted us to design and synthesize a series of rimonabant analogue compounds by the method of Analogue Based Drug Research² to obtain more effective derivatives. As a result of this study almost 200 new CB1 chemical entities were synthesized in this field. The lead compound exhibited very promising pharmacological profile, in particular: low nanomolar CB1 affinity, full CB1 antagonist with CB2 selectivity over 100 fold, similarly efficacy in the food intake test to rimonabant, in the diet-induced obesity test (disease model) body weight decreasing effect was dose-dependent and similar to reference compound, rimonabant. These biological data and structure-activity relationship (SAR) will be presented and discussed.



Rimonabant (Acomplia®)

References

- [1] (a) Rinaldi-Carmona, M.; Barth, F.; Héaulme, M.; Shire, D.; Calandra, B.; Congy, C.; Martinez, S.; Maruani, J.; Néliat, G.; Caput, D.; Ferrara, P.; Soubrié, P.; Brelière, J.C.; Le Fur, G. SR141716A, a potent and selective antagonist of the brain cannabinoid receptor. *FEBS Lett.* 1994, 350, 240-244. (b) Sorbera, L. A.; Castaner, J.; Silvestre, J. S. Rimonabant hydrochloride. *Drugs Future* 2005, 30, 128-137.
- [2] Fischer J.; Ganelli C. R. Analogue Based Drug Discovery. Wiley-VCH Verlag GmbH & Co. KGaA: New York, 2006.

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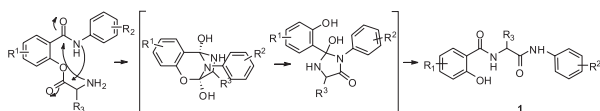
SALICYLANILIDE AMINO ACID ESTERS REARRANGEMENT LEADS TO ANTIMYCOBACTERIAL ACTIVE DI- AND TRIAMIDES

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Multidrug resistance to antimicrobial agents is an unavoidable side effect and goes hand in hand with the relentless drive of bacterial evolution. The ongoing combat against drug-resistant bacteria leads to the search for new types of active molecules, with different or novel mechanisms of action. Salicylanilides may provide the answer. They have a wide range of biological effects, principle of which is antifungal and antibacterial activity.¹ They serve as inhibitors of the protein tyrosine kinase, which is the epidermal growth factor receptor.² Generally, they are suggested to compete with ATP for binding at the catalytic domain of tyrosin kinase.³ We have found significant *in vitro* antimycobacterial activity against *Mycobacterium tuberculosis*, as well as non tuberculosis strains *M. avium* and *M. kansasii* (MIC 1-4 µg/ml).^{4,5} To improve their bioavailability and decrease toxicity, amino acid ester prodrugs were designed. In the course of blocking the phenolic hydroxyl group by esterification, with *N*-protected amino acids, we have obtained esters that have significant antimycobacterial activity. After *N*-deprotection, esters immediately rearranged to *N*-(2-anilino-2-oxoethyl)-2-hydroxybenzamide derivatives ("diamide") **1** (Scheme 1).



Scheme 1

The fact that, during the molecular rearrangement, a five membered imidazolinone ring is formed as an intermediate is supported by our results. Its dehydrated form, 1*H*-imidazol-5(4*H*)-one, was isolated and then confirmed by MS, IR, ¹H NMR, ¹³C NMR and ¹⁵N NMR in 2D experiments. As a general rule, all of the salicylanilide esters underwent the same rearrangement. This is demonstrated by the same type of intermediate being isolated during the glycine and L-phenylalanine esterification of 5-chloro-*N*-(1-(3-chlorophenylamino)-3-methyl-1-oxobutan-2-yl)-2-hydroxybenzamide ("triamide" products were obtained). All rearranged products of type **1** demonstrated the required antimycobacterial activity.

Acknowledgements

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References

- [1] Vinšová J.; Imramovský A. *Ces. Slov. Farm.* 2004, 53, 6.
- [2] Kamath S.; Buolamwini J. K. *Med. Res. Rev.* 2006, 26, 569.
- [3] Liechti C.; Séquin U.; Bold G.; Furet P.; Meyer T.; Traxler P. *Eur. J. Med. Chem.* 2004, 39, 11.
- [4] Waisser, K.; Bures, O.; Holy, P.; Kunes, J.; Oswald, R.; Jiraskova, L.; Pour, M.; Klimesova, V.; Kubicova, L.; Kaustova, J. *Arch. Pharm.* 2003, 336 (1), 53-71.
- [5] Vinsova J.; Imramovsky A.; Buchta V.; Ceckova M.; Dolezal M.; Staud F.; Jampilek J.; Kaustova J. *Molecules* 2007, 11, 1.

P384

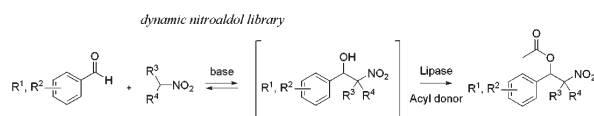
DYNAMIC COMBINATORIAL RESOLUTION: DIRECT ASYMMETRIC LIPASE-MEDIATED SCREENING OF A DYNAMIC NITROALDOL LIBRARY

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Using a new method to adaptive enrichment processes combining the DCL chemistry, reversible reactions/interactions under thermodynamic control, with kinetically controlled selective steps, the chemical diversity can easily be targeted. Such combined processes exert an area of particular promise, since these in principle can be efficiently applied to drug - and biocatalyst - discovery campaigns. An advantage with this concept is the potential use of catalytic amounts of target species, avoiding stoichiometric amounts of enzymes/receptors that can be difficult to obtain.

In the present investigation, the nitroaldol (Henry) reaction was developed as an efficient C-C bond-forming route to dynamic combinatorial libraries (DCLs) from a series of aldehydes and nitro compounds. These DCLs generated under thermodynamic control were coupled in a one-pot process with kinetically controlled lipase-mediated transesterification (see scheme 1). The asymmetric resolution of the DCLs by the enzyme led to enantiomerically pure β-nitroacetates in high yield.¹ Moreover, the asymmetric synthesis of β-nitroalkanol derivatives was simply achieved by a combined nitroaldol (Henry) reaction with lipase-catalyzed transesterification in high yield and enantiomeric purity (up to 92% and 99% ee) through a direct one-pot procedure.²



[1] P. Vongvilai, M. Angelin, R. Larsson, O. Ramström, *Angew. Chem. Int. Ed.* 2007, 46, 948–950.

[2] P. Vongvilai, R. Larsson, O. Ramström, *Adv. Synth. Catal.* 2008, 350, 448–452.

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SYNTHESIS OF D-RIBO-PHYTOSPHINGOSINE BASED ON REGIOSELECTIVE AND DIASTEREOSELECTIVE AMINATION WITH USE OF CHLOROSULFONYL ISOCYANATE

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Sphingosines which are important membrane components have a long-chain that forms the backbone of sphingolipids. Sphingolipids (e.g. ceramides, sphingomyelins, gangliosides, cerebroside), the second large class of membrane lipids, have a polar head group and two nonpolar tails. They are related to various cellular roles such as cell growth, differentiation and have a direct bearing on cancer.

Phytosphingosine which contains a hydroxyl at position 4 but lacks the double bond was first isolated from mushrooms in 1911 and extracted from fungal, plant, and animal sphingolipids. It is a bioactive lipid that has potential heat stress signal in yeast cell. Because it is hard to obtain homogeneous material from natural sources, the development of synthetic method is needed.

Lately, we have developed a short and efficient synthetic method for regioselective and diastereoselective amination with use of chlorosulfonyl isocyanate and have extended its application to various syntheses of (2*R*,5*S*)-dihydroxymethyl-(3*R*,4*R*)-dihydroxypyrrolidine (DGDP), indolizidine alkaloid (–)-lentiginosine and so on.

As a program part of our research aimed at developing enantioselective syntheses of chiral 1,2-amino alcohols, we envisaged that our CSI-mediated stereoselective amination of polybenzyl ethers could be readily applied to the synthesis of D-ribo-phytosphingosine

In this presentation, we described the total synthesis of D-ribo-phytosphingosine from the readily available D-ribose in 8 steps. The key step in this route is the diastereoselective amination of anti,anti-1,2,3-tribenzyl ether using our CSI methodology.

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TOTAL SYNTHESIS OF (–)-LENTIGINOSINE AND ITS ANALOGUES AS GLYCOSIDASE INHIBITORS

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Polyhydroxylated pyrrolizidine and indolizidine alkaloids are powerful glycosidase inhibitors leading to the development of new drugs for the treatment of diabetes,

viral infections (e.g., HIV and influenza), malaria, and cancer. These compounds with the 5,5- and 5,6-heterocyclic ring system have been often found in natural plants and microorganisms, and have been extensively studied as the subjects of intensive research in synthetic and pharmacological fields. However, we were surprised to discover that compounds with the 5,7-heterocyclic ring system, i.e. 1*H*-pyrrolo [1,2-*a*]azepines, have been received little attention.

Recently we reported regioselective and diastereoselective amination of tribenzyl ethers using chlorosulfonyl isocyanate (CSI) and its application to the total synthesis of pyrrolidine alkaloid 1,4-dideoxy-1,4-imino-D-arabinitol (DAB1), and indolizidine alkaloid (–)-lentiginosine.

As a part of our research program aimed at developing enantioselective synthesis of polyhydroxylated alkaloids, we envisaged that the pyrrolidine intermediate used in the preparation of (–)-lentiginosine could be readily applied to the synthesis of polyhydroxylated ring systems with other ring size combinations, such as the 5,5- and 5,7-heterocyclic ring systems of (1*R*,2*R*,7*aR*)-1,2-dihydroxypyrrolizine and (1*R*,2*R*,9*aR*)-1,2-dihydroxypyrroloazepine.

Herein we describe stereoselective total synthesis of three (–)-lentiginosine analogues, e.g. 5,5-, 5,6-, 5,7-bicyclic dihydroxylated alkaloids, from commercially available D-lyxose via CSI-mediated stereoselective amination of polybenzyl ether, ring-closing metathesis (RCM), and intermolecular olefination employing the second generation Grubbs catalyst.

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SYNTHESIS AND BIOLOGICAL EVALUATION OF N-ACYLHYDRAZONES AS INHIBITORS OF MUR LIGASES

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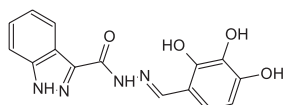
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Cell wall biosynthesis is an essential process that is exclusive to bacteria and thus it remains an attractive target for antimicrobial action. The early cytoplasmic steps of peptidoglycan precursor synthesis, which are catalyzed by the enzymes MurA to MurF, are still relatively underexploited. The Mur ligases constitute a series of four ATP-dependent enzymes (MurC to MurF) that are responsible for the step-wise addition of the pentapeptide side-chain onto the D-lactoyl group of the uridine diphosphate-*N*-acetylmuramic acid (UDP-MurNAc) initially formed via MurA and MurB. In *Escherichia coli*, the first amino acid of the growing peptide stem is L-Ala (MurC),

followed by D-Glu (MurD), *meso*-diaminopimelic acid (MurE), and finally the D-Ala-D-Ala dipeptide (MurF).^[1,2]

A series of compounds with an *N*-acylhydrazone scaffold was synthesized and screened for inhibition of MurC-F enzymes. Compounds with micromolar inhibitory activities against Mur enzymes were identified, and some of them also showed antibacterial activity.



IC₅₀ = 123 μM (MurC)
 = 230 μM (MurD)
 = 70 μM (MurE)
 = 10 μM (MurF)

References

- [1] J. van Heijenoort, *Nat. Prod. Rep.* 2001, 18, 503-519.
 [2] H. Barreateau, A. Kovac, A. Boniface, M. Sova, S. Gobec, D. Blanot, *FEMS Microbiol. Rev.* 2008, 32, 168-207.

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AUTOMATION AND APPLICATION OF A MINIATURIZED RADIOLIGAND ASSAY ON GPCR SCREENING

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The G-protein coupled receptor (GPCR) superfamily is regarded as the largest known class of molecular targets with proven therapeutic value, since 30 to 45% of currently available drugs act through these receptors (Davies et al. 2007). Human α_2 -adrenergic receptors (α_2 -AR) belong to the rhodopsin-like family of GPCRs, and there are three different human α_2 -AR subtypes: α_2A , α_2B and α_2C , with slightly different pharmacological properties. These receptors are known to mediate a diverse spectrum of physiological functions, thus giving potential therapeutic indications to α_2 -AR- agonists and antagonists (Gentili et al., 2007). In addition, only a few subtype-selective ligands exist which can be used to determine the physiological and pathophysiological significance of these individual receptor subtypes. In that direction, the screening for subtype-selective ligands could provide with excellent molecular probes for further drug discovery. The aim of this project was to optimize and automate a radioligand- based assay for screening of α_2B -adrenoceptors interacting- compounds (agonist or antagonists) using 96-well microtiter plates in Combi Multidrop dispenser and Biomek 3000 environments. Miniaturization of this competitive binding assay included a first sequence of experiments where total reaction volume, optimal reading time and optimal protein concentration of the receptor- containing membrane fractions were established. Assay

quality was monitored and acceptance limits of the automated assay were set as: $Z' > 0.5$, $S/B > 3$ and $S/N > 8$. Other parameters typically used as indicators of assay performance were also measured: well-to-well; plate-to-plate and day-to-day variations and DMSO tolerability. Furthermore, a chemical library was screened including a subset of 2112 FDA- approved drugs and a second subset of 17952 diverse compounds (from the collection of ChemDiv Company, San Diego, US). A total of 180 compounds were declared active (0.9% of the total) at 10 μM and most of the positive hits were found on the FDA-subset (110). When retested at 1 μM, 87 compounds were reconfirmed as highly active, producing more than 50% inhibition of radioligand binding and being more active than adrenaline, used as the agonist control compound. This specific group of molecules is now under further investigation to establish their IC₅₀, K_i values as well as their specificity on the interaction against other α_2 -AR subtypes. In summary, this project shows the feasibility of using a competitive binding assay in an automated screening environment aimed for the bioactivity profiling of large libraries.

- [1] Davies MN, Gloriam DE, Secker A, Freitas AA, Mendao M, Timmis J and Flower DR. *Proteomics* (2007). 7: 2800-2814.

- [2] Gentili F, Pigini M, Piergentili A and Giannella M. *Curr Top Med Chem.* (2007).7(2):163-186.

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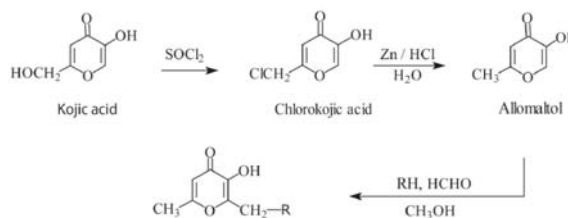
SYNTHESIS AND ANTIMICROBIAL ACTIVITIES OF NOVEL MANNICH DERIVATIVES OF 4H-PYRAN-4-ONE'S

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In the past years kojic acid and its derivatives maltol and allomaltol were investigated and proved to be active for their antimicrobial [1-3] and antitumoral [4] activities. Since Yabuta [5] first synthesized allomaltol from kojic acid in 1924 and was improved by Ellis [6], many researchers studied over the mannich reactions of kojic acid and chlorokojic acid with various amines [7,8].

In this study we first to prepared allomaltol using commercially available kojic acid according to given literature and with appropriate amines (RH) prepared some mannich bases using formaldehyde and methanol in room temperature using the synthetic procedure given below.



RH: substituted piperidine, piperazine, morpholine derivatives

Purity control and structural elucidation of new compounds were controlled by using elemental analysis and IR, ¹H, ¹³C-NMR, Mass spectrometers, respectively.

Final compounds were tested and for their antimicrobial activities

References

- [1] M. Veverka, Chem. Papers 1992, 46(3), 206-210.
- [2] P. A. Wolf, W. M. Westveer, Arch. Biochem, 1950, 28,201-206.
- [3] T. Kotani, I. Ichimoto, C. Tatsumi, T. Fujita, Agr. Biol.Chem. 1975, 39(6), 1311-1317.
- [4] M. Yamato, Y. Yasumoto, J. Sakai, R. F. Luduena, A. Baneerjee, T. Tashiro, J. Med. Chem. 1987, 30, 1897-1900.
- [5] T. Yabuta, J. Chem. Soc. 1924, 125, 575-587.
- [6] B. L. Ellis, A. K. Duhme, R. C. Hider, M. B. Hossain, S. Rizvi, D. van der Helm, J. Med. Chem. 1996, 39, 3659-3670.
- [7] G. O'Brien, J. M. Patterson, J. R. Meadow, J. Org.Chem. 1960, 25, 86-89.
- [8] L. L. Woods J. Am. Chem. Soc. 1946, 68, 2744-2745.

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DESIGN AND SYNTHESIS OF SUBSTITUTED-N-[2-(3H-IMIDAZOL-4-YL) ETHYL]BENZAMIDE DERIVATIVES AS PHOSPHODIESTERASE IV B INHIBITORS

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Phosphodiesterase-4 isoenzymes have absolute specificity for cyclic adenosine-3',5'-monophosphate and are considered potential therapeutic targets for the treatment of chronic inflammatory disorders, such as chronic obstructive pulmonary disease, with small-molecule inhibitors. Several selective phosphodiesterase-4 inhibitors are in clinical trials of chronic obstructive pulmonary disease, including cilomilast and roflumilast. Despite some encouraging data from phase III clinical trials, the current generation of phosphodiesterase-4 inhibitors is hampered by a low therapeutic ratio. Indeed, a major obstacle is their propensity to evoke non-steroid-like side effects, of which nausea, diarrhea, abdominal pain, vomiting, and dyspepsia are the most common [1]. Consequently, research into new, isoenzyme-selective PDE IV inhibitors is seen as important.

In 2004, Card et al. described the high-resolution crystal structures of the catalytic domains of PDE4B, PDE4D, and PDE5A with ten different inhibitors, including the drug candidates cilomilast and roflumilast, for respiratory diseases. (PDB code: 1XMU for PDE4B) [2].

In this study, first with FTrees XL software (BiosolveIT GmbH, Germany) we performed a similarity match-search between roflumilast and 62517 compounds depoted in the "lead-like subset" of the Zinc database using Jarvis-Patrick parameters as clustering algorithm and filtered the

200 of them in range of > 0.8 overall similar to roflumilast. We then measured the docking/scoring functions of these compounds in the active site of catalytic domain of human phosphodiesterase 4B (PDB code: 1XMU) using Surflex-Dock mode in Sybyl 7.3. 2-Ethoxy-N-[2-(3H-imidazol-4-yl) ethyl]benzamide had the greatest score in C-Score calculations.

With these findings we decided to synthesize a series of substituted-N-[2-(3H-imidazol-4-yl)ethyl]benzamide derivatives to perform enzymatic inhibition assay to evaluate further selective PDE IV B antagonists where the major therapeutic areas are investigated for asthma and COPD (chronic obstructive pulmonary disease) [3,4].

References

- [1] Giembycz MA; Proc Am Thorac Soc. 2005; 2(4):326-33.
- [2] Card GL, England BP, Suzuki Y, Fong D, Powell B, Lee B, Luu C, Tabriziad M, Gillette S, Ibrahim PN, Artis DR, Bollag G, Milburn MV, Kim SH, Schlessinger J, Zhang KY.; Structure. 2004 Dec; 12(12):2233-47.
- [3] Barnes, P. J. J.; Allergy Clin. Immunol., 2001; 108: S72-S76.
- [4] Barnette, M. S.; Prog. Drug Res., 1999; 53:193-229.

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DESIGN, SYNTHESIS AND MODELING STUDIES OF SIGMA RECEPTORS LIGANDS*

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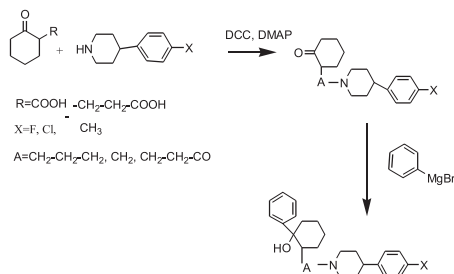
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Sigma (σ) receptors are functional, membrane-bound proteins distributed throughout the brain and peripheral organs. $\sigma 1$ and $\sigma 2$ receptor types are clearly established, and further pharmacological differentiation may be possible. $\sigma 1$ receptors are implicated in central nervous system (CNS) disorders such as depression, schizophrenia and dementia. Further, $\sigma 1$ receptor agonists have value as neuroactive agents, while antagonists may help alleviate cocaine addiction. The significance of the σ receptor system to human health is augmented through the overexpression of σ sites by a number of cancers. Thus, σ receptor ligands might be used for the detection and treatment of malignant neoplastic diseases. $\sigma 2$ receptors may be of particular prognostic relevance, as the extent of their expression seems indicative of the proliferative status of tumors [1]. Structurally, σ ligands are represented by wide variety of chemical scaffolds, for example (+)-benzomorphans, phenylpiperidines, (+)-pentazocine and haloperidol [2].

Based on medicinal-chemistry-guided approach, a new series of 4-hydroxy-4-substituted-phenylpiperidine derivatives were synthesized according to previous modeling studies [3] (Scheme). Purity control and structural elucidation of new ligands were controlled by using ele-

mental analysis and IR, ¹H, ¹³C-NMR, Mass spectrometers, respectively.

Binding characteristics will be evaluated in further studies.



Scheme: Synthetic pathway for the compounds

- [1] Lever, J.R., Gustafson, J.L., Xu, R., Allmon, R.L., Lever, S.Z., Synapse 59; 350-358 (2006).
- [2] Vangveravong, S., Xu, J., Zeng, C., Mach, R.H., Bioorg. Med. Chem. 14, 6988-6997 (2006).
- [3] Gund, T. M.; Floyd, J.; Jung, D. J. Mol. Graphics Modell. 22, 221-230 (2004).

*This work is supported by Novartis

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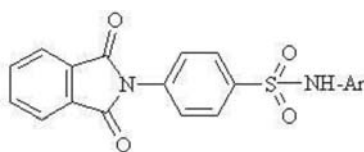
DESIGN, SYNTHESIS AND TNF α MODULATORS ACTIVITY OF NOVEL PHYTALIMIDE DERIVATIVES, STRUCTURALLY RELATED TO THALIDOMIDE

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Thalidomide was first synthesized as an antihistaminic drug in 1954 but withdrawn from the market because of its catastrophic teratogenicity [1, 2]. Despite this effect of thalidomide, there is remarkable interest over this compound due to its usefulness for the treatment of ENL, inflammatory and autoimmune diseases such as Behçet's diseases, rheumatoid arthritis and wasting syndrome [3, 4]. The effectiveness of the drug in these diseases has mostly been attributed to its specific inhibitory activity on TNF- α productions. TNF- α is a key cytokine produced primarily by monocytes and macrophages which is involved in the host immune response [5].

In this work a new series of new phthalimide derivatives below were synthesized according to previous studies [6]. Structural elucidations of the compounds were performed by using elemental analysis, IR, ¹H-NMR and mass spectroscopy.



Ar: Heterocyclic rings

References

- [1] McBride, W. G., *Lancet* 1961,2,1358.
- [2] Sheskin, J., *Clin. Pharmacol. Ther.* 1965,6, 303.
- [3] Sommer, C. *Drugs Future* 1999, 24, 67.
- [4] Zwingenberger, K., Wnendt, S. *J. Inflamm.* 1996, 46, 177.
- [5] Gütschow, M. Hecker, T., Thiele, A., Eger, K., *Bioorganic & Med. Chem.* 2001, 1059.
- [6] Machado L. A., Lima, L.M., Araujo, J. x., Fraga, C., A., M., Koatz, V.L.G., Barreiro, E. J., *Bioorganic & Med. Chem. Letters*, 2005, 15, 1169.

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LOSARTAN DERIVATIVES FOR MOLECULAR IMAGING OF ANGIOTENSIN-II RECEPTOR

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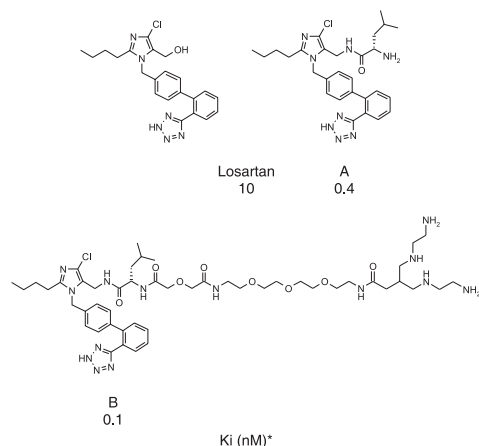
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Angiotensin-II is an active component of the renin-angiotensin system playing an important role in regulation of cardiovascular parameters. Expression of angiotensin-II type 1 receptor is involved in pathological processes like cardiac hypertrophy, fibrosis and remodelling, and angiotensin-II receptor antagonists, like Losartan, Valsartan and Candesartan are widely used drugs for treatment of hypertension and heart failure. Non-invasive molecular imaging of this receptor may have value in detecting pathophysiological conditions at an early stage and have clinical implications in therapy decisions.

Losartan, the first angiotensin-II receptor antagonist introduced to the market, was transformed into a radio-pharmaceutical comprising a ^{99m}Tc label for SPECT imaging. Substitutions were made at the 5-position of the imidazole ring by converting the hydroxymethyl group into an amine as anchoring point for further derivatisation using peptide coupling techniques. The syntheses were carried out on a trityl modified solid support by attaching Losartan through the tetrazole function leaving the hydroxyl group open for chemical modification.

One part of the programme focussed on developing a derivative with enhanced receptor affinity compared to Losartan itself and it was demonstrated that the hydrophobic side chain of leucine was preferable, increasing potency by at least one order of magnitude (A). Furthermore, it was seen that introducing Tc-chelators, like the tetraamine moiety, was well tolerated (B). The short PEG linker was introduced as a biomodifier to obtain a more favourable pharmacokinetic profile, more rapid blood clearance and increased renal excretion as compared to the parent drug molecule.

In conclusion, it was demonstrated that Losartan successfully could be transformed from a therapeutic drug into an agent suitable for *in vivo* imaging of the angiotensin-II receptor.



*Determined by competition of 125-I [Sar¹,Ile⁸]angiotensin-II binding to angiotensin-II receptor containing cell membranes.

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ENANTIOSELECTIVE SULFOXIDATION OF TRI- AND TETRA-SUBSTITUTED 2-THIOIMIDAZOLES

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A series of recently published tri-and tetrasubstituted 2-thioimidazoles^[1] are efficient inhibitors for the p38 mitogen-activated protein (MAP) kinase, which is a promising target for the treatment of a number of inflammatory and autoimmune diseases like rheumatoid arthritis, psoriasis and inflammatory bowel disease. The p38 MAPK are a family of serine/threonine protein kinases that play important roles in many cellular processes including inflammation, cell differentiation, cell growth and death. In response to extracellular signals like osmotic shock, mechanic stress and lipopolysaccharide exposure^[1], the activation of p38 MAPK can lead to increased activities of proinflammatory cytokines, such as TNF- α and IL-1 β . Therefore p38 MAPK is an important key enzyme in the biosynthesis and in the regulation of the expression of inflammatory mediators.

CYP-mediated metabolism studies of the pyridinyl-2-thioimidazole derivative ML3403 showed a fast and high conversion to its active sulfoxide phase I metabolite ML3603^[2], which has a centre of asymmetry in the sulphur atom meaning that two sulfoxide enantiomers exist. In general, two enantiomers of a chiral drug can differ in pharmacodynamic and/or pharmacokinetic properties like absorption, distribution, metabolism and excretion as a consequence of the stereoselective interaction with an enzyme or receptor^[3]. To prove possible advantages of one enantiomer of a chiral drug instead of the racemate, it

is of interest for us to develop new procedures for the enantioselective synthesis of our heterocyclic sulfoxides.

The metal catalyzed asymmetric oxidation of sulfides is an efficient synthetic method for the preparation of chiral sulfoxides. In this context, transition metals such as Ti or Fe are utilised as catalyst precursors in the presence of a chiral ligand and a hydroperoxide as oxidant. By the use of the system Ti(O-*i*Pr)₄ / D-DET (Diethyl D-tartrate) / CHP^[4] the sulfoxides of selected 2-thioimidazoles could be obtained with moderate to good yields (>40 %) and with an enantiomer ratio up to 88.3 % : 11.7 %, which was determined by chiral, analytical HPLC. Furthermore, a change of configuration was observed by the use of L-DET (Diethyl L-tartrate), instead of D-DET. The absolute configuration of both enantiomers could be determined by x-ray crystal structure analysis. The selected 2-thioimidazoles could also be oxidized enantioselectively by chiral iron complexes, generated in situ from Fe(acac)₃ and chiral Schiff base ligands using H₂O₂ as terminal oxidant^[5]. The best results of enantiomeric excess were achieved by use of the systems Ti(O-*i*Pr)₄ / R-BINOL / TBHP and Ti(O-*i*Pr)₄ / S-BINOL / TBHP which yielded the second enantiomer respectively. Under these conditions, the reaction proceeded nearly enantiospecific (97.4 % : 2.6 %) with good to high conversions up to 90 %. We will present the synthetic procedures and the results of the enantioselective sulfoxidation of our tri-and tetrasubstituted 2-thioimidazole derivatives.

References:

- [1] Laufer S., Hauser D., Domeyer D., Kinkel K. and Liedtke A., *J. Med. Chem.*, 2008, in press
- [2] Kammerer B., Scheible H., Albrecht W., Gleiter C. H. and Laufer S., *Drug Metab Dispos*, 2007, 35(6), 875-883
- [3] Hong Lu, *Expert Opin. Drug Metab. Toxicol.*, 2007, 3(2), 149-158
- [4] Seenivasaperumal M., Federsel H. J., Ertan A., Szabo K. J., *Chem. Commun.*, 2007, 2187-2189
- [5] Legros J., Bolm C., *Chem. Eur. J.*, 2005, 11, 1086-1092

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THE ENANTIOMERS OF EPIBOXIDINE: SYNTHESIS AND BINDING AFFINITY AT NEURONAL NICOTINIC ACETYLCHOLINE RECEPTOR SUBTYPES

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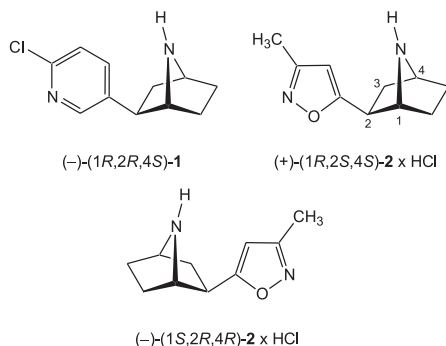
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Neuronal nicotinic acetylcholine receptors (nAChRs) belong to a heterogeneous family of ligand-operated ion channels, differently expressed in the central and periph-

eral nervous systems, which are assembled in a pentameric architecture to form a pore permeable to cations such as Na⁺, K⁺, or Ca⁺⁺. Of the seventeen different nAChR subunits which have been cloned, twelve ($\alpha 2$ to $\alpha 10$ and $\beta 2$ to $\beta 4$) were found in the neuronal receptors of vertebrates. Different subunit combinations characterize the nAChRs expressed within the diverse areas of the central nervous system (CNS), and affect their biophysical and functional properties, such as ion selectivity, conductance, mean open-channel time, rate of desensitization as well as the sensitivity to neurotoxins.

Ongoing investigations on the pharmacology and neurobiology of CNS nAChRs allowed a better understanding of their involvement in various neuropsychiatric pathologies, such as Alzheimer's and Parkinson's diseases, mild cognitive impairment (MCI), Tourette's syndrome, schizophrenia, depression, anxiety, attention-deficit hyperactivity disorder (ADHD), and nicotine addiction. More than 90% of the heteromeric channels localized in the CNS contain the $\alpha 4$ and $\beta 2$ subunits, whereas the most abundant homomeric channel is the $\alpha 7$ pentamer. Thus, research efforts have mainly focused on the discovery of selective $\alpha 4\beta 2$ or $\alpha 7$ ligands to ascertain the physiological and pathophysiological relevance of these receptor subtypes.

A significant impulse to the development of selective $\alpha 4\beta 2$ nAChR ligands was given by the discovery of Epibatidine (-)-**1**, a natural toxin possessing an analgesic potency roughly one hundred times higher than that of morphine. Among the numerous Epibatidine-related analogues investigated so far, ($\pm$)-Epiboxidine **2**, characterized by the presence of a 3-methylisoxazole ring, emerged as a potent $\alpha 4\beta 2$ nicotinic receptor agonist, 10-fold less potent than Epibatidine as antinociceptive agent but 20-fold less toxic. Since we aimed at deepening the pharmacological profile of Epiboxidine, we developed a novel synthetic sequence for the racemate and extended this approach to the preparation of the enantiomers (1*R*,2*S*,4*S*)-**2** and (1*S*,2*R*,4*R*)-**2** in enantiomeric excess higher than 99%.



The synthesis of target derivatives took advantage of a palladium-catalyzed Stille reaction involving two enantiomeric enoltriflates and the suitable tributylisoxazolylstannane. The synthetic details, the affinity profile of (+)-**2** and (-)-**2** and that of related analogues at $\alpha 4\beta 2$ and $\alpha 7$ nAChRs will be presented and discussed.

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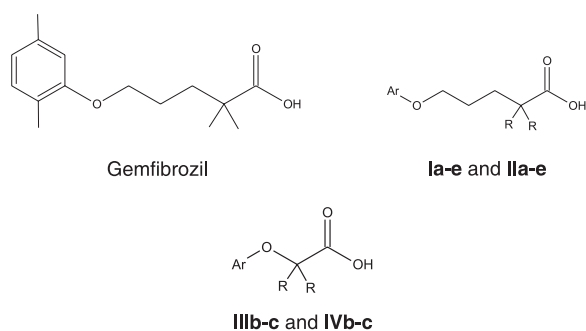
SYNTHESIS AND PHARMACOLOGICAL EVALUATION OF FIBRATES ANALOGUES AS CARDIOVASCULAR AGENTS

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Atherosclerosis is a progressive disease involving metabolic abnormalities in both lipoprotein and artery wall metabolism. Several clinical trials indicate a benefit of fibrate treatment in cardiovascular risk reduction and improvement of lipid profiles.¹ Their clinical effects are mediated principally by the peroxisome proliferator-activated receptor, PPAR α , a member of the nuclear hormone receptor superfamily of transcription factors, that plays a central role in regulating the storage and catabolism of dietary fats.²

We have recently reported preliminary studies carried out on gemfibrozil and some of its analogues (**I a-e** and **Ila-e**) in which the pentanoic chain of gemfibrozil was kept but the aromatic portion was more hindered. The studies displayed good interaction profiles of these molecules toward the PPAR α protein;³ some of these derivatives showed EC₅₀ values comparable to gemfibrozil and GW7647, a potent human α -agonist.⁴ On this basis, we have synthesized and tested compounds where the pentanoic chain was substituted by an acetic (**IIb-c**) or 2,2-dimethylacetic chain (**IVb-c**) and compounds where one or more heteroatoms (N, S) was introduced in the planar and aromatic moiety (Ar) of compounds listed below.



		Ar				
Acids	R	a	b	c	d	e
I	H					
II	Me					
III	H					
IV	Me					

Therefore, docking calculations have been carried out to shed some light onto the structure-binding relationships affecting the PPAR α ligands of the 2,2-dimethylacetic subset.

Description and discussion of the synthesis and both biological and computational results will be given in more details in the poster session.

References

- [1] S.J. Robins, *J. Cardiovasc. Risk* 2001, 8, 195-201
- [2] S. Verste, B. Desvergne, W. Wahli, *Nature* 2000, 405, 421-424
- [3] A. Ammazalorso, R. Amoroso, G. Bettoni, B. De Filippis, M. Fantacuzzi, L. Giampietro, A. Giancrisofaro, C. Maccalini, A. Moschetta, "Sintesi e valutazione biologica di analoghi eterociclici del gemfibrozil a potenziale attività cardiovascolare", XVIII Convegno Nazionale della Divisione di Chimica Farmaceutica della Società Chimica Italiana, Chieti, 16-20 Settembre 2007
- [4] P. J. Brown, L. W. Stuart, K. P. Hurley, M. C. Lewis, D. A. Winegar, J. G. Wilson, W. O. Wilkinson, O. R. Ittoop, T. M. Willson, *Bioorganic & Medicinal Chemistry Letters* 2001, 11, 1225-1227

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CHARACTERIZATION OF IMIDAZOLE-BASED LIGANDS WITH DIFFERENT FUNCTIONALITIES AT HISTAMINE H₄ RECEPTOR

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Histamine as an ubiquitous chemical messenger mediating its physiological functions by binding at four known histamine receptor subtypes. Its role in various (pro-)inflammatory disorders, e.g. allergy, asthma and autoimmune diseases was long time thought to be mediated mainly by binding at histamine H₁ receptor subtype. Since the recent discovery of the histamine H₄ receptor and its detection of distinct expression on various immune and inflammatory cells, studies underlined its potential role as an attractive target in medicinal chemistry. Selective H₄ receptor ligands and synergism of histamine H₁ and H₄ receptor modulation could be more effective in the treatment of inflammation, allergy, asthma and immunological disorders.^[1]

First studies comparing the strongly related receptor pharmacology at human histamine H₃ and H₄ receptors, showed remarkable structure-activity relationships of histamine derivatives and sulphur-containing ligands like thioperamide or clobenpropit.^[2]

Based on recent studies we expanded ligand binding data at H₄ receptor on a library screening with imidazole-based histaminergic ligands structurally defined as amides and carbamates, which have firstly been described as potent H₃ receptor antagonists.^[3,4] Investigation on pharmacological profiling and selection of

ligands with appropriate binding data we performed a two step screening method based on in vitro radioligand binding studies using membranes from SF9 cells expressing the human H₄ receptor. We started with a rough one concentration testing; selected ligands with promising affinities were further characterized in a detailed competition binding assay. Carbamate and amide functionalities maintain affinity in H₃/H₄ receptor ligands, whereas slight structural variations in substituents may cause larger changes in H₄ receptor affinity.

References:

- [1] M. Zhang et al., *Pharmacol. Ther.* 2007, 113, 594-606.
- [2] F. Gbahou et al., *Br. J. Pharmacol.* 2006, 147, 744-754.
- [3] H. Stark et al., *J. Med. Chem.* 1996, 39, 1157-1163.
- [4] H. Stark et al., *Eur. J. Pharm. Sci.* 1995, 3, 95-104.

P400

NEW DERIVATIVES OF ANTI-INFLAMMATORY DRUGS, DESIGNED AS POTENTIAL AGENTS FOR ALZHEIMER'S DISEASE, HAVE ACCESS TO THE CENTRAL NERVOUS SYSTEM (CNS) AND PROTECT ISCHEMIC BRAIN FROM RADICAL ATTACK.

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Alzheimer's Disease (AD) is a chronic neurodegenerative condition that affects an increasing number of individuals as life expectancy rises. Neurofibrillary tangles and senile plaques in the brain are main pathological features of AD. Inflammatory reactions, surrounding cerebral microvasculature, are often observed, and inflammation products are found in the CNS of AD patients. Furthermore, recent evidence has linked altered cholesterol metabolism to AD development, and high LDL-cholesterol concentrations in the blood of AD sufferers. Brain capillaries are also affected in the course of AD. Thickened capillaries reduce vessel elasticity, eventually influencing cerebral blood flow and transport of compounds and nutrients such as glucose into the brain. In addition, cerebral ischemia seems to contribute to AD pathogenesis. Moreover, there is a significant role of oxidative stress in the evolution of AD. Glutathione metabolism is altered and its levels are decreased in affected brain regions from AD patients. The function of blood brain barrier (BBB) is impaired in AD and reactive oxygen species released in the CNS after an ischemic event influences BBB permeability. Thus, although there is still enough uncertainty about the molecular mechanism in AD pathogenesis and there has not been a really effective

therapeutic intervention yet, the above pathobiochemical changes in the demented brain could be used as a starting point for rational design of multifunctional molecules for the medicinal treatment of cognition disorders.

In this research, we synthesised and studied derivatives of known non steroidal anti-inflammatory drugs (ibuprofen, naproxen, indomethacin) connected, via an amide bond, with a proline moiety, expected to offer nootropic properties, since structurally related amides or esters of proline have nootropic action and proline dipeptides have been found to be about 2000 times more potent than piracetam. Proline carboxylic group has been converted to an amide group by reaction with cysteamine and cystein ethyl ester, known antioxidants with low toxicity. The structures of the compounds were confirmed spectroscopically and by elemental analysis.

The access of these compounds to the CNS has been validated by monitoring their concentration in rat brain homogenates for several time intervals after an intraperitoneal administration. The determination has been performed by developing a solid phase extraction preparation followed by HPLC analysis. Blood concentrations at the same time periods have also been determined for one compound by the HPLC method, in order to calculate blood-brain concentration ratio. The protective effect of representative compounds against radical attack after rat brain ischemia/reperfusion, expressed as malondialdehyde formation and glutathione depletion, has also been verified.

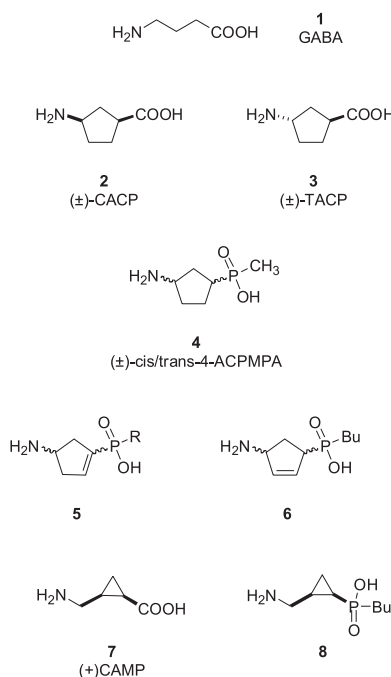
The synthesised compounds were also found to inhibit in vitro rat hepatic microsomal membrane lipid peroxidation, induced by Fe(II)/ascorbate, to exert anti-inflammatory activity, assessed as paw edema reduction, and to reduce total cholesterol, LDL-cholesterol and triglyceride levels in the plasma of dyslipidemic rats. Furthermore, they seem to acquire low gastrointestinal toxicity, a property especially desired for chronic administration, in view of the presence of the non steroidal anti-inflammatory drug and the known gastrointestinal toxicity of this class of compounds.

In conclusion, our results indicate that the design of agents able to be directed to different, selected biological targets, through different pharmacophores properly integrated in a single molecule, may be proven useful for the treatment of complex neurodegenerative conditions

Alzheimer's disease and schizophrenia; as well as a role in memory, learning, pain and visual processing.²

(±)-*cis/trans*-3-ACMPA (**4**, Figure), a conformationally restricted, phosphinic acid analogue of GABA, which has a similar structure to (±)-CACP and (±)-TACP (*cis*-3-aminocyclopentanecarboxylic acid and *trans*-3-aminocyclopentanecarboxylic acid, respectively, **2** and **3**, Figure 1), have been synthesised³ and were found to be potent and selective GABA_C receptor antagonists.⁴ An insertion of a double bond as shown in **5** exhibited selective antagonistic characters at GABA_C receptors,⁵ which has prompted further investigation of the effect of the double position at GABA_C receptors. Compound **6** was chosen as the target compound. The synthesis of **6** and related results will be presented.

(+)-CAMP ((+)-*cis*-2-aminomethylcyclopropanecarboxylic acid, **7**, Figure 1), another conformationally restricted GABA analogue, is a selective GABA_C antagonist.² We envisaged that the replacement of the carboxylic moiety with a phosphinic acid to give **8** (Figure 1) would be of interest to study on GABA_C receptors. The synthesis of **8** and related results will be presented.



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INVESTIGATION OF NOVEL CYCLIC ALKYL PHOSPHINIC ACID ANALOGUES OF GABA

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γ-Aminobutyric acid (GABA, **1**) is the major inhibitory neurotransmitter in the CNS. It acts as an agonist at ionotropic GABA_A and GABA_C receptors, as well as the metabotropic GABA_B receptors.¹ GABA receptors play a role major role in neurodegenerative diseases, such as

References

- [1] Chebib, M.; Johnston, G.A.R.; *Clin. Exp. Pharmacol. Physiol.*, 26, p937 – 940, 1999
- [2] Chebib, M. *et al.*; *E. J. Pharm.*, 430, p185 – 192, 2001
- [3] Hanrahan, J. *et al.*; *Org. Biomol. Chem.*, 4, p2642 – 2649, 2006
- [4] Chebib, M. *et al.*; *Neuropharmacology*, 52, p779 – 787, 2007
- [5] Chebib, M.; Kumar, R.J.; Johnston, G., Hanrahan, J.; *PCT Int. Appl.*, WO 2006000043 A1, 2006

P402

NATURAL BUTENOLIDES: A WAY TO NEW ANTI-FUNGALS?

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The past two decades have witnessed a significant increase in fungal infections, mainly due to the use of immunosuppressive agents, broad-spectrum antibiotics, antineoplastics and anti-HIV agents. Given the rising resistance of fungal strains to classical azole antifungals and the poor pharmacokinetics of polyene antibiotics, there is a high, but as yet unmet need for the development of new antifungal agents. In this regard, several natural products and related molecules seem to provide potentially useful leads (Fig. 1).

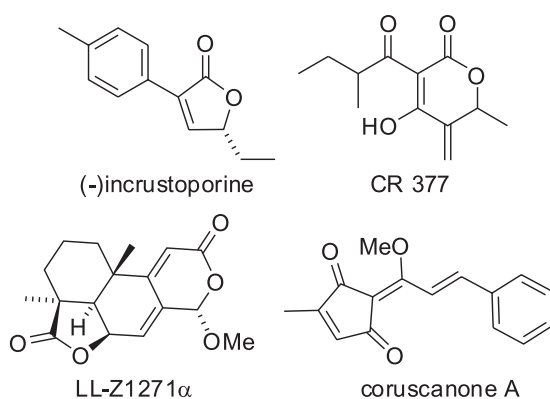


Fig. 1

An apparent feature common to all structures is the presence of one or more double bonds conjugated to a carbonyl group that is built in a cycle or may be a part of a lactone ring. Thus, biological screening^{1,3,4} of several small libraries of (-)-incrustoporine analogues has revealed that the endocyclic double bond is necessary for antifungal effect of the compounds and that the effect may be further boosted by the substitution of the aryl ring with halogens. Further development² led to 5-acyloxymethyl-3-(halogenated phenyl)-2,5-dihydrofuran-2-ones (**1**, Fig. 2), comparable to amphotericin B in antifungal potency in vitro. Interestingly, ketone⁴ (**2**) and pentenolide⁵ (**3**) analogues of these butenolides possess no antifungal activity at all, which seems to testify against simple Michael addition to the β-carbon of the double bond as a possible mechanism of action. A recent flow cytometric study⁶ showed that these compounds interact with an as yet unknown target in the fungal cell wall.

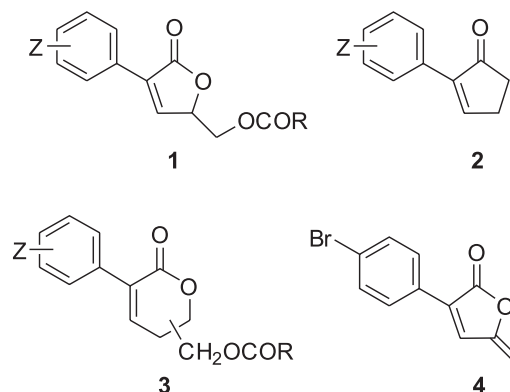


Fig. 2

Most recently, a metabolic study⁷ of 5-acetoxymethyl-3-(4-bromophenyl)-2,5-dihydrofuran-2-one in a mouse model indicated that the 5-methylene derivative (**4**) formed via in vivo elimination of acetic acid is the most probable active species. This compound retains excellent in vitro antifungal activity (IC₈₀ 0.49 to 15.62 μmol/L against *Candida spp.*, 1.95 to 7.81 μmol/L against *A. fumigatus*), and possesses a selective cytostatic effect (marginal effect on L1210 and HL60 cells, IC₅₀ 2.09 μmol/L against HeLa cells). Apart from an overview of the structure activity-relationships, the development of the lactone-based antifungals will be discussed with emphasis on the application of Pd-mediated reactions to the syntheses of the target butenolides.

References:

- [1] *Bioorg. Med. Chem. Lett.* 2000, 10, 1893.
- [2] *J. Med. Chem.* 2001, 44, 2701.
- [3] *Collect. Czech. Chem. Commun.* 2001, 66, 1809.
- [4] *Bioorg. Med. Chem.* 2003, 11, 2843.
- [5] *Collect. Czech. Chem. Commun.* 2007, 72, 1472.
- [6] *Bioorg. Med. Chem. Lett.* 2006, 16, 2492.
- [7] *J. Chrom. B* 2007, 853, 10.

P403

USING MOLECULAR FIELDS FOR RATIONAL DRUG DESIGN AGAINST GPCRS: APPLICATION TO CCK₂ ANTAGONISTS

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The targets for 40% of all marketed drugs are G-protein couple receptors (GPCRs) that have no experimental structural data on which to base rational drug design (though several groups have used homology models) and

their ligands are often structurally diverse. These shortcomings make such targets challenging candidates for most existing quantitative structure-activity relationship (QSAR) methods. Here, we propose that rational drug design can be applied to such targets by using molecular fields (electronic, steric and hydrophobic) to describe the molecules [1].

Specifically, the following approach has been successfully exploited to rationalize the activity of a highly diverse set of 21 Cholecystokinin 2 (CCK₂) receptor antagonists, comprising 7 different chemical series [2]:

- Find the 3D bioactive conformation. Compounds which bind to the same protein active site must display similar field patterns in their bioactive conformation because the protein requires a complementary field pattern in the ligand to enable binding. Therefore, by looking for a common pattern among conformation populations of active compounds, hypotheses for the bioactive conformation can be made. In this example, a single highly active representative from 3 of the series was selected to create the bioactive conformation model.
- Fit compounds to the 3D model. Active analogues of these 3 compounds and members of the 4 other compound series were fitted to the model using their molecular fields; not structure.
- Test the model. The field similarity of all 21 compounds to the model was calculated and plotted against activity. A linear relationship was found, so giving a quantitative measure of activity ($r^2 = 0.75$, rms error = 0.68 pKb units)

The advantages of this approach are that it does not require protein X-ray data, it automatically derives a 3D molecular alignment, and needs only a small number of diverse actives to derive a model, so making it suitable for the initial stages of the drug discovery process.

References

- [1] Cheeseright, T. et al 'Molecular field extrema as descriptors of biological activity: Definition and validation', J. Chem. Inf. Model., 46, 665-676 (2006)
- [2] Low, C.M.R. and Vinter, J.G. 'Rationalizing the activity of cholecystokinin 2 receptor antagonists using molecular field points', J. Med. Chem., 51, 565-573 (2008)

P404

AN AUTOMATED PLATFORM FOR ANTI-BIOFILM SCREENING USING A LIBRARY CONTAINING NATURAL AND SYNTHETIC COMPOUNDS

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The scientific community is facing the need for novel reliable techniques allowing the testing of antimicrobial

compounds against infections caused by biofilms, since they have been regarded a key and still unsolved problem in nowadays biomedical sciences [1]. The impact of natural products in antimicrobials drug discovery has continued to be substantial over the last years, and in order for them to continue as successful choices, new and innovative approaches are required, including appropriated screening platforms [2]. Previously, our group has optimized an automated method suitable for screening of anti-biofilm compounds using crystal violet staining in a 96- microtiter well plate format [3]. The experimental set-up consisted of a combination of Biomek 3000 and Thermo Combi Multidrop dispenser environments. The particular aim of this project was to evaluate the suitability of this automated assay for screening inhibitory activity against *Staphylococcus aureus* (ATCC 25923) biofilms using natural and synthetic compounds. An in-house library consisting of 114 compounds was used, 81 of natural origin and 33 synthetic, in which coumarins, furanocoumarins, phenolic acids, flavonoids and gallates were represented, among other chemical classes. The screening was performed in two different approaches; prevention of biofilm formation and destruction of formed biofilms. In the prevention approach the compounds were added simultaneously with bacteria solution onto the plates, incubated for the whole growing period in optimal conditions (18 h in 37°C, 200 rpm) and stained with crystal violet using the automated set-up. In the destruction approach the biofilms were first formed in optimal growth conditions, then tested substances were added, incubated in optimal growth conditions for 2 h, and finally crystal violet- stained. Results showed 4 positive hits in the prevention approach and 5 positive hits in the destruction approach, which were all natural, except for nordihydroguaiaretic acid, found positive in the destruction approach. From all actives, the natural farnesol and lauryl gallate, were the only two compounds found positive in both approaches. Since these results are in accordance with previous literature reports [4, 5], the reliability of this assay to screen natural as well as synthetic libraries was confirmed. In summary, current project provided results favouring the use of an automated 96- well plate model of *S. aureus* biofilms, with specific relevance as a screening tool in the bioactivity profiling of compounds of different kinds, including natural ones.

- [1] Lasa I. *International Microbiology* (2006) 9:21-28.
- [2] Payne D et al. *Nature Reviews Drug Discovery* 2007;6:29-40.
- [3] Sandberg M et al. *International Journal of Antimicrobial Agents* (2008) Sub. 01/08.
- [4] Jabra-Rizk MA et al. *Antimicrobial Agents and Chemotherapy* (2006) 50(4):1463- 69.
- [5] Saginur R et al. *Antimicrobial Agents and Chemotherapy* (2006) 50(1): 55-61.

P405

DOCKING STUDIES ON hERG POTASSIUM CHANNELS - TRAPPING OF PROPAFENONE DERIVATIVES.

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The hERG channel is expressed in various cells and plays an important role on the 3rd phase of the heart repolarization. Delay in this repolarization phase prolongs the QT interval and might cause the long QT syndrome (LQTS). LQTS could result in arrhythmia called Torsade de Pointes (TdP), which finally might end lethal. In the past decade several drugs had to be withdrawn from the market because they interact with the hERG channel causing the LQTS. The hERG channel is polyspecific in its ligand recognition and thus targeted by many classes of compounds, like antiarrhythmic, psychiatric, antimicrobial and antihistaminic drugs. There is evidence that gating structures may trap drug molecules during channel closure. A hallmark of drug trapping is "ultraslow" recovery of hERG channels from block at rest. We speculate that the resting state dissociation (i.e. recovery from the "trapping state") may be crucial because profound drug trapping will result in sustained HERG channel inhibition and prolongation of the cardiac action potential.

We used a series of propafenone derivatives which bear N-substituents of different size and investigated their pharmacological behaviour with respect to trapped/non trapped. Biological data show that GPV0001, GPV0005, GPV0009 and GPV0180 are trapped, GPV0576 and GPV0574 are not trapped. In order to get insights into the molecular determinants for trapping and non-trapping, docking studies were performed on the protein homology model published by Tseng et al. [1].

The docking results show that Phe656 shows a π - π interaction with the phenyl ring of the propafenone scaffold. This interaction seems to be of major importance for binding of the compounds. The positively charged nitrogen group interacts with Tyr652 via a π -cation interaction. Drug trapping is mainly determined by the size of the substituent on the nitrogen atom. In order to get deeper insights into the importance of Phe656 and Tyr652 for drug trapping, docking studies were also applied on selected mutants.

Acknowledgments

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References

- [1] Gea-Ny Tseng,* Kailas D. Sonawane,y Yuliya V. Korolkova,z Mei Zhang,* Jie Liu,* Eugene V. Grishin,z and H. Robert Guy, *Probing the Outer Mouth Structure of the hERG Channel with Peptide Toxin Footprinting and Molecular Modeling*, Biophysical Journal Volume 92 May 2007 3524–3540.

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(1,2,3,5-TETRAHYDRO-4,1-BENZOXAZEPINE-3-YL)-PURINES AS TEMPLATES FOR IMPROVE ANTI-CANCER ACTIVITY

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Advance in the knowledge of molecular biology has thrown light on many aspects of the cell- cycle regulation mechanisms. This has allowed a change in the anti-cancer therapy trends, from the classic cytotoxic strategies to new non-harmful therapies which target the cell cycle regulation. One of them is the differentiation therapy, searching for the survival of the cells once the cancerous phenotype is eliminated. Despite these advances, the successful treatment of cancer still remains a significant challenge. We have previously reported the synthesis and biological activity of a series of *O,N*-acetals with antitumoural activity, which included 7-membered benzoxaheterocyclic moieties and different nitrogen bases. The most potent structures obtained ($IC_{50} = 0.67$ - $6.75 \mu M$ on MCF-7 cells)^{1,2} can be compared to that of drugs currently used in therapy, such as the cytotoxic agent 5-fluorouracil (5-FU, $IC_{50} = 4.32 \pm 0.02 \mu M$, *in vitro* studies on MCF-7 human breast cancer cells).

Extended studies on the synthesis and pharmacological evaluation of (1,2,3,5-tetrahydro-4,1-benzoxazepine-3-yl)-purines are presented. The microwave-assisted organic synthesis has provided faster access to the target compounds with the advantage of regiospecific preparation of the *N*-9' isomers. The anticarcinogenic potency of the target molecules is reported against four cancer cell lines and two non-tumoural ones. The most active compound presents and IC_{50} of $0.355 \pm 0.011 \mu M$, being the most potent structure so far reported. These results provide promising information for further development of potent antiproliferative agents. At present, studies are being carried out to determine the mechanism of action at the molecular level of the most active compounds.



Figure 1

References

- [1] Núñez, M.C.; Rodríguez-Serrano, F.; Marchal, J.A.; Caba, O.; Aránega, A.; Gallo, M.A.; Espinosa, A.; Campos, J.M. *Tetrahedron* 2007, 63, 183-190.
- [2] Díaz-Gavilán, M.; Gómez-Vidal, J.A.; Rodríguez-Serrano, F.; Marchal, J. A.; Caba, O.; Aránega, A.; Gallo, M. A.; Espinosa, A.; Campos, J.M. *Bioorg. Med. Chem. Lett.*, 2008, 18, 1457-1460.

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THE INFLUENCE OF GARLIC (*ALLIUM SATIVUM* L.) EXTRACTS ON HYPNOTIC EFFECT OF DRUGS

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The appearance of common usage of various spices and herbal preparations in everyday practice and life imposes the question of possible interactions with different drugs. The aim of the study was to examine the effects of methanol extracts derived from garlic (*Allium sativum* L.) on pentobarbitone and thiopentane induced sleeping time in mice. The examined extracts were obtained from immature garlic plant (I), grounded and air-dried garlic bulbs (II-prepared as an Aged Garlic Extract) and fresh garlic bulbs (III). The animals were divided according to pretreatment regime (peroral application of 0.1 mL/kg of particular extract for p.o. use, during 5 consecutive days) into 5 groups: control (water), extract I, II and III group. Pentobarbitone (40 mg/kg) or thiopentane (65 mg/kg) was intraperitoneally injected 2 h after the particular extract application on 5th day. Pretreatment with all extracts produce changes in both, pentobarbitone and thiopentane induced sleeping time. Also, examined extracts exhibited notable changes in induction time after application of both hypnotic drugs. Thus, the treatment with extracts I and III caused significant increase of the induction time after the application of pentobarbitone (36.47% and 42.21%, respectively) while extract II decreased it for 8.82%. On contrary, application of extract I with thiopentane decreased the induction time (8.33%). Extracts I and III decreased the pentobarbitone induced sleeping time for 48.5% and 24.16%, respectively, while extract II increased it for 43.35%. On the other hand, extract II decreased the thiopentane induced sleeping time for 32.06% and extracts I and III increased it (8.69% and 12.28%). Regarding the fact that garlic alone do not induce sleep and that its usage produce changes in pentobarbitone and thiopentane effects, it can be recommended that the interaction between drugs and phyto-preparations containing garlic should be additionally examined.

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PREDICTIVE MODELS FOR THE CLASSIFICATION OF ABCG2 AND ABCC1 SUBSTRATES/NON-SUBSTRATES TO IMPROVE ADME/TOX PROFILING

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ATP-binding cassette (ABC) transporters represent an important class of transmembrane proteins responsible for extrusion of xenobiotics out of mammalian cells. Therefore, these proteins have a impact on the pharmacokinetic properties of drugs and play a critical role in chemo-defense mechanisms. The ABC transporter family members ABCG2 (MXR/BCRP) and ABCC1 (MRP1) are sophisticated for their highly polyspecific substrate recognition pattern. ABCG2 and ABCC1 are known to transport a variety of chemical unrelated, hydrophobic compounds, and ABCC1 additionally extrudes anionic compounds and drug conjugates. In order to limit off-target effects of novel drug candidates, computational classification algorithms may provide versatile tools for identification of potential ABCG2 and ABCC1 substrates,

In this study we utilize data derived from the NCI60 screen on the ABC-transporters ABCG2 and ABCC1. This screening data comprise Pearson's correlation coefficients of the mRNA levels of the indicated transporter and the cytotoxicity of drugs over a panel of 60 tumour cell lines. Therefore, we select negative correlated compounds to be substrates and non-correlated compounds to be non-substrates. This procedure yields a set of 198 compounds for ABCG2 and a set of 227 compounds for ABCC1.

Three different types of descriptor sets have been calculated as input for the classification algorithms used. The first one is comprised of 11 simple and intuitive physicochemical parameters, that are widely used to represent ADME properties of compounds (ADME). The other two descriptor types, 32 Van-der-Waals surface area (VSA) descriptors and 77 Autocorrelation (ACor) descriptors, represent rather complex and abstract descriptions of the molecules.

The classification algorithms applied in this study range from "state-of-the-art" classifiers widely used in chemoinformatics, such as support vector machines (SVM), k-nearest neighbor (kNN), decision trees (DT) to more sophisticated ensemble classifiers, like random forest, bagging, and boosting. The characteristic of ensemble classifiers is to aggregate the predictions of multiple models, and therefore, they are known to derive more predictive, albeit less interpretative models.

The best model for the ABCG2 data set is derived from a boosting classifier in conjunction with a SVM as base classifier, with a total prediction accuracy of 92.5% on an external test set, whereas the most predictive model for the ABCC1 data set only shows a total prediction accuracy of 78%. This discrepancy in classification performance suggests that a correct description of ABCC1/substrate interaction is more difficult than for ABCG2. The classification results for both data sets indicate that the complex descriptor sets (VSA and ACorr) show better classification results than the simple ADME descriptors.

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APPLYING WEB SEARCH ALGORITHMS FOR ADME/TOX PROFILING OF ABCB1 (P-GP) SUBSTRATES

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Computational classification techniques provide versatile and useful tools in shaping chemical drug candidate libraries in order to identify potential compounds harbouring unfavourable properties that minimize their bioavailability. Characterization of drug candidates on basis of their chemical similarity has been shown to represent a promising approach [1, 2]. Since web search methods are also based upon finding similarities within text documents, it seems to be feasible to adapt these algorithms in order to provide new strategies for ADME/TOX profiling.

ABCB1 (MDR1, P-Glycoprotein), a member of the ABC-transporter drug efflux protein family, is notorious for its impact on the pharmacokinetics, tissue penetration and ADME of drug candidates. A characteristic hallmark of ABCB1 is its polyspecific substrate recognition pattern. We utilize two different data sets containing ABCB1 substrate liability in binary format (substrate/non-substrate). One embodies 249 compounds collected from the literature and the other one is comprised of 216 compounds derived from the NCI60 cancer screen.

In this study we apply two methods originating from web search and also applied in text mining and spam filtering [3], LSI and VSM, for ABCB1 substrate classification. In contrast to similar previous attempts [4] we are focusing more on dimensionality reduction aspects for very large problem settings. In information retrieval, a vector space model (VSM) is widely used for representing information where documents and queries are represented as points in a vector space. The document vectors are stored in a *term-document* matrix M , and the distance to a query vector q can be measured in terms of the cosines of the angles. Latent semantic indexing (LSI) is a variant of the basic VSM, which integrates dimensionality reduction. LSI operates on an approximation M_k of M . The benefits are the reduction of both, storage requirements and computational cost for determining distances between the query vector and the documents.

In the case of drug classification we are dealing with numeric representations of molecular structures instead of text documents. To apply VSM for this specific task, we replace the *term-document* matrix M with a *descriptor-compound* matrix M^{DC} and measure the distance between an unlabeled compound (query vector) and M^{DC} . For predicting biological activity we use a *k*-nearest-neighbor (*k*-NN) approach based on a majority vote among the *k* nearest compounds. For carefully selected parameter settings the obtained results using an external test set clearly outperform other models with a sensitivity of 96% and a Specificity of 100%.

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- [1] Keiser, M.J., et al., *Relating protein pharmacology by ligand chemistry*. Nat Biotech, 2007. 25(2): p. 197-206.
- [2] Zdrazil B, et al., *Similarity-Based Descriptors (SIBAR) as Tool for QSAR Studies on P-Glycoprotein Inhibitors: Influence of the Reference Set*. QSAR & Combinatorial Science, 2007. 26(5): p. 669-678.
- [3] Gansterer, W., A. Janecek, and R. Neumayer, *Spam Filtering Based on Latent Semantic Indexing*, in *Survey of Text Mining II: Clustering, Classification, and Retrieval*. 2007, Springer.
- [4] Hull, R.D., et al., *Latent semantic structure indexing (LaSSI) for defining chemical similarity*. J Med Chem, 2001. 44(8): p. 1177-84.

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NOVEL BENZO[D]THIAZOLIMINO-5-ARYLIDEN-4-THIAZOLIDINONES AS CYCLOOXYGENASE/LIPOXYGENASE INHIBITORS. ESTIMATION OF STRUCTURE-ACTIVITY RELATIONSHIP

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Inflammation is a multifactorial process. Although, it is a protective mechanism against physical injuries, pathogenic microorganisms and other factors, it is related to a number of serious and chronic disorders such as asthma and arthritis which require repeated or prolonged treatment. Cyclooxygenase (COX) and lipoxygenase (LOX) enzymes are involved in inflammation response by producing two groups of arachidonic acid metabolites, prostaglandins (COX products) and leucotrienes (LOX products). Non-steroidal anti-inflammatory drugs (NSAIDs), act via the inhibition of cyclooxygenase isoenzymes (COX-1, COX-2). Although COX-2 is concerned to be the main isoenzyme related to inflammation, most NSAIDs block both forms of COX enzyme. Since, COX-1 or COX-2 inhibition has been associated with side effects such as gastrointestinal or cardiovascular problems, balanced inhibition of COX-1/2 isoenzymes combined with LOX inhibition seems to be the choice of preference for some scientists.

Various thiazole and thiazolidinone [1, 2] derivatives, synthesized by our team have been found to have LOX as well as COX inhibitory action. Recently, a number of 2-thiazolylimino-5-arylidene-4-thiazolidinones were evaluated for dual COX/LOX inhibitory action [1]. In an effort to produce compounds with higher LOX and COX inhibition, twenty seven benzothiazolimino – 5 – aryliden – 4 – thiazolidinone derivatives were synthesized and evaluated for their activity.

COX-1 and COX-2 activities of the compounds were measured using ovine COX-1 and human recombinant COX-2 enzymes included in the "COX Inhibitor Screening Assay" kit provided by Cayman (Cayman Chemical Co., Ann Arbor, MI, USA). The assay directly measures PGF_{2a} produced by SnCl₂ reduction of COX-derived PGH₂. The prostanoid production was quantified via enzyme immunoassay using a broadly specific antibody that binds to all the major prostaglandin compounds

For the determination of LOX inhibition, different concentrations of the tested compounds, dissolved in DMSO, were incubated at 28°C with 0.1 mM sodium linoleate and 1000 U of soybean lipoxygenase in a total volume of 1 ml. The conversion of sodium linoleate to 13-hydroperoxylinoleic acid at 234 nm was recorded and compared with nordihydroguaretic acid, an appropriate standard inhibitor.

All new compounds had improved LOX inhibitory activity compared to the 2- thiazolylimino-5-arylidene-4-thiazolidinone analogues. Some of the compounds exhibited better COX inhibition as well. In order to analyze the mode of binding of different derivatives, docking studies for selected compounds were performed

References

- [1] Athina A. Geronikaki, Alexey A. Lagunin, Dimitra I. Hadjipavlou-Litina, Phaedra T. Eleftheriou, Dmitrii A. Filimonov, Vladimir V. Poroikov, Intekhab Alam, Anil K. Saxena (2008) *J. Med. Chem.*
- [2] Hadjipavlou-Litina, D., Geronikaki, A., Mgonzo, R. and Doytchinova, I. (1999). *Drug Develop. Res.* 48: 53–60.

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NEW PDE7 INHIBITORS LEADS FOR NEURODEGENERATIVE DISEASES DISCOVERED BY USING A PHARMACOPHORIC MODEL

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Phosphodiesterases (PDE) are the enzymes responsible for the hydrolysis of intracellular cyclic adenosine (cAMP) and guanosine monophosphate and their activity is associated with a wide variety of diseases, such as those affecting central nervous system function (e.g. depression), cardiovascular function, cell adhesion, metabolic processes and inflammatory cells/immune system.[1] From the large PDE family, PDEs 3, 4 and 7 are predominant in immune cells, and among them, T cells appear to rely on PDE7 for regulation of cAMP levels. Moreover, expression of PDE4B2 and PDE7A was found in the brain of EAE rats, a well-known paradigm of multiple sclerosis.[2] Thus, an intense effort toward the development of PDE7 inhibitors has been generated for the last years because inhibition of this enzyme could be an approach to treating T cell dependent disorders and neurodegenerative diseases.[3-5]

Starting with chemically diverse families of active compounds a pharmacophoric model for specific PDE7 inhibitors were defined.[6] From the pharmacophoric features of this model a search in 3D databases was achieved finding new leads structurally diverse from those previously known.

The biological evaluation of these hits confirms their PDE7 inhibition in the submicromolar range. Considering

the Lipinski index and others in vitro ADME properties, the new identified PDE7 inhibitors have a good profile to be considered for further drug development in the neurodegenerative field.

References

- [1] Conti, M.; Jin, S. L. C. The molecular biology of cyclic nucleotide phosphodiesterases. *Prog. Nucleic. Acid Res. Mol. Biol.* 1999, 63, 1-38.
- [2] Reyes-Irisarri, E.; Sanchez, A. J.; Garcia-Merino, J. A., Mengod, G. Selective induction of cAMP phosphodiesterase PDE4B2 expression in experimental autoimmune encephalomyelitis. *J. Neuropathol. Exp. Neurol.* 2007, 66, 923-931.
- [3] Castro, A.; Jerez, M. J.; Gil, C.; Martínez, A. Cyclic nucleotide phosphodiesterases and their role in immunomodulatory responses: Advances in the development of specific phosphodiesterase inhibitors. *Med. Res. Rev.* 2005, 25, 229-244.
- [4] Menniti, F. S.; Faraci, W. S.; Schmidt, C. J. Phosphodiesterases in the CNS: targets for drug development. *Nat. Rev. Drug Discov.* 2006, 5, 660-670.
- [5] Giembycz, M. A.; Smith, S. J. Phosphodiesterase 7 (PDE7) as a therapeutic target. *Drugs Fut.* 2006, 31, 207-229.
- [6] Jerez, M. J.; Castro, A.; Gil, C.; Martínez, A. Development of a pharmacophoric model for specific PDE7 inhibitors. *Drugs Fut.* 2004, 29 (Suppl. A), 129.

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NOVEL APPROACHES FOR NEURODEGENERATIVE DISEASES THERAPY: PDE7 INHIBITORS

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Phosphodiesterase (PDE) 7 is a high affinity cAMP-specific PDE whose functional role in T-cells has been the subject of some controversy. Recent findings on tissue distribution, however, support the hypothesis that PDE7 could be a good target for the treatment of airway diseases, T-cell related diseases or even Central Nervous System (CNS) disorders being its inhibitors a new approach to be explored for the treatment of neurodegenerative disorders.[1,2]

Therefore, the identification of selective inhibitors targeted against PDE7 enzyme has become an attractive area of research.[3,4] We show here the first use of the descriptors generated by the CODES program for ligand-based virtual screening and its application to the discov-

er of new PDE7 inhibitors.[5] This program codifies molecules from a topological point of view and the generated descriptors are related to the chemical nature of the atoms, the atomic bonds and the connectivity with the rest of the molecule. They are also able to distinguish among stereoisomers. By using this approach, 173 compounds were codified, and their similarity with the reference compound analysed. Based on the analysis, new potential PDE7 inhibitors have been virtually identified.

The synthesis of the new virtual discovered compounds and their biological evaluation confirms the inhibition of PDE7 at the micro molar range and the ability to increase the AMPc in lymphocytes with a good therapeutic window.

This study demonstrates that CODES descriptors are valid for ligand-based virtual screening and provides new lead compounds for further optimization as potent and selective PDE7 inhibitors as a potential neurodegenerative therapy.

References

- [1] Brandon, N.J.; Rotella, D.P. Potential CNS applications for PDEs inhibitors. *Ann. Rep. Med. Chem.* 2007, 42, 3-12.
- [2] Menniti, F. S.; Faraci, W. S.; Schmidt, C. J. Phosphodiesterases in the CNS: targets for drug development. *Nat. Rev. Drug Discov.* 2006, 5, 660-670.
- [3] Giembycz, M. A.; Smith, S. J. Phosphodiesterase 7 (PDE7) as a therapeutic target. *Drugs Fut.* 2006, 31, 207-229.
- [4] Castro, A.; Jerez, M. J.; Gil, C.; Martínez, A. Cyclic nucleotide phosphodiesterases and their role in immunomodulatory responses: Advances in the development of specific phosphodiesterase inhibitors. *Med. Res. Rev.* 2005, 25, 229-244.
- [5] Castro, A.; Jerez, M. J.; Gil, C.; Calderon, F.; Doménech, T.; Nueda, A.; Martínez, A. CODES, a novel procedure for ligand-based virtual screening: PDE7 inhibitors as an application example. *Eur. J. Med. Chem.* 2008, doi: 10.1016/j.ejmech.2007.10.027.

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TOWARDS NOVEL THERAPIES FOR NEURODEGENERATIVE DISEASES – CASE STUDIES OF NON-COMPETITIVE GLUR5/6 LIGANDS

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The aim of the presented work was to conduct a synthesis of and studies on the novel indole derivatives which are potential partial antagonists of GluR5/6 receptors and might exhibit neuroprotective activity. Novel indole derivatives were designed by modification of the lead compound, E099-25011 (1-ethyl-5-methoxy-2-(4-

methoxyphenyl-3-methylindole). 6 new compounds exhibited antagonistic activity to GluR6 receptor and 2 substances – to GluR5 receptor. The values of EC_{50} were in micromolar range. It should be stressed that novel indole derivatives are among most active selective GluR5 non-competitive antagonists and are the only known GluR6 selective non-competitive antagonists. Applying theoretical methods it was stated that the compounds investigated also had affinity to other molecular targets engaged in neurodegenerative diseases, including cholinesterase which is in agreement with contemporary tendency for searching drugs fulfilling simultaneously requirements of several pharmacophores.

Next, estimation of drug-likeness of considered compounds was carried out, applying Lipinski's rule and calculating constitutional, geometrical, physicochemical and topological descriptors and subsequently placing novel compounds in chemical space determined by chemical compounds collected in known databases of medicinal substances. The investigated indole derivatives were characterized with favourable values of many parameters, but have borderline values of lipophilicity. Furthermore, the elements of ADMET analysis were performed and skin permeation, crossing blood-brain barrier, bioavailability after oral administration, intestinal absorption according to Caco-2 and MDCK models as well as % of absorption were calculated. Most of them easily cross blood-brain barrier. One of the unfavourable parameter turned out to be the high degree of binding with plasma proteins.

The integral part of the studies was the construction of GluR5 and GluR6 receptor models in the closed form with the application of homology modelling with Modeller 9v2 [1-2]. The obtained models were the first complete models of any ionotropic glutamatergic receptor. They displayed the correct symmetry: two-fold in N-terminal domain, ligand binding domain and the extracellular part of transmembrane domain and four-fold in the main part of a channel which is in agreement with single-particle electron microscopy images of iGluR and with the results of the studies by Sobolevsky et al. The constructed models were applied to investigate interactions of novel indole derivatives with these receptors. Taking into account experimental studies by Balannik et al. it was stated, that binding pocket for kainic acid receptors non-competitive antagonists is situated in the transduction domain, between two subunits in the receptor dimer. The following residues were recognized as crucial for interactions: Arg663A, Arg663B (M3-S2 linker), Ser809B (S2-M4 linker) and Phe553A (S1-M1 linker).

The characteristics of the binding pocket as well as the constructed pharmacophore model allowed to suggest further modifications of the lead compound. The modified compounds exhibited lower lipophilicity, lower toxicity and had more anchor points for interactions with the receptors. Their activity towards GluR5/6 receptors as well as the neuroprotective properties will be confirmed by experimental studies.

[1] Kaczor, A.; Kijkowska-Murak, U.; Matosiuk, D. *Curr. Pharm. Des.* 2008, accepted

[2] Kaczor, A.; Kijkowska-Murak, U.; Matosiuk, D. *J. Med. Chem.* 2008, accepted

This work was funded by the Polish Ministry of Science and Higher Education, grant number 405 021 31/1121. Computations were performed in the framework of computational grant by Interdisciplinary Centre for Mathematical and Computational Modelling, Warsaw, Poland, grant number G30-18.

P414

NOVEL BINDING SITE OF GLUR6 RECEPTOR – MOLECULAR DOCKING OF NON-COMPETITIVE ANTAGONISTS

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Antagonists of ionotropic glutamate receptors are particularly important as potential medicinal substances, because blocking of glutamate receptors, together with enhancing γ -aminobutyric acid (GABA) neurotransmission, is a strategy against excitotoxicity and thus – one of the ways of neuroprotective therapy. Moreover, only antagonists of iGluRs are at present in clinical use. Most of them, like memantine or amantadine, are NMDA channel blockers of moderate affinity, as high affinity uncompetitive antagonists (MK-801, phencyclidine) are characterized with undesirable psychotomimetic side effect and high toxicity in vitro and in vivo. Currently, the available data for kainate receptor antagonists, especially for those which do not compete with an agonist for the binding site, indicates that they may be more promising than NMDA receptor ligands because they are better tolerated.

The aim of presented work was to investigate molecular interaction of novel indole derivatives which are non-competitive antagonists of GluR6 receptors with their molecular targets. The binding pocket was identified taking into account experimental results for AMPA non-competitive antagonists obtained by Balannik et al. [1]. It was stated that the binding cavity for kainic acid receptors non-competitive antagonists is situated in the transduction domain, between two subunits in the receptor dimer. The following residues were recognized as crucial for interactions: Arg663A, Arg663B (M3-S2 linker), Ser809B (S2-M4 linker) and Phe553A (S1-M1 linker). The binding cavity is closed properly by Ile755A, Pro558B and Leu559B [2].



[1] Balannik, V.; Menniti, F.S.; Paternain, A.V.; Lerma, J.; Stern-Bach, Y. *Neuron* 2005, 48, 279-288

[2] Kaczor, A.; Kijkowska-Murak, U.; Matosiuk, D. unpublished results

This work was funded by the Polish Ministry of Science and Higher Education, grant number 405 021 31/1121. Computations were performed in the framework of computational grant by Interdisciplinary Centre for Mathematical and Computational Modelling, Warsaw, Poland, grant number G30-18.

P415

STRUCTURAL DETERMINANTS OF NMDA CHANNEL BLOCKADE – MOLECULAR DOCKING OF UNCOMPETITIVE TRAPPING BLOCKERS

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The aim of presented work was to investigate molecular interactions of several uncompetitive blockers with NMDA receptor channel. The ion channel of NMDA receptor interacts with organic cations and dications which bind inside the pore. Compounds of this type may be classified into two subfamilies: trapping blockers, like MK-801 or phencyclidine which become trapped insight the pore and 'foot-in-the door' blockers, like aminoacridine which prevent channel closure [1].

The NMDA channel model was constructed as earlier described [2]. The docking results obtained in this work are consistent with commonly acceptable model of NMDA channel blockers interaction which indicates N/Q/R site as the main anchoring point. These follow the idea of the previous studies of Magazannik's group as the ligands were placed in the center of a channel vestibule. The additional argument for such ligand position may be supplied by the analysis of interactions of a tetrabutylammonium ion with KcsA potassium channels in the experimentally solved complexes (which were used as templates in this study). The blocking ion in these crystals is situated exactly in the center of the pore or moved slightly towards one subunit. The positively charged nitrogen of tetrabutylammonium ion interacts with Thr75 of the potassium channel which is homologous with Asn615/

616 in NMDA channel. Moreover, the binding pocket in the potassium channel is formed by Phe101 residues, similarly as in the NMDA channel. The obtained docking results are also consistent with site-directed mutagenesis data.

It is worth emphasizing that all the four ligands (memantine, (-)-MK-801, (+)-MK-801 and IEM-1925) occupy exactly the same binding pocket. The variants of receptor models differ only slightly with side chain conformations. The binding cavity is constituted by residues of M2 and M3 helices. The main contact is hydrogen interaction between oxygen atom of amide group of Asn616 (NR1) or Asn615 (NR2B) from Q/R/N site and the protonable nitrogen atom of the ligand. Residues from M3, namely Met641 (NR1) (Val640 in NR2B) close properly the binding pocket and are engaged in the hydrophobic interaction with the aryl or alicyclic moiety of the ligand. Val644 in NR1 (Leu643 in NR2B), also from M3 transmembrane region, are especially significant for ligand binding as their branched side chains acts as a kind of switch and direct ligands into interaction with Asn.

The proposed binding mode of IEM-1925 is, however, in contrary to the literature data [3] which indicate that the long alkyl chain of the ligand inserts the crevice formed by P loops and the part of the ligand responsible for the interactions with Asn is the nitrogen atom closer to cyclohexyl ring. In the presented model Asn residue interacts with the nitrogen atom situated at the end of alkyl chain. In such a binding mode, the threonine residues of the SSY-TANLAAF motif which were also found as crucial for interaction with uncompetitive blockers, close properly the binding pocket and may be treated as a part of the trapping gate. The proposed IEM-1925 pose in the NMDA channel explains why the larger and nitrogen-ethylated ligand, IEM-2041, exhibits 100-fold lower affinity and prevents channel closure ('foot-in-the-door-blocker').

[1] Bolshakov, K.V.; Gmiro, V.E.; Tikhonov, D.B.; Magazannik, L.G. *J. Neurochem.* 2003, 87, 56-65

[2] Kaczor, A.; Kijkowska-Murak, U.; Matosiuk, D. *J. Med. Chem.* 2008, accepted

[3] Magazannik, L.G.; Tikhonov, D.B.; Bolshakov, K.V.; Gmiro, V.E.; Buldakova, S.L.; Samoilova, M.V. *Neurosci. Behav. Physiol.* 2003, 33, 237-245

Computations were performed in the framework of computational grant by Interdisciplinary Centre for Mathematical and Computational Modelling, Warsaw, Poland, grant number G30-18.

P416

MOLECULAR MODELLING OF NOVEL DERIVATIVES OF 3,5-DIOXO-4-AZATRICYCLO[5.2.2.0^{2,6}]UNDECANES

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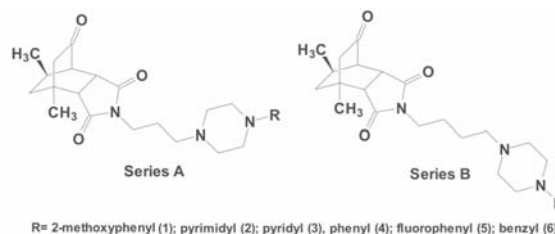
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Arylpiperazine derivatives represent one of the most important classes of 5-HT_{1A} receptor ligands. However, because of the significant conformational freedom of the alkyl linker between arylpiperazine moiety and second pharmacophoric group, the problem of bioactive conformation for this group of medicinal substances has not been solved yet.

The aim of presented work was molecular modelling of three-dimensional structure 12 novel N-substituted 3,5-dioxo-4-azatricyclo[5.2.2.0^{2,6}]undecanes. The starting conformation was found in Cambridge Crystallographic Database (code MAXNAA). The common fragments (imide moiety) were frozen for further studies. The energy and geometry of novel compounds were optimized by *ab initio* method applying Hartree-Fock approximation and 6-31G* basis set. The obtained structures in the local energy minimum of the considered derivatives were further subjected to conformational analysis. The conformational analysis was performed with molecular mechanics (MMFF94 force field) by systematic search method with the simulation of solvent as well as with semi-empirical methods (PM3) and was repeated till the final conformation was the same as the initial conformation. The conformational analysis was carried out both for neutral and protonated molecules.

The resulted populations of conformers were analysed taking into consideration the relationship between the molecule energy, the distance between protonable nitrogen atom and the no-imide nitrogen atom and the dihedral angle between planes defined by the protonable nitrogen atom and the neighbouring carbon atom from alkyl chain and the last carbon atom of alkyl chain (plane A), and the imide nitrogen atom, the neighbouring carbon atom of alkyl chain, the the last carbon atom of alkyl chain (plane B).

The obtained lowest-energy conformers with differentiated values of the investigated dihedral angle and the protonable nitrogen - the non-imide oxygen atom distance were finally optimized by *ab initio* method applying Hartree-Fock approximation and 6-31G* basis set and will be docked to 5-HT_{1A} receptor model. The 5-HT_{1A} receptor model binding pocket will be earlier validated by molecular docking of non-arylpiperazine 5-HT_{1A} receptor ligands.



Computations were performed in the framework of computational grant by Interdisciplinary Centre for Mathematical and Computational Modelling, Warsaw, Poland, grant number G30-18.

P417

DEVELOPMENT OF NEW ACETYLCHOLINESTERASE REACTIVATORS: FROM THE SYNTHESIS TO THE MILITARY AND CIVILIAN USE

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Organophosphorus compounds (pesticides and nerve agents) belong to the acetylcholinesterase (AChE; EC 3.1.1.7) inhibitors. AChE is an enzyme influencing the acetylcholine level at the synaptic clefts. After its inhibition, AChE is unable to fulfill its physiological function and subsequent cholinergic crisis occurs in intoxicated organism within few minutes. AChE reactivators (called oximes) are group of antidotes designed for the reactivation of the inhibited enzyme. Five reactivators (pralidoxime, trimedoxime, obidoxime, methoxime and HI-6) are commercially available at present. None of them is able to serve as a universal antidote ("broad-spectrum antidote") in case of intoxication by every organophosphorus compound. Due to this, the development of new reactivators still continues. There were synthesized and tested more than 500 structurally different AChE reactivators within last five years at our department. Many other international institutions (Korea, France, USA, Croatia, Brazil, UAE, etc.) are involved in evaluation of these compounds. The most promising candidates will be shown in this presentation.

The work was supported by the grant of Grant Agency of Ministry of Education, Youth and Sports (Czech Republic) – grant no. ME865.

P418

SYNTHESIS AND DETERMINATION OF PERMEABILITY COEFFICIENTS OF NOVEL DIASTEREOISOMER ESTERS OF TOPICAL ANTI-INFLAMMATORY STEROIDS

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Anti-inflammatory steroids are “drugs of choice” in treatment of afflictions ranging from the moderate skin rash to severe acute inflammatory disorders. However, these compounds have serious systemic adverse effect when they come in circulation [1]. Recently developed topical anti-inflammatory steroids in clinical use are monoesters, diesters, acetanilides and esters of cortienic acid. The increase of logP, number of donor/acceptor of hydrogen bonds, molecular weight and polar surface area of steroid compounds decrease adverse effect [2]. Our goal was to synthesise novel ester derivatives of topical anti-inflammatory steroids with modified physico-chemical properties. 30 novel diastereoisomeric esters have been synthesised and studied.

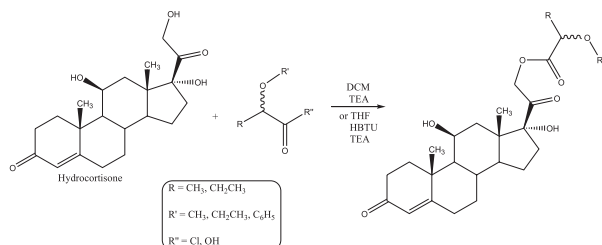


Figure 1. Synthesis of novel steroidal esters

In vitro permeability coefficients for these products have been determined by using PAMPA (Parallel Artificial Membrane Permeability Assay) tests [3].

A novel rapid RP-HPLC method has been employed to determine logP values. This chromatographic method has been validated with logP values, determined with classic “shake-flask” method, for 15 standard corticosteroid compounds and has been tested with set of 30 novel diastereoisomer esters. Number of donors and acceptors of hydrogen bond and polar surface area (PSA) were determined using MarvinSkech software [4].

Permeability coefficients (logKp) and membrane retention coefficients (R) have been correlated with partition coefficients (logP), molecular weight, number of donors and acceptors of hydrogen bonds and PSA. These results will be discussed.

References

- [1] Todd G., Dunlop K., McNaboe J, Ryan M.F., Carson D, Shields M.D. Growth and adrenal suppression in asthmatic children treated with high-dose fluticasone propionate *Lancet* (1996) 348, 27-29
- [2] Wepierre J., Marty J.P. Percutaneous absorption of drugs, *Trends Pharmacol. Sci.*, (1979) 1, 23-26
- [3] Ottaviani G., Martel S., Carrupt P.A. Parallel Artificial Membrane Permeability Assay: A New Membrane for the Fast Prediction of Passive Human Skin Permeability, *J.Med.Chem* (2006) 49, 3948-3954
- [4] PSA plugin, Marvin 4.1.13, 2007, ChemAxon (<http://www.chemaxon.com>)

P419

NEW NATURAL PHARMACEUTICAL PRODUCTS USED IN THE REDUCING OF THE AGING PROCESS

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The preoccupation of the scientists to clear up and manage the ageing phenomenon led to the development of anti-ageing medicine's concept, which is based on the idea of modelling through prevention, the ageing processes. The ageing process effects have intrinsic, organic causes, but are also the result of the environment factor's attack, which are based on the oxidative stress, generated by the free radicals activity.

In the anti-ageing treatments, an important role is occupied by using of some compounds with antioxidant properties for the oxidative stress control.

Current tendencies are oriented towards the study of some natural compounds obtained from plants, with high antioxidant pharmaceutical potential and with minimal secondary effects: phenol carboxylic acids, flavonoids, betacarotens etc.

The paper presents the studies have been effectuated for isolation and characterization of some new natural pharmaceutical products of the *Ginkgo biloba* L and *Rosmarinus officinalis* species. For obtaining of the total extracts, the specific methods were used and for the enriched fractions, the selective extracting methods and purification or the hydrolysis and purifications were used. For realisation of pharmaceutical products, the total extracts have been conditioned as capsules (pelletes). The obtained products have been characterized by some analysis techniques (HPLC, HPTLC, UPLC-MS).

P420

DISCOVERY AND OPTIMIZATION OF AN ETHYLPYRIDAZINE SERIES OF POTENT AURORA A/B DUAL INHIBITORS

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The Aurora kinases (A, B, and C) are a family of serine/threonine kinases that play essential roles in cell division, including the regulation of chromosome segregation and cytokinesis. Aurora A and B have been implicated in tumorigenesis, with overexpression levels correlating to clinical staging of cancers and poor prognosis. There has been considerable interest in developing small molecule Aurora inhibitors for the treatment of a range of cancers, and there are currently several Aurora inhibitors in clinical trials. A series of pyridinyl pyrimidine phthalazines was identified whose members are potent Aurora A and B dual inhibitors that possess excellent kinase selectivity. Here we report on structure-activity relationships around the phthalazine moiety and optimization of a series of compounds in which the phthalazine has been replaced by alkylpyridazines, providing lower molecular weight compounds that maintain the benefits of the phthalazine leads.

P421

STICKY HELPERS: NOVEL LIPOPHILIC AS WELL AS BOLAAMPHIPHILIC IMINOALDITOL DERIVATIVES SUPPORT MUTANT LYSOSOMAL GALACTOSIDASE FOLDING

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It could be shown that iminoalditols as well as aminocyclitols [1] with large N-substituents such as *N*-butyl-1-deoxynojirimycin, *N*-nonyl-1-deoxynojirimycin [2] as well as *N*-octyl-epivalienamine [3] aid the correct folding of mutant lysosomal glycosidases which exhibit highly reduced catalytic activities.

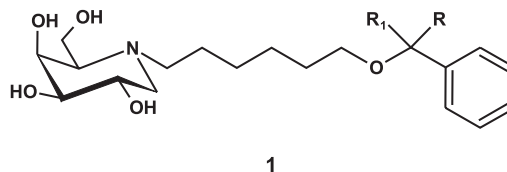
Based on our previous work on structure-activity relationships in iminoalditols, we have recently prepared a range of *N*-modified iminoalditol derivatives (1) featuring highly lipophilic moieties attached via medium length spacer arms, which show excellent inhibitory

power against a range of galactosidases from various sources.

These new compounds could also be shown to dramatically improve residual β -galactosidase activity in GM1-gangliosidosis fibroblasts.

Most recent results indicate a strong dependency of their inhibitory activities as well as their template properties as a function of the N-substituent and the latter's chemical nature and distal functionalisation pattern.

Details of the synthetic approaches, the inhibitory activities of compounds as well as of preliminary results with GM1-gangliosidosis cell lines will be discussed.



1

[1] P. Compain, O. R. Martin, *Iminosugars From Synthesis to Therapeutic Applications*, Wiley & Sons, Chichester, 2007.
b) A. E. Stütz, *Iminosugars as Glycosidase Inhibitors*, Wiley-VCH, Weinheim, 1999.

[2] B. Brumshtein, H.M. Greenblatt, T.D. Butters, Y. Shaaltiel, D. Aviezer, I. Silman, A.H. Futerman, J.L. Sussman, *J. Biol. Chem.* 2007, 282(39), 29052-29058.

[3] Y. Suzuki, *Cellular and Molecular Life Sciences* 2008, 65(3), 351-353.

P422

NOVEL N-MODIFIED DERIVATIVES OF 1-DEOXY-GALACTONOJIRIMYCIN ARE POWERFUL INHIBITORS OF GALACTOSIDASES AND RESTORE MUTANT GALACTOSIDASE ACTIVITY IN A GM1-GANGLIOSIDOSIS CELL LINE

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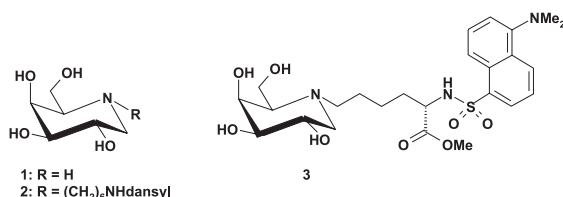
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1,5-Dideoxy-1,5-imino-D-galactitol (1, 1-deoxy-galactonojirimycin, galactostatin) has been known as a powerful inhibitor of α - as well as β -galactosidases. In context with a project aiming at the synthesis of strong galactosidase inhibitors for diagnostic applications, we have prepared a range of *N*-modified derivatives of compound 1, for example, compounds 2 and 3, featuring various moieties attached to 1 via medium lengths spacer arms.



These compounds could be shown to exhibit excellent inhibitory activities against a range of galactosidases of various sources.

Furthermore, some of the structures under consideration improve residual β -galactosidase activity in GM1-gangliosidosis mutant human fibroblasts considerably pointing to their possible viability as chemical chaperones in this context.

Synthetic approaches, the inhibitory activities of compounds as well as of preliminary results with GM1-gangliosidosis cell lines will be discussed.

P424

¹⁸F LABELING OF BIOMOLECULES USING CLICK CHEMISTRY FOR PET APPLICATIONS : SYNTHETIC DEVELOPMENTS

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Molecular imaging has been defined as the in vivo characterization and measurement of biological process at the cellular and molecular level. By example, molecular imaging creates the possibility of in vivo imaging of gene expression, optimizing drug therapy and imaging drug effect at molecular and cellular level or detecting pathology at a « predisease » state¹.

Thanks to its high sensitivity (10⁻⁹ -10⁻¹²M) and “quantitatively”, positron emission tomography (PET) is ideally suited to image molecular processes. Among a number of positron-emitting nuclides, fluorine-18 appears to be the best candidate for labeling biomolecules in regards of its favourable physical and nuclear properties.

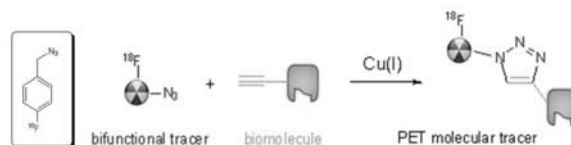
The challenge for the radiochemist is to introduce a short half-life radioisotope (18-fluorine, $t_{1/2}$ 109.7 min) onto biomolecules which are specific to the biological process studied. In this context, the goal of our work is to develop a general and simple method that permit to label a large library of biological compounds. To achieve this aim, we have selected a fast, selective and efficient reaction that permits to graft ¹⁸F tagged molecules onto compounds of biological interest (peptides, oligonucleotides, lipids, ...). « Click » reactions and more particularly Huisgen 1,3 dipolar cycloaddition of alkynes with azides are well adapted to the preparation of radiopharmaceuticals as they require only benign reaction conditions, simple workup and purification procedures^{2,3}. This strategy implies the introduction of either alkyne or azide groups

onto the biomolecules and to prepare a bifunctional tracer featuring the complementary function.

Two ways of functionalization used in the laboratory will be presented:

- Direct peptide functionalization at the N terminal position
- Multi-step procedure based on aromatic amino acids alkynated through Sonogashira reaction

In the other hand, an original four steps, fully automated radiosynthesis of an ¹⁸F labeled azide has been developed. Final functionalization is obtained by means of nucleophilic substitution using an azide reagent supported on an anionic resin. At last, preliminary results obtained in « Click » chemistry will be demonstrated. These results confirm that « Click » chemistry for fluorine-18 labeling in well selected conditions has the potential to develop into a general labeling tool thanks to its rapidity and selectivity. The next step must be in vivo studies to determine both stability and toxicity.



References

- [1] Massoud, T.F., Gambhir, S.S. Molecular Imaging in Living Subjects: Seeing Fundamental Biological Processes in a New Light. *Genes & Development* 17, 545, 2003.
- [2] Marik, J., Sutcliffe, J.L. Click for PET: rapid preparation of [¹⁸F]fluoropeptides using Cu^I catalysed 1,3-dipolar cycloaddition. *Tetrahedron Letters* 47, 6681-6684, 2006.
- [3] Glaser, M., Arstad, E. “Click Labeling” with 2-¹⁸F-fluoroethylazide for Positron Emission Tomography. *Bioconjugate Chemistry* 18, 989-993, 2007.

P425

FC-GAMMA RECEPTOR BINDING PEPTIDE CHIMERAS AND CONJUGATES AGAINST AUTOIMMUNE DISEASES

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The IgG binding Fc γ receptors (Fc γ Rs) play a key role in defence against pathogens by linking humoral and cell-mediated responses. The Fc γ RI, IIa and III are activating receptors, while Fc γ RIIb is responsible for the immune complex mediated inhibition of B cell activation. Impaired

expression and function of the FcγRs may result in the development of pathological autoimmunity, resulting in autoimmune diseases like rheumatoid arthritis, systemic lupus erithematosus, etc. Previously we have found three peptides capable to bind FcγR (Uray et al 2004, *J. Mol. Recognition* **17**, 95-105), and one of these exhibited functional activity as well (Medgyesi et al 2004, *Eur. J. Immunol.* **34**, 1127-1135). Based on our earlier studies and on the known crystal structure of the IgG Fc – FcγR complex, we are planning peptides, peptide chimeras and conjugates effectively binding to FcγRs. The individual chains of IgG and autoantibody epitopes were synthesised by solid phase peptide synthesis method. The peptides were cleaved from the resin with TFA or liquid HF, purified with HPLC, and characterised by MS and amino acid analysis. The peptides were conjugated to polylysine backbone branched chain polymer or protein carrier, producing conjugates containing both the ligand of BCR and FcγR. We expect that these conjugates may become parts of future immunomodulatory drugs.

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P426

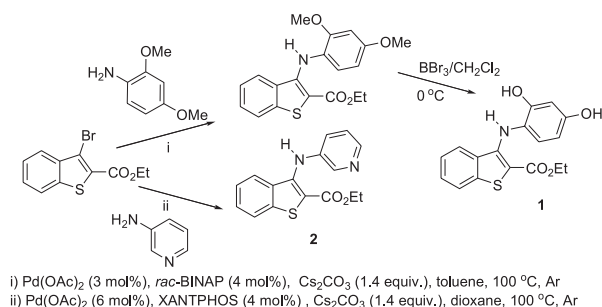
ANTIMICROBIAL ACTIVITY OF NEW SYNTHETIC DI(HETERO)ARYLAMINES IN THE BENZO[B]THIOPHENE SERIES

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There is a constant need for the development of new alternatives in antimicrobial therapy, accompanying the rising incidence of certain infections and/or the emergence of resistant isolates to the available molecules. Two new di(hetero)arylamines (**1** and **2**) were previously prepared by us [1] using Buchwald-Hartwig coupling and, in the case of compound **1**, also demethylation of the C-N coupling precursor (Scheme 1).



Scheme 1

The antifungal and antibacterial activities of compounds **1** and **2** were evaluated using the reference CLSI

(formerly NCCLS) broth microdilution protocols [2], followed by the study of their influence on the dimorphic transition in *Candida albicans*. Both compounds were found to have antifungal activity, with minimum inhibitory concentrations (MICs) ranging from 25 to 100 µg/mL, against several *Candida* spp., including isolates with decreased susceptibility to fluconazole, like *C. krusei*, *C. glabrata*, and a resistant *C. albicans* strain. They were further shown to inhibit germ tube formation in *C. albicans*, an attribute which has generally been associated with pathogenicity, with test concentrations of MIC/2 resulting in up to 100% inhibition. Besides the antifungal activity, they were also shown to have antibacterial activity against Gram-positive bacteria, namely *Staphylococcus aureus*, *Bacillus cereus*, and *Enterococcus faecalis* (MICs ≤ 64 µg/mL), while being inactive against the Gram-negative bacteria tested (*Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*).

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[1] Queiroz M.-J. R. P. et al. *Eur J Org Chem.* 2004;3679-3685.

[2] Clinical and Laboratory Standards Institute. Approved standard M7-A7, Wayne, Pa, USA, 2006 and National Committee for Clinical Laboratory Standards. Approved standard M27-A2, Wayne, Pa, USA, 2002.

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HELPING GLYCOSIDASES WITH GLYCOSIDASE INHIBITORS: DESIGN AND EVALUATION OF INHIBITORS AS POTENTIAL CHEMICAL CHAPERONES BASED ON ACTIVE SITE STRUCTURE DATA OF LYSOSOMAL GLYCOSIDASES

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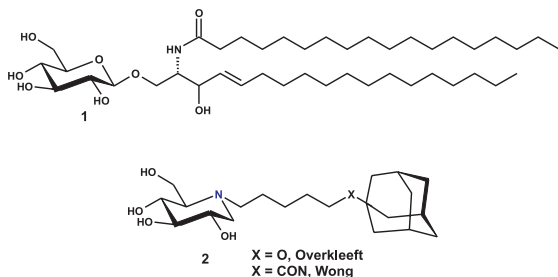
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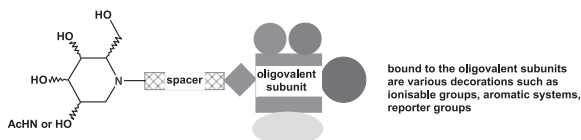
Small molecules, such as iminosugars for Gaucher's disease or cyclitol derivatives for GM1-gangliosidosis, have been introduced as pharmacological chaperones for the treatment of hereditary lysosomal storage diseases (LSD) [1]. Compared to enzyme replacement therapy (ERT) which is very cost intensive on the health system and substrate reduction therapy (SRT) which suffers from having side effect, chaperone mediated therapy (CMT) is thought to be able to cross the blood brain barrier and

therefore may also be beneficial for the treatment of neurodegenerative symptoms of LSDs.

Iminoalditols [2] are natural compounds in which the ring oxygen of carbohydrate molecules is replaced by a basic, trivalent nitrogen. Such compounds are powerful competitive inhibitors of glycosyl hydrolases. Recently, they have been introduced to CMT: The structural information of the respective human enzymes, as well as crystal structures of the proteins in complex with the natural substrate (1) as well as inhibitors (2), are supporting the rational design of iminosugar derivatives, as was shown by Wong and others. The synthetic *N*-modified iminoalditols such as 2 turned out to exhibit unexpected and pharmacologically interesting interactions with the respective enzyme.[3]



Following our synthetic approaches, all important configurations of the sugar moiety, such as D-glucosyl, D-galactosyl, L-idosyl, D-mannosyl, 2-acetamido-D-glucosyl were made available. Based on pieces of structural information of enzymes, functionally modified iminosugars can be designed which we believe have potential as pharmacological chaperones for the treatment of LSDs such as Gaucher's disease, GM1-gangliosidosis, Sandhoff and Tay-Sachs diseases.



The strategy for the design of iminoalditol derivatives as potential chaperones with emphasis on synthetic details as well as K_i -values and chaperone activities will be presented.

- [1] Grabowski, G. A. *Expert Opinion on Emerging Drugs* 2008, 13(1), 197-211.
- [2] Compain, P.; Martin, O. R. *Iminosugars From Synthesis to Therapeutic Applications*, Wiley & Sons, Chichester, 2007. b) Stütz, A. E. *Iminosugars as Glycosidase Inhibitors*, Wiley-VCH, Weinheim, 1999;
- [3] Suzuki, Y. *Cellular and Molecular Life Sciences* 2008, 65(3), 351-353. Wennekes, T. et al, *J. Org. Chem.*, 2007, 72, 1088-1097; Yu, Z. et al, *J. Med. Chem.*, 2007, 50, 94-100.

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SYNTHESIS AND APPLICATIONS OF FLUORINATED CARBOHYDRATES

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The increasing interest in fluorinated organic compounds is due to the fact that these derivatives not only exhibit challenging biological and physico-chemical properties, but also allow the application of high end NMR techniques as a result of the highly sensitive ¹⁹F nucleus.

In recent years, we have witnessed significant improvements in NMR spectroscopy especially as a powerful tool for studying protein-protein interactions.^{1,2,3} The benefit of fluorine (¹⁹F) NMR detection alleviates most of the problems often encountered with ¹H observation, e.g. signal overlap, dynamic range problems due to intense solvent peaks. Additionally, the ¹⁹F nucleus has 100% natural abundance and is highly sensitive and, due to its large CSA (chemical shift anisotropy), very responsive to molecular weight changes accompanied by binding events.

Consequently, we focused our efforts on the preparation of fluorinated mono- and disaccharides^{4,5} to study relative binding affinities of carbohydrates to proteins and to investigate enzymatic reactions. Various maltose derivatives and their binding behaviour towards the maltose binding protein (MBP) will be presented.

References

- [1] Zuiderweg, E. R. P. *Biochemistry* 2002, 41, 1-7.
- [2] Bonvin, A. M. J. J.; Boelens, R.; Kaptein, R. *Curr. Opin. Chem. Biol.* 2005, 9, 501-508.
- [3] Ludwiczek, M. L.; Baminger, B.; Konrat, R. *J. Am. Chem. Soc.* 2004, 126, 1636-1637.
- [4] Dax, K.; Albert, M.; Ortner, J.; Paul, B. J. *Carbohydr. Res.* 2000, 327, 47-86.
- [5] Nyffeler, P. T.; Gonzales Duran, S.; Burkart, M. D.; Vincent, S. P.; Wong, C.-H. *Angew. Chem. Int. Ed.* 2005, 44, 192-212.

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DETERGENTS BASED ON HETEROCYCLES AS NEW CHOLINESTERASE INHIBITORS

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Detergents based on quaternary nitrogenous heterocycles are already known for many years. Some of these compounds are used as disinfectant or micellar catalysts. In our study, we have focused our attention on the synthesis and testing of biological activity towards cholinesterases. Three sets of these compounds were chosen. Pyridinium, quinolinium and isoquinolinium salts differing in the length of the side alkylating chain were tested. The rat brain homogenate was used as a source of cholinesterase. The inhibition activities were obtained by titration method. The dependence of inhibition activity on the length of the side chain was compared. The change of the heterocyclic ring towards inhibition activity was also evaluated.

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SCAFFOLD HOPPING TO GENERATE NEW CPLA₂α INHIBITORS: BEYOND INDOLES

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Cytosolic phospholipase A₂α (cPLA₂α) selectively cleaves the *sn*-2 position of arachidonyl glycerophospholipids to generate arachidonic acid. Arachidonic acid is converted by downstream enzymes to a variety of inflammatory mediators, including leukotrienes, prostaglandins, and thromboxanes. The lysophospholipid remaining after arachidonic acid cleavage can be acetylated to form yet another inflammatory mediator, platelet activating factor (PAF). Selective inhibition of cPLA₂α could provide a novel therapeutic with applications in many disease states including rheumatoid arthritis, osteoarthritis, asthma, multiple sclerosis, and atherothrombosis. We have previously disclosed indole inhibitors of cPLA₂α; here we present our work on new cores. In this presentation, we will present the various synthetic routes employed to prepare these new cPLA₂α inhibitors and data from isolated

enzyme and whole blood assays, pharmacokinetic studies, and animal models.

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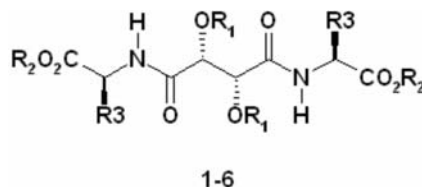
DESIGN AND BIOLOGICAL ACTIVITY OF POTENTIAL PEPTIDOMIMETIC INHIBITORS OF SECRETED ASPARTYL PROTEASES (SAP) FROM *CANDIDA ALBICANS*

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Candida albicans infections are an emerging health problem, especially for immunodeficient patients. One of the major factors contributing to the pathogenicity of this fungus is its ability to secrete some aspartyl proteases (SAP1-10), being SAP-2 the most abundant isoform. In order to develop new potential peptidomimetic inhibitors of these enzymes, amides derived from tartaric acid and α-amino acids were prepared by to different routes, either by a classical sequence involving protection/deprotection of tartaric acid hydroxyl moieties², or by one-step microwave promoted methodology.



The compounds 1-6 were tested against *Candida albicans*, and all compounds inhibited cellular growth, with compounds 3, 5 and 6 showing the highest activity (about 60%). On another assay, the inhibition of *Candida* proteolytic activity was measured and compounds 2 and 5 were the most potent, and are equipotent to pepstatin, a hexapeptide displaying high aspartyl protease inhibition activity.

In order to analyze the potential interaction of these compounds with SAP-2 and SAP-3, a molecular modeling study was performed using MM+ (HyperChem) force field. The crystal structures of A70450-SAP2 (1EAG)³ and pepstatin-SAP3 (2H6T)⁴, obtained from Protein Data Bank (PDB), were chosen as the reference structures. After measuring the complexation energy of these ligands, they were changed for the optimized structure of 1-6, followed by a multiple step geometry optimization. These docking analyses revealed that compounds 1,2,4

and 6 showed a better interaction with SAP-3, while 3 and 5 had a better fit with SAP-2.

The current results show that peptidomimetics 1-6 are inhibitors of *C. albicans* growth, probably by interfering with its proteolytic activity and further structural modifications can lead an interesting hit compound for development of a new class of antifungal compounds.

Acknowledgement:

CAPES, CNPq, FAPERJ

- [1] Naglik J.R.; Challacombe S.J.; Hube B. 2003 *Microbiology and Molecular Biology Reviews* 67: 400.
- [2] Barros J.C.; Silva J.F.M.; Calazans A.R.; Tanuri A.; Brindeiro R.M.; Williamson J.S.; Antunes O.A.C. 2006 *Letters in Organic Chemistry* 3: 882; Resende G.O.; Sequeira L.C.; Cotrim B.A.; Silva J.F.C.; Antunes O.A.C. 2007 *Letters in Organic Chemistry* 4: 168.
- [3] Cutfield S.M.; Dodson E.J.; Anderson B.F.; Moody P.C.; Marshall C.J.; Sullivan P.A.; Cutfield J.F. 1995 *Structure* 3: 1261.
- [4] Borelli C.; Ruge E.; Schaller M.; Monod M.; Korting H.C.; Huber R.; Maskos K. 2007 *Proteins* 68: 738.

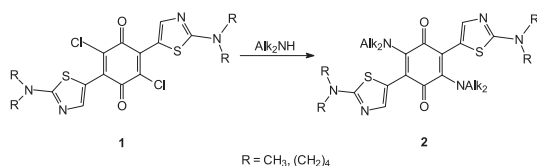
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SYNTHESIS OF BISHETEROARYLBENZOQUINONE-BEARING AMINES

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The natural and non-natural quinone derivatives exhibit a diverse array of biological activities. Their bioactivity may result from free radical-induced damage through redox cycling, a process well recognised in quinone systems. On the other hand a large number of thiazole derivatives showed marked activity in many biological systems. Previously, we have reported a method for the synthesis of 2,5-bisheteroaryl-3,6-dichloro-1,4-benzoquinones ¹. It is expected that the substitution of chlorine atoms will enhance biological activity of compound 1. Recently has been shown that polyamine-quinone skeleton showed promising properties in preclinical investigations against Alzheimer's disease ². This fact prompted us to investigate reaction of compound 1 with cyclic and alicyclic amines.



Our progress towards the synthesis of compound 2 and properties of target compounds will be presented in the poster session.

References

- [1] N. Batenko, G. Karlivans, R. Valters, *Heterocycles*, 2005, 65, 1569.
- [2] M. Bolognesi, R. Banzi, M. Bartolini, A. Cavalli, A. Tarozzi, V. Andrisano, A. Minarini, M. Rosini, V. Tumiatti, C. Bergamini, R. Fato, G. Lenaz, P. Hrelia, A. Cattaneo, M. Recanatini, C. Melchiorre, *J. Med. Chem.*, 2007, 50, 4882.

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HTS-IDENTIFICATION AND SAR-BASED OPTIMIZATION OF NEW ANTIFUNGAL LEAD-STRUCTURES

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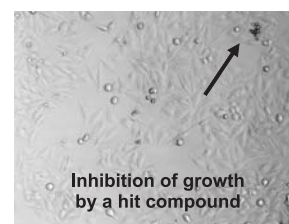
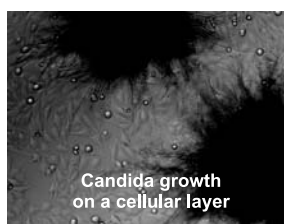
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Antifungals currently on the market show lack in bioavailability, strong side effects (polyenes) or restricted activity. Additionally resistance against antifungals (azoles) by biofilm formation on implants is described. The aim of the study was to identify potent and tolerable substances that inhibit targets essential for fungal growth or virulence factor expression and to develop new drugs for improving the therapy of fungal infections in transplant recipients and intensive care patients.

Thus, a highly diverse compound collection of more than 100,000 synthetic compounds was screened employing a new fluorometric high-throughput tissue culture based screening assay. As a generally applicable screening test, the assay covers all potential in vitro targets of both the pathogen and the host simultaneously and thereby allows for the determination of cytotoxicity in host cells and activity against the pathogen in one step.[1]



Based on the initial hit compounds comprising e.g. oxazoles, pyrazolones, isoxazoles and thiomorpholines, focussed compound collections were rationally designed and synthesized. A detailed structure-activity based lead optimization including the examination of stereoisomers is presented on an example hit compound.

The most promising lead structures were further optimised with respect to stereochemistry and biocompatibility in tissue models.[2].

Literature:

- [1] G. Kleymann, H.-O. Werling, J. Biomol. Screen. 2004, 9, 578-587.
- [2] Patent DE 10 2007 037 579.6.

P435

TOWARD A BETTER DESCRIPTION OF HYDROPHOBICITY IN PROTEIN BINDING SITES: VALIDATION OF THE MOLECULAR LIPOPHILICITY POTENTIAL (MLP-FILTER) WITHIN A GOLD DOCKING STRATEGY

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GOLD software is widely used for docking small molecules into macromolecules. It explores the conformational landscape of ligands with a genetic algorithm and using a fitness function mainly based on hydrogen-bonding and steric interactions. At early stages of docking, GOLD defines "hydrophobic fitting points", generated by a steric Lennard-Jones potential, to guide the placement of ligand hydrophobic atoms into the binding site. Since intermolecular forces encoding hydrophobicity are not only of steric nature, a different molecular interaction field (MIF), namely in-house Molecular Lipophilicity Potential (MLP) based on *n*-octanol/water partition coefficients, was investigated to better characterize hydrophobic regions in protein pockets.

Precise MLP analysis of binding sites from different protein crystal structures revealed that a significant number of points considered as hydrophobic by GOLD were actually polar. Special refinements were performed to carefully define the hydrophobicity based on MLP calculations and a new filter was generated to retain only real hydrophobic points and applied during GOLD processes.

Preliminary docking experiments with and without MLP-filter were conducted on a set of 224 ligand/protein complexes - the CCDC/ASTEX clean test set - to evaluate the real benefits of a better hydrophobicity definition of cavities. The results showed an encouraging impact of the MLP-filter upon the docking performance of GOLD. However, due to the relatively low crystallographic quality of the complexes, no definitive conclusions could be drawn.

A second test set of better quality - the ASTEX Diverse Set, comprising 85 complexes - was thus investigated with a refined methodology and provided substantially positive results, showing that a better description of hydrophobicity with the MLP-filter can enhance the quality of the docking with GOLD.

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MITOCHONDRIAL AMIDOXIME REDUCING COMPONENTS MARC1 AND MARC2: NEW ENZYMES INVOLVED IN DRUG METABOLISM AND ACTIVATION OF PRODRUGS

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The purification of the mitochondrial enzyme responsible for the reduction of *N*-hydroxylated prodrugs led to the identification of two newly discovered mammalian molybdenum containing proteins named mitochondrial Amidoxime Reducing Components mARC1 and mARC2^[1]. These 35-kDa proteins represent a novel group of molybdenum proteins in eukaryotes as they form the catalytic part of a three-component enzyme complex consisting of separate proteins.

In continuation of our investigations we present the cloning of mARC1 and studies in reconstituted systems with the recombinant enzyme.

The recombinant protein showed an *N*-reductive ability after reconstitution with its electron transport proteins cytochrome b₅ and b₅ reductase.

The reconstituted enzyme system was fully capable of reducing amidoximes, the *N*-hydroxylated prodrugs of amidines, representing a prodrug principle leading to decreased basicity and improved oral bioavailability compared to the active amidines^[2]. The *N*-reductive activity was demonstrated for the model substrate benzamidoxime as well as the drugs pentamidine and diminazene amidoximes and *N*-hydroxymelagatran. Further examples are the *N*-hydroxylated guanidine prodrugs guanoxabenz and *N*-hydroxydebrisoquine which are reduced to their antihypertensively acting metabolites^[3,4].

Beside this bioactivation of prodrugs, mARC may also contribute to an important detoxification mechanism, as it showed an activity in reducing toxic aromatic hydroxylamines to their non-toxic aromatic amines. The undesirable effects of the antimicrobial agents sulfamethoxazole and dapsone are attributed to enzymatic formation of such hydroxylamine metabolites^[5]. The retroreduction of these metabolites was shown of being efficiently catalysed by this recombinant enzyme system.

Thus, a recombinant *in vitro* system with the newly discovered enzyme mARC can be used to predict if further *N*-hydroxylated products are reduced *in vivo*. Furthermore, the *N*-reduction of different prodrugs and toxic compounds catalysed by these molybdenum containing proteins present a novel step in the development and metabolism of drugs and prodrugs.

References

- [1] Havemeyer, A., Bittner, F., Wollers, S., Mendel, R., Kunze, T., and Clement, B. (2006) *J Biol Chem* 281, 34796-34802
- [2] Clement, B. (2002) *Drug Metab Rev* 34, 565-579
- [3] Clement, B., Schultze-Mosgau, M. H., and Wohlers, H. (1993) *Biochem Pharmacol* 46(12), 2249-2267
- [4] Clement, B., Demesmaeker, M., and Linne, S. (1996) *Chem Res Toxicol* 9(4), 682-688
- [5] Kurian, J. R., Bajad, S. U., Miller, J. L., Chin, N. A., and Trepanier, L. A. (2004) *J Pharmacol Exp Ther* 311(3), 1171-1178

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SYNTHESIS AND EVALUATION OF THE CHEMICAL STABILITY OF A SERIES OF PRODRUGS OF THE POTENT DOPAMINE D₂ AGONIST 5-OH-DPAT, COMPOUNDS DESIGNED FOR IONTOPHORETIC TRANSDERMAL DELIVERY

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Dopamine (DA) replacement therapy is still considered the most efficacious treatment for Parkinson's disease, but dopamine agonists, formerly prescribed as adjunctive therapy, are emerging as useful initial therapy. However, L-dopa induces severe long-term motor complications. These side-effects are believed to be the result of the disease progression and the pulsating character of L-dopa plasma concentration.

To obtain a constant plasma concentration we have synthesized a series of prodrugs of the potent DA D₂ agonist 5-hydroxy-2-(N,N'-di-*n*-propylamino)tetralin (5-OH-DPAT) and have tested these compounds for their usefulness in transdermal iontophoretic delivery.

The importance of the chemical stability, pK_A-values and valence of the prodrugs will be discussed. The most suitable candidate was chosen for further pharmacokinetic research.

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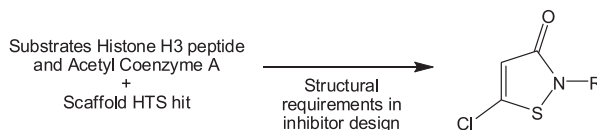
DEVELOPMENT OF SMALL MOLECULE HISTONE ACETYL TRANSFERASE INHIBITORS AS TOOLS FOR CHEMICAL-BIOLOGICAL INVESTIGATIONS ON HISTONE ACETYLATION

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Posttranslational modifications of histones play a crucial role in chromatin structure and regulation of gene

transcription. However their precise roles and the applicability of the involved enzymes as drug targets remain to be elucidated. Dynamic acetylation and deacetylation is a key regulatory event and inhibitors of deacetylation are currently entering the clinic for therapeutic applications. Histone acetylation is equally important as deacetylation, however very little histone acetyl transferase inhibitors have been reported so far. We exploited substrate similarity as inspiration source for structure based design of new inhibitors of the histone acetyl transferases PCAF and GCN5. The inhibitors were tested *in vitro* for inhibition of these histone acetyl transferases and structure activity relationships were derived. The inhibitors were studied for inhibition of histone acetylation and growth inhibition in cancer cell lines. The results demonstrate that it is possible to develop inhibitors for histone acetyl transferases and that these inhibitor can be used to investigate the role of these enzymes. It is a future challenge to investigate the influence of these inhibitor on other acetylation events in the cell.



P440

IMPACT OF STEREOCHEMISTRY ON THE ACTIVITY AND THE TISSUE-SELECTIVITY OF K_{ATP} CHANNEL OPENERS BELONGING TO 3-ALKYLAMINO-4H-PYRIDO-1,2,4-THIADIAZINE 1,1-DIOXIDES

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ATP-sensitive potassium channels (K_{ATP} channels) are trans-membrane structures regulating the flow of potassium ions through the cell membrane in response to variation of intracellular concentrations of nucleotides such as ATP and ADP. These channels play important roles in diverse physiological processes and their activation lead to an hyperpolarization of the plasma membrane. As a result, agents able to open the K_{ATP} channels (PCOs) have been shown to reduce the activity of different excitable cells. The biological consequences of this activation are, for example, the inhibition of insulin release from pancreatic B-cells or the relaxation of vascular smooth muscle tissues. Such drugs could be very useful in the treatment of pathologies such as diabetes, obesity, hyperinsulinemia, arterial hypertension.

In search for new potent and tissue-selective PCOs, we described several series of benzo- and pyridothiadiazine

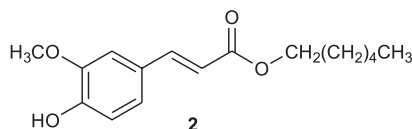
The figure displays the chemical structures of BPDZ 44 and BPDZ 79, along with their enantiomers. BPDZ 44 is a pyrazoloquinazolinone derivative with a 4-isobutyl-2-methyl-1H-pyrazol-5-yl group. BPDZ 79 is a pyrazoloquinazolinone derivative with a 4-tert-butyl-2-methyl-1H-pyrazol-5-yl group. Below each main structure, a pair of enantiomers is shown, separated by a vertical line, indicating the chiral nature of the compounds.

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BIOCHEMICAL ACTIVITY OF PHENOLIC DERIVATIVES ON TUMOUR CELL PROLIFERATION AND ISOLATED MITOCHONDRIA

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Phenolic compounds such as caffeic and ferulic acids, with well-known antioxidant properties have also been associated to anti-neoplastic effects. However, their therapeutic potential is limited, since they do not cross cell membranes easily, due to its high polarity. Accordingly, we have designed and synthesized new molecules based on the caffeic and ferulic acid structure, but with increased lipophilicity, conferred by an additional alkyl chain. For this purpose comparative studies were made between the parent caffeic and ferulic acids and its amide and ester derivatives **1** and **2**. The compounds under study have been tested in different tumour cells lines in monolayer, namely human oral squamous carcinoma (HSC-3) and human breast cancer cells (MCF-7 and 578T). Non-tumor cells BJ fibroblasts were also used to test the selectivity and toxicity of the compounds. The parent acid compounds as well as its derivatives **1** and **2** were incubated (25-75 μ M) with the cells for periods between 12 and 48 hours, and variations in cell proliferation were evaluated through the sulforhodamine B assay. The results obtained indicate that although the acids are devoid of growth-inhibiting activity, their ester and amide derivatives (**1** and **2**) display a significant anti-proliferative effect. Besides the cell proliferation assays, isolated mitochondrial fractions were also incubated with the compounds above-mentioned and no significant effect has been detected on bioenergetic parameters, namely oxygen consumption, transmembrane electric potential and induction of the calcium-induced permeability transition.

CCCCCN=Cc1ccc(O)c(O)c1

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DESIGN OF BENZOTHIADIAZINE 1,1-DIOXIDES ACTING AS AMPA POTENTIATORS: NEW PHARMACOMODULATIONS LEADING TO AN IMPROVEMENT OF PHARMACOKINETIC PROPERTIES

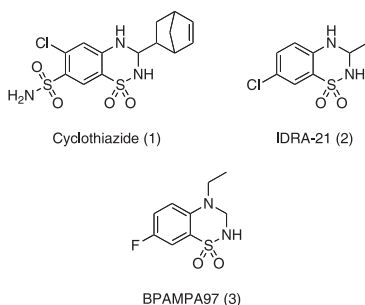
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Amongst the ionotropic receptors responding to glutamate appears the AMPA subtype, which mediates fast excitatory transmission in the brain. It is now well established that AMPA receptors are involved in the expression and the maintenance of long-term potentiation, a phenomenon that has been closely linked to learning and memory processes. Hence the AMPA receptors are considered as an attractive target to develop cognitive enhancers. AMPA potentiators have been found also to be of interest for the management of schizophrenia or depression.

Contrary to AMPA agonists that may cause severe adverse effects such as neurotoxicity, the AMPA potentiators seem to be a more interesting and less toxic pharmacological class because of their properties: they are allosteric positive modulators able to potentiate the AMPA signals in the presence of glutamate having no effect on the receptor without the endogenous neurotransmitter.

Benzothiadiazine 1,1-dioxides constitute a chemical class currently investigated for the discovery of new AMPA PAMs. Starting from the structure of cyclothiazide (1) and IDRA-21 (2), our team previously synthesized *in vitro* active 3,4-dihydro-2H-benzothiadiazine 1,1-dioxides from which emerged BPAMPA97 (3), bearing a short alkyl chain at the 4-position¹.



In the attempt to enhance the pharmacokinetic behaviours of the previously described compounds, our present work focused on the insertion of new substituted alkyl chains at the 4-position of the thiadiazine ring. Biological evaluations are realized *in vitro* on *Xenopus* oocytes expressing AMPA receptors. The *in vitro* screening permitted to select several compounds for *in vivo* studies.

References

[1] Francotte *et al.*, *J. Med. Chem.* 2007, 50, 3153-7.

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DISCOVERY OF MK-0952, A SELECTIVE PDE4 INHIBITOR FOR THE TREATMENT OF LONG-TERM MEMORY LOSS AND MILD COGNITIVE IMPAIRMENT

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Abstract—The structure-activity relationship of a series of naphthyridinone acting as type 4 phosphodiesterases (PDE4) inhibitors is described herein. This effort led to the identification of MK-0952, a potent and selective PDE4 inhibitor ($IC_{50} = 0.4 - 1.7$ nM for isozymes A, B, C and D) for the treatment of CNS related diseases such as long-term memory loss and mild cognitive impairment. MK-0952 was found to be orally active in two pre-clinical models assessing memory integrity; the *Novel Object Recognition* (MED = 0.01mpk) and the *Water Maze Delayed Matching to Position* (MED = 0.1mpk) tests. The ability of MK-0952 to cause malaise in rats was evaluated using the *Conditioned Taste Aversion* (MED = 10mpk) model, while its susceptibility to produce an emetic response was determined by performing pharmacokinetic studies in squirrel monkeys.

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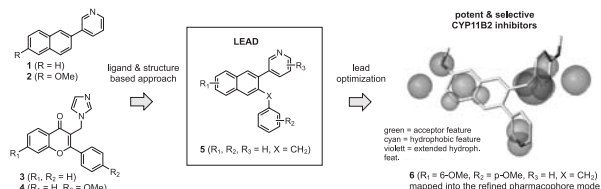
POTENT AND HIGHLY SELECTIVE CYP11B2 INHIBITORS AS NOVEL THERAPEUTICS FOR THE TREATMENT OF HEART FAILURE AND MYOCARDIAL FIBROSIS: A COMBINED LIGAND AND STRUCTURE BASED DESIGN APPROACH

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Aldosterone is the most important circulating mineralocorticoid and its crucial physiological role is well known. Abnormally increased plasma levels of aldosterone have been diagnosed in different cardiovascular diseases such as congestive heart failure and myocardial fibrosis.¹ The therapeutic benefit of reducing aldosterone dependent effects by administration of mineralocorticoid receptor (MR) antagonists has been demonstrated in two recent studies (RALES and EPHESUS).^{2,3} However, the use of these drugs is accompanied by severe side effects due to its affinity to other steroid receptors. Additionally, a correlation between the use of MR antagonists and hyperkalemia-associated mortality was observed.⁴ As postulated by our group, a novel therapeutic strategy for the treatment of diseases affected by elevated aldosterone levels (hyperaldosteronism) with potential to overcome the drawbacks of MR antagonists is to block the production of

aldosterone by inhibition of the key enzyme of its biosynthesis, aldosterone synthase (CYP11B2).^{5,6} For this approach, however, selectivity towards other CYP enzymes is a prerequisite, especially with regard to the highly homologous (> 93%) 11 β -hydroxylase (CYP11B1), the key enzyme of the glucocorticoid biosynthesis.



In our search for new lead compounds as CYP11B2 inhibitors that are structurally differing from the previously discovered pyridylnaphthalenes (**1**, **2**)⁷ imidazolymethylene substituted flavones (e.g. **3**, **4**) were identified by compound library screening. These compounds that originally have been described as aromatase inhibitors⁸ display moderate to high inhibitory potencies (IC_{50} = 10 – 100 nM) without any selectivity towards the highly homologous CYP11B1. The SAR information of both classes, flavones and naphthalenes, together with the application of a combined *in silico* drug design approach using our CYP11B2 protein model⁹ and our earlier pharmacophore model¹⁰ resulted in the new lead structure **5**. Consequent computer-aided lead optimization led to a series of highly potent CYP11B2 inhibitors with an extended carbocyclic scaffold, displaying inhibitory potencies in the low nanomolar concentration range and remarkable selectivities towards the highly homologous CYP11B1 (selectivity factors 120 – 1370), e.g. compound **6** shows an IC_{50} value of 11 nM at the target enzyme whereas CYP11B1 was only inhibited at an inhibitor concentration of IC_{50} = 4330 nM (selectivity factor = 394). A selected set of compounds was additionally tested for inhibitory potency towards other steroidogenic (CYP17, CYP19) and important hepatic CYP enzymes. Pharmacokinetic profiles of promising inhibitors were investigated in cassette dosing experiments in rats.

- [1] Brilla, C. G., *Cardiovasc. Res.* 2000, 47, 1–3. Lijnen, P. *et al.*, *J. Mol. Cell. Cardiol.* 2000, 32, 865–87.
- [2] Pitt, B. *et al.*, *N. Engl. J. Med.* 1999, 341, 709–17.
- [3] Pitt, B. *et al.*, *N. Engl. J. Med.* 2003, 348, 1309–21.
- [4] Juurlink, D.N. *et al.*, *N. Engl. J. Med.* 2004, 351, 543–51.
- [5] Hartmann, R. W., *Eur. J. Pharm. Sci.* 1994, 2, 15–16.
- [6] Hartmann, R. W. *et al.*, *Eur. J. Med. Chem.* 2003, 38, 363–66.
- [7] Voets, M. *et al.*, *J. Med. Chem.* 2005, 48, 6632–42.
- [8] Gibbi, S. *et al.*, *J. Med. Chem.* 2006, 49, 4777–80.
- [9] Ulmschneider, S. *et al.*, *J. Med. Chem.* 2005, 48, 1563–75.
- [10] Ulmschneider S. *et al.*, *Bioorg. Med. Chem. Lett.* 2005, 16, 25–30.

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SPECIFIC INHIBITORS OF THE ONCOGENIC PROTEIN TYROSINE PHOSPHATASE SHP2 IDENTIFIED BY HIGH-THROUGHPUT DOCKING

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The protein tyrosine phosphatase Shp2 is a positive regulator of growth factor signaling. Gain-of-function mutations in several types of leukemia define Shp2 as a *bona fide* oncogene. We performed a high-throughput *in silico* screen for small molecular weight compounds that bind the catalytic site of Shp2. We have identified the phenylhydrazonopyrazolone sulfonate PHPS1 as a potent and cell-permeable inhibitor, which is specific for Shp2 over the closely related tyrosine phosphatases Shp1 and PTP1B. PHPS1 inhibits Shp2-dependent cellular events such as hepatocyte growth factor/ scatter factor (HGF/SF)-induced epithelial cell scattering and branching morphogenesis. PHPS1 also blocks Shp2-dependent downstream signaling, namely HGF/SF-induced sustained phosphorylation of the Erk1/2 MAP kinases and dephosphorylation of paxillin. Furthermore, PHPS1 efficiently inhibits activation of Erk1/2 by the leukemia-associated Shp2 mutant, Shp2-E76K, and blocks the anchorage-independent growth of a variety of human tumor cell lines. The PHPS compound class is therefore suitable for further development of novel therapeutics for the treatment of Shp2-dependent diseases.

References

- [1] Birchmeier, C., Birchmeier, W., Gherardi, E., and Vande Woude, G.F. (2003). *Nat Rev Mol Cell Biol* 4, 915-925.
- [2] Hellmuth, K., Grosskopf, S., Lum, C. T., Würtele, M., Röder, N., von Kries, J. P., Rosario, M., Rademann, J. and Birchmeier, W. (2008), *Proc. Natl. Acad. Sci. U S A*, 105, 7275-7280

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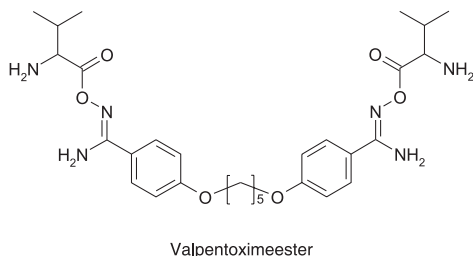
SYNTHESIS, *IN VITRO* AND *IN VIVO* BIOTRANSFORMATION OF NOVEL WATER-SOLUBLE PRODRUGS OF AMIDINES

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The esterification of amidoximes with amino acids represents a newly developed prodrug-principle in order to improve the aqueous solubility of amidoxime prodrugs and

thus, the oral bioavailability of amidines. Valbenzamidoxime represents a model compound for this principle. Furthermore the prodrug-principle was transferred to antiprotozoic pentamidine. Owing to the highly ionic nature of this diamidine, the drug does not pass the blood-brain-barrier to achieve therapeutic levels in the brain [1]. Therefore another goal was to allow penetration of the blood-brain-barrier by amino acid transporters. New synthetic methods for the compounds were established and their biotransformation was studied *in vitro* and *in vivo*. The activation of valbenzamidoxime via benzamidoxime to benzamidine could be demonstrated *in vitro* by porcine and human subcellular enzyme fractions, the mitochondrial benzamidoxime reducing system, and porcine hepatocytes. *In vivo*, the bioavailability of benzamidine after oral application of valbenzamidoxime to three rats was about 88 %. After oral application of the valpentoximester to three rats, respectively, the prodrug was activated and high concentrations of the active form pentamidine were found mainly in liver and kidney. Low concentrations of pentamidine could be found in the brain, too.



References

- [1] Barrett M P, Fairlamb A H, *Parasitology today*, 1999, 15, 4, 136-40

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EVALUATION OF A NUMBER OF NOVEL COMPOUNDS WITH ANTIOXIDANT ACTIVITY ON LIVER INTOXICATION AFTER PARACETAMOLE OVERDOSE

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Xenobiotic toxicity and particularly hepatotoxicity is often caused by their metabolic transformation to active radical intermediates. High doses of paracetamol induce renal but especially liver toxicity. This toxicity is due to the reactive electrophilic metabolite, N-acetyl-p-quinone-imine (NAPQI), formed via cytochrom P450 through a radical intermediate. NAPQI causes glutathione (GSH) depletion, oxidative modification of proteins and hepatic damage.

A number of novel antioxidant agents with combined anti-inflammatory, hypolipidemic and neuroprotective properties have been designed and synthesised by our team during the past years¹⁻⁴.

In the present study, five of these compounds are tested for their protecting potential against paracetamol hepatotoxicity. Four of the compounds are derivatives of cysteamine or cysteine ethyl ester, known to be potent antioxidants with low toxicity. Furthermore, these compounds bear a moiety of a known non steroidal anti-inflammatory drug. One of them is a novel derivative of trolox, the water soluble analogue of vitamin E, with established antioxidant properties.

The compounds were administered to rats after paracetamol intoxication. Liver protection was estimated by measuring activities of the hepatic enzymes GPT, GOT and LDH in plasma as well as liver GSH content and from histological examination. Animal groups receiving only paracetamol, paracetamol and N-acetylcysteine, the commercially available antidote, or paracetamol and guaiazoulene, a non-toxic natural product, found to cause remarkable inhibition of lipid peroxidation, were also used for comparison. The used intoxication protocol caused extended liver damage, expressed as a statistically very significant increase of hepatic enzymes in plasma, and a severe decrease of liver GSH levels. Histological examination of the liver revealed a serious hepatic degeneration.

Antioxidant activity of the compounds was estimated by their interaction with the stable radical DPPH, by their effect on rat hepatic microsomal membrane lipid peroxidation and by their influence in oxidation of 2,2'-azino-bis(3-ethylbenzthiazoline)-6-sulfonic acid by the ferryl myoglobin -H₂O₂ system. Among tested compounds, the cysteine ethyl ester derivative of tolfenamic acid exhibited the best activity, comparable to that of N-acetylcysteine. An effort to correlate protective action with antioxidant activity and other characteristics of the compounds as well as an evaluation of the methods used for estimation of antioxidant activity is made.

References

- [1] Ziakas, G.N.; Rekka, E.A.; Gavalas, A.M.; Eleftheriou, P.T.; Kourounakis, P.N. *Bioorg. Med. Chem.* 2006, 14(16), 5616-5624.
- [2] Ziakas, G.N.; Rekka E.A.; Gavalas A.M.; Eleftheriou P.T.; Tsiakitsis K.C.; Kourounakis, P.K. *Bioorg. Med. Chem.* 2005, 13 (23), 6485-6492.
- [3] Doulgkeris CM, Galanakis D, Kourounakis AP, Tsiakitsis KC, Gavalas AM, Eleftheriou PT, Victoratos P, Rekka EA, Kourounakis PN. *Bioorg Med Chem Lett.* 2006 Feb 15;16(4):825-9. Epub 2005 Nov 23.
- [4] Galanakis D, Kourounakis AP, Tsiakitsis KC, Doulgkeris C, Rekka EA, Gavalas A, Kravaritou C, Charitos C, Kourounakis PN. *Bioorg Med Chem Lett.* 2004 Jul 16;14(14):3639-43.

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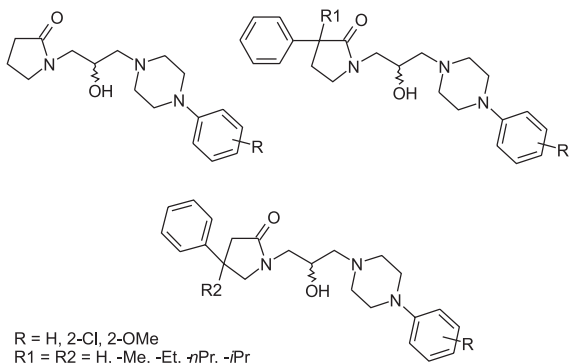
DESIGN, SYNTHESIS AND α -ADRENOCEPTORS AFFINITY OF ENANTIOMERS OF 1-[2-HYDROXY-3-ARYLPYPERAZINYL]-PROPYL 3,3- OR 4,4-SUBSTITUTED PYRROLIDIN-2-ONE DERIVATIVES

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α_1 -Adrenoceptors antagonists belong to a various chemical groups thus, quinazolines, phenylalkylamines, piperidines, dihydropyridines, and arylpiperazines. Currently, the arylpiperazines represent one of the most studied class of their antagonists. The typical α_1 -antagonist contains arylpiperazine moiety, connects with one or two additional fragments of molecule. It was found that the protonatable nitrogen atom of piperazine ring, play a crucial role in interaction with the receptor site. Moreover, an aryl moiety (preferentially substituted at the *ortho*-position with halogen or alkoxy group) connected to the second nitrogen atom of piperazine, is the second structural feature which is needed for interacting with receptor. Finally, the basic nitrogen atom is bound to the spacer which is usually represented by polymethylene chain (substituted or not) with different number of methylene units. It is also important to note that the arylpiperazine and the terminal group should be located at the proper distance to interact with the receptor counterparts [1].

In the course of our studies, on α_1 -adrenoceptor antagonists [2], we designed and synthesized new chiral arylpiperazine derivatives found to have affinity towards α_1 -adrenoceptors. We have chosen compounds, whose common structural features are substituted phenylpiperazine, the second terminal group is represented by 3,3- or 4,4-phenyl,alkyl-substituted and unsubstituted pyrrolidin-2-one moiety bounds to the second piperazine nitrogen atom with 2-hydroxy substituted three methylene units chain. The optical purity of obtained enantiomers was determined using HPLC methods.



In order to better define the structure-activity relationships within the investigated compounds, a molecular modeling study was undertaken. The three-dimensional structures of the pyrrolidin-2-ones derivatives at their neutral state were obtained through full optimization based on the AM1 quantum chemical procedure. The aim of this approach was the identification of a pharmacophore, which is a template derived from the structure of these compounds, and represents the geometry of the receptor site as a collection of functional groups in 3D space.

Acknowledgement:

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References

- [1] R. Barbato, L. Bett, M. Botta, F. Corelli, G. Giannaccini, L. Maccari, F. Manetti, G. Strappaghetti, F. Cordano, *Bioorg. Med. Chem.* 2002, 10, 361
- [2] K. Kulig, J. Sapa, D. Maciag, B. Filipek, B. Malawska: *Arch. Pharm. Chem. Life Sci.*, 2007, 340, 466

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CELL CYTOTOXICITY OF NOVEL 5 β ,6 β -EPOXY STEROIDS

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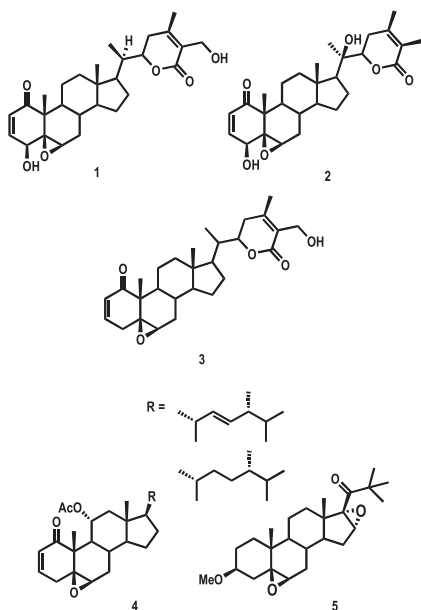
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Natural occurring compounds such as Withaferin A **1** (Figure 1), some Withanolides **2**, Jaborosalactone **3** and marine steroids isolated from *C. Viridis* **4** all possess 5 β ,6 β -epoxide functions and have demonstrated antitumor activity against several cancer cell lines¹. Based on our previous knowledge that steroidal 5 β ,6 β -epoxides can easily be obtained from Δ^5 -steroids by the use of numerous potassium permanganate-inorganic salts for the oxidation of C-C double bonds², we have prepared a panel of novel compounds and tested them for cytotoxic activity towards HT-29, PC-3 and LNCaP cells.

Compound **5** (Figure 1) was found to have EC₅₀ values of 20 and 55 μ M for HT-29 and PC-3 cells, respectively. The tested compounds, however, were non-inhibitory towards LNCaP cells.



References

- [1] (a) Kupchan, S. M. et al. *J. Org. Chem.* 1969; 34: 3858. (b) Gamoh, K., Hirayama, M., Ikekawa, N. *J. Chem. Soc. Perkin Trans. 1* 1984; 449. (c) Tschesche, R., Schwang, H., Fehlhaber, H. W., Snatzke, G. *Tetrahedron* 1966; 22: 1129. (d) Watanabe, K., Iwashima, M., Iguchi, K. *Steroids* 1996; 61: 439.
- [2] (a) Salvador, J. A. R., Sá e Melo, M. L., Campos Neves, A. S. *Tetrahedron Lett.*, 1996, 37: 687; (b) Salvador, J. A. R., Hanson, J. R. *J. Chem. Res. (S)*, 2002, 576; (c) Salvador, J. A. R., Hanson, J. R. *J. Chem. Res. (S)*, 2004, 513.

Acknowledgments

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4-BROMPHENYL- ω -DIMETHYLAMINO BUTYROPHENONE- HCL, A NOVEL INHIBITOR OF NITRIC OXIDE SYNTHASES

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Nitric oxide (NO) is involved in numerous biological events such as vasodilatation, neurotransmission and immune response. Alongside these biological effects there are various pathological processes associated with

its overproduction, for example stroke, migraine, septic shock and multiple sclerosis.

NO is generated by nitric oxide synthase (NOS) during oxidation of L-arginine to L-citrulline. There are three mammalian NOS isoforms: endothelial NOS (eNOS), neuronal NOS (nNOS) and inducible NOS (iNOS) which play very different physiological roles due to the type of cell where they are located. eNOS and nNOS are regulated by intracellular Ca²⁺/calmodulin levels. In contrast, iNOS is expressed with calmodulin tightly bound and regulated transcriptionally in response to cytokines. Because of the various physiological effects it is important to develop isoform-specific nitric oxide synthase inhibitors to reduce the pathophysiological effects associated with increased NOS activity.

Within the scope of developing NOS inhibitors for the therapy of migraine, 4-Bromophenyl- ω -dimethylaminobutyrophenone-HCl (BDAB) was found to be an inhibitor of NOS. During the present work BDAB was tested for its ability to inhibit human nNOS and eNOS as well as murine iNOS in a standard *in vitro* assay. The studies showed that BDAB is an inhibitor of mainly nNOS and eNOS with IC₅₀-values of 60 μ M (nNOS, human) and 59 μ M (eNOS, human). iNOS (murine) is less affected with an IC₅₀-value of 676 μ M. Furthermore, we were interested in the mechanism of action. The inhibitory effect of BDAB diminishes with increasing amounts of calmodulin in the incubation medium, indicating that the inhibitor might interfere with the interaction between nNOS and calmodulin. BDAB inhibits the reduction of cytochrome c and ferricyanide as well as the oxidation of NADPH through the reductase domain by disrupting the intraprotein electron transfer. Additionally the calcineurin activity was measured after incubation with the inhibitor in order to determine whether BDAB inhibits other Ca²⁺/calmodulin-dependent enzymes and a decreased activity was found.

The obtained results clearly indicate that BDAB is mainly an eNOS and nNOS inhibitor which achieves this effect through interaction with calmodulin.

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VIRTUAL SCREENING AND DESIGN OF SELECTIVE LIGANDS: AN MFTA APPROACH

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Despite the wide application of 3D methods in drug design and virtual screening, 2D techniques in this area could be quite successful. Molecular Field Topology Analysis (MFTA) [1] is based on the construction of a so-called molecular supergraph – a simple graph such that the molecular graphs of all training set structures can be represented as its subgraphs. Uniform descriptor set for statistical analysis is obtained by superimposing the structure onto the molecular supergraph. Each super-

graph vertex is assigned the values of effective atomic charge, van der Waals radius, H-bond donor and H-bond acceptor ability, local lipophilicity and/or other parameters for the corresponding atom. For unoccupied vertices the neutral descriptor values are used.

MFTA provides a powerful and efficient tool for modeling and understanding of the structure-activity relationships as well as for the virtual screening of new promising structures. It is especially useful for the series of congeneric organic compounds whose activity is based on specific interactions with one or more biotarget proteins. In spite of the 2D nature of this approach, the results are consistent with the molecular models of ligand-biotarget interactions and in many cases outperform the 3D QSAR approaches in terms of model quality, computational efficiency and chemical interpretability. As a source of structures for virtual screening the databases of available compounds as well as the generated libraries of structures can be used. For the latter case, an efficient structure generator has been developed that takes into account the features and constraints of an MFTA model [2, 3]. Consideration of a full set of activity and selectivity models supports the optimal screening of potential candidate drug structures.

[1] Palyulin V.A., Radchenko E.V., Zefirov N.S. *J. Chem. Inf. Comp. Sci.* 2000, 40, 659-667.

[2] Melnikov A.A., Palyulin V.A., Zefirov N.S. *J. Chem. Inf. Model.* 2007, 47, 2077-2088.

[3] Melnikov A.A., Palyulin V.A., Radchenko E.V., Zefirov N.S. *Doklady Chemistry* 2007, 415, 196-199.

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IDENTIFICATION OF NOVEL, NON-ACIDIC, EP₁ RECEPTOR ANTAGONISTS

Price, H.S. Billinton, A. Bristow, A.K. Brown, S.H. Chowdhury, A. D'Souza, A. Giblin, G.M.P. Goldsmith, P. Hall, A. Hayhow, G.C. Healy, M.P. Hurst, D.N. Kilford, I.R. Naylor, A. Scoccitti, T.

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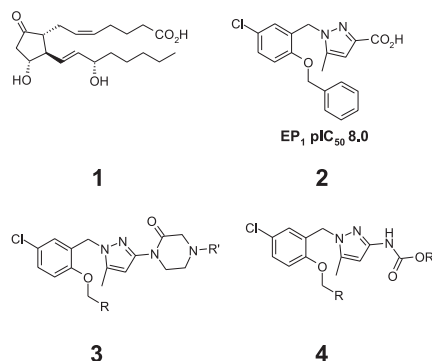
The EP₁ receptor is a seven transmembrane G-protein coupled prostanoid receptor. The natural ligand for the EP₁ receptor is an arachidonic acid metabolite, prostaglandin E₂ (PGE₂) **1**.

Preclinical evidence has shown that EP₁ receptor antagonism could provide a potential treatment for several conditions including inflammatory and neuropathic pain. More recently, an EP₁ antagonist was shown to be effective in a model of visceral pain in the clinic.

Literature evidence suggests that the carboxylic acid group on acidic prostanoid ligands, forms a key binding interaction (salt bridge) with a highly conserved arginine residue on prostanoid receptors. Hence, most of the known EP₁ antagonists contain acidic groups, which are essential to bind to the receptor.

GSK have discovered novel, highly potent EP₁ antagonists such as **2**. Due to the paucity of known non-acidic EP₁ receptor antagonists, we were interested in identifying compounds where the carboxylic acid moiety was replaced by non-acidic groups.

This disclosure shows the approach taken to replace the carboxylic acid of **2** with hydrogen bond acceptor groups. This led to the identification of a number of non-acidic derivatives, including the piperazinones **3** and the carbamates **4**. Compounds of substructure **3** have also incorporated a basic group in the vicinity of the key binding interaction, and retained EP₁ activity.



This poster will describe the chemistry, SAR, DMPK and *in vivo* efficacy data for compounds within this series.

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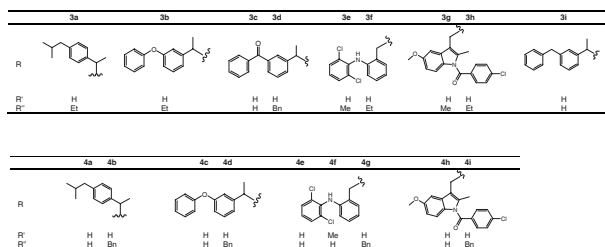
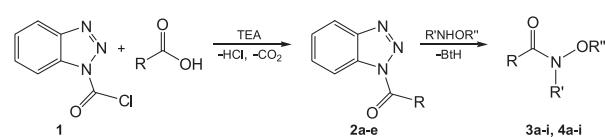
ANTIBACTERIAL AND ANTIUREASE EVALUATION OF NSAID HYDROXAMIC ACIDS

Zrinka Rajic, Ivana Perkovic, Ivan Butula, Branka Zorc, Ivan Kosalec, Stjepan Pepeljnjak

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Ibuprofen and oxamethacin, hydroxamic acid derivatives of the nonsteroidal anti-inflammatory drugs (NSAID) ibuprofen and indomethacin, are efficient anti-inflammatory drugs registered internationally. On the other hand, hydroxamic acids have been reported as highly specific and potent inhibitors of bacterial, jack bean and sword bean urease [1]. One of them, acetohydroxamic acid (Lithostat®), is used for treating chronic urinary infections (urolithiasis, pyelonephritis) in combination with surgery or antibiotic medicines. Urease inhibitors have been regarded as targets for treatment of ulcer because of the crucial role of inhibiting *Helicobacter pylori* urease during colonization. Here we report synthesis of *O*-methyl and *O*-ethyl NSAID hydroxamic acids, their antibacterial and antiurease evaluation and comparison with NSAID hydroxamic acids with free and *O*-benzyl substituted hydroxy group.

NSAID hydroxamic acid derivatives **3a-i** and **4a-i** were prepared from appropriate NSAID benzotriazolides **2a-e** and corresponding hydroxylamine.



Antimicrobial activity was determined against following microbial species *B. subtilis*, *S. aureus*, *E. faecalis*, *K. rhizophila*, *E. coli*, *S. enterica* subsp. *enterica*, *P. aeruginosa* and *C. albicans*. The results of the hole-plate diffusion method showed that only NSAID hydroxamic acids with free and *O*-ethyl substituted hydroxy group possessed noticeable antimicrobial activity at concentration 29.6 mg mL⁻¹. The minimal inhibitory and the minimal microbicidal concentrations were determined by the microdilution broth method.

The potential of NSAID hydroxamic acid derivatives **3** and **4** to inhibit urease was tested and the results showed that only NSAID hydroxamic acids bearing free hydroxy group inhibited urease activity.

- [1] Z. Amtul, Atta-ur-Rahman, R. A. Siddiqui, M. I. Choudhary, Chemistry and mechanism of urease inhibition, *Curr. Med. Chem.* 2002, 9, 1323-1348.

P456

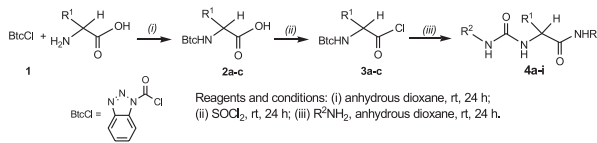
PREPARATION OF HYDROPHILIC UREIDO AMIDES

Ivana Perkovic, Ivan Butula, Zrinka Rajic and Branka Zorc

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Hydroxyurea is an anticancer agent used to treat leukemia and other malignant tumor diseases. It inhibits ribonucleotide reductase as well as cellular DNA synthesis during the S phase of cell division [1]. *N*-hydroxyureas previously synthesized by our group were evaluated for their antiviral and cytostatic activity against malignant tumor cell lines and normal human fibroblasts and showed significant activity [2].

In this paper we report the synthesis of the new ureido amides **4a-i**, derivatives of *N*-hydroxyureas, bearing hydroxy groups separated by two, three or five carbon atoms from terminal nitrogen atom in urea moiety. These compounds are more hydrophilic compared to previously synthesized *N*-hydroxyureas [2].



4	a	b	c	d	e	f	g	h	i
R ¹	<i>i</i> -Bu	<i>i</i> -Bu	<i>i</i> -Bu	Ph	Ph	Ph	Bn	Bn	Bn
R ²	(CH ₂) ₂ OH	(CH ₂) ₃ OH	(CH ₂) ₄ OH	(CH ₂) ₂ OH	(CH ₂) ₃ OH	(CH ₂) ₄ OH	(CH ₂) ₂ OH	(CH ₂) ₃ OH	(CH ₂) ₄ OH

New compounds **4a-i** were prepared by reaction of one equivalent of *N*-(1-benzotriazolecarbonyl)-amino acid chlorides **3a-c** with three equivalents of hydroxyamines (ethanolamine, propanolamine or pentanolamine). New compounds were obtained in high yields and their structures were deduced from analysis of their IR, ¹H and ¹³C NMR spectra.

- [1] E. Mutschler, H. Derendorf, *Drug Actions, Basic Principles and Therapeutic Aspects*, Medpharm Scientific Publishers, Stuttgart, 1995, p. 612.
- [2] I. Perkovic, I. Butula, B. Zorc, S. Kraljevic Pavelic, K. Pavelic, E. De Clercq, J. Balzarini and M. Mintas, Novel Lipophilic Hydroxyurea Derivatives: Synthesis, Cytostatic and Antiviral Activity Evaluations, *Chem. Biol. Drug Des.* (in press)

P457

DRUG TARGET IDENTIFICATION BY CHEMICAL PROTEOMICS: TARGET PROFILE COMPARISON OF THE BCR-ABL INHIBITORS

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Many drugs currently used in the clinics are more promiscuous than originally anticipated. This harbors the inherent potential for additional medical uses, but also the danger of serious side effects. Furthermore, in spite of great efforts of the scientific community, the disease-relevant target proteins for a large proportion of drugs remain unknown. In the age of upcoming personalized medicine and systems biology it becomes more and more necessary as well as feasible to determine comprehensive drug-target interaction profiles and map these on signalling and metabolic pathways, be they disease-related or not. With our drug affinity-chromatography based chemical proteomics approach it is feasible to identify the protein interactors of small molecule drugs. We pursue a combined approach utilizing our chemical capabilities and cutting-edge mass spectrometry in a pathway proteomics setting that provides the necessary biological background

as well as follow-up assaying and orthogonal experimental capabilities.

We have established this technology in our laboratory and previously applied it to the paradigmatic BCR-ABL tyrosine kinase inhibitors imatinib (gleevec), nilotinib (tasigna) and dasatinib (sprycel), which are in use for Chronic Myeloid Leukemia (CML). In our current study we describe the target profile of the next-generation BCR-ABL tyrosine kinase inhibitor bosutinib (SKI-606) in CML using K562 and primary patient cells from chronic and accelerated disease stages. As for dasatinib we found a wide range of protein kinases binding to bosutinib in CML and validated their inhibition through the Millipore Kinase Profiler. Although bosutinib is, like dasatinib, a multitargeted ABL- and SRC-family tyrosine kinase inhibitor, their target profiles differed to a considerable extent, which might also explain the different side effect patterns of these drugs observed in CML patients. Furthermore, the target profile of bosutinib in K562 cells and the CML patient samples displayed significant differences, which underline the importance of investigating primary patient cells in order to obtain a more thorough understanding of the drug's effects and side effects.

P458

DESIGN, SYNTHESIS AND DOCKING STUDIES OF HYDROXYETHYLAMINE AND HYDROXYETHYL-SULFIDE BACE-1 INHIBITORS

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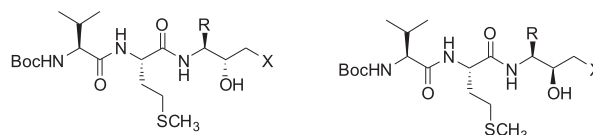
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Siena Biotech S.p.A - ITALY

We have previously reported BACE-1 inhibition by hydroxyethylamine (HEA) transition-state isostere, and showed that the (*R*)-HEA stereoisomer is preferred by BACE-1.[1] This stereopreference is peculiar since for aspartyl proteases the most active stereoisomer is typically the (*S*) isomer of the central $\psi(\text{CH-OH})$. This inversion has been observed also in others BACE-1 and HIV protease (HIV-Pr) inhibitors. The origins of this selectivity have been investigated in the case of HIV-Pr by molecular modeling and X-ray crystallography, and it has been demonstrated that the central $\psi(\text{CH-OH})$ of the (*R*)-HEA inhibitors interacts only with one catalytic aspartate of HIV-Pr while the amino group binds to the second aspartate; differently to the (*S*) stereoisomer where the hydroxyl group interacts with both aspartates.[2] Not all HEA inhibitors of HIV-Pr display the same stereoselectivity, the preference for one or the other stereoisomer is determined by substituents. For example, compound JG365 with a Pro in P1' shows a stereopreference for the (*S*)-HEA isostere, while compounds having larger ring systems display a reverse selectivity. X-ray crystallography of (*R*)-HEA inhibitors bound to BACE-1 showed a binding

mode of the HEA isostere in the catalytic site similar to HIV-Pr inhibitors, but no information is available about (*S*)-HEA inhibitors and the change in stereopreference has not been observed yet.

Considering these observations we decided to further investigate the molecular basis for the peculiar behaviour of the (*R*)-HEA isostere by synthesizing both stereoisomer of hydroxyethylamine (HEA) and hydroxyethylsulfide (HES) transition-state isostere inhibitors of BACE-1.

The *syn*-HEA epimer resulted always more active than the anti stereoisomer independently from the P₁ and the P₁' substituents. On the contrary, the anti epimer of the HES isostere resulted more active than the *syn* stereoisomer. The change of stereopreference was studied by molecular modelling.



R	X
i-Bu	AlaValNHBN
Bn	AlaValNHBN
i-Bu	ValValNHBN
i-Bu	ProValNHBN
i-Bu	ThidactValNHBN

References

- [1] L. Rizzi, Romeo, S., *Lett. Drug Des. & Disc.* 2005, 2, 184-193.
- [2] D. H. Rich, C. Q. Sun, J. V. N. V. Prasad, A. Pathiaseril, M. V. Toth, G. R. Marshall, M. Clare, R. A. Mueller, K. Houseman, *J. Med. Chem.* 1991, 34, 1222-1225.

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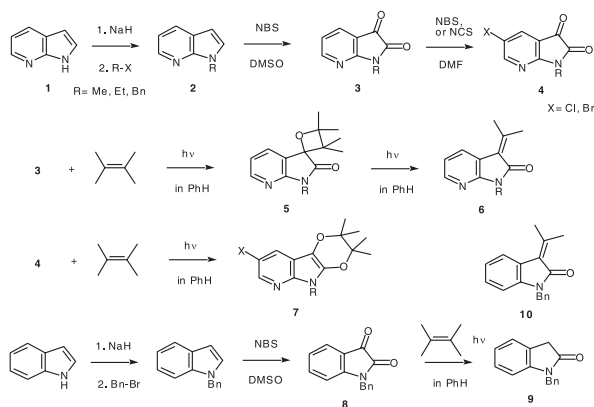
PHOTOCHEMICAL REACTIONS OF I-SUBSTITUTED 7-AZAIASATINS AND ISATIN WITH ALKENES : THE FORMATION OF 3-ISOPROPYLIDENE DERIVATIVES

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During our studies on photochemical reactions of cyclic vicinal polycarbonyl compounds, we need to prepared 1-alkyl-7-azaistatins (**3**) and 1-alkyl-5-halo-7-azaistatins (**4**). The compounds **3** were prepared from commercially available 7-azaindole (**1**) via 1-alkyl-7-azaindoles (**2**) which were oxidized with NBS-DMSO reagent. Halogenation of **3** with NBS or NCS in DMF under mild conditions gave the corresponding 1-alkyl-5-halo-7-azaistatins in moderate yields. In order to clarify the photochemical intrinsic behaviour of heterocyclic vicinal dicarbonyl compounds, we have examined the photochemical reactions of **3** and **4** with 2,3-dimethylbut-2-ene or 2-methylpent-2-ene in degassed benzene. The pho-

toreactions of **3** with the alkene gave the corresponding adducts as a product, which took place photodecomposition to isopropylidene derivatives (**6**), of Paterno Büchi reaction in moderate yields. On the other hand, the photochemical reactions of **4** afforded dioxene derivatives (**7**) as photoproducts in good yields. The photoreactions of 1-benzylisatin (**8**) with 2,3-dimethylbut-2-ene in degassed benzene gave 1-benzylloxindole (**9**) as a major product together with the corresponding of oxetane derivative (**10**).



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STRUCTURE-ACTIVITY RELATIONSHIPS ON 4-ANDROSTENES AND 5 α -ANDROSTAN-4-ONES AS AROMATASE INHIBITORS

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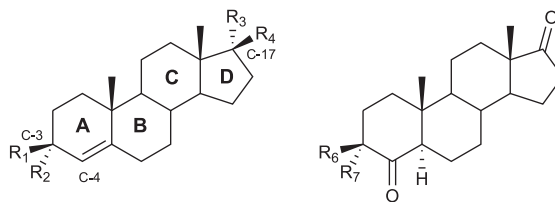
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Aromatase is a cytochrome P-450 enzyme that catalyses the aromatization of androgens, the final step in the biosynthesis of estrogens. Aromatase inhibitors (AIs) reduce the synthesis of estrogens and offer a therapeutic alternative for the treatment of estrogen-dependent cancers such as breast cancer.¹

Among the most recent AIs designed, steroids resembling androstenedione, the natural androgen substrate of the enzyme, revealed to be potent inhibitors, and some of them have been clinically approved. Nevertheless, the active site of aromatase, its over-expression in the tumour tissues, and its inhibition mechanism still remains unknown, in spite of the intensive research done in this subject. The design, synthesis and evaluation of new androstenedione-based inhibitors, containing into the A- and D-rings chemical key-features for the drug-receptor interaction, are greatly contributing for establishing new structure-activity relationships (SAR). These SAR are

considered valuable tools for understanding the enzyme mechanism, to find more selective and potent AIs, and to improve the knowledge of the molecular recognition basis of aromatase inhibitor.²⁻⁶

From the established SAR for steroidal AIs, it is known that some planarity in the A-ring as well as the presence of oxygen functions at C-3, C-4 and C-17 positions, seems to be important for anti-aromatase activity.^{6,7} Steroid compounds having planar sp² C-4 atoms, as in olefin and carbonyl functions, can confer the required planarity in the A/B-ring region. Based on this chemical statement, we have synthesized and evaluated biologically a set of steroids with the above-mentioned features and with oxygen groups at C-3/C-4 and C-17 positions.



1 R₁ (OH); R₂ (H); R₃, R₄ (=O)

2 R₁ (H); R₂ (OH); R₃, R₄ (=O)

3 R₁ (OH); R₂ (H); R₃ (H); R₄ (OH)

4 R₆ = R₇ (H)

5 R₆ (H); R₇ (OH)

References

- [1] a) Chen, S. *Frontiers of Bioscience* 1998, 3(9), 22. b) Blickenstaff, R. T. *Antitumor Steroids*, Academic Press (NY); 1992.
- [2] Bordell, M.M.; Fernandez Mateos, A; Rubio Gonzalez, R. *J. Chem. Soc., Perkin Trans I*. 1995, 569.
- [3] Tavares da Silva, E. J.; Sá e Melo, M. L.; Campos Neves, A. S.; Paixão, J. A.; Andrade, L. R. C.; Almeida, M.J.M.; Costa, M.M.R. *J. Chem. Soc., Perkin Trans.1*, 1997, 3487.
- [4] Tavares da Silva, E.J.; Fernandes Roleira, F.M.; Sá e Melo, M.L.; Campos Neves, A.S.; Paixão, J. A. Almeida, M.J.; Costa, M.M.R.; Andrade, L.C.R. *Steroids*, 2002, 67(3-4), 311.
- [5] Gasi, K.M.P.; Stankovic, S.M.; Csanadi, J.J.; Djurendic, E.A.; Sakac, M.N.; Mijacevic, L.M.; Arcson, O.N.; Stojanovic, S.Z.; Andric, S.; Gabor, D.M.; Kovacevic, R. *Steroids*, 2001, 66(8), 645.
- [6] Nagaoka, M.; Watari, Y.; Yajima, H.; Tsukioka, K.; Muroi, Y.; Yamada, K.; Numazawa, M. *Steroids*, 2003, 68(6), 533.
- [7] Cepa, M. D. S., Tavares da Silva, E.J., Correia-da-Silva, G., Roleira, F.M.F., Teixeira, N.A.A. *J. Med. Chem*, 2005, 48, 6379.

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SYNTHESIS OF PROSTAMIDE STRUCTURAL ANALOGS AS POTENTIAL ANTIGLAUCOMA DRUGS

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Anandamide (arachidonylethanolamide, AEA) is a polyunsaturated fatty acid amide that was identified as an endogenous ligand for brain cannabinoid receptor. Some synthetic analogs of anandamide are enzymatically stable and decrease eye pressure. Such anandamide-type compounds provide clues to a new pharmacological strategy for glaucoma treatment. AEA is effectively oxygenated by human cyclooxygenase COX-2 but not COX-1 and the products are similar to those formed with arachidonic acid. Starting from AEA, the formed metabolites are prostanoid 1-ethanolamides (prostamides) which represent a novel class of prostaglandins. Studies to date indicate potent ocular hypotensive activity and a pharmacological activity profile, which is distinct from known prostaglandin receptors.

Starting from a receptor selective analog of PGF_{2α} obtained by total stereocontrolled synthesis, D-cloprostenol, some new prostamide F_{2α} analogs were obtained in a two step process: transformation of the terminal carboxy group into the corresponding methyl ester followed by aminolysis of the methyl ester group obtaining the desired 1-N-substituted prostamide with potential antihypertensive activity. Aminolysis reaction were successfully performed with ethanolamine, D-phenylglycinol and 6-aminohexanol, obtaining the corresponding prostamide F_{2α} analogs. Purification of prostamide compounds was performed by pressure column chromatography on silica gel and the structure confirmed especially by ¹H-NMR and ¹³C-NMR.

Investigation of pharmaco-toxicological activity profile of the obtained prostamides and especially of ocular hypotensive activity useful for antiglaucoma drugs is in progress.

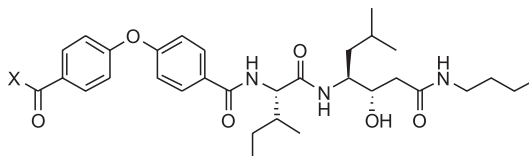
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NOVEL INHIBITORS OF PLASMEPSINS, A FAMILY OF HAEMOGLOBIN PROTEASES OF *P. FALCIPARUM*

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Plasmeprins (PLMs) are a family of plasmodial aspartic proteases involved in the degradation of haemoglobin during the blood stage of the parasite. Several potent inhibitors have been obtained, but effectiveness in killing the parasite was limited (IC₅₀ range 2-20 μM). The low potency against cultured parasites may be explained by the plasmeprins' redundancy which implies that blockage of this enzyme family may not be lethal.[1] We developed new inhibitors based on the double-drug approach. [2-4]



X: 6-Methoxyquinoline, 7-Chloroquinoline, Atovaquone.

Compounds developed were tested in vitro for anti-PLM I/PLM II/PLM IV activities and against chloroquine-sensitive (D10) and chloroquine-resistant (W2) strains of *P. falciparum*. All compounds inhibited PLM I, PLM II and PLM IV in a nanomolar range (K_i 1-700 nM). Antiplasmodial activity (IC₅₀) as low as 0.1 μM was obtained, an excellent improvement in comparison with inhibitors previously reported (IC₅₀ range 2-20 μM). The killing activity was equally directed against both *P. falciparum* strains and was correlated with lipophilicity (calculated as LogP). The most promising compounds were not cytotoxic against human fibroblasts at 100 μM and were highly selective for PLMs vs human cathepsin D. In addition they showed curative efficacy in the murine malaria model of *Plasmodium berghei*. The most active compound determined a 96% (CI95%: 89% – 100%) reduction of the parasitemia in mice treated at a dose of 25 mg/kg.

References

- [1] Liu J., Istvan E. S., Gluzman I. Y., Gross J., Goldberg D. E. Proc. Natl. Acad. Sci. U S A 2006, 103, 8840-5.
- [2] Dell'Agli M., Parapini S., Galli G., Vaiana N., Taramelli D., Sparatore A., Liu P., Dunn B.M., Bosisio E., Romeo S. J. Med. Chem. 2006, 49, 7740-9.
- [3] Romeo S., Parapini S., Dell'Agli M., Vaiana N., Magrone P., Galli G., Sparatore A., Taramelli D., Bosisio E. ChemMedChem, 2008, 3:, 418-420.
- [4] The financial support from the EU 018834 ANTIMAL is acknowledged.

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DESIGN AND SYNTHESIS OF RDEA119 A POTENT AND ORALLY BIOAVAILABLE MEK INHIBITOR

Shunqi Yan, Andreas Maderna, Eric Hanson, Hassan Abdellaoui, Varaprasad Chamakura, Cory Iverson, Chon Lai, Dongmei Zhou, Ryan Dick, Li-Tain Yeh, Jean-Michel Vernier

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Protein kinase-mediated pathways have become key targets for the treatment of numerous diseases including cancer and inflammation. The Ras/Raf/MEK/ERK mitogen-activated protein (MAP) kinase is one of the most prevalent signal transduction pathways involved in the formation, progression, and survival of tumors. This pathway consists of a cascade of three enzymes. Activation, by growth factor receptors, of the GTP-bound RAS triggers the activation of Raf kinase which then phosphorylates MEK1 and MEK2. Activated MEK kinases play a key role where the signal transduction cascade is extremely specific and are the only known enzymes to activate ERK1/2. Phosphorylated ERK dimerizes and migrate to the nucleus where they are involved in cellular function such as cell proliferation. Furthermore, genetic mutation in this pathway, such as the BRAF(V600E) mutation (present in ~ 60% of melanomas) are highly dependent on MEK activity and have been found to be associated with cancers. Therefore novel MEK inhibitors have recently emerged and have advanced into clinical development as anticancer drugs. We wish to report the discovery of a novel class of potent and bioavailable non competitive MEK inhibitors designed to interact in the allosteric binding pocket previously described¹. In this presentation we will discuss the design, synthesis and structural-activity relationship that led to the discovery of RDEA119, a novel highly potent and selective MEK inhibitor, with favorable human PK profile.

References

- [1] Ohren, J. F. C., H.; Pavlovsky, A.; Whitehead, C.; Zhang, E.; Kuffa, P.; Yan, C.; McConnell, P.; Spessard, C.; Banotai, C.; Mueller, W. T.; Delaney, A.; Omer, C.; Sebolt-Leopold, J. S.; Dudley, D. T.; Leung, I. K.; Flamme, C.; Warmus, J.; Kaufman, M.; Barrett, S.; Tecle, H.; Hasemann, C. A. Structures of human MAP kinase kinase1 (MEK1) and MEK2 describe novel non-competitive kinase inhibition. *Nature Struct. Mol. Biol.* 2004, 11, 1192-1197.

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ANTIDEGENERATIVE ACTIVITY OF HETEROARYL-IMINO-4-THIAZOLIDINONES ON HUMAN CHONDROCYTE CULTURES STIMULATED BY IL-1 β

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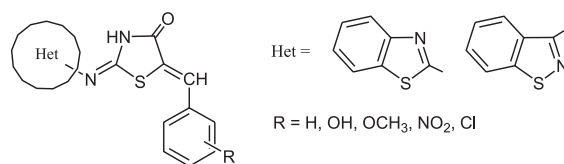
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Osteoarthritis (OA) is a painful and debilitating joint condition that affects hundreds of millions worldwide [1]. Despite the prevalence of the disease the current therapeutic options have significant limitations. The non-steroidal anti-inflammatory drugs (NSAIDs), which block cyclo-oxygenase (COX), provide symptomatic relief but do not abrogate the underlying disease process [2]. Indeed, it is well appreciated that cartilage destruction can proceed unabated despite suppression of inflammation [3]. Cartilage loss is a hallmark of OA and follows activation of catabolic processes concomitant with a disruption of anabolic pathways. Chondrocytes, unique cellular population in this tissue, play a crucial role in the inflammatory response leading to structural damage in OA.

During inflammation or injury, chondrocytes release MMPs, which in turn degrade the cartilage matrix, releasing glycosaminoglycans (GAGs), which can then be readily quantified as an index of catabolic events in cartilage. Indeed, these events are reproduced in this study with human chondrocytes treated with interleukin-1 β (IL-1 β) that is considered pivotal cytokine in the process of human osteoarticular diseases. It is responsible of degradation of cartilage matrix, in fact it induces connective tissue cells, including chondrocytes, to produce inflammatory mediators such as nitric oxide (NO), prostaglandins E₂ (PGE₂) and MMPs.

In the present study, we assay the anti-inflammatory/chondroprotective effect of some 2-benzo[d]thiazolyl- and 2-benzo[d]isothiazolyl-imino-5-benzylidene-4-thiazolidinones bearing well know bioactive heterocyclic systems, namely the 4-thiazolidinone ring and the benzo/benzisothiazole system [4,5].

The effects of the title compounds on NO production, GAGs release, presence of MMPs on stimulated chondrocytes with IL-1 β will be discussed.



- [1] Yellin E, Callahan LF. The economic cost and social and psychological impact of musculoskeletal conditions. *Arthritis Rheumatism*. 1995;38:1351-1362.
- [2] Kraan PM, van den Berg WM. Anabolic and destructive mediators in osteoarthritis. *Curr Opin Nutr Metab Care*. 2000;3:205-211.
- [3] Van den Berg WB. Joint inflammation and cartilage destruction may occur uncoupled. *Springer Semin Immunopathol*. 1998;20:149-164.

[4] Ottana R, Maccari R, Ciurleo R, Vigorita MG, Panico A M, Cardile V, Garufi F, Ronsisvalle S, *Bioorg. Med. Chem.* 2007; 15: 7618-7625.

[5] Vicini P, Geronikaki A, Incerti M, Zani F, Dearden J, Hewitt M. *Bioorg. Med. Chem.* 2008; 16: 3714–3724.

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ANTIMICROBIAL AND ANTIOXIDANT PROPERTIES OF PORTUGUESE PROPOLIS

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Propolis is a resinous substance collected by honeybees from the bud and bark of certain trees and plants and stored inside their hives. It has been used in folk medicine from ancient times in many countries. More recently, it has been reported to possess various biological activities, such as antibacterial, antiviral, anti-inflammatory, anti-cancer, antifungal, and antitumoral properties.

Propolis usually contains a variety of chemical compounds such as polyphenols (flavonoids, phenolic acids and their esters), terpenoids, steroids and amino acids, being its composition qualitatively and quantitatively variable depending on vegetation at the area from which it was collected. Because of geographical differences, propolis from Europe, South America and Asia have different chemical composition and also distinct biological activities.

Chemical composition and biological activity of propolis have been studied extensively in many countries. However, there are no reports on the Portuguese propolis. In this study, we investigated the *in vitro* antioxidant and antimicrobial activities of different extracts of two different colored samples of Portuguese propolis. Propolis samples were collected as the crude materials and were extracted with ethanol at room temperature. The ethanol suspension was filtered and the filtrate was concentrated to give the ethanolic extract of propolis (EEP). The EEP was further fractioned with hexane and chloroform solvents. The fractions obtained, HEX-Fr and CHLO-Fr, and the residual ethanolic extract were concentrated. Total polyphenol and flavonoid contents in EEP, HEX-Fr and CHLO-Fr were determined by using aluminum nitrate and Folin–Ciocalteu colorimetric methods. The quantitative levels of phenolic and flavonoid components of these extracts can be used for evaluation of propolis quality and its properties and a search for a correlation between these parameters was performed.

Active free radicals, together with other factors, are responsible for cellular ageing and many conditions such

as cardiovascular diseases, cancer, diabetes, arthritis, Parkinson disease and Alzheimer. Several reports have proved the usefulness of the free-radical scavenging properties of antioxidants and their general benefits to human health but development and utilization of more effective antioxidants of natural origin are desired. We used two assay systems for evaluating the antioxidant activity of propolis extracts: the free radical-scavenging activity on 1,1-diphenyl-2-picrylhydrazyl (DPPH), and the scavenging activity on 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation.

The antimicrobial properties of propolis extracts were screened by a disk diffusion assay using a panel of Gram-positive/Gram-negative bacteria and yeasts with clinical interest or importance in food industry.

To our knowledge, this is the first report describing a study on Portuguese propolis. The present work examines the potential of two propolis samples as sources of natural antioxidants and new antimicrobials agents.

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SYNTHESIS AND EVALUATION OF DERIVATIVES OF PHENYL-PROPIOPHENONE AS ANTIPROLIFERATIVE AGENTS

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Cytotoxic and anticancer activity of a number of chalcones (1,3-diaryl-1-oxo-2-propen derivatives) has been described in literatures [1,2]. A series of chalcones and analogs of propafenone has been synthesized and evaluated for antiproliferative activity against the human cervical carcinoma HeLa cells.

Chalcon (1-(2-hydroxyphenyl)-3-phenyl-1-oxo-2-propen) is intermediary substance in the synthesis of propafenone. Propafenone (2-(2-hydroxy-3-propylamino-propoxy)-3-phenyl-propiofenone) as hydrochloric salt is antiarrhythmic drug with beta-adrenergic – antagonist properties, which is effective in the treatment of supraventricular and ventricular arrhythmias [3]. A range of propafenone analogs with changes on amino group have been synthesized. These compounds have been presented in the literature as propafenone type modulators of cancer multidrug resistance [4].

The aim of this work was to synthesize a class of compounds with antiproliferative activity with modifications in the benzyl moiety (R = -CH₃, -OCH₃, -F, -Cl, -OH, -NO₂) on the basis of pre SAR and pharmacophoric studies.

The proposed derivatives were prepared in four steps. The first step in the synthetic route consisted in the condensation of acetophenone with *o*-, *m*- or *p*- mono substituted benzaldehyde to give an α,β -unsaturated ketone (chalcone). The reduction of α,β -unsaturated ketone with

triethylammonium formate produced saturated ketone. The addition of phenolic nucleophiles to epichlorohydrin in basic media afforded aryloxyepoxypropane derivatives that was subjected to aminolysis with propylamine to give aryloxypropanolamine derivatives.

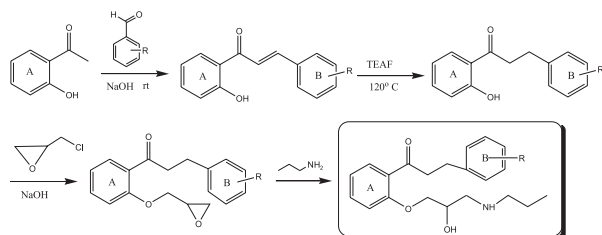


Figure 1. Synthesis of derivatives of phenyl-propiophenone

Physicochemical properties of the proposed compounds have been showed as well.

The IC₅₀ values for all compounds correlated with energy of HOMO orbitals as well as hydrophobic fields.

References

- [1] Yit C. C., Das N. P., Cancer Lett. 1994, 82, 65-72
- [2] Satomi Y., Int.J.Cancer, 1993, 55, 506-514
- [3] Porterfield J.G., Porefield L.M., Am. J. Cardiol., 63,114-116 (1989)
- [4] Pajeva I.K., Wiese M., Quantitative Structure-Activity Relationships, 1998, 178(4), 301-312

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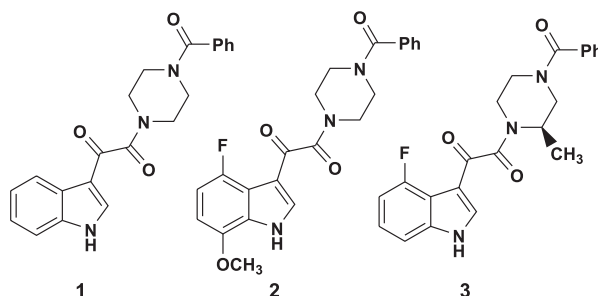
INDOLE-BASED INHIBITORS OF HIV ATTACHMENT: AN INITIAL SURVEY OF INDOLE AND PIPERAZINE SUBSTITUTION PATTERNS

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A cell-based screening assay conducted against the Bristol-Myers Squibb compound inventory identified 1-(4-benzoylpiperazin-1-yl)-2-(1*H*-indol-3-yl)ethane-1,2-dione (**1**) as an anti-HIV inhibitor that was subsequently determined to interfere with the interaction between the HIV surface protein gp120 and the host cell receptor CD4. A survey of the effects of substitution of the indole and piperazine moieties was found to exert a significant impact on HIV inhibitory activity and modulate pharmaceutical properties. The examination of indole substitu-

tion patterns revealed that antiviral activity could be markedly enhanced by substituents at either the 4- or 7-positions and particularly by combinations of 4,7-disubstitution, with 1-(4-benzoylpiperazin-1-yl)-2-(4-fluoro-7-methoxy-1*H*-indol-3-yl)ethane-1,2-dione (**2**) one of the most potent antiviral agents prepared as part of this study. The SAR associated with the piperazine linkage indicated that the introduction of a methyl group on the carbon atom proximal to the glyoxal element provided the best combination of overall activity and physicochemical properties with (*R*)-1-(4-benzoyl-2-methylpiperazin-1-yl)-2-(4-fluoro-1*H*-indol-3-yl)ethane-1,2-dione (**3**) 10-fold more potent than the des-methyl analogue.



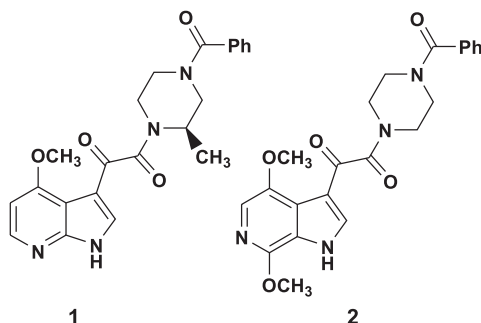
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AZAINDOLE INHIBITORS OF HIV ATTACHMENT: THE DISCOVERY OF BMS-488043, AN INHIBITOR OF THE GP120-CD4 INTERACTION THAT DEMONSTRATES ANTIVIRAL ACTIVITY IN HIV INFECTED PATIENTS

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A family of indole derivatives derived from the screening lead 1-(4-benzoylpiperazin-1-yl)-2-(1*H*-indol-3-yl)ethane-1,2-dione was characterized as inhibitors of HIV-1 that function by interfering with the interaction between the HIV surface protein gp120 and the host cell receptor CD4. Replacement of either the C-6 or C-7 carbon atoms in the phenyl ring of the indole moiety by a nitrogen atom led to a series of azaindole derivatives that demonstrate potent antiviral activity accompanied by marked improvements in pharmaceutical properties. Optimization of these azaindole leads resulted in the identification of two clinical compounds, (*R*)-1-(4-benzoyl-2-methylpiperazin-1-yl)-2-(4-methoxy-1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)ethane-1,2-dione (BMS-377806, **1**) and 1-(4-benzoylpiperazin-1-yl)-2-(4,7-dimethoxy-1*H*-pyrrolo[2,3-*c*]pyridin-3-yl)ethane-1,2-dione (BMS-488043, **2**). BMS-488043 was advanced into clinical phase IIa studies that established the potential of this class of compounds to reduce viremia in HIV-infected individuals.



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PYRIDAZIN-3(2H)-ONES AS POTENT PLATELET AGGREGATION INHIBITORS: DESIGN, SYNTHESIS AND SAR STUDIES

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Platelets, or thrombocytes, are anucleated cells that circulate in blood as sentinels of vascular integrity,¹ they show no interaction with the inner surface of normal vessels but adhere promptly where endothelial cells are altered or extracellular matrix substrates are exposed. Since under pathological conditions platelets are a major contributor to thrombosis and heart disease the inhibition of platelet function represents a well established approach to prevent these diseases.² The therapeutic family of antiplatelet agents is represented by drugs as ticlopidine, clopidogrel, aspirin, dipyridamole and tirofiban, being the combination of clopidogrel and aspirin the current gold standard of antiplatelet therapy.³

In the course of our studies in the search for novel pyridazinone-based antiplatelet agents we have recently identified⁴ a novel family of pyridazinone-based antiplatelet agents that can be formally considered as hybrid structures since they incorporate at the 5 position of the heterocyclic nuclei an α,β unsaturated moiety present in chalcones, another chemotype eliciting antiplatelet activity. In the present communication we describe the synthesis, biological evaluation as well as the structure-activity relationships of a series of pyridazin-3(2H)-ones incorporating 1-oxo-3-arylpropenyl or 1-oxo-1-arylpropenyl fragments at position 5 of the heterocyclic core (figure 1).

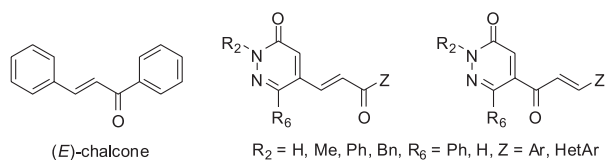


Figure 1. General structure of compounds described.

References

- [1] Stormoken, H., In *Platelets Responses and Metabolism*, vol 3, ed. by Holmsen, H., CRS Press Boca Raton, Florida, 1986. Ruggeri Z. M. *Nat Med.* 2002; 8, 1227.
- [2] Clutton, P.; Folts, J. D.; Freedman, J. E.; *Pharmacol. Res.* 2001, 44, 255. Davies, M. J.; Thomas, A. C.; *Br. Heart J.* 1985, 53, 363. Fuster, V.; Badimon, L.; Badimon, J. J.; Chesebro, J. H. *N. Engl. J. Med.* 1992, 326, 242.
- [3] Bennet, J. S. *Annu. Rev. Med. Chem.* 2001, 52, 161.
- [4] Coelho, A., Raviña, E., Fraiz, N., Yáñez, M., Laguna, R., Cano, E. and Sotelo, E., *J. Med. Chem.*, 2007, 50, 6476-6484. Meyers, C., Yáñez, M., Elmatougi, A., Verhest, T., Coelho, A., Fraiz, N., Lemiére, G. L. F., García-Mera, X., Laguna, R., Cano, E., Maes, B. U. W., Sotelo, E., *Bioorg. & Med. Chem. Lett.* 2008, 18, 793-797. Coelho, A., Sotelo, E., Fraiz, N., Yáñez, M., Laguna, R., Cano, E., Raviña, E., *Bioorg. and Med. Chem. Lett.*, 2004, 14, 321-324.

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BINDING FREE ENERGY OF DNA LIGANDS USING THE MM/PBSA AND MM/GBSA METHODOLOGIES.

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Ligands that interact with DNA have the unique ability to affect processes occurring at the genetic level. DNA minor groove binders are such ligands, which are commonly used as molecular probes and have the potential to alleviate problems arising from genetic disorder.¹ The synthesis of such ligands is not trivial, and gaining insights into their molecular aspects of binding to DNA through computer simulation is an important challenge in their development as drug candidates. The University of Strathclyde is at the forefront of chemical novelty in this area, and is currently developing these ligands as a new class of antimicrobial agents.²

The use of Molecular Dynamics simulations to derive thermodynamic properties of binding events between ligands and DNA has been reported for relative binding free energies for different DNA sequences,³ but determination of absolute binding free energies has remained elusive. Using the thiazotropsin-A:5'GCGACTAGTCGC system⁴ as a test case, we have simulated a binding free energy

closely matching that of our analytical calorimetric data. We achieved this by running separate Molecular Dynamics trajectories for the ligand-DNA complex, free DNA duplex and free ligand using the Molecular Mechanics/Poisson-Boltzmann Surface Area (MM/PBSA) and Generalized Born Surface Area (MM/GBSA) methodologies within the AMBER suite of software. We are currently applying this protocol to other related sequences to assess its robustness.

References

- [1] Burnett, R.; Melander, C.; Puckett, J. W.; Son, L. S.; Wells, R. D. et al. DNA sequence-specific polyamides alleviate transcription inhibition associated with long GAA center dot TTC repeats in Friedreich's ataxia. *Proceedings of the National Academy of Sciences of the United States of America* 2006, 103, 11497-11502.
- [2] Anthony, N. G.; Breen, D.; Clarke, J.; Donoghue, G.; Drummond, A. J. et al. Antimicrobial lexitropsins containing amide, amidine, and alkene linking groups. *Journal of Medicinal Chemistry* 2007, 50, 6116-6125.
- [3] Cheatham, T. E. Simulation and modeling of nucleic acid structure, dynamics and interactions. *Current Opinion in Structural Biology* 2004, 14, 360-367.
- [4] Anthony, N. G.; Johnston, B. F.; Khalaf, A. I.; Mackay, S. P.; Parkinson, J. A. et al. Short Lexitropsin that Recognizes the DNA Minor Groove at 5'-ACTAGT-3': Understanding the Role of Isopropyl-thiazole. *Journal of the American Chemical Society* 2004, 126, 11338-11349.

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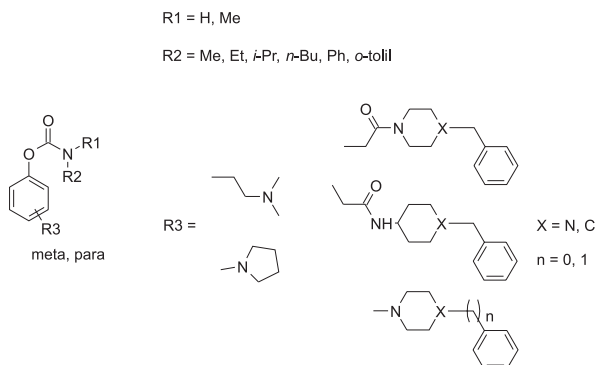
DESIGN, SYNTHESIS AND BIOLOGICAL ACTIVITY OF NOVEL CARBAMATE DERIVATIVES AS CHOLINESTERASE INHIBITORS

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Alzheimer's disease (AD), a progressive, degenerative disorder of the brain is associated with the neurotransmitters deficits, particularly the deficits in cholinergic system in the brain areas related to memory and learning. Hence, the most important drugs approved for the treatment of AD include a group of indirect cholinomimetic agents (rivastigmine, donepezil, galantamine) which enhance central cholinergic neurotransmission by inhibiting the acetylcholinesterase (AChE) – the enzyme that hydrolyses acetylcholine. Lately there has been a growing number of evidence that butyrylcholinesterase (BuChE) plays an important role in the pathogenesis of AD [1].

Our research group has managed to synthesize potential new inhibitors of cholinesterases. Into their structure we incorporated carbamoyl moiety attached directly to the phenyl ring, which are crucial for the activity of rivastigmine. Those fragments were modified by different alkyl and aryl substituents.



The activity of the obtained products against AChE and BuChE was tested *in vitro* with the spectrometric method of Ellman. Among all tested compounds the most potent was butyl-carbamic acid 3-[2-(4-benzylpiperazin-1-yl)-2-oxo-ethyl]-phenyl ester with $IC_{50} = 9,83 \mu M$ (BuChE). Using molecular modelling technique, docking of the new structures to the active site of acetylcholinesterase was performed and the most important interactions that may be responsible for the inhibition of the enzyme were described.

References

- [1] Ezio Giacobini, Butyrylcholinesterase, its function and inhibitors, Martin Dunitz, 2003

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NOVEL INHIBITORS OF FATTY ACID SYNTHASE (FASN) WITH ANTITUMOR ACTIVITY

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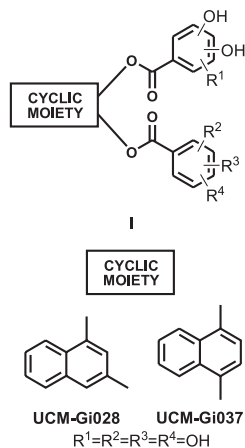
Cancer is a leading cause of death worldwide.¹ Fatty acid synthase (FASN) has recently emerged as a promising target for antitumor drug development, since it is highly expressed in breast carcinoma and other human cancers.² However, its therapeutic exploitation has been limited by the lack of pharmacological inhibitors that are potent, specific and stable enough. Accordingly, the identification of small-molecule inhibitors of FASN is an area of intense research.

Targeting FASN inhibition, in the present work we have designed and synthesized a new series of compounds of general formula I. The derivatives are structurally not complicated, and their synthesis is straightforward in few steps. The synthesized derivatives were

evaluated for their cytotoxic capacity in a panel of human breast cancer cell lines (SK-Br3, MCF-7, and MDA-MB-231) and in two types of non-malignant cells (fibroblasts and adipocytes), as well as for their effect in FASN activity.³

Our data show a strong correlation between FASN expression and cell growth inhibition in the studied cell lines, suggesting that direct FASN inhibition plays a predominant role in the cytotoxicity of the compounds. Indeed, higher degree of FASN activity inhibition correlated with increased cytotoxicity. The biological evaluation has resulted in the identification of two potent FASN inhibitors (**UCM-Gi028** and **UCM-Gi037**, with 90% and 69% inhibition of FASN activity at 25 μ M), that are highly cytotoxic against SK-Br3 cells (IC_{50} = 21 and 29 μ M, respectively) without affecting the growth of non-malignant cells. Importantly, they do not stimulate carnitine palmitoyltransferase-1 (CPT-1) activity nor they induce weight loss *in vivo*, in contrast to previously reported FASN inhibitors (such as C75).

In summary, the novel compounds developed herein represent a new class of potent and selective synthetic inhibitors of FASN, which do not exhibit the limiting toxicity effects of weight loss, and therefore may hold promise as suitable antitumor drugs, in particular against breast cancer. We are currently exploring their full potential by assessing their *in vivo* antitumor effect in a breast cancer model, and we are also involved in the synthesis and pharmacological evaluation of a larger structural field focused on an in-depth structure-activity relationship (SAR) study. These results will enable the definitive validation of FASN as a useful target-directed therapeutic strategy for the treatment of cancer, one of the most widespread diseases in the world nowadays.



This work was supported by grants from the Ministerio de Educación y Ciencia (MEC, SAF-2007/67008-C02-01), the Instituto de Salud Carlos III (FIS PI04/1417, RD06-0020-0028, ISCIII-RETIC RD06), the Comunidad Autónoma de Madrid (CAM, S-SAL-249-2006), and the Susan G. Komen Breast Cancer Foundation (PDF-0504073), and by the Juan de la Cierva (TP), Ramón y Cajal (SO-G), and FPU (CT) programs from MEC.

References

- [1] World Health Organization, <http://www.who.int/mediacentre/factsheets/fs297/en/>.
- [2] Kuhajda, F. P. *Cancer Res.* 2006, 66, 5977.
- [3] López-Rodríguez, M. L.; Benhamú, B.; Turrado, C.; Ortega-Gutiérrez, S.; Puig, T.; Brunet, J.; Colomer, R. Patent EP07110956.

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BENZIMIDAZOLE DERIVATIVES AS NOVEL SEROTONIN 5-HT₆ RECEPTOR ANTAGONISTS. A COMPUTATIONAL AND MUTAGENESIS STUDY OF LIGAND-RECEPTOR INTERACTION

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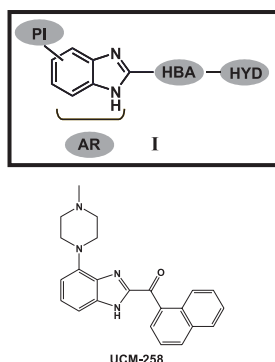
The G protein-coupled 5-HT₆ receptor (GPCR, 5-HT₆R) is one of the most recently added subtypes to the serotonin receptor family. Since it is almost exclusively localized in the brain, this receptor has generated considerable interest as a promising target for central nervous system-mediated diseases such as obesity, cognitive dysfunction, and certain neurological and neuropsychiatric disorders, including schizophrenia and depression.¹ In the past few years several 5-HT₆R agents have been identified, mainly by high throughput screening of pharmaceutical companies, but few compounds have entered clinical trials. Therefore, significant efforts are currently focused on a rational drug discovery in this research area.

In a project aimed at the development of new 5-HT₆R ligands, we have generated a pharmacophore and a 3-D computational model of the ligand-receptor interaction.² Using these models, in the present work we have designed *de novo* a series of new benzimidazole derivatives of general structure **I** that have been synthesized and evaluated for their binding affinity at the human 5-HT₆R. Most of the benzimidazole-4-substituted derivatives (**1a**) show high 5-HT₆R affinity, whereas 5-substituted analogues (**1b**) are inactive. Indeed, the structural elements in compounds **1a** optimally fit the pharmacophoric features of the 3-D model, validating our previous proposal. The new ligands have been characterized as antagonists in COS7 cells transfected with the human 5-HT₆R, representing a novel structural class of antagonists of this receptor.

In order to get a better insight in the molecular details of ligand recognition by the 5-HT₆R, site-directed muta-

genesis assays were performed using different agonists and antagonists, including **UCM-258** identified in this work. A computer-developed mode of interaction between these ligands and a β_2 -based model of the 5-HT₆R predicted Cys^{3.36}, Ser^{5.43}, Trp^{6.48}, Phe^{6.52}, and Asn^{6.55} as the ligand binding sites, which were independently mutated to Ala in the human 5-HT₆R. The functional activity of the ligands was determined in the five mutants constructed, and their values were compared to the wild type.

This experimental and computational study has permitted to understand how ligand binding is ultimately translated into receptor activation or inactivation, which is a key subject in medicinal chemistry. Thus, we will report valuable information that should help in the optimization of 5-HT₆R ligands, which may contribute to the rational design of new compounds with predetermined pharmacological properties at the recently identified serotonin 5-HT₆R.



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References

- [1] Holenz, J.; Pauwels, P. J.; Diaz, J. L.; Mercè, R.; Codony, X.; Buschmann, H. *Drug Discov. Today*, 2006, 11, 283-299.
- [2] López-Rodríguez, M. L.; Benhamú, B.; de la Fuente, T.; Sanz, A.; Pardo, L.; Campillo, M., *J. Med. Chem.*, 2005, 48(13), 4216-4219.

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BIOTIN TAGGED BETA-CYCLODEXTRIN: A POTENTIAL NEW DRUG TARGETING SYSTEM FOR TUMOR THERAPY

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Antitumor drugs are characterized by several side-effects due to a wide distribution in healthy tissues and organs. Moreover, several drugs present poor solubility,

low stability in biological fluids and low absorption level. Thus, the specific targeting of therapeutic agents to a given organ, tissue, or cell as well as the increase of their solubility, stability and absorption represents one of the main challenges in drug development.^{1,2} In the particular case of the cancer treatment, we have approached the problem using tagged cyclodextrin as drug carrier system.

Cyclodextrins (CDs) are water soluble cyclic oligosaccharides composed of six to eight D-glucose units (α -, β -, γ - CD, respectively). These units are arranged in a truncated cone with hydrophilic outside and a hydrophobic cavity. Due to these structural features, CDs are able to form host-guest inclusion complexes with hydrophobic molecules possessing the appropriate size and shape. CDs were widely used in pharmaceutical formulations generally to improve drug properties such as solubility, stability and absorption.³ In order to drive the host-guest complex, we have modified the primary crown with a specific recognition pattern, biotin. This conjugate can recognize streptavidin coupled to a monoclonal antibody targeting cancer cells.

Herein, we described the monofunctionalization of β -CD and its coupling to biotin.⁴ The evaluation of the biological receptor recognition properties of the new conjugate was determined as well as its inclusion ability.

Such system can find applications as tumor-targeted drug delivery system via recognition of streptavidin-antibody and via host-guest complexes with anticancer drug such as paclitaxel, doxorubicin.⁵

- [1] Petrak, K. *Drug Discovery Today* 2005, 10, 1667-1673
- [2] Houshmand, P.; Zlotnik *Current Opinion in Cell Biology* 2003, 15, 640-644
- [3] Loftsson, T.; Duchêne, D. *International Journal of Pharmaceutics*, 2007, 329, 1-11
- [4] Byun, H.-S.; Zhong, N.; Bittman, R. *Organic Syntheses* 2000, 77, 225-230
- [5] Al-Omar, A.; Abdou, S.; De Robertis, L.; Marsura, A.; Finance, C. *Bioorg. Med. Chem. Lett.* 1999, 19, 1115

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ADENOSINE RECEPTOR BINDING AND 3D-QSAR-OF 4-SUBSTITUTED 2-(2'-FURYL)-1,2,4-TRIAZOLO-[1,5-A]QUINOXALINES

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Interaction of adenosine with a set of specific receptors, classified as A₁, A_{2A}, A_{2B} and A₃, may regulate different physiological functions.¹ For this reason, adenosine receptors (AR) are currently considered as attractive targets in the treatment of diverse pathophysiological disorders,² particularly, AR antagonists are sought as renal protective, anti-Parkinson, antiasthmatic, antidiabetic, or antiischemic agents. In the present report we will discuss the synthesis of a collection of 2-(2'-furyl)-1,2,4-triazolo[1,5-a]quinoxalines (figure 1) incorporating different substitution pattern at position 4, their binding affinity toward human adenosine receptors (hA₁, hA_{2A}, hA_{2B} and hA₃) as well as a 3D-QSAR modelling study (GRIND methodology) performed on the hA₁ receptor.³

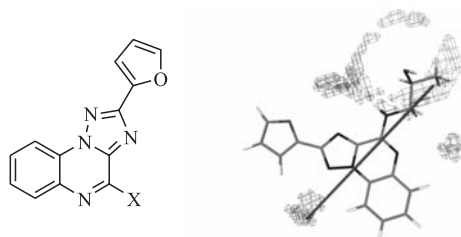


Figure 1. General structure of derivatives studied and representation of compound **9f** [X = NH-CH₂-CH(OH)CH₃] with the corresponding molecular interaction fields used to generate the 3D-QSAR models with the GRIND/ALMOND methodology.

References

- [1] Von Lubitz, D. K. J. E., Jacobson, K. A., Behavioral Effects of Adenosine Receptors Stimulation. In *Adenosine and Adenine Nucleotides: From Molecular Biology to Integrative Physiology*, Bellardinelli, L., Pelleg, A., Eds.; Kluwer: Norwell, 1995, pp.489-498.
- [2] Jacobson, K. A., Gao, Z-G., *Nature Reviews Drug Discovery*, 2006, 247. Baraldi, P.G., Tabrizi, M. A., Gessi, S., Borea, P. A., *Chem. Rev.*, 2008, 108, 238.
- [3] Martínez, A., Gutiérrez de Terán, H., Brea, J.M., Raviña, E., Loza, M.I., Cadavid, M.I., Sanz, F., Vidal, B., Segarra, V., Sotelo, E., *Bioorg. Med. Chem.* 2008 16, 2103

P476

ARYLSULFONAMIDE CB2 RECEPTOR AGONISTS: ANTI-INFLAMMATORY ACTIVITY AND SAR STUDIES DIRECTED TOWARDS THE OPTIMIZATION OF METABOLIC STABILITY

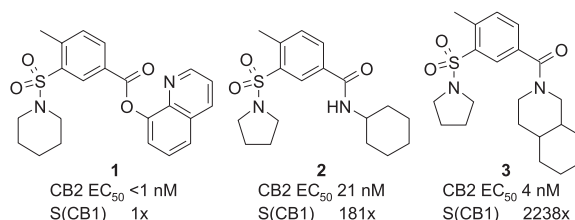
Monika Ermann¹, Doris Riether², Edward Walker¹, Innocent Mushi¹, James E. Jenkins¹, Beatriz Noya-Marino¹, Stephen East¹, Malcolm Taylor¹, Patricia Amouzegh¹, Mark Gemkow³, Sabine Löbbe³, Andreas Ebne³, Diane Thome², Kathy O'Shea², Roger Dinallo², Ernest Raymond², Daw-tsun Shih², and David Thomson²

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Literature evidence suggests that selective cannabinoid receptor 2 (CB2) agonists are potentially valuable therapeutics for the treatment of inflammatory diseases and chronic pain. We have identified a novel series of CB2 selective agonists¹ and our goal was the evaluation of the pharmacokinetic and pharmacological properties of these compounds and to direct SAR studies towards the optimization of oral bioavailability.

Commercially available arylsulfonamide **1** was identified in a high-throughput screen as a CB1/CB2 dual agonist. CB2 and CB1 agonistic activity is determined by measurement of the inhibition of cAMP production in forskolin stimulated CB2 and CB1 transfected CHO cells, respectively. Hit-compound **1** served as a starting point for an extensive SAR study with the primary objective of introducing selectivity for CB2 over CB1. Within the scope of this exploration, we identified numerous amides that possess the combination of potent CB2 activity (cAMP EC₅₀ <10 nM) and high selectivity (> 1,000 fold) versus the CB1 receptor.¹ Compounds, e.g. **3** were found to exhibit selectivity over CB1(S(CB1)) of greater than 2000-fold.

The *in vitro* ADMET properties of compounds **2** and **3** were assessed and metabolic stability and low plasma concentrations were identified as key issues responsible for poor oral bioavailability. Using metabolite identification studies, metabolically labile site were identified and various approaches to introduce metabolic stability will be discussed. Furthermore *in vivo* data for representative arylsulfonamides **2** and **3** will be presented, which demonstrated anti-inflammatory efficacy in a mouse zymosan induced paw inflammation (ZIPI) model after i.p. dosing.



- [1] Ermann, M.; Riether, D.; Walker, E.R.; Mushi, I.F.; Jenkins, J.E.; Noya-Marino, B.; Brewer, M.L.; Taylor, M.G.; Amouzegh, P.; East, S.P.; Dymock, B.W.; Gemkow, M.J.; Kahrs, A.F.; Ebne, A.; Löbbe, S.; O'Shea, K.; Shih, D.-T.; Thomson, D.; *Bioorg. Med. Chem. Lett.* 2008, 18 (5), 1725-1729

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FLUORINATED HISTAMINE hH₃ RECEPTOR ANTAGONISTS AS POTENTIAL PET LIGANDS

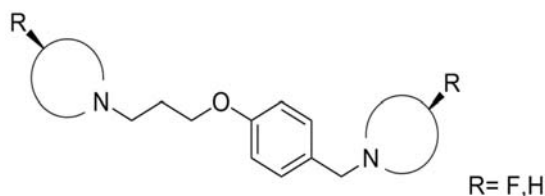
Isensee, K.¹; Ligneau, X.²; Camelin J.-C.²; Capet, M.²; Schwartz, J.-C.²; Stark, H.¹

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Histamine hH_3 receptor ligands exhibit an attractive pharmaceutical potential in the treatment of different CNS disorders like schizophrenia, epilepsy, narcolepsy or Alzheimer's disease because of the modulation of several central functions, e.g. vigilance, attention or learning. The histamine hH_3 receptor does not only control synthesis and release of histamine as an autoreceptor, but also modulates several other neurotransmitters in his function as heteroreceptor on non-histaminergic neurons (e.g. acetylcholine, dopamine, glutamate, noradrenalin, serotonin, γ -aminobutyric acid, some peptides and co-transmitters)^[1].

Here, radioactive labelled histamine hH_3 receptor antagonists/inverse agonists may be used to get information on localization of the receptor, but also on the mechanism and the behaviour of ligand binding, the occupancy and the interactions. So, these pharmacological tools offer a possibility for a faster and more efficient drug development in pre-clinical and clinical studies. The radioactive labelling by ^{18}F for PET (positron emission tomography) studies has been shown to be useable radiotracer with a half life of 110 minutes.



Based on the two lead structures FUB 637^[2] and FUB 880^[3] different fluorinated histamine hH_3 receptor antagonists were designed. The compounds vary in the basic centers (piperidine or pyrrolidine) and are fluorinated in different positions of the basic ring. For a facilitated [^{18}F]-labelling different leaving groups have been introduced on the most affine compound, ST-889. Data on histamine H_3 affinities were obtained by [^{125}I]iodoproxyfan displacement on CHO-K1 cells stably expressing the hH_3 receptor^[4]. Most newly designed ligands show high affinity in subnanomolar to nanomolar concentration range as well as antagonist/inverse agonist functionality and seem to be promising PET ligands.

References

- [1] S. Celanire, F. Lebon, and H. Stark. *Drug Discovery: From Hits to Clinical Candidates. The Third Histamine Receptor: Selective Ligands as Potential Therapeutic Agents in CNS Disorders* (ed. D. S. Vohora), Taylor & Francis CRC Press Inc. 2008, Boca Raton, Fla., in press. (ISBN: 9781420053920 - 1420053922).
- [2] G. Meier et al., *Eur. J. Pharm. Sci.*, 2001, 13, 249-259.
- [3] T. Miko et al., *J. Med. Chem.*, 2003, 46, 1523-1530.
- [4] X. Ligneau et al., *Br. J. Pharmacol.*, 2000, 131, 1247-50.

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PHARMACOLOGICAL ACTIVITY OF HUMAN HISTAMINE H_3 RECEPTOR LIGANDS AND COMPARISON WITH HUMAN HISTAMINE H_4 RECEPTOR POTENCIES

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Histamine has been found to exert tremendous influence on a variety of physiological processes. So far, there are known four histamine receptor subtypes: H_1 , H_2 , H_3 and H_4 . The H_1 and H_2 receptor antagonists are in clinical use, in treating disorders e.g. allergic disorders and peptic ulcers, respectively. The histamine H_3 receptor (H_3R) has been known since 1983. At the beginning, histamine H_3R antagonists were imidazole-containing compounds. Now, the class without the imidazole ring, is rapidly developing having large structural variations. Piperidine and piperazine moieties, among others proposed, have been shown to be a suitable bioisosteric replacement of the imidazole ring. Some of the non-imidazole H_3R antagonists have reached clinical studies and results from these trials are eagerly awaited.¹

The histamine H_4 receptor (H_4R) has only recently (2000) been discovered independently by several research group. The human histamine H_4R is closely related to the human H_3R . The two proteins have a sequence identity of 31%. Their homology in the transmembrane region is 58% and both have a long third intracellular loop. It is not surprising, that most imidazole-containing H_3R ligands display also considerable affinity for the H_4R like thioperamide with K_i (nM): 43 ± 3 for hH_4R and 60 ± 12 for hH_3R .

The first potent and selective H_4R antagonist is a non-imidazole derivative JNJ 7777120 which is used for the characterization of the recombinant and native H_4R .²

Our research group is involved in the searching for histamine H_3R antagonists. So far, we have prepared and tested both imidazole and non-imidazole compounds.³ Some of them were defined as highly selective (considering histamine H_1 and H_2 receptors) and potent antagonists tested at rat or guinea-pig H_3Rs . As a continuation of these previous works, we have chosen various H_3R ligands (mostly imidazole derivatives) and tested at the recombinant human H_3R by measuring the displacement curves of [^{125}I]iodoproxyfan binding at human H_3R

expressed in CHO-K1 cells. The evaluated human H₃R affinities were comparable or lower from those tested earlier. For comparison, affinity at the recombinant human H₄R was also determined. The assays were performed using proteins from SF9-cell and ³[H]-histamine x 2HCl as radioligand.

Most of the tested compounds showed preference on the human histamine H₃R.

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References

- [1] Celanire, S.; Lebon, F.; Stark, H. In *The third histamine H₃ receptor: selective ligands as potential therapeutic agents in CNS disorders*. (ed. D.S. Vohora) Taylor & Francis CRC Press Inc, 2008 – in press.
- [2] Jablonowski, J.A.; Grice, C.A.; Chai, W.; Dvorak, C.A.; et al *J. Med. Chem.* 2003, 46, 3957.
- [3] Lazewska, D.; Ligneau, X.; Schwartz, J.-C.; Schunack, W.; Stark, H.; Kiec-Kononowicz, K. *Bioorg. Med. Chem.* 2006, 14, 3522.

P480

FROM FRAGMENTS TO LEADS FOR NEW ALZHEIMER MEDICINES – STRUCTURE-BASED DESIGN AND SYNTHESIS OF NOVEL BETA-SECRETASE INHIBITORS

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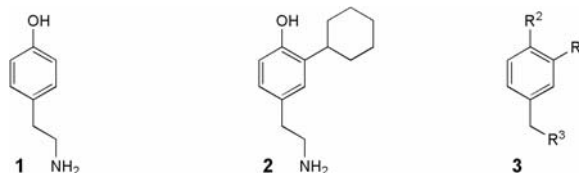
Alzheimer's disease (AD) is a severe neurodegenerative disorder and the most common cause of dementia in elderly people. To this day there is no cure for AD. Existing treatments offer – if at all – only small therapeutic benefits, therefore there is an urgent need for new medicines.

According to the amyloid hypothesis of AD, accumulation of amyloid β-peptide (Aβ) is the primary influence driving AD pathogenesis.¹ Aβ is formed by the sequential processing of the amyloid precursor protein (APP) by β-secretase (BACE-1) and γ-secretase.² Being the key enzyme that initiates the formation of Aβ, BACE-1 represents an attractive drug target.³

A fragment screening performed by F. Hoffmann-La Roche revealed that tyramine (**1**) binds to the active site of BACE-1 with a high ligand efficiency (LE = 0.37, K_D = 2000 μM). Further tyramine derivatives with improved binding affinities have been discovered using surface plasmon resonance (SPR) measurements. X-ray structures of BACE-1 complexed with these compounds showed that the amine moiety of the tyramine fragments forms hydrogen bonds to the catalytic dyad and the residues located in *ortho*-position of the phenol moiety occupy the S3 pocket.⁴

The fragment with the highest binding affinity of which an X-ray structure was available (**2**, LE = 0.31, K_D = 220 μM, PDB code: 3BUH) served as our starting point for the structure-based design of novel BACE-1 inhibitors.⁴ In the first part of the project, a series of compounds (**3**) with different residues addressing the S1/S3 pocket have been designed, synthesized and tested in an SPR assay to study the SAR. In addition, an X-ray structure of one of the compounds complexed with BACE-1 has been solved.

In the second part of the project, different binding motifs for the catalytic dyad of BACE-1 have been investigated and a promising novel binding motif has been discovered.



- [1] J. Hardy, D. J. Selkoe, *Science* 2002, 297, 353-356.
- [2] M. P. Mattson, *Nature* 2004, 430, 631-639.
- [3] R. Vassar, *Adv. Drug Delivery Rev.* 2002, 54, 1589-1602.
- [4] A. Kuglstatter, M. Stahl, J.-U. Peters, W. Huber, M. Stihle, D. Schlatter, J. Benz, A. Ruf, D. Roth, T. Enderle, M. Hennig, *Bioorg. Med. Chem. Lett.* 2008, 18, 1304-1307.

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A NEW SYNTHESIS OF BETA AMINO ACIDS VIA THE MILLER REARRANGEMENT

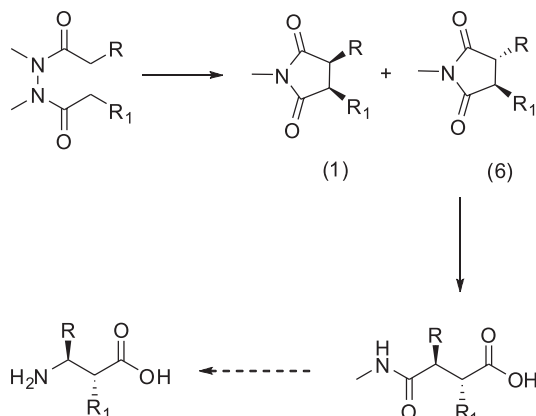
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Over the past decade, considerable interest has been shown in developing chains of β-amino acids for use as potential therapeutic agents, protein mimics, and ultimately for the design of macromolecules with novel biological function. This interest has been heightened by the discovery that short chains of β-amino acids (β-peptides, foldamers) are able to assume highly stable conformations analogous to the secondary structure adopted by proteins. The resistance of β-peptides to protease degradation within the human body has added to their attraction. As β-amino acids are nearly structurally identical to their alpha counterparts, inclusion into protease resistant peptidomimetics is an attractive option as shown in prior group work.¹ Not surprisingly, the enantioselective synthesis of β-amino acids has received considerable attention over the last few years. Our overall strategy for preparing such structures is achieved via three major steps: a Miller rearrangement², hydrolysis then Curtius rearrangement. The Miller rearrangement involves a

diastereoselective intramolecular enolsilane coupling, which converts both symmetrical and unsymmetrical hydrazides to succinimide heterocycles. This key step efficiently establishes the initial stereochemistry of the molecule. Hydrolysis to the amide acid, followed by a Curtius rearrangement, amide hydrolysis and enantiomeric resolution leaves us with ready access to a variety of highly functionalized, complex β -amino acids. These can then be strategically placed into specific peptide sequences for utility as specificity and stability enhancers in novel peptidomimetics.



[1] Aguiar M.I., Perlmutter, P., et. al. *Organic and Biomolecular Chemistry*. 2007 (5) 2884.

[2] Miller, S., Bayne, C. *Journal of Organic Chemistry*, 1997 (62) 5680.

P482

CARBOXYLIC R- α -PHENYLALANINE DERIVATIVES AS A POTENT LIGANDS OF GLUTAMATE RECEPTORS

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Glutamate is the principal excitatory neurotransmitter in the central nervous system acting on glutamate receptors in synapses. Glutamate receptors have been divided into two families: ionotropic receptors opening cation-permeable channels, and G-protein-coupled metabotropic glutamate (mGlu) receptors. Ionotropic receptors have been categorised according to their selective ligands: NMDA (*N*-methyl D-aspartate), AMPA (α -amino-3-hydroxy-5-methyl-4-izoxazolepropionate) and KA (kainate) receptors. Eight mGlu receptors subtypes have been subdivided into three groups according to their sequence homology. These receptors play important role in regulating the activity of many synapses and are involved in many pathological processes including neurodegeneration, ischaemic brain damage and epilepsy¹. In the past few years intensive studies have led to the identification of ionotropic glutamate receptors AMPA and

group III mGlu receptors as a potent targets for drug therapy in epilepsy².

The large number of compounds with specific agonists or antagonists actions at glutamate receptors consists of amino acids. Chirality of amino acids plays a significant role in acting at either ionotropic or metabotropic glutamate receptors. The R-isomer of amino acid - 3,4-dicarboxyphenylglycine (3,4-DCPG) has recently been shown as a competitive ionotropic glutamate receptor (AMPA) antagonist, whilst S-isomer is selective mGlu8 receptor agonist².

In our work several carboxylic R-phenylalanine derivatives were designed and obtained. The only one described glutamate receptor active, phenylalanine derivative, (S)- α -methyl-3-carboxyphenylalanine, mGlu-8 antagonist, was treated as leading structure³.

In our research two-steps enzymatic hydantoinase method was applied for the biosynthesis of enantiomers of chiral amino acids (Fig.1). The first step involved a stereoselective hydrolysis of racemic (un)substituted with acidic groups in benzene ring 5-benzylhydantoin derivatives to optically pure *N*-carbamoyl-R-amino acids. Hydrolysis was performed by immobilized, cloned and expressed in *Escherichia coli* hydantoinase. In the second step *N*-carbamoyl-R-amino acids were converted into the corresponding R-amino acids by diazotization or by cloned and expressed in *Escherichia coli* *N*-carbamoyl-R-amino acid amidohydrolase (carbamoylase)⁴. Substrates for enzymatic hydantoinase method - racemic phenyl ring substituted 5-benzylhydantoin were synthesized using at the first step Knoevenagel condensation and at the next step the reduction of double bond at 5-position of hydantoin.

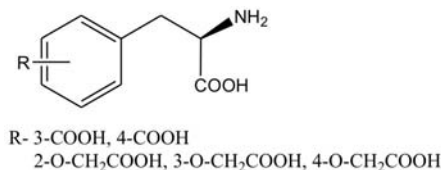


Fig.1

Acknowledgement

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References

- [1] Pin, J-P., De Colle, C., Bessis, A.-S., Acher, F., *Eur. J. Pharmacol.* 1999, 375, 277-294.
- [2] Lee, J.J., Jane, D.E., Croucher, M.J., *Brain Res.*, 2003, 977, 119-123
- [3] Moldrich, R. X., Chapman, A. G., De Sarro, G., Meldrum, B.S., *Eur J Pharmacol*, 2003, 476, 3-16
- [4] Chao Y.-P., Fu H., Lo T.-E., Chen P.-T., Wang J.-J., *Biotechnol Prog*, 1999, 15, 1039-1045

P483

AN EXPERT SYSTEM APPROACH TO THE PREDICTION OF MAMMALIAN XENOBIOTIC METABOLISM

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In the domain of mammalian xenobiotic metabolism there are many approaches to the in silico prediction of biotransformation. Such approaches include two- and three-dimensional quantitative structure-activity relationships (QSARs), quantum-mechanical calculations, molecular modeling of enzymes and ligand docking, and the application of expert systems. One of the advantages of an expert system approach includes the supporting evidence, collated by human experts, which accompanies each prediction. This evidence can play an important role in engendering confidence in, and promoting understanding of, predictions made by the system. The types of supporting evidence which can be incorporated include comments detailing the scope and applicability of biotransformations, primary literature citations, example compounds, enzyme information, and the presentation of mechanistic pathways and their associated intermediate metabolites. Characteristics of metabolites such as their molecular mass may also be automatically calculated and compared with available experimental data. As a result, an expert system approach to the prediction of mammalian xenobiotic metabolism offers the possibility to extend beyond simply predicting the structure of potential metabolites.

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EVALUATION OF STEM BARK EXTRACTS FROM MEDICINAL PLANTS OF CAMEROON: ANTIBACTERIAL AND ANTIOXIDANT ACTIVITIES AND PROANTOCYANIDINS COMPOSITION

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Three medicinal plants, *Bridelia grandis* (Pierre ex Hutch) (Euphorbiaceae), *Piptadeniastrum africanum* (Hook. F.) Brenan (Fabaceae) and *Celtis tessmannii* Rendle, (Ulmaceae), traditionally used in Cameroon by pygmies Baka for oral cavity affection, diarrhoea and stomach pain respectively⁽¹⁾, were analyzed for their antibacterial and antioxidant activity.

Stem bark extracts were screened against pathogenic bacteria; the Folin-Ciocalteu method⁽²⁾, the DPPH and nButanol/HCl assays^(3,4) were used to determine the total phenolic content, the antioxidant activity and the proantocyanidins composition.

In order to validate the traditional use of each plant extracts, the antimicrobial activity, evaluated as the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC), was tested against oral Streptococci strains for *Bridelia grandis*, *Staphylococcus aureus*, *Escherichia coli* and *Salmonella choleraesuis* for *Piptadeniastrum africanum* and *Celtis tessmannii*.

Bridelia grandis and *Piptadeniastrum africanum* extracts showed some activity against the tested microorganisms while *Celtis tessmannii* resulted completely inactive. Polyphenols were found either in *Bridelia grandis* and *Piptadeniastrum africanum* but *Bridelia grandis* had the highest antioxidant activity. The same samples were also characterised using the nButanol/HCl assay which highlighted the presence of proantocyanidins.

Results suggest that these plants are not only interesting sources for antimicrobial activity but also potential sources of natural antioxidant compounds.

References

- [1] Brisson, R., 1999, Etudes pygmées, SELAF n 376, Ed Peeters, Paris.
- [2] Singelton, V.L. et al., 1999, Methods Enzymol. 299 :152-177
- [3] Bondet, V. et al., 1997, Lebensm. Wiss. U. Technol., 30, 609-615
- [4] Nitao, J.K. et al., 2001, J. Agric. Food Chem., 49, 2207-2214

P485

VIRTUAL SCREENING BY QUALITY-DRIVEN BIOACTIVE CONFORMATIONS AND PHARMACOPHORE-BIASED CREATIVE PHARMACOPHORE SEARCH. PART II_III - SCALING-UP AND VIRTUAL SCREENING APPLICATIONS.

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The problem of generation of bioactive conformations have been attracting continuous interest [1, 2]. There are controversial opinions on which approach gives better results: diversity-oriented, rule-based or low-conformation-directed [1, 2]

In our previous study[3] we introduced the concept of creative pharmacophore search and quality-driven conformations generated by ChemoSoft 3D Tools which form a part of ChemoSoft™ software[4]. Based on the compounds investigated in [1] (32 in total) we demonstrated that for this dataset:

1. Low-energy conformation approach implemented in ChemoSoft's 2D-3D Converter™ gives a considerably better first approximation of actual bioactive conformations as compared to rule-based 2D-3D molecule conversion tools Corina™ and Concord™.
2. The creative pharmacophore search methodology implemented in ChemoSoft's 3D Pharmacophore

Search Engine™ does adjust generated conformations to bioactive conformations from which corresponding pharmacophore queries are created.

3. The conformations generated with the use of the creative pharmacophore search targeted approach are of from considerably to dramatically better quality than those obtained by the other techniques (Catalyst™, Confort™, and Omega™) including conformational diversity-oriented approach (Catalyst™).

In our present work we scale up our tests to the part of compounds studied in [2] that are publicly available (100 in total). We are also presenting results of virtual screening of publicly available databases (particularly, ZINC, PubChem, and NCI).

Summary

Novel ChemoSoft™ software tools addressing the problem of generation of bioactive conformations have been created. The ability of quality-driven conformations and creative pharmacophore search to compete with previously published results have been evaluated.

[1] Bostrom J. // *J. Comp.-Aided. Mol. Design*, (2001), V.15, P.1137-1152

[2] Perola E., Charifson P. S. // *J. Med. Chem.* (2004), V.47, P.2499-2510

[3] Nasonov, A. F., Pletnev I. V. Tackling the Problem of Hit Rate Enrichment by Virtual Screening: Quality-Driven Bioactive Conformations and Pharmacophore-Biased Creative Search // Poster presentation at Virtual Discovery, London, October 23-26 2007

[4]. www.chemosoft.com

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DYNAMIC LIGATION SCREENING: A METHOD FOR THE SITE-DIRECTED DISCOVERY OF INHIBITORY FRAGMENTS

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Specific protein ligands are crucial for the modulation of protein activities in medicinal chemistry and chemical biology. We present a concept for the discovery and development of small molecule fragments binding to defined protein sites: Dynamic Ligation Screening enables the rapid and site-directed identification of low-affinity binders in protein activity and fluorescence polarization experiment by exploiting template-assisted fragment assembly.^[1]

Dynamic Ligation Screening was conducted with libraries of 200-6000 fragments consuming only minor

amounts of enzyme: A fluorogenic protease substrate competed with an equilibrium of nucleophilic fragments and a designed peptide electrophile for the active-site.^[2,3] Decreased initial rates of product formation in the fluorophore-based enzyme assay indicated the inhibitory activity of the reversibly formed ligation product. One selected hit was modified synthetically in order to verify the binding site. Via an iterative scanning of different binding sites on the protein surface, moderately active peptidic inhibitors could be transformed into entirely non-peptidic inhibitors with a low μM K_i .

The concept was established for the development of a non-peptidic SARS coronavirus main protease (SARS-CoV M^{pro}) inhibitor. This enzyme has been identified as a drug target of SARS being essential for replication of the virus inside the infected host cell. Thermodynamics of protein-assisted fragment ligations were studied for caspase 3, the cellular switch for apoptosis, the programmed cell death. As a result a model for the additivity of binding contributions of reversibly ligated fragments is proposed. The method can be further extended to other protein targets such as phosphatases^[4] and to protein-protein interactions.

References

[1] M.F. Schmidt, A. Isidro-Llobet, M. Lisurek, R. Hilgenfeld, J. Rademann, *Angew. Chem. Int. Ed.* 2008, 47, 3275-3278.

[2] A. El-Dahshan, S. Weik, J. Rademann *Org. Lett.* 2007, 9, 949-952.

[3] Al-Gharabli, S.I., Ali Shah, S.T., Weik, S., Schmidt, M.F., Mesters, J.R., Kuhn, D., Klebe, G., Hilgenfeld, R., Rademann, J., *ChemBioChem* 2006, 7, 1048-1055.

[4] K. Hellmuth, S. Grosskopf, C. T. Lum, M. Würtele, N. Röder, J.P. von Kries, M. Rosario, J. Rademann, W. Birchmeier, *Proc. Nat. Acad. Sci. USA* 2008, in print.

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EXPERIMENTAL DATA GUIDED LIGAND DOCKING – A TOOL FOR ELUCIDATING THE BINDING MODE OF BENZODIAZEPINES

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Ligand/protein docking uses energetic scoring functions for evaluating and ranking poses. However, if the precise induced fit structure is not known, docking results

are often unsatisfying and do not lead to plausible binding hypotheses. Main strategies to overcome these problems currently focus on improving scoring functions, applying induced fit docking and speeding up binding free energy calculations. In addition, lack of structural data can, in principle, be compensated by biological data. In this study we wish to report a novel approach utilising a statistical evaluation of docking poses with respect to their ability to fit experimental data derived from both mutagenesis studies and labelling with chemically modified ligands.

The FlexE docking tool was applied to dock five benzodiazepines into an array of 14 homology models of the benzodiazepine binding site of the $\alpha_1\beta_2\gamma_2$ GABA_A-receptor. Docking into an array of models with FlexE takes into consideration both receptor uncertainty and side chain flexibility. Subsequently, we screened for ligand orientations which support the maximum common binding mode hypothesis [1] and which are in accordance with biological data. In order to enable comparison of ligand poses derived from different homology models, substructure Protein Ligand Interaction Fingerprint (PLIF) was applied. This derivation of the MOE built-in tool PLIF [2] enables the predefinition of substructures and determines the interaction pattern of these substructures. Thus we used the results from substructure PLIF in order to cluster the generated poses. Those clusters which harbour all five benzodiazepines are evaluated in the light of consistency with labelling data of NCS-analogues of ligands at Cysteine mutants of the binding site [3]. This procedure enabled us to identify one single homogenous cluster which harbours five different benzodiazepines.

Acknowledgement

Lars Richter is grateful to the Austrian Academy of Sciences for financial support under the framework of the DOC programme (grant 22473)

References

- [1] S. Renner, S. Derksen, S. Radestock, F. Mörchen, (2008): Maximum Common Binding Modes (MCBM): Consensus Docking Scoring Using Multiple Ligand Information and Interaction Fingerprints. *J. Chem. Inf. Model.*, 48(2): 319-332
- [2] MOE.2007.09 <http://www.chemcomp.com>
- [3] D. Berezhnoy, Y. Nyfeler, A. Gonthier, H. Schwob, M. Goeldner, E. Sigel, (2004): On the benzodiazepine binding pocket in GABAA receptors. *J. Biol. Chem.*, 279(5): 3160-3168

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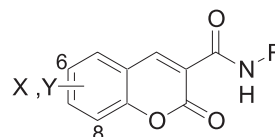
NOVEL 3-CARBOXAMIDE-COUMARINS AS POTENT AND SELECTIVE FXIIa INHIBITORS

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Recently, factor XIIa (FXIIa), a trypsin-like serine protease implicated in the intrinsic pathway of the coagulation cascade, was highlighted as an original attractive target for the development of new anticoagulant drugs for the treatment and prevention of thromboembolic disorders.¹⁻³ Indeed, FXII-deficient mice were found to be protected against arterial thrombosis, collagen- and epinephrine-induced thromboembolism⁴ and ischemic brain stroke⁵. In all these *in vivo* models, the protection was abolished by infusion of human FXII into FXII-null mice. Moreover, as their human counterparts, the FXII-null mice do not suffer from impaired haemostasis.⁶ Consequently, it is anticipated that the use of FXIIa inhibitors in clinic would be associated with relatively low rates of therapy-related haemorrhages, a well known adverse drug reaction of the majority of anticoagulant agents.

In this work, we successfully developed original 3-carboxamide-coumarins as potent and selective inhibitors of FXIIa. Among these newly designed coumarins, the best compounds were **1** and **2** with IC₅₀ values for inhibition of FXIIa of 4.3 µM and 4.4 µM, respectively. Moreover, they were selective towards structurally related coagulation factors (thrombin, FXa, TF/FVIIa and kallikrein). To our knowledge, these compounds are the first potent and selective non peptidic inhibitors of FXIIa described in the literature to date. Their uses as anticoagulant agents are currently evaluated. They also constitute very interesting pharmacological tools to investigate the role of FXIIa in thrombosis and haemostasis.



- 1:** X, Y = 6,8-dichloro, R = 3,5-dimethyl-phenyl
2: X, Y = 8-bromo-6-chloro, R = phenyl

References

- [1] Gailani, D.; Renne, T. Intrinsic pathway of coagulation and arterial thrombosis. *Arterioscler Thromb Vasc Biol* 2007, 27, 2507-2513.
- [2] Gailani, D.; Renne, T. The intrinsic pathway of coagulation: a target for treating thromboembolic disease? *J Thromb Haemost* 2007, 5, 1106-1112.
- [3] Renne, T.; Gailani, D. Role of Factor XII in hemostasis and thrombosis: clinical implications. *Expert Rev Cardiovasc Ther* 2007, 5, 733-741.
- [4] Renne, T. *et al.* Defective thrombus formation in mice lacking coagulation factor XII. *J Exp Med* 2005, 202, 271-281.
- [5] Kleinschnitz, C. *et al.* Targeting coagulation factor XII provides protection from pathological thrombosis in cerebral ischemia without interfering with hemostasis. *J Exp Med* 2006, 203, 513-518.
- [6] Pauer, H. *et al.* Targeted deletion of murine coagulation factor XII gene-a model for contact phase activation in vivo. *Thromb Haemost* 2004, 92, 503-508.

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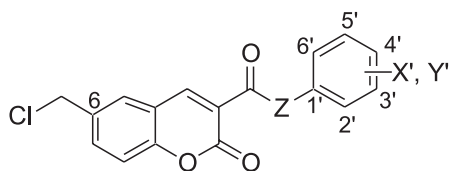
STRUCTURAL INSIGHTS INTO THE INHIBITION MODE OF THE ACTIVATED COAGULATION FACTOR XII BY COUMARINS

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Blood coagulation involves a series of plasmatic proteins, called **coagulation factors**, which are sequentially activated through a complex cascade to form a fibrin network. The majority of the coagulation factors are **trypsin-like serine proteases** that circulate in blood as inactive enzyme precursors (zymogens). At each step of the cascade, a coagulation factor is activated into a functional enzyme that will catalyze the specific hydrolysis of the next coagulation factor. In this way, the extrinsic and intrinsic coagulation activation pathways converge to produce **thrombin** that ultimately converts fibrinogen into fibrin.¹⁻²

Excessive and unregulated activation of coagulation cascade may lead to pathological thrombus formation that may occlude the vessel i.e. **thrombosis**. Most of the antithrombotic drugs are currently designed to target key coagulation factors such as thrombin or factor Xa. However, recently, **activated factor XII (FXIIa)**, also called *Hageman Factor*, was highlighted as an original attractive target for the treatment and prevention thrombosis disorders. It is anticipated that the use of selective FXIIa inhibitors in clinic would be associated with relatively low rates of therapy-related haemorrhages, a well known adverse drug reaction of the majority of anticoagulant agents.³⁻⁴ Moreover, designing specific FXIIa inhibitors as pharmacological tools would be also an interesting strategy to give new insights into the controversial roles of FXII in physiopathology.⁵⁻⁶



Z = O or NH

X', Y' = H, 3'-chloro or 2',5'-dichloro

In this work, we evaluated the inhibitory potency against FXIIa and thrombin of **original coumarins** designed and synthesized in our lab. In order to understand structure-activity relationships, a structure-based drug design approach was used. The crystal structures of some coumarins were determined in order to evaluate the restraints induced by the amide or the ester function. These data were then compared to structural information from the CSD databank and from a theoretical conformational scan. Moreover, **a homology model of the catalytic domain of human FXIIa**, not yet available in the PDB databank, was build and was used to docked

coumarins into the enzyme. Docking studies were also conducted into the active site of the crystallized thrombin. All together these data allowed us **to understand the inhibition mode of FXIIa by coumarins and to highlight its specificity in comparison with thrombin**.

References

- [1] Davie, E.W. *et al. Biochemistry* 1991, 30, 10363-10370.
- [2] Mann, K.G. *Thromb Haemostasis* 1999, 82, 165-174.
- [3] Gailani, D.; Renne, T. *Arterioscler Thromb Vasc Biol* 2007, 27, 2507-2513.
- [4] Renne, T.; Gailani, D. *Expert Rev Cardiovasc Ther* 2007, 5, 733-741.
- [5] Gailani, D.; Renne, T. *J Thromb Haemost* 2007, 5, 1106-1112.
- [6] Cooper, J.A. *et al. Circulation* 2000, 102, 2816-2822.

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LEAD STRUCTURE IDENTIFICATION BY VIRTUAL SCREENING AND SCAFFOLD MODIFICATION FOR NEW HISTAMINE H₄ RECEPTOR LIGANDS

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Histamine as an important neurotransmitter and chemical messenger is involved in the regulation of many physiological processes which are mediated *via* four different G-protein coupled receptors. The most recently discovered histamine H₄ receptor is expressed on various immune cells plays a key role in chemotaxis, upregulation of inflammatory cells and therefore, in inflammatory signalling. Due to this prominent behaviour it became a challenging target in medicinal chemistry: antagonists could be effective in the treatment of inflammatory diseases such like asthma, allergy and immunological disorders^[1].

Taking different heterocyclic structures published as potent and selective histamine H₄ receptor antagonists as starting point, ligand-based virtual screening^[2] on fragment libraries and commercially available compound databases led to the finding of a variety of scaffolds that all contain a methylpiperazine moiety linked to a (substituted and/or annelated) five-membered heterocycle, e.g. imidazole and triazole. In competition binding assays some hits showed affinities in the low micromolar range. Here, we report the synthetic steps and preparative modification of a triazolopyrimidine scaffold which resulted in

a novel lead structure for the development of histamine H₄ receptor ligands.

References

- [1] Zhang M et al. *The histamine H₄ receptor: a novel modulator of inflammatory and immune disorders*. Pharmacol. Ther. 2007, 113, 594-606.
- [2] Schneider G, Baringhaus K-H. *Molecular Design – Concepts and Applications*. Weinheim, New York 2008.

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PLASMODIUM DNA-DIRECTED ENDOPEROXIDES

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Malaria remains one of the most devastating endemic diseases in the world killing over a million people each year and infecting around 500 million, mostly those living in the world's poorest countries. The situation has worsened over recent years, as resistance against the more common drugs used in malaria chemotherapy such as chloroquine has been developed by *Plasmodium falciparum*, in most of the more than 100 endemic countries [1]. To fight back this fact the development of new strategies for malaria chemotherapy became a matter of utmost importance and urgency.

In this project we aim to develop synthetic routes to produce DNA-directed endoperoxides, combining a DNA binding moiety [2] with a known antimalarial function. This strategy is based on the ability of Fe (II) to selectively cleave the peroxide bridge of the artemisinin type of drugs, generating radicalar species capable of damaging parasitic biomolecules [3].

We propose to build different classes of endoperoxide hybrids (Fig. 1) and exploit their mechanism and site of action as well as their biological activity.

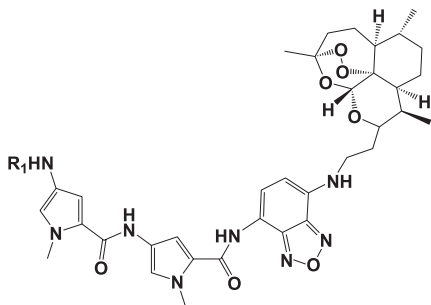


Fig. 1. Target hybrid-drug.

References

- [1] <http://www.who.int/mediacentre/factsheets>.
- [2] Tanada M., Tsujita S. and Sasaki S., *J. Org. Chem.* 2006, 71, 125-134; Woynarowski, J.M. et al., *Mol. & Bioch. Paras.*, 154, 2007, 70; Romagnoli, R. et al., *Europ. J. Med. Chem.*, 40, 2005, 1123.
- [3] Posner, G. and O'Neill, P. M., *J. Med. Chem.*, 2004, Vol. 47, No. 12, 2945-2964.

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We acknowledge FCT for the grant SFRH / BPD/ 20587 / 2004.

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DESIGN, SYNTHESIS, AND EVALUATION OF ALPHA-GALACTOSYL CERAMIDE (KRN7000) ANALOGUES FOR NKT CELL SELECTIVE ACTIVATION

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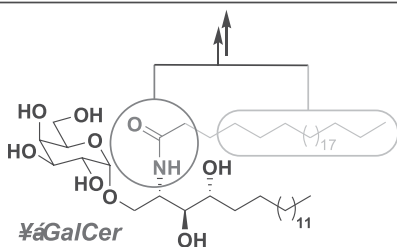
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Unlike other T cells, NKT cells recognize glycolipid antigens presented by CD1d (1). Upon recognition of the glycolipid/CD1d complex through the T cell receptor, NKT cells rapidly produce large amounts of Th1 and Th2 cytokines, such as IFN-gamma and IL-4, respectively (2). The released cytokines play critical roles in inducing a series of cellular activation events leading to the activation of innate and adaptive immune cells. In this cellular process, the Th1/Th2 balance is believed to be importantly related to the target of the immune response (3).

The first defined and most potent agonistic antigen of the TCR is alpha-GalCer (also known as KRN7000), which release Th1 and Th2 cytokines without preference. However, there are some limitations on the potential clinical usefulness of alpha-GalCer because of its low solubility and the reciprocal inhibition exhibited by Th1 and Th2 cytokines. Therefore, compounds which increase the solubility as well as the selectivity toward either Th1 or Th2 cytokines responses may be more advantageous.

To modulate the solubility and the avidity of alpha-GalCer, we designed and synthesized the analogues in which the lipid part and amide moiety was modified (4). Some of synthesized analogues have a comparable stimulatory effect on cytokine production as alpha-GalCer, and exhibit a stronger Th2 cytokine response. Furthermore, their solubility was much improved. To understand the altered cytokine profiles and the interaction between the synthesized analogues and CD1d, molecular modeling studies were also performed. Since IL-4 is a key cytokine for the control of autoimmune diseases such as type 1 diabetes and multiple sclerosis, our analogues might be more useful than alpha-GalCer for the treatment of these diseases.

Modification for NKT selective activation



References

- [1] Brigl, M.; Brenner, M. B. *Annu. Rev. Immunol.* 2004, 22, 817-890.
- [2] Kronenberg, M. *Annu. Rev. Immunol.* 2005, 23, 877-900.
- [3] Van Kaer, L. *Nat. Rev. Immunol.* 2005, 5, 31-42.
- [4] Lee, T.; Cho, M.; Ko, S.; Youn, H.; Baek, D. J.; Cho, W.; Kang, C.; Kim, S. J. *Med. Chem.* 2007, 50, 585-589.

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SPECTROPHOTOMETRICAL EVALUATION OF Cu, Zn-SUPEROXIDE DISMUTASE (SOD) AND GLUTATHIONE PEROXIDASE (GPx) ENZYMES IN PREGNANCY WITH PATHOLOGICAL CARIOTYPE

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The present work investigated the activity of Cu/Zn superoxide dismutase enzyme (SOD) and glutathione peroxidase (GPx) in red blood cells (hemolysate) by spectrophotometrical methods (RAN-SOD, RAN-SEL Randox). The purpose of this work was to study the level of main antioxidant enzyme SOD and GPx considering three facts: the gene for SOD synthesis is located on chromosome 21; the rate of trisomy 21 is in correlation with mother's age as well as the level of oxidative stress; and it has been discovered that increased levels of Cu, Zn-SOD occur in the extra-cellular body fluid, in particular in amniotic fluid with a trisomy 21 fetus. The study included hemolysate samples obtained from 14 patients (pregnant women) with pathological cariotype-trisomy 21, gestational age 12 to 22 weeks and their 15 matched controls. Patients were divided into three groups: complete trisomy 21 (group 1), translocations (group 2) and control group (normal cariotype). Our results showed that in the population with complete trisomy and translocations, Cu, Zn-SOD and GPx activities were increased comparing to

control group. These findings suggest impairment in antioxidant system and possible role of SOD in early biochemical diagnostic of trisomy 21.

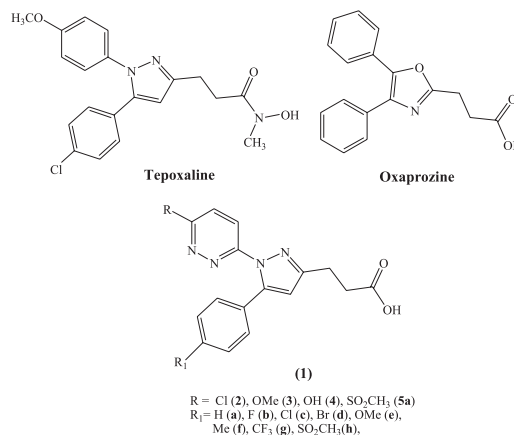
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SYNTHESIS, BIOLOGICAL ACTIVITY AND MOLECULAR DOCKING STUDIES OF CYCLOOXYGENASE/5-LIPOXYGENASE INHIBITOR 1,5-DIARYLPYRAZOLE-3-PROPANOIC ACID DERIVATIVES

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The therapeutic value of drugs able to block equally well the synthesis of both PGs and LTs was recently shown with their superior anti-inflammatory efficacy and with their almost complete lack of gastric toxicity. Therefore, the dual inhibition of cyclooxygenases (COX) and 5-lipoxygenase (5-LOX) emerged as a new strategy to provide effective and safer anti-inflammatory drugs. Taking into account this information, we aimed to design a new class of tepoxaline (dual COX/5-LOX inhibitor) and oxaprozine (nonselective COX inhibitor) analogs of general structure of **1** wherein we systematically modified the *p*-substituent on the 5-phenyl ring of three groups of compounds characterized by the presence of 6-chloropyridazine, 6-methoxypyridazine and pyridazin-3(2H)-one respectively, at 1-position of the pyrazole nucleus. Among the synthesized compounds, **2g** showed nonselective COX inhibition with a selectivity index of 0.93 (IC₅₀ COX1=1,5 µm, IC₅₀ COX2=1,6 µm), whereas compounds **2b** and **2f** resulted in the inhibition of 5-LOX at 14 µm and 12 µm IC₅₀ values, respectively.



To further investigate the behaviour of the newly synthesized compounds, molecular docking studies were also carried out and basically two binding modes to COX-2 active site are predicted for the whole set of compounds while one major binding mode is observed to 5-LOX active site which was similar for both 5-LOX inhibitor compounds. The results based on biological and docking data will be discussed herein, which might lead to design of better dual inhibitors.

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SYNTHESIS OF 6-(SUBSTITUE THIAZOLE-4-YL)-2-OXO-3H-BENZOXAZOLE DERIVATIVES AS POTENTIAL INHIBITORS OF COX-2 AND THROMBOXANE SYNTHASE

Seyma Cankara, Erden Banoglu, Serdar Ünlü

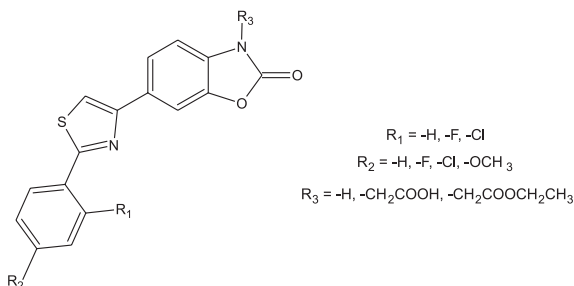
Gazi University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Ankara, Turkey.

Although COX-2 inhibitors are better tolerated than classical NSAIDs with improved gastric safety profile, their prolonged use causes cardiovascular side effects which limit their clinical use. Researchers suggested that COX-2 inhibitors may increase the risk of thrombotic effects by reducing the production of prostaglandin I₂ (PGI₂), while exerting a negligible effect on Thromboxane A₂ (TxA₂) level. PGI₂ formation is associated with vasodilatation and reduction in platelet aggregation. In contrast, TxA₂ promotes vasoconstriction and platelet aggregation. While traditional NSAIDs were known to inhibit both PGI₂ and TxA₂ formation, the more focused inhibition of COX-2 by selective inhibitors leaves TxA₂ unaffected tipping the balance toward a prothrombotic effect¹.

Based on these results, dual inhibition of COX-2 and thromboxane synthase emerged as a new strategy to provide effective and safer NSAIDs lacking the drug associated GI and cardiovascular side effects.

The thiazole ring system is a useful structural motif found in numerous biologically active molecules for the treatment of inflammation, allergies, hypertension, bacterial and HIV infections². Additionally, 1,3-diaryl heterocyclic compounds without sulfonyl group has been reported as selective COX-2 inhibitors³.

Thus, we have synthesized eighteen new compounds as possible dual inhibitors of COX-2 and Tx Synthase hoping that these molecules may be further explored as powerful anti-inflammatory and analgesic agents without cardiovascular side effects.



The results of inhibitory potency against COX-2 was evaluated in an enzymatic assay using human whole blood⁴. Further biological studies are under progress.

References

- [1] Gabay M, Slover C. COX-2 inhibitors-Cardiovascular and Gastrointestinal Safety. University of Illinois Medical Center at Chicago. Vol 5, no.5 2004
- [2] Narender M, Reddy MS, Sridhar R, Nageswar YVD and Rama Rao K. Aqueous phase synthesis of thiazoles and aminothiazoles in the presence of β -cyclodextrin. Tetrahedron Letters 46: 5953-5955, 2005.
- [3] Chakraborti AK, Thilagavathi R. Computer-Aided Design of Non Sulphonyl COX-2 Inhibitors: An Improved Comparative Molecular Field Analysis Incorporating Additional Descriptors and Comparative Molecular Similarity Indices Analysis of 1,3-Diarylisindole Derivatives. Bioorganic & Medicinal Chemistry 11: 3989-3996, 2003
- [4] Patrignani P et al. Biochemical and Pharmacological Characterization of the Cyclooxygenase Activity of Human Blood Prostaglandin Endoperoxide Synthases. The Journal of Pharmacology and Experimental Therapeutics 271: 1705-1712, 1994.

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SYNTHESIS OF DOXAZOSIN ANALOGUES AS α_1 -ADRENOCEPTOR ANTAGONISTS WITH PROSTATE ANTITUMOR ACTIVITY

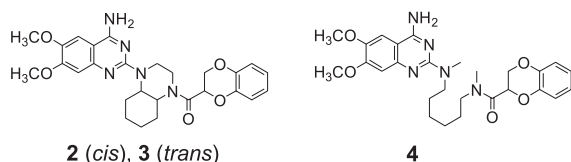
Dario Giardinà,^a Daniele Martarelli,^b Gianni Sagratini,^a Piero Angeli,^a Dario Ballinari,^d Ugo Gulini,^a Evelin Medori,^a Carlo Melchiorre,^c Pierluigi Pompei^b

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Doxazosin (**1**), bearing a quinazoline moiety and a 2,3-dihydro-1,4-benzodioxine-2-carbonyl group linked to the piperazine nitrogen atoms, is a potent and selective α_1 -adrenoceptor antagonist.¹ Notwithstanding its failure to discriminate among α_1 -adrenoceptor subtypes, it has been used in therapy as effective antihypertensive agent. Recently, **1**, because of its relaxant activity on prostate smooth muscle and ability to increase apoptosis in prostate cells, leading to a reduction of both urinary obstructive and irritative symptoms, has been used in elderly for the treatment of benign prostatic hyperplasia (BPH). More interestingly, recent *in vitro* studies have shown that **1** exerts a potent antigrowth effect against two highly aggressive androgen-independent human prostate cancer cell lines, PC-3 and DU-145, and the androgen-sensitive human prostate LNCaP cancer cells, via an apoptotic induced mechanism.^{2,3} This antitumor activity was confirmed by an *in vivo* study in mice, bearing a PC-

3 induced prostate cancer, where **1** significantly inhibited the tumor growth.² The above studies suggest an emerging therapeutic significance of the use of quinazoline-based compounds, not only for the BPH management, but also as potential antitumor agent for the treatment of prostate cancer.³

In our continuing research for quinazolinyl derivatives, we studied the doxazosin analogues **2-4** to evaluate: a) the selectivity towards α_1 -adrenoceptor subtypes, b) the *in vitro* antitumor activity against the PC-3, DU-145, and LNCaP cancer cells. Owing to the observed cytotoxic activity of **2**, it was also studied *in vivo* in mice, bearing a subcutaneous tumor implanted by human prostate PC-3 cancer cells. Interestingly, compound **2** displayed an anti-cancer effect 10 fold higher than doxazosin towards the PC-3 prostate tumor.



References

- [1] Campbell, S.F.; Davey, M.J.; Hardstone J.D.; Lewis, B.N.; Palmer, M.J. *J. Med. Chem.* 1987, **30**, 49-57.
- [2] Kyprianou, N.; Benning, C.M. *Cancer Res.* 2000, **60**, 4550-4555.
- [3] Benning, C.M.; Kyprianou, N. *Cancer Res.* 2002, **62**, 597-602.

P497

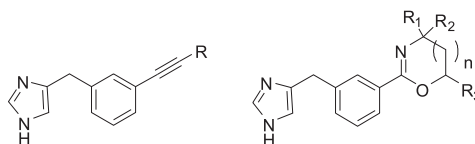
4-BENZYL-1H-IMIDAZOLES WITH OXAZOLINE- AND ALKYNE-TERMINI AS HISTAMINE H₃ RECEPTOR AGONISTS

M. Wijnmans, S. Celanire, E. Snip, E. Gelens, R. Bakker, H. van der Goot, S. Hulscher, M. Gillard, P. Collart, B. Christophe, B. Lallemand, F. Lebon, H. Timmerman, R. Leurs, P. Talaga, I. de Esch.

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Therapeutic research on the histamine H₃ receptor (H₃R) has focused mostly on antagonists/inverse agonists and to a lesser extent on agonists. However, H₃R agonists may be useful in treating ischemic arrhythmias, migraine, asthma, obesity, diabetes mellitus and liver cholestasis. Not only has the lower availability of agonists hampered full exploration of their therapeutic potential, it has also prevented an unequivocal understanding of the structural requirements for receptor activation. We present our findings in the development of rigid H₃R agonists based on the 4-benzyl-1H-imidazole template. Starting

from a high throughput screening hit, the benzyl sidechain was altered with lipophilic groups. Alkyne- or oxazolino-substituents gave excellent affinities and activities up to the single digit nM range. Our findings further substantiate the growing notion that basic ligand sidechains are not necessary for receptor activation and reveal the oxazolino group as a hitherto unexplored functional group in H₃R research.



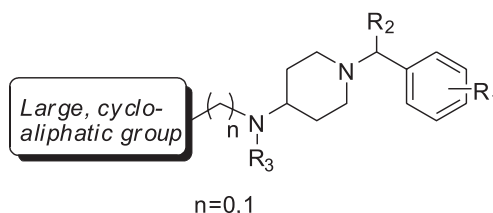
P498

NOVEL CXCR3 ANTAGONISTS: 4-AMINO-PIPERIDINES FLANKED BY LIPOPHILIC GROUPS

Maikel Wijnmans, Dennis Verzijl, Leontien Bosch, Rob Leurs, Martine J. Smit, Iwan J. P. de Esch

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Small-molecule antagonists for the CXCR3 chemokine receptor are attracting much interest as a possible means to therapeutically combat inflammatory diseases and certain types of cancer. In an attempt to identify novel types of antagonist chemotypes, we applied a strategic merger of an existing class of patented, relative large antagonists with an in-house discovered adamantane-based small-molecule hit. This led to a novel class of amino-piperidine based compounds that were readily accessible by only a handful of synthetic transformations (2-4 steps). The synthetic convenience allowed a rapid exploration of the Structure-Activity Relationship at many positions of the structure. In general, a substituted benzyl group on the NH-terminus and a highly lipophilic, cycloaliphatic group (such as a bornyl or adamantyl group) on the NH₂-terminus seem preferred, leading to affinities around 130 nM. The full outcome of the synthetic and pharmacological studies on this series will be discussed.



P500

STRUCTURE-BASED DESIGN OF NACHR LIGANDS USING ACHBP AS A TEMPLATE

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Neuronal nicotinic acetylcholine receptors (nAChRs) are potential therapeutic targets for the treatment of pain, epilepsy and a wide variety of neurodegenerative and psychiatric disorders e.g. Alzheimer's disease, Parkinson's disease, schizophrenia and depression. Several important physiological processes appear to be regulated by a single or a few nAChR subtypes.¹ Subtypes selective ligands are needed to aid target validation and enable the development of safe and selective drugs. However, due to high homology between the binding sites of the different subtypes, the development of selective ligands poses an interesting challenge.

Recently, high-quality X-ray structures of several acetylcholine-binding proteins (AChBPs) co-crystallized with ligands, have become available.^{2,3} These proteins are structural homologs of the ligand-binding domain of nAChRs.⁴ An hierarchical *in silico* screening procedure using our proprietary compound database and a AChBP X-ray structure, has resulted in the identification of novel nAChR ligands.

[1] Jensen et al. J. Med. Chem. 2005, 48, 4705-4745 2) Celie et al., Neuron 2004, 41, 907-14 3) Hansen et al. The EMBO journal 2005, 24, 3635-46 4) Sixma et al. Annu. Rev. Biophys. Biomol. Struct. 2003, 32, 311-34

P501

MACROMOLECULAR LIGANDS FOR G PROTEIN-COUPLED RECEPTORS: POLY(AMIDOAMINE) DENDRIMER CONJUGATES OF ADENOSINE RECEPTOR AGONISTS

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Activation of the A_{2A} adenosine receptor (AR), a G protein-coupled receptor (GPCR), by extracellular adenosine is antiaggregatory in platelets and antiinflammatory. An approach we have used in GPCR ligand design has been to apply structural modification at permissive distal

sites, which led to the concept of functionalized congeners (1). For instance, the pharmacological profile of ligands for GPCRs may benefit from attachment to a carrier molecule for delivery, and the chemical functional groups and geometry of a linker moiety can be varied, as guided by receptor affinity. Small-molecule ligands for a given GPCR generally bind within its heptahelical transmembrane domain to trigger intracellular signaling. Accordingly, a secondary interaction of a distal structural unit of a bound ligand, away from the pharmacophore region, with a GPCR at/near its extracellular domain may impart additional selectivity and affinity. Dendrimers are treelike macromolecules, which possess characteristics advantageous to their use as drug carriers and imaging agents (2). These advantages include structural integrity, controllable component functional groups and physical properties by chemical synthesis, conjugation of multiple functional units at the periphery and the interior, and low enzymatic degradation rates. Poly(amidoamine) (PAMAM) dendrimer is composed of aliphatic amino and amido groups, and its biocompatibility has led to many applications in biomedicine. Here, we report the first example of covalent drug conjugates of dendrimers to induce intracellular signal transduction across GPCRs (3). In many of the earlier applications of dendrimers as drug carriers, the active drug is endocytosed for intracellular action and/or released *in vivo*. By our approach, the intact covalent conjugate is intended to act at GPCRs on the cell surface. Multiple copies of an antiaggregatory A_{2A} AR agonist, the nucleoside CGS21680, and other adenosine derivatives were coupled covalently to PAMAM dendrimers and characterized spectroscopically. Fluorescent PAMAM-nucleoside conjugates inhibited aggregation of washed human platelets induced by ADP (acting at proaggregatory P2Y nucleotide receptors), and in some cases increased A₃ AR selectivity was noted. Multivalent dendrimer conjugates are envisioned to improve overall pharmacological profiles compared to the monovalent GPCR ligands. The multivalent conjugation to PAMAM dendrimers alters the pharmacological properties and provides targeting options. The binding of multivalent dendrimer conjugates simultaneously to multiple sites on receptor dimers and higher order structures may prove possible using this approach. The bivalent binding of PAMAM dendrimer conjugates of AR agonists to receptor dimers has been simulated using molecular modeling (4).

[1] Jacobson, K.A., et al., J. Mol. Recognition, 1989, 2:170.

[2] Tomalia D.A., et al., Biochem. Soc. Trans., 2007, 35:61.

[3] Kim, Y., et al., Bioconjugate Chem., 2008, 19:406.

[4] Ivanov, A.A., Jacobson, K.A., Bioorg. Med. Chem. Lett., 2008, doi : 10.1016/j.bmcl.2008.06.087.

P502

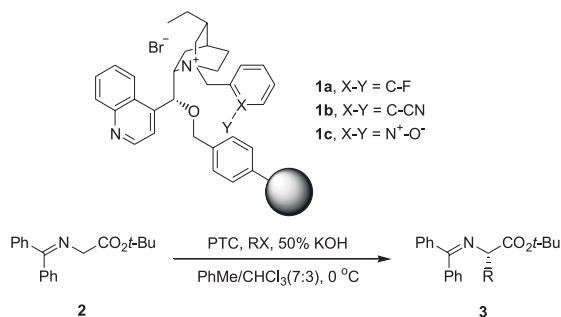
LIBRARY CONSTRUCTION OF UNNATURAL AMINO ACID BY POLYMER SUPPORTED PHASE-TRANSFER CATALYSTS (I)

Hyeyeong Lee, Qinghua Shi, Yeon-Ju Lee, Mi-Jeong Kim, Mi-Kyung Park, Kyoungyim Lee, Taek-soo Kim, Hongrui Song, Maosheng Cheng, Byeong-Seon Jeong, Hyeung-geun Park, Sang-sup Jew

Research Institute of Pharmaceutical Sciences and College of Pharmacy, Seoul National University, Seoul 151-742, Korea

Quaternary ammonium salts derived from cinchona alkaloids have been developed as efficient chiral phase-transfer catalysts (PTCs) which have been successfully applied to various organic reactions, such as alkylation, Michael reaction, aldol condensation, and epoxidation. Especially, intensive studies have been performed on asymmetric alkylation of *N*-(diphenylmethylene)glycine *tert*-butyl ester (**2**) affording a variety of natural and non-natural α -amino acid derivatives.

We have proposed that the high enantioselectivities might be due to more rigid conformations of catalysts caused by hydrogen bonding (or induced dipole-dipole interaction) involving water between C(9)-O and 2'-F, 2'-CN, or 2'-N⁺-O⁻ in each catalyst. We made an attempt to apply the electronic functional group effect into polymer supported phase-transfer catalysts.



Among the catalysts, N-oxy derivative **1c** showed the highest enantioselectivity (**1a**, 91% ee; **1b**, 93% ee; **1c**, 95% ee). These cumulative findings along with our previous results strongly support that the electronic effects of the functional groups play integral role of increasing of enantioselectivity in asymmetric phase-transfer catalytic alkylation. The best catalyst **1c** was chosen for investigation of the optimal amount of catalyst loading. The optimal amount of **1c** was 20 mol%. Generally, the larger amount of the catalyst showed the faster reaction rate with slightly increased enantioselectivity except for 40 mol% loading (93% ee), which showed slightly decreased enantioselectivity than 20 mol% (95% ee).

In conclusion, we developed a series of Merrifield resin supported hydrocinchonidine-derived ammonium salts **1a-c** incorporated the functional groups having an ability of hydrogen bonding. Very high enantioselectivities were obtained in the alkylation of *N*-(diphenylmethylene)glycine *tert*-butyl ester (**2**) under mild reaction condi-

tions, and **1c** showed the highest catalytic efficiency. The easy preparation and recyclable capability without decrease of enantioselectivity of **1c** make this catalyst very practical for the synthesis of optically active α -amino acids in industrial process. Further application to other phase-transfer catalytic reactions is currently under investigation.

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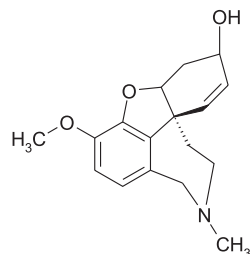
INSIGHTS INTO MECHANISM OF ACTION LEAD TO A NEW GENERATION OF GALANTHAMINE-DERIVED DRUGS

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The plant alkaloid galantamine has been described as a cholinesterase inhibitor (ChE-I) and as a nicotinic acetylcholine receptor (nAChR) sensitizing agent (APL; allosterically potentiating ligand), and galantamine has been proposed for the treatment of several human brain diseases, including Alzheimer's disease (AD).



Galantamine

Presently, the compliance of Alzheimer patients to treatment with ChE-I and APL is rather low, of the order of 20%, a key reason being the adverse effects nausea, diarrhea, vomiting, anorexia and muscle cramps. In the case of galantamine, the majority of these adverse effects is due to rather limited permeation through the blood-brain barrier (BBB) into the brain.

We will present the synthesis of pro-drug candidates resulting from novel insights into mechanism of action.

P504

DESIGN, SYNTHESIS, AND EVALUATION OF POLYNYE-CONTAINING SPHINGOID BASE PROBES

Yun Mi Lee, Jihee Cho, Songhee Ahn, and Sanghee Kim*

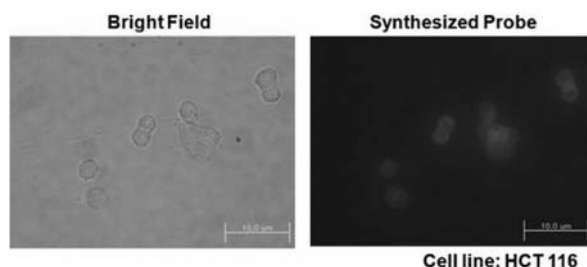
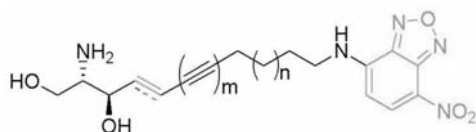
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Sphingolipids are a structurally diverse class of compounds, considered to be composed of three principal moieties: a sphingoid base, an amide-linked fatty acid, and a polar head group. They are important components of the plasma membranes of essentially all eukaryotic

cells (1). The main structural role of sphingolipids in membranes is the regulation of the fluidity and subdomain structure of the lipid bilayer. In addition to their structural functions, they also play critical roles in many fundamental biological processes.

As part of our ongoing research focused on the preparation of the sphingolipid analogues and libraries, we were particularly interested in the chemical tools that might be useful on the identification and elucidation of sphingolipid pathways. Recently, we reported on the synthesis of polyyn-containing sphingoid base analogues via an iterative acetylene homologation sequence (2). We envisaged that the incorporated rigid polyyn moiety in the sphingoid base could alter the physicochemical properties of the sphingolipid, thereby affecting the biological behaviors that would be useful in the sphingolipid research.

Herein, we wish to report our successful synthesis and preliminary evaluation of newly designed fluorescence labeled polyyn-containing sphingoid bases, in which the fluorescent NBD group was situated in the terminal of the sphingoid backbone. The first syntheses of fluorescence labeled polyyn-containing sphingoid base analogues were achieved by employing our iterative strategy that uses a two-step acetylene homologation sequence. Repeating this homologation sequence followed by cross-coupling with the fluorescence labeled long-chained terminal acetylene provides access to the polyyn framework in good overall yields. As a demonstration of their usefulness and a preliminary evaluation of the chemical tools, these obtained probes were evaluated for cytotoxic activity against various cancer cells. In order to evaluate their possible application as bioimaging probes, we also investigated the sphingoid probe uptake in cancer cell by fluorescence microscope.



References

- [1] Merrill, A. H., Jr.; Sandhoff, K. Sphingolipids: Metabolism and Cell, Signaling. In *Biochemistry of Lipids, Lipoprotein, and Membranes*; Vance, D. E., Vance, J. E., Eds.; Elsevier: New York, 2002; pp 373-407.
- [2] Kim, S.; Lee, Y. M.; Kang, H. R.; Cho, J.; Lee, T.; Kim, D. *Org. Lett.* 2007, 9, 2127 - 2130.

P505

SYNTHESIS AND EVALUATION OF THE ANTI-DYSKINETIC ACTIVITY OF THE ENANTIOMERS OF MDMA AND KW101, A NOVEL DRUG CANDIDATE FOR THE TREATMENT OF PARKINSON'S DISEASE

Lewis K.,* Johnston T.,[§] Skelton B.,* Brochie J.,[§] Piggott M.*

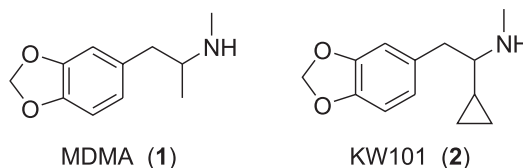
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The mainstay symptomatic treatment for the neurodegenerative disorder Parkinson's disease (PD) is the dopamine precursor levodopa. Several problems are associated with long term levodopa therapy, the most serious being the emergence of involuntary movements known as levodopa induced dyskinesia (LID). 3,4-Methylenedioxymethamphetamine (MDMA, 'ecstasy', **1**) is a reported anti-LID agent¹ and is thus a lead for an adjunctive therapeutic for the treatment of PD.²

We have identified KW101 (**2**) as a novel anti-LID agent in reserpine-treated rodent and MPTP-lesioned primate models of LID. In addition, KW101 significantly prolongs the anti-parkinsonian action of levodopa. Subsequent biological testing has demonstrated that KW101 lacks an *in vivo* MDMA-like psychoactivity profile and, unlike MDMA, is non-toxic to serotonergic and dopaminergic cell lines.

We hypothesised that the mode of action of KW101, with respect to anti-dyskinetic activity, mimics that of MDMA. To better understand this, it was pertinent to determine which of the enantiomers of MDMA and KW101 are the more efficacious, or if the observed activities are derived from synergism in both instances. This presentation details our investigations into this area including the preparation of (*R*) and (*S*)-KW101 via a diastereoselective reductive amination, novel syntheses of (*R*) and (*S*)-MDMA, and their evaluation as anti-LID agents in MPTP-lesioned primates.



- [1] Iravani, M. M.; Jackson, M. J.; Kuoppamaki, M.; Smith, L. A.; Jenner, P. 3,4-Methylenedioxymethamphetamine (ecstasy) inhibits dyskinesia expression and normalizes motor activity in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-treated primates. *J. Neurosci.* 2003, 23, 9107-9115.
- [2] Schapira, A. H. V.; Bezard, E.; Brochie, J.; Calon, F.; Collingridge, G. L.; Ferger, B.; Hengerer, B.; Hirsch, E.; Jenner, P.; Novère, N. L.; Obeso, J. A.; Schwarzschild, M. A.; Spampinato, U.; Davidai, G. Novel pharmacological targets for the treatment of Parkinson's disease. *Nat. Rev. Drug Discovery* 2006, 5, 845-854.

P506

MULTI-SITE-DIRECTED MOLECULES AS A THERAPEUTIC APPROACH FOR ATHEROSCLEROSIS

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Elevated as well as oxidized levels of LDL-cholesterol and lipids in the plasma are major factors in the pathogenesis of atherosclerosis, the consequences of which are the leading causes of death in developed countries. In particular, high levels of oxidized LDL are a major factor contributing to the formation of atherosclerotic plaques. Several research studies of the last two decades have indicated that atherosclerosis is a chronic inflammatory condition that frequently turns into an acute thrombosis condition due to rupture of the atherosclerotic plaque.

The aim of this study is the design and evaluation of new compounds, for the treatment of atherosclerosis, that possess a multifunctionality i.e. they are designed to simultaneously act on the multiple mechanisms involved in this disease. Towards this direction, new compounds that combine structural moieties with potential antihypercholesterolaemic, antihyperlipidaemic, antioxidant as well as anti-inflammatory activity were synthesized and their activity was determined *in vitro* and *in vivo*.

The new derivatives were synthesized employing modifications of existing methods and their structures were confirmed by spectroscopic techniques and elemental analysis. Subsequently, we examined their *in vitro* antioxidant activity (prevention of hepatic microsomal lipid peroxidation induced by Fe²⁺/ascorbate) as well as the activity of squalene synthase, which is the enzyme that catalyzes the first committed step towards cholesterol biosynthesis. The antihypercholesterolaemic and antihyperlipidaemic activity of the new compounds was evaluated *in vivo* based on the reduction of experimentally induced elevated plasma levels of total cholesterol, LDL-cholesterol and triglycerides in hyperlipidaemic rats. Moreover, the anti-inflammatory activity of the novel compounds was assessed *in vitro* based on their inhibition of lipoxygenase activity and *in vivo* by their ability to inhibit rat paw-oedema induced by carrageenin.

The newly synthesized derivatives combined considerable antioxidant activity (IC₅₀ values of the more potent inhibitors ranged from 0.17-0.21 µM), a significant inhibitory activity towards squalene synthase (IC₅₀ = 0.7-10 µM) and a very good antihypercholesterolaemic and antihyperlipidaemic effect (the plasma levels of total cholesterol, LDL-cholesterol and triglycerides were reduced by 40-80%). At the same time, they demonstrated a significant anti-inflammatory activity with the more active compounds inhibiting lipoxygenase activity with IC₅₀ values between 20 and 84 µM (*in vitro*) and reducing rat paw-oedema, induced by carrageenin, by c.a. 60%. Furthermore, exploration of structure-activity relationships and especially correlation of lipophilicity with activity was carried out.

Our results demonstrate that each of these molecules exhibits multiple pharmacological properties which may lead to synergistic effects beneficial for the treatment of atherosclerosis.

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SYNTHESIS, CYTOSTATIC AND ANTIVIRAL ACTIVITY EVALUATIONS OF THE NOVEL UNSATURATED ACYCLIC NUCLEOSIDE ANALOGUES

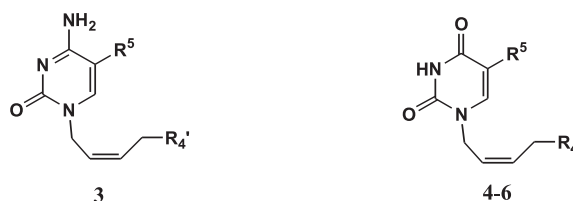
Karlo Wittine,^a Krešimir Benci,^a Sandra Kraljevic Pavelic,^b Krešimir Pavelic,^b Jan Balzarini,^b and Mladen Mintas^{a*}

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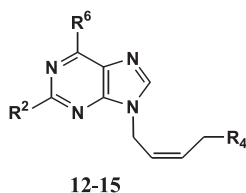
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The novel pyrimidine (**3-6**) and purine (**12-19**) acyclic nucleoside analogues containing (*Z*) 4-amino or 4-amino-hydrochloride-2-butenyl side chain (Fig.) were synthesized to evaluate their antiviral and cytostatic activity potency. The pyrimidine (**3, 4**) and purine (**12**) derivatives with free amino group were obtained using Gabriel amine synthetic methodology while the corresponding aminohydrochloride salts (**5** and **6, 12-19**, respectively) were prepared either from their amines or directly by acidifying their *N*-phtalimido derivatives. Results of antiviral and cytostatic activity evaluation will also be presented.

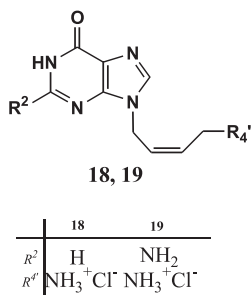


	3	4	5	6
R ⁵	H	CH ₃	CH ₃	H
R ^{4'}	NH ₂	NH ₂	NH ₃ ⁺ Cl ⁻	NH ₃ ⁺ Cl ⁻



12-15

	12	13	14	15
R ²	NH ₂	H	NH ₂	NH ₂
R ⁶	NH ₂	NH ₂	NH ₂	Cl
R ^{4'}	NH ₂	NH ₃ ⁺ Cl ⁻	NH ₃ ⁺ Cl ⁻	NH ₃ ⁺ Cl ⁻



P509

RATIONAL EXPLORATION OF STRUCTURAL DATABASES: THE CASE OF H₃ LIGANDS.

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^a Institut de Recherches SERVIER – 11, rue des Moulineaux – 92150 Suresnes, France

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The histamine H₃ receptor has been portended as a drug target in the central nervous system (CNS) in different pathological disorders such as dementia, cognition impairments, obesity and ADHD.

In connection with our drug discovery program using rational exploration of structural databases, we wish to report in this poster the emergence of a new antagonist H₃ series. We will briefly describe first the strategy for database mining, then the application to our in-house database leading to the identification of a hit. Finally, we will highlight the different pharmacomodulations giving rise to the lead candidate (figure 1).

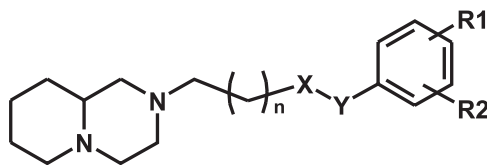


Figure 1

Synthesis, biological and pharmacokinetic data will be presented.

P510

BRAIN-SELECTIVE ESTROGEN THERAPY

Laszlo Prokai^a, Katalin Prokai-Tatrai^a, Peter Koulen^a, Istvan Merchantaler^b, Angela Brodie^b, Heather Bimonte-Nelson^c

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^b University of Maryland, School of Medicine, Baltimore, MD, USA,

^c Arizona State University, Tempe, AZ, USA

Estrogens are potent neuroactive/neuroprotective compounds. However, treatment of maladies affecting the central nervous system (CNS) with estrogens is hampered by undesirable side-effects such as peripheral feminization, stimulation of estrogen-sensitive malignancies and risk of venous thrombosis. Differential expression of distinct estrogen-receptor (ER) subtypes has been implicated in the pleiotropic and tissue-specific effects of estrogens, and a plethora of non-genomic actions has been brought into connection with estrogens' neuroprotective action. This presentation focuses on prodrug design from naturally occurring estrogens to confine the action of the steroid to the CNS and, thereby, reduce or even eliminate systemic side-effects of hormone treatment. The prodrug approach may be considered an alternative strategy that is not burdened by the excessive costs, enormous challenges and high risks associated with an extensive screening and discovery program to find neuroselective estrogen-receptor modulators with an optimal activity profile. Specifically, estrogen-derived *para*-quinols will be highlighted as novel brain-selective site-activated prodrugs for the hormone with details on chemistry, physicochemical and biopharmaceutical properties of 3-hydroxyestra-1,3,5(10)-trienes, evidence for CNS target engagements and absence of off-target impact via a novel strategy of tissue proteomics, assessment of efficacy against a variety of neurological maladies such as stroke, postmenopausal hot flashes, depression and cognitive impairment in rodent models, as well as demonstration of the lack of uterotrophic and mammatrophic effects after treatment.

P511

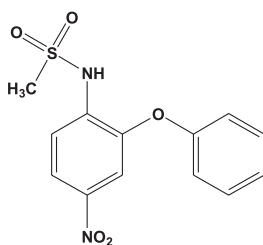
NEW PYRIDINIC ANALOGUES OF NIMESULIDE AS POTENT COXS INHIBITORS

Renard J.-F.^{*}, Gérard X.^{*}, Garbacki N.[†], de Leval X.^{*} and Pirotte B.^{*}

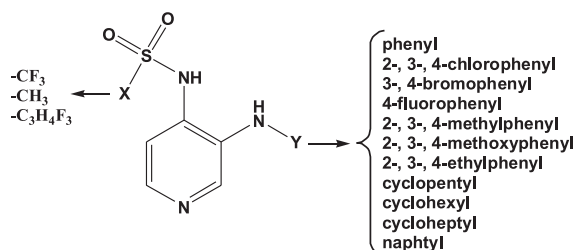
^{*}Drug research center, Laboratory of Medicinal Chemistry, Université de Liège, Av. de l'Hôpital 1, B-4000 Liège, Belgium.

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NSAIDs represent a class of compound widely used in medicine. They act as cyclooxygenase inhibitors. Two isoforms of cyclooxygenases have been described. COX-1 is involved in homeostatic processes while COX-2 is rather expressed in pathological states. This second isoform of cyclooxygenase was discovered in the early 90's. Since that time, many researches have been undertaken to obtain compounds acting more selectively towards COX-2. Nimesulide is known as a COX-2 preferential compound. We have modulated this compound and discovered a pyridinic analogue bearing an amino bridge and a trifluoromethanesulfonamide moiety (FJ 29) expressing preferential COX-2 inhibitory activity *in vitro* and anti-inflammatory activity *in vivo* (1). As a result, we considered the synthesis of original analogues of FJ 29 as shown in figure 1.



Nimesulide



Pyridinic analogues

Figure 1. Structure of Nimesulide and its pyridinic analogues.

The COX-1 and COX-2 inhibitory potencies of our compounds were evaluated using a human whole blood assay. These original compounds have shown a strong COXs inhibitory potency with IC_{50} comprised for most of them between 0.1 and 1 μ M. Moreover, some of these derivatives present a good selectivity towards COX-2 with a selectivity ratio similar to that of nimesulide and sometimes to that of celecoxib which is known as a COX-2 selective compound.

Consecutively to these *in vitro* studies we have selected the most potent compounds in order to evaluate their putative anti-inflammatory effect *in vivo*. We have carried out these experiments in a model of acute inflammation: the rat carrageenan-induced pleurisy. All compounds tested have shown a marked anti-inflammatory effect ranging up to 70% at the dose of 20 mg/kg ip.

So, we have realized the synthesis and pharmacological evaluation of original pyridinic derivatives of nimesulide. These compounds expressed a strong COXs inhibitory potency with a preferential inhibitory activity of COX-2 coupled with a strong anti-inflammatory effect *in vivo*.

References

- [1] Julemont, F., et al., Design, synthesis, and pharmacological evaluation of pyridinic analogues of nimesulide as cyclooxygenase-2 selective inhibitors. *J Med Chem*, 2004. 47(27): p. 6749-59.

P512

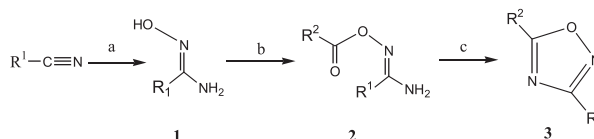
MICROWAVE-ASSISTED ONE-POT SYNTHESIS OF 3,5-DISUBSTITUTED 1,2,4-OXADIAZOLES UNDER SOLVENT-FREE CONDITIONS USING POTASSIUM FLUORIDE AS CATALYST

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Nitrogen–oxygen heterocycles are of synthetic interest because they constitute an important class of natural and non-natural products, many of which exhibit useful biological activities.[1] The interest in five-membered systems containing one oxygen and two nitrogen atoms (positions 1, 2, and 4) stems from the occurrence of saturated and partially saturated 1,2,4-oxadiazoles in biologically active compounds and natural products.[2] 1,2,4-Oxadiazoles have recently received considerable attention as heterocyclic amide and ester bioisosteres. Bioisosteric replacement of the amide moiety represents an area that is currently a center of focus because of its implications in peptide chemistry and the development of peptidomimetics.[3,4]

Potassium fluoride (KF) was found to be a mild and efficient catalyst for the synthesis of 3,5-disubstituted-1,2,4-oxadiazoles. In this process, started from nitriles, the corresponding amidoximes were prepared *in situ* by addition of hydroxylamine hydrochloride and KF under microwave irradiation. Then, benzoyl chloride was added and the reaction mixture was irradiated for the specified time to furnish the formation of desired product (Scheme 1). Unlike solid supports or conventional methods, the work up of this method is simple because KF dissolves easily in water in which the products are insoluble and isolated by simple filtration.



Scheme 1. Efficient KF catalyzed microwave mediated synthesis of 1,2,4-oxadiazoles. (a) KF, Hydroxylamine hydrochloride, MW. (b) R^2COCl , r.t. (c) MW

References

- [1] Swinbourne, J. F.; Hunt, H. J.; Klinkert, G. *Adv. Heterocycl. Chem.* 1987, 23, 103.
- [2] Jochims, J. C. In *Comprehensive Heterocyclic Chemistry II*; Katritzky, A. R., Rees, C. W., Scriven, E. V. F., Eds.; Pergamon Press: London, 1996; Vol. 4, pp 179–228, Chapter 4 and references cited therein.

[3] Vu, C. B.; Corpuz, E. G.; Merry, T. J.; Pradeepan, S. G.; Bartlett, C.; Bohacek, R. S.; Botfield, M. C.; Lynch, B. A.; MacNeil, I. A.; Ram, M. K.; van Schravendijk, M. R.; Violette, S.; Sawyer, T. K. J. *Med. Chem.* 1999, 42, 4088–4098

[4] Clithrow, J. W.; Beswick, P.; Irving, W. J.; Scopes, D. I. C.; Barnes, J. C.; Clapham, J.; Brown, J. D.; Evans, D. J.; Hayes, A. G. *Bioorg. Med. Chem. Lett.* 1996, 6, 833–838.

P513

NOVEL AMINOPTEROIL-SULFONAMIDE DERIVATIVES AS INHIBITORS OF DIHYDROFOLATE REDUCTASE AND CARBONIC ANHYDRASE, WITH POTENTIAL ANTI TUMOR ACTIVITY

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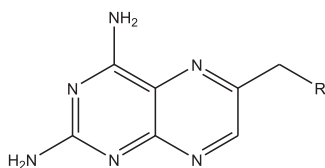
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Recent evidence suggest that combination of conventional chemotherapeutics, which are usually cytotoxic, with cytostatic agents can provide improved cancer treatment options. As a continuation of our previous work on methotrexate derivatives¹ we have designed, synthesized and evaluated novel methotrexate (MTX) analogues, aminopteroyl-based benzenesulphonamide derivatives, with potential bifunctional inhibitory activity against carbonic anhydrase (CA) and dihydrofolate reductase (DHFR). Both of these enzymes are often overexpressed in tumors and are considered to be prosurvival factors. The designed dual specificity drugs (Fig 1) thus target two unrelated cellular pathways, important for tumor progression that should be beneficial in terms of enhanced anti-tumor activity.



R = -SpacerC₆H₄-SO₂NH₂

Herein we present the design, the synthetic scheme of five sulfonamide aminopteroil derivatives, which differ in the length and the nature of spacer between the two key molecular moieties (pterin ring and sulphonamide group), and the results of their inhibitory effects against three isozymes of carbonic anhydrase (CA I, II and IX). Our preliminary results revealed high potency of several compounds against CA isozymes II and IX with IC₅₀ in

the nanomolar range. Another isozyme CA I revealed a weaker inhibition by the same compounds with IC₅₀ from high nanomolar to low micromolar range. The docking experiments using CA IX as a model provided the rationale for the inhibitory studies from the point of view of protein-ligand interactions. Ongoing evaluation of antiproliferative activity of the synthesized compounds in the cell culture model, as well as DHFR inhibition assays, should provide further information regarding the potency of these MTX analogues as dual target drugs.

[1] M. A. Santos, E. A. Enyedy, E. Nuti, A. Rossello, N. I. Krupenko, S. A. Krupenko, *Bioorg. Med. Chem.* 15 (2007) 1266-1274.

P514

SYNTHESIS AND BIOLOGICAL EVALUATION OF FOLIC ACID DERIVATIVES AS HISTONE DEACETYLASE INHIBITORS

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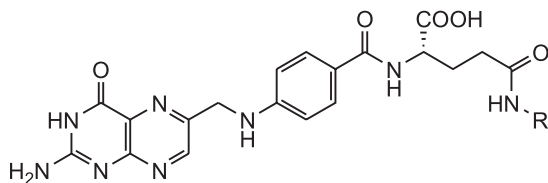
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Histone deacetylases (HDACs) are a family of enzymes involved in the regulation of gene expression, DNA repair and stress response. These processes are often altered in tumours and HDAC inhibitors have emerged as new targets for cancer therapy with promising results in clinical trials.

HDACs are divided into three structural classes depending on sequence identity and domain organization of the protein. HDAC8 represents Class I, that includes zinc-dependent amidohydrolases with a conserved catalytic core but different in size, domain structure and tissue expression patterns. HDAC8 is localized in the cytoplasm and its expression is restricted to smooth muscle.^{1,2} The physiological function of HDAC8 is under a thorough investigation. It seems to play a role in one of the most frequent types of acute myeloid leukaemia (AML) and, for this reason, selective inhibitors of HDAC8 are highly sought.

To address this, we synthesized several new folic acid derivatives bearing different linear chains as a linker and a hydroxamic acid as zinc binder. These compounds were tested on human recombinant HDAC8 and showed a good inhibitory potency against the enzyme. Docking studies confirmed the favourable features of these compounds and prompted us to further investigate their biological activity, namely their effect on proliferation of cultured cells.



References

- [1] Krennhrubec K, Marshall BL, Hedglin M, Verdin E, Ulrich SM, *Bioorg Med Chem Lett*, 2007, 17, 2874-2878.
- [2] Vannini A, Volpari C, Filocamo G, Casavola EC, Brunetti M, Renzoni D, Chakravarty P, Paolini C, De Francesco R, Gallinari P, Steinkühler C, Di Marco S., *Proc Natl Acad Sci*, 2004, 101, 15064-15069.

P515

DESIGN, SYNTHESIS AND EVALUATION OF 2,3,6,8-TETRA SUBSTITUTED CHROMONE DERIVATIVES AS SOMATOSTATIN MIMETICS

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Physico-chemical properties and enzymatic hydrolysis limit the use of peptides as drugs [1]. To overcome these problems, peptidomimetics are designed to be more stable and at the same time retain the effect of the parent peptide. A scaffold approach is one way to develop these kinds of structures. Chromone derivatives are frequently found in nature and in this project the chromone ring system is used as a scaffold for peptidomimetics. Incorporation of amino acid side chain moieties in the 2-, 3-, 6-, and 8-positions of the chromone structure makes it possible to mimic a β -turn of a peptide chain. The peptide hormone somatostatin is known to adopt a β -turn conformation when interacting with its receptor(s). In this study computer based modelling has been used to design chromone based mimetics of somatostatin (figure 1). Appropriate substituents have been introduced in the chromone system using efficient reactions such as Pd-mediated coupling reactions [2] and organocatalyzed reactions. Factors such as sterical hindrance, use of different amines, catalysts, solvents, reaction times and temperatures have been studied and evaluated in order to optimise the reaction. Synthesized chromone based somatostatin mimetics have been tested for their somatostatin receptor binding properties.

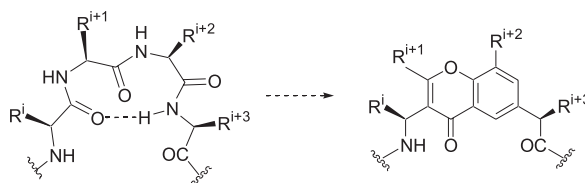


Figure 1. 2,3,6,8- Tetrasubstituted chromone as a potential β - turn mimetic.

References

- [1] Adessi, C. et al. *Curr. Med. Chem.*, 9, 963-978, (2002).
- [2] Dahlén, K. et. al. *J. Org. Chem.*, 71, 6863-6871, (2006).

P517

DOCKING COMPARISON OVER THE GLUCOCORTICOID AND ANDROGEN RECEPTORS OF GINSENO-SIDES

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The glucocorticoid (GR) and androgen (AR) receptors are members of the nuclear super family of intracellular receptors. The GR is expressed in almost every cell in the body and regulates either directly or indirectly genes controlling a wide variety of processes including the development, metabolism, and immune response of the organism. Glucocorticoids are capable of exerting both genomic and non-genomic actions in target cells of multiple tissues. The glucocorticoids participate in the resolution of inflammatory conditions by suppressing a variety of immune and inflammatory functions by inhibition of inflammatory cytokines such as IL-1, IL-2, IL-6, and TNF- α , as well as the expression of adhesion molecules on endothelial cells, and the non-genomic action of glucocorticoids results in part by the rapid steroid actions that inhibit the production of arachidonic acid and downstream pro-inflammatory prostaglandins.¹

Ginsenosides (GSs), the main component of *Panax ginseng* C.A. Meyer, are known for their anti-inflammatory and anti-proliferative activities, inhibiting the production of proinflammatory cytokines such as TNF- α , IL-1 β , IL-8, GM-CSF and IL-6. The mechanism of action is based mainly on interference with the activity of transcription factors, such as nuclear factor κ B (NF- κ B) and activator protein-1 (AP-1).^{2,3}

A molecular docking study was performed in order to investigate the interactions of ginsenosides in the ligand-binding domains of the glucocorticoid and the androgen receptors (GR-LBD, PDB: 1p93 and AR-LBD, PDB: 2am9, respectively). The receptor-binding energy of a series of ginsenosides: (20S)-Rb1, (20S)-Rb2, (20R)-Rg3, (20S)-Rgh2, (20S)-Rd, (20S)-Re and metabolites

like compound K (C-K, a metabolite of ginsenoside Rb1), protopanaxatriol (PPT) and protopanaxadiol (PPD), was measured. The PPT and PPD aglycones docked to the GR-LBD and AR-LBD show high receptor-binding affinity; on the other hand, the saponins (except C-K), show unfavorable sterical interactions (Figure 1). The information rendered by this study suggests a possible binding mode of these compounds in the ligand-binding domain of the receptors in question.

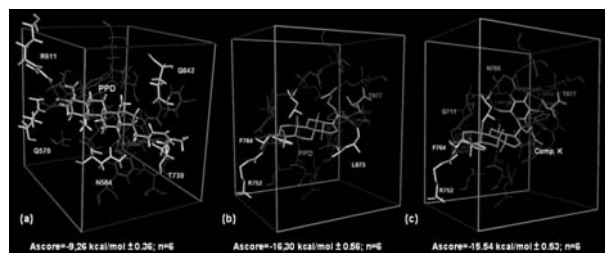


Figure 1. Docking results for: **a.** PPD into the GR-LBD; **b.** PPD into the AR-LBD; **c.** C-K into the AR-LBD

References

- [1] De Bosscher K., Van Craenenbroeck K., Meijer O. and Haegeman G. *Eur. J. Pharmacol.* 2008; 583: 290–302.
- [2] Leung K.W., Leung F.P., Huang Y., Mak N.K. and Wong R. *FEBS Lett.* 2007; 581: 2423–2428.
- [3] Choi K., Kim M., Ryu J. and Choi C. *Neurosci. Lett.* 2007; 421: 37–41.

P518

LIBRARY CONSTRUCTION OF β -AMINO CARBONYL SYSTEM VIA PHASE-TRANSFER CATALYTIC AZA-MICHAEL ADDITION

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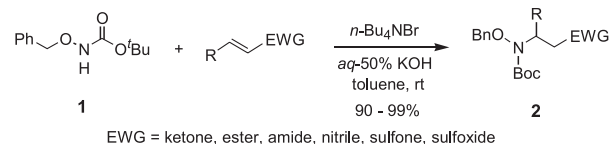
Since the β -amino carbonyl functionality has been recognized as not only a key structure of biologically important natural products including β -lactams but also a versatile nitrogen-containing intermediates such as amino alcohols, diamines, and β -amino acid derivatives, numerous methods have been developed for the efficient synthesis of β -amino carbonyl compounds.

Among the less reactive nucleophiles, carbamates also usually need Brønsted acids or several expensive transition metal-based Lewis acids. Furthermore, the scope of the substrate was restricted to α,β -unsaturated ketones (enones) or α,β -unsaturated aldehydes (enals). In this abstract, a new convenient aza-Michael reaction system using carbamates as nucleophile with a wide range of substrate scope is reported.

To develop a new efficient and versatile aza-Michael reaction, we attempted to use a phase-transfer catalytic

reaction system because it provides various advantages in terms of operational simplicity as well as economic- and environmental-concerns. Fortunately, as we expected, **1** afforded the aza-Michael adduct **2** even though the yield (44%) and the reaction time (10 h) needed to be improved. We presumed that the modest chemical yield and the slightly longer reaction time might be due to the self-aldol condensation of enone and a retro-aza-Michael reaction of the initially formed product under the basic conditions. A dramatic increase of chemical yield (99%) without the self-aldol condensation and a remarkable decrease in reaction time (10 min) were finally accomplished both by employing 50% aqueous KOH solution as a base instead of solid KOH, and by reducing the amount of the base from 2.0 equiv. to 1.2 equiv.

In summary, we developed a highly efficient phase-transfer catalytic aza-Michael reaction with commercially available tert-butyl benzyloxycarbamate as a protected nitrogen nucleophile. The notable features of this novel aza-Michael reaction system lie, especially, in the wide scope of substrates and the advantages came from phase-transfer catalysis. We are currently investigating an asymmetric version of this methodology, and it will be reported in the near future.



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LIBRARY CONSTRUCTION OF UNNATURAL AMINO ACID BY POLYMER SUPPORTED PHASE-TRANSFER CATALYSTS (II)

Sangah Lee, Qinghua Shi, Yeon-Ju Lee, Minwoo Ha, Hongrui Song, Maosheng Cheng, Sang-sup Jew, Hyeung-geun Park, and Byeong-Seon Jeong

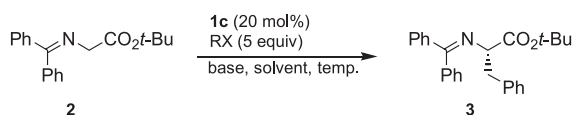
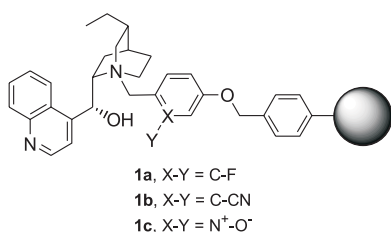
Research Institute of Pharmaceutical Sciences and College of Pharmacy, Seoul National University, Seoul 151-742, Korea

Cinchona alkaloid-derived quaternary ammonium salts have been frequently employed as chiral phase-transfer catalysts (PTCs) in the enantioselective phase-transfer catalytic alkylation of glycine imine ester affording a variety of optically active natural and nonnatural α -alkyl- α -amino acid derivatives. In addition, considerable efforts have been made on the immobilization of cinchona PTCs on various polymer supports. The most advantageous aspect to use a polymer-supported PTC (PS-PTC) is that it can be readily recoverable and recyclable, which makes PS-PTC more suitable for a large-scale production than unsupported PTCs from both practical and economical points of view.

Newly designed type-2 cinchona PS-PTCs (**1a-c**) were prepared from (-)-hydrocinchonidine and the corresponding Merrifield resin-bound benzylic bromides. The catalytic efficiency of the newly prepared PS-PTCs was

evaluated by catalytic asymmetric benzylation of **2** under the phase-transfer conditions. As there are some factors influencing enantioselectivity, such as catalyst, base, solvent, and reaction temperature, we performed this reaction with PTC **1c** under different conditions as described. 50% KOH base provided the highest enantioselectivity and good chemical yield in proper reaction time compared to other bases. It was also found that both a mixed solvent of toluene-chloroform (7:3) and 0 °C of reaction temperature gave better results than others.

In summary, we developed a series of new cinchona PS-PTCs in which hydrogen bonding inducing functional group is incorporated. All of the newly prepared PS-PTCs were found to have an ability to catalyze the asymmetric alkylation of **2** under phase-transfer conditions in good chemical/optical yields.



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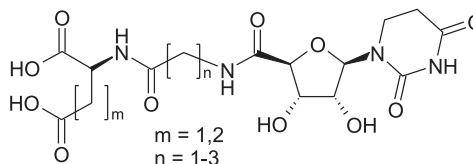
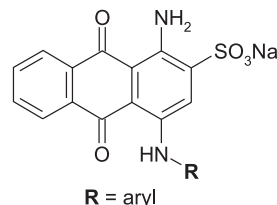
DEVELOPMENT OF ECTO-5'-NUCLEOTIDASE INHIBITORS AS POTENTIAL NEW ANTI-CANCER DRUGS

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A major part of the extracellular adenosine which activates adenosine A₁, A_{2A}, A_{2B} and A₃ receptor subtypes, is produced from adenine nucleotides (ATP, ADP, AMP) by ectonucleotidases. Ecto-nucleoside triphosphate diphosphohydrolases (NTPDases 1, 2, 3, and 8) or ecto-nucleotide pyrophosphatases (NPP 1-3) can hydrolyze the P2 receptor agonists ATP and/or ADP yielding AMP. AMP can then be further dephosphorylated by ecto-5'-nucleotidase (ecto-5'-NT, CD73) yielding adenosine. Overexpression of ecto-5'-NT by tumour cells has been implicated in cancer progression. Inhibition of ecto-5'-NT may represent a novel approach to cancer and immunostimulatory therapy. Therefore we were interested in developing potent and selective ecto-5'-NT inhibitors.

As a first step we developed a suitable test system using capillary electrophoresis for the separation and quantitative determination of AMP and its product adenosine.¹ Subsequently, we identified two novel lead structures for the development of competitive ecto-5'-NT inhibitors: (i) anthraquinone derivatives related to Reactive Blue 2, and (ii) nucleotide mimetics.



The lead structures were optimized yielding ecto-5'-NT inhibitors with K_i values below 200 nM which belong to the most potent ecto-5'-NT inhibitors described to date. For example, the anthraquinone derivatives sodium 1-amino-4-[2-anthranylamino]-9,10-dioxo-9,10-dihydroanthracene-2-sulfonate (PSB-0863) had a K_i value of 150 nM, and the nucleotide mimetic N-[4-((2*S*,3*R*,4*S*,5*R*)-5-(2,4-dioxo-3,4,5,6-tetrahydropyrimid-1(2*H*)-yl)-3,4-dihydroxytetrahydrofuryl-2-carboxamido)butyl]-(*S*)-aspartic acid (PSB-08193) had a K_i value of 180 nM; it was inactive at P2Y₂, 4, 6, 12 receptors. In conclusion, we identified new, potent and selective inhibitors of ecto-5'-NT from two different structural classes.

[1] Iqbal, J., Jirovsky, D., Lee, S. Y., Zimmermann, H., Müller, C. E., *Anal. Biochem.* 2008, 373, 129-140.

P522

ON THE PRODUCTS OBTAINED DURING THE HBR TREATMENT OF A CONDENSED NITROPYRIDONE

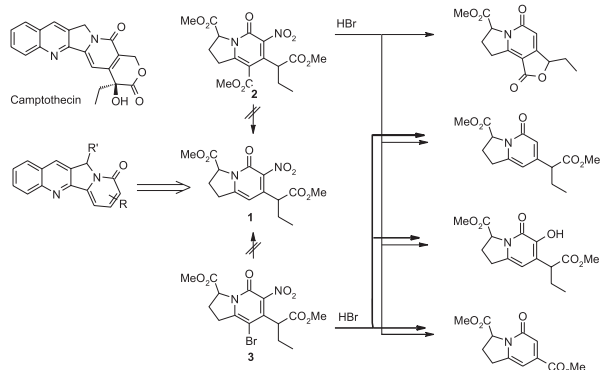
L. Gavara^a, B. Rigo^a, P. Gautret^a, J.-P. Hénichart^b

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The formation of side products in chemical synthesis can hardly be avoided. In spite of this problem, a systematic examination of the products obtained in few quantities is not only interesting from a chemical point of view¹ but also for the design of innovating structures. One of the main research topics of our laboratory is the preparation of potential anticancer compounds targeting topoisomerases I and II.² In order to produce camptothecin

analogs, we tried to get the key compound **1** by decarboxylation of **2** or **3**. Actually, we did not obtain the desired compound **1**, but a mixture of different products. In a second time, we were able to produce each unexpected structure in a better yield by changing the experimental conditions.



Purification of these products by preparative chromatography (direct and reverse phase) allowed their separation and their characterization. Elucidation of structures was carried out by NMR (¹H, ¹³C, DEPT, COSY and NOESY), mass spectrometry (LC/MS) and IR. Mechanisms were elucidated,³ and the isolated compound will be tested on therapeutic targets.

[1] Akué-Gédu R.; Bourry A.; Camus F.; Norberg B.; Durant F.; Couturier D.; DeBacker M.; Rigo B., *Heterocycles* 2005, 63, 1855.

[2] Brunin T.; Legentil L.; Hénichart J.-P.; Rigo B., *Tetrahedron* 2006, 62, 3959; Brunin T.; Hénichart J.-P.; Rigo B. *Tetrahedron*, 2005, 61, 7916; Malecki N.; Houssin R., Hénichart J.-P.; Couturier D.; Petra F.; Legentil L.; Rigo B. *J. Heterocyclic Chem.* 2003, 40, 45.

[3] Gavara L.; Rigo B.; Couturier D.; Goossens L.; Hénichart J.-P. *Tetrahedron* 2007, 63, 9456.

P523

COMPUTER AIDED DRUG DESIGN IN THE SYNTHESIS OF LIPOXYGENASE INHIBITORS WITH ANTIOXIDANT ACTIVITY AND BIOLOGICAL INTEREST

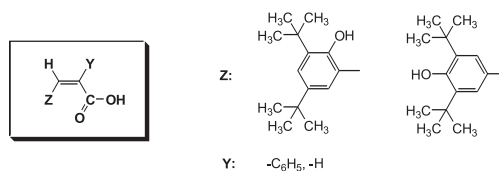
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Eicosanoids are formed from arachidonic acid (AA) by the cyclooxygenase, lipoxygenase and epoxigenase pathways. Eicosanoids such as prostanoids and leukotrienes have a wide range of biological actions including potent effects on inflammation and immunity. The cascade involves two major pathways: a) the lipoxy-

genase (LOX) is the first enzyme in a cascade which produces leukotrienes (LTs) while cyclooxygenase (COX) initiates the cyclic pathway leading to prostanoids. PGs and LTs produced in these COX and LOX pathways have been implicated as pro-inflammatory mediators in numerous inflammatory diseases, in allergic disorders, in cell proliferation and in neoangiogenesis. Especially, leukotrienes have been implicated as important mediators in several diseases including asthma, arthritis, psoriasis, inflammatory bowel disease and others.

Recently, we have reported a series of substituted acrylic acids, hydroxamic acids and amides, having potent inhibitory activity against soybean lipoxygenase and possessing anti-oxidant and anti-inflammatory activities¹⁻⁴. In continuation to our effort towards highly effective antioxidants, inhibitors of LOXs, according to the QSAR study we synthesized some new acrylic acids combining the appropriate physicochemical features in order to inhibit soybean lipoxygenase and to present antioxidant and anti-inflammatory activities. Since the role of 3,5-ditert-butyl-4-hydroxyphenyl group as a redox inhibitor, or antioxidant, to interfere with the redox cycle of the 5-LOX isozyme has been investigated extensively in the search for new dual COX/LOX inhibitors, we found interesting to synthesize phenyl-substituted or not (3,5-ditert-butyl-2-hydroxyphenyl) and (3,5-ditert-butyl-4-hydroxyphenyl) acrylic acids for further investigation.



The compounds have been identified using IR, ¹H-NMR, ¹³C-NMR spectra and elemental analyses. Lipophilicity as R_m values was determined using RPTLC.

The derivatives were screened for their biological activity *in vitro* on: a) soybean lipoxygenase inhibition, b) interaction with 1,1-diphenyl-2-picryl-hydrazyl (DPPH) stable free radical, c) the HO[•] mediated oxidation of DMSO, d) antiradical activity as loss of ABTS[•] radical, e) inhibition of lipid peroxidation using the AAPH protocol, f) scavenging of superoxide anion radicals, g) *in vivo* for the inhibition of carrageenin induced rat paw edema.

The compounds have shown potent antioxidant activity, good anti-inflammatory activity and high inhibition of soybean lipoxygenase as a result of their physicochemical features. The results are discussed in terms of the structural and physicochemical characteristics of the compounds.

Acknowledgements

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References

- [1] Pontiki E. and Hadjipavlou-Litina D., *Arzneim. Forsch./Drug Res.*, 2003, 53, 780.
- [2] Pontiki E. and Hadjipavlou-Litina D., *Med. Chem.*, 2006, 2(3), 251.
- [3] Pontiki E. and Hadjipavlou-Litina, D. *Med. Chem.*, 2007, 3(2), 175.
- [4] Pontiki E. and Hadjipavlou-Litina, D. *Biorg. & Med. Chem.*, 2007, 15 (17), 5819.

P524

UTILITY OF SALIVARY SIGA AS A MARKER FOR ASSESSING STRESS INDUCED BY DENTAL TREATMENT

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During dental treatment, children can become apprehensive due to various stimuli, such as environmental noises and surgical procedures, resulting in the onset of psychological or physical stress. These stresses can subsequently lead to an avoidance of opportunities for early dental care and of treatment. Changes in the secretory immunoglobulin A (slgA) level in saliva have received much attention in recent years as an index of mental stress. Herein, we examined the utility of this parameter for assessing stress caused by dental treatment through measuring the salivary slgA levels in children before and after dental treatment. We then assessed their levels of stress by analyzing changes in salivary slgA following treatment. Moreover, a comparison was also carried out regarding cortisol and alpha-amylase activities which are parameters of other stress indices. A decrease in salivary slgA after treatment was detected in 83% of the subjects. Furthermore, the overall level of slgA had decreased significantly following dental treatment ($p < 0.01$), indicating that the children experienced stress during treatment, since increased stress results in decreased salivary slgA. The level of slgA was significantly decreased after a prolonged treatment time (≥ 20 min; $p < 0.01$), the use of infiltration anesthesia ($p < 0.01$), and surgical procedures ($p < 0.01$). Moreover, the level of salivary slgA was significantly lower in children who were cooperative with treatment ($p < 0.01$), indicating that stress may be reduced, in part, by cooperative behavior. Changes in salivary slgA were similar with those in cortisol or alpha-amylase levels in various categories of medical records.

These findings indicate that changes in the salivary slgA level may provide an index for monitoring the stress of dental treatment, by determining changes in salivary slgA following treatment. Taken together, as a non invasive and convenient method for evaluating stress induced by dental treatment, the level of salivary slgA represents a promising, clinically relevant marker similar to cortisol and alpha-amylase levels.

P525

DISCOVERY OF ANTI-INFLAMMATION DRUG: SELECTIVE REPRESSION OF COX-2 ACTIVITY AND INFLAMMATORY GENE EXPRESSION IN RAW 264.7 MACROPHAGE CELLS

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3-(benzo[d]-1,3-dioxol-5-yl)-4-phenylfuran-2,5-dione (BPD) was designed and synthesized for evaluation as selective COX-2 inhibitor. BPD significantly inhibited the lipopolysaccharide (LPS)-induced prostaglandin E₂ (PGE₂) production in RAW 264.7 cells. Consistent with these findings, BPD not only reduced the LPS-induced expression of COX-2 at the protein and mRNA levels but also inhibited COX-2 activity. In addition, the release of pro-inflammatory mediators such as nitric oxide (NO), tumor necrosis factor- α (TNF- α), interleukin-1 β (IL-1 β) and interleukin-6 (IL-6) were reduced by BPD via down-regulation of their gene expression. BPD attenuated the LPS-induced DNA binding activity and the transcription activity of nuclear factor-kappa B (NF- κ B), and this was associated with a decrease in inhibitory kappa B- α (I κ B- α) phosphorylation and in the subsequent blocking of p65 and p50 protein translocations to the nucleus, whereas BPD produced a weak inhibition on AP-1 activation. Furthermore, BPD suppressed the phosphorylations of TAK1, IKK, ERK1/2, and p38, whereas the phosphorylation of JNK1/2 was unaffected. Other TLRs ligands (peptidoglycans and double-stranded RNA)-induced PGE₂ production and COX-2 protein expression were also inhibited by BPD. Moreover, the PGE₂ production from mouse peritoneal macrophages in response to LPS was diminished by BPD. These results suggest that the anti-inflammatory properties of BPD are caused by iNOS, COX-2, TNF- α , IL-1 β and IL-6 down-regulation due to NF- κ B inhibition via the suppression of TAK1, IKK, ERK1/2 and p38 phosphorylation in RAW 264.7 cells, also particularly reduction of PGE₂ production due to inhibition of COX-2 activity and cPLA₂ phosphorylation. These anti-inflammatory effects are not restricted to TLR4 activation and immortalized macrophage-like cells.

P527

DEVELOPMENT OF NON-PEPTIDE INHIBITORS OF HER2-GRB2 INTERACTION USING MOLECULAR MODELING AND NMR SPECTROSCOPY

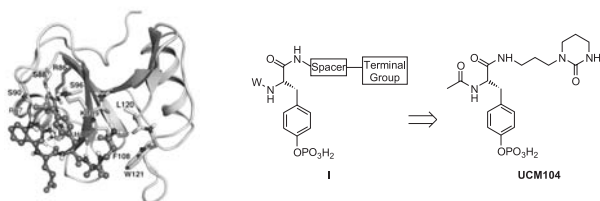
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The human epidermal growth factor receptor 2 (HER2 or ErbB-2) is an important therapeutic target in breast cancer. HER2 gene is overexpressed in around the 25% of the invasive breast cancers and it is associated with poor disease-free survival and resistance to chemotherapy drugs. Hence, the blockade of HER2 activation by inhibiting its interaction with the Src homology 2 (SH2) domain of the growth factor receptor-bound protein 2 (Grb2) has emerged as a new strategy in drug discovery to identify novel therapeutic agents in oncology.¹ Nonetheless, targeting SH2 domain with small molecules, similarly to other protein-protein interactions, remains as challenging as promising strategy. Although a number of potent peptide inhibitors have been reported to date, they show important pharmacokinetic limitations that preclude their use as drugs. Therefore, the development of small molecules capable of disrupting binding between HER2 and Grb2 is of outmost importance.

Here, we disclose a structurally novel series **I** of non-peptide molecules that block the interaction between HER2 and Grb2. These structures have been designed using molecular modeling techniques based on the crystal structure of the Grb2-SH2 domain complexed with the pentapeptide inhibitor 2-Abz-EpYINQ-NH₂.² We have successfully reproduced the key interactions determined in the computational models, and we have identified compounds that bind Grb2 with IC₅₀ values in the micromolar range. Among the synthesized derivatives, **UCM104** (IC₅₀ = 174 µM) was identified as a new lead compound. Additionally, NMR chemical shift perturbation experiments enabled experimental confirmation of the binding site of **UCM104** in the SH2 domain of Grb2.



This study sets the bases for further structure optimization aided by molecular modeling. The molecules synthe-

sized herein represent a new family of Grb2-SH2 binders devoid of peptide nature. They will serve as new scaffolds to obtain more efficient HER2-Grb2 binding blockers as tools for the validation of this anticancer therapy.

This work was supported by grants from MEC (SAF-2007/67008) and CAM (S-SAL-249-2006), by a CAM predoctoral fellowship (to A.L.O.), by a MEC postdoctoral fellowship (to P.S.) and by the MEC Ramón y Cajal program (to S.O.-G.). A.V. is currently at Instituto de Química Orgánica General, CSIC, E-28006 Madrid, Spain.

References

- [1] Hynes, N. E. *et al. Nat. Rev. Cancer* 2005, 5, 341.
- [2] Rahuel, J. *et al. J. Mol. Biol.* 1998, 279, 1013.

P528

6-SUBSTITUTED BENZOPYRAN DERIVATIVES AS POTENT K_{ATP} CHANNEL OPENERS: SYNTHESIS AND BIOLOGICAL IN VITRO EVALUATION

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ATP-sensitive potassium channels (K_{ATP} channels) are trans-membrane structures involved in the passive transport of potassium ions according to gradient concentration. K_{ATP} channels control membrane potential of a wide variety of excitable cells. As a result, they are able to regulate several physiological processes such as insulin release from pancreatic β-cells, smooth muscle tone and contractility.

Potassium channel openers (PCOs) are able to open K_{ATP} channels. These compounds can be expected to be used as new drugs in many therapeutic applications but, to avoid side effects, they need to express a powerful activity associated with a high tissue selectivity. PCOs which are selective for the pancreatic endocrine tissue have been proposed as new therapeutic agents in the treatment of hyperinsulinemia, diabetes and obesity. In this context, we have developed new cromakalim derivatives as potent and selective pancreatic K_{ATP} channel openers.

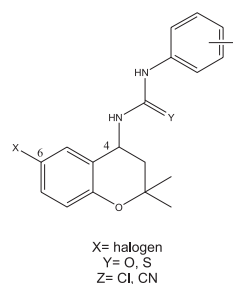


Figure 1: last synthesized compounds

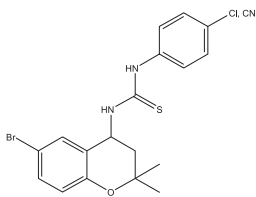


Figure 2 : BPDZ 490 (Cl) and 568 (CN)

Recent works on benzopyran derivatives were conducted on molecules bearing a halogen atom at the 6-position and a phenylurea or a phenylthiourea group at the 4-position (figure 1). Some of them, like BPDZ 490 and 568 (figure 2), were found to be relatively potent and selective pancreatic K_{ATP} channel openers. These compounds, however, are characterized by a poor hydrosolubility. To improve the physico-chemical properties of these compounds, we decided to replace the halogen atom at the 6-position by some new groups expected to improve hydrosolubility.

The first biological results showed that modifications around the benzopyran's 6-position can highly influence the activity of these compounds on the pancreatic endocrine tissue. With these new results, we can propose some additional pharmacomodulations to improve the activity and the selectivity for the pancreatic tissue.

This study was supported by grants from the Formation to Research in Industry and Agriculture (F.R.I.A., Belgium).

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SYNTHESIS AND STRUCTURE-ACTIVITY RELATIONSHIP STUDIES OF NOVEL 6-UREA/THIOUREA-2-OXINDOLES AS POTENTIAL RECEPTOR TYROSINE KINASE INHIBITORS

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Angiogenesis plays an important role in the growth of most solid tumors and the progression of metastasis. Inhibition of angiogenesis represents a promising new approach to cancer therapy. In tumor angiogenesis, the receptor tyrosine kinases (RTKs) of vascular endothelial growth factor receptor (VEGFR) and platelet-derived growth factor receptor (PDGFR) are thought to play prominent roles. As a result of the complex and redundant cellular signaling network associated with RTKs, broad-acting, multitargeted RTK inhibitors may be even more advantageous than selective agents. This is evidenced by the FDA's recent approval of two multitargeted kinase inhibitors, SU11248 (Sutent) and BAY 43-9006

(Sorafenib), as new drugs for cancer treatment. In the search for novel and potent multitargeted RTK inhibitors, a series of 6-urea/thiourea-2-oxindole derivatives have been synthesized. Structure-activity relationship (SAR) studies led to the identification of some compounds as potent inhibitors of kinases of both VEGFR and PDGFR families (IC_{50} values in the low nanomolar range). The synthesis and SAR studies of this series of compounds will be presented.

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NITROBENZYL PRODRUGS OF ISOPHOSPHORAMIDE MUSTARD

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The toxicity related to conventional anticancer chemotherapy is mostly caused by the lack of selectivity of cytostatics used against target tumor cells. The desired selectivity can be obtained by, *inter alia*, the use of gene therapy. One method of such anticancer experimental therapy is enzymatic activation of pro-drug in genetically modified tumor cells, so-called Gene-Directed Enzyme Pro-drug Therapy (GDEPT) [1, 2]. In recent years several enzyme/pro-drug systems were elaborated, some now under clinical trial. The possibility of the use of anticancer alkylating drug Ifosfamide (IFO) [3] and of cytochrome P450 isoforms in GDEPT was intensively examined in recent years [4]. Studies were also undertaken to examine the possibility of the use in GDEPT derivatives of isophosphoramidate mustard (iPAM), the final IFO metabolite [5, 6]. Here we present a new class of iPAM analogues, namely *p*-nitrobenzyl triesters. These compounds are activated by a bacterial nitroreductase and a kinetics of this activation strongly depends on the substituents present in an aromatic ring. Obtained results suggests possibility of the use of such type of compounds in the anticancer GDEPT.

References

- [1] Niculescu-Duvaz, I.; Spooner, R.; Marais, R.; Springer, C.J. *Bioconjugate Chem.*, 1998, 9, 4-22.
- [2] Niculescu-Duvaz, I.; Friedlos, F.; Niculescu-Duvaz, D.; Davies, L.; Springer, C.J. *Anti-Cancer Drug Design*, 1999, 14, 517-538.
- [3] Misiura, K. *Mini-Rev. Med. Chem.*, 2006, 6, 395-400.
- [4] Chen, L.; Waxman, D.J. *Curr. Pharm. Design*, 2002, 8, 1405-1416.
- [5] Misiura, K.; Szymanowicz, D.; Kusnierczyk, H.; Wietrzyk, J.; Opolski, A. *Acta Biochim. Pol.*, 2002, 49, 169-176.
- [6] Misiura, K.; Szymanowicz, D.; Wietrzyk, J.; Opolski, A. *Acta Pol. Pharm.-Drug Res.*, 2003, 60, 109-112.

P531

DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION OF NOVEL CALPAIN INHIBITORS.

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Calpains are a family of cystein-proteases involved in multiple cellular processes. Their de-regulation has been implied in several pathologies such as cataracts in humans and altered calpain activity (upregulation) has also been observed in several types of cell death. In mammals, calpain 2 was localized to the acrosomal cap, and the presence of a calpain-calpastatin system in human spermatozoa has been demonstrated.

Growing evidence supporting caspase-independent cell death in testis after traumatic injury and a suggestive result showing that heat-induced apoptosis of mouse meiotic cells is suppressed by ectopic expression of testis specific calpastatin, prompted us to evaluate the role of these enzymes in both physiological and heat-induced apoptosis during puberty.

For drug design purposes, docking experiments on a set of reported ligands of calpains were conducted on a molecular model of calpain 2 (m-Calpain) that was generated by comparative modelling. The obtained interaction models allow the identification of the residues involved in enzyme-ligand molecular interactions, and provide a structural basis for ligand design.

The designed peptide-like ligands were synthesized, characterized, and evaluated in their capacity to inhibit calpain 2 in vitro, using a recombinant calpain from porcine kidney. The most promising compounds were then evaluated in vivo model of heat-induced apoptosis of testis.

Preliminary results obtained by our group have demonstrated that a series of compounds designed by means of an integrated approach comprising bioinformatics, medicinal chemistry and molecular biology techniques, are able to inhibit in vitro calpain 2 activity, and capable to significantly inhibit in vivo apoptosis in rat testis germ cells, suggesting that these chemical entities may have relevant clinical application as a new therapeutics.

References

[1] Goll DE, Thompson VF, Li H, Wei W, Cong J. The calpain system. *Physiol Rev* 2003; 83: 731-801.

[2] Ben-Aharon I, Brown PR, Etkovitz N, Eddy EM, Shalgi R. The expression of calpain 1 and calpain 2 in spermatogenic cells and spermatozoa of the mouse. *Reproduction* 2005; 129: 435-442.

[3] Rojas FJ, Brush M, Moretti-Rojas I. Calpain-calpastatin: a novel, complete calcium-dependent protease system in human spermatozoa. *Mol Hum Reprod* 1999; 5: 520-526.

[4] Somwaru L, Li S, Doglio L, Goldberg E, Zirkkin BR. Heat-induced apoptosis of mouse meiotic cells is suppressed by ectopic expression of testis-specific calpastatin. *J Androl* 2004; 25: 506-513

[5] Strobl S., Fernández-Catalán C., Braun M., Huber R., Masumoto H., Nakagawa K., Irie A., Sorimachi H., Bourenkow G., Bartunik H., Susuki K. and Bode W. The crystal structure of calcium-free m-calpain suggests an electrostatic switch mechanism for activation by calcium. *Proc Nat Acad Sci USA* 2000; 97: 588-592.

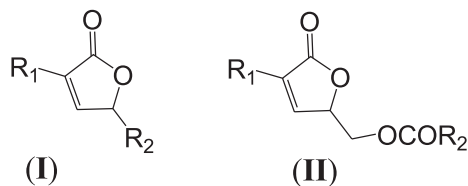
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5-ALKYLIDENE BUTENOLIDES WITH ANTIFUNGAL AND CYTOSTATIC ACTIVITY

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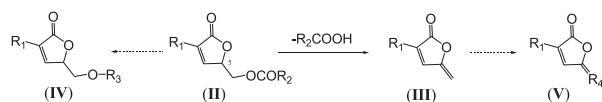
For several years, our research team has been running a broad research programme aimed at design and practical synthesis of new biologically active compounds based on naturally occurring substances. These natural precursors possess initial biological activity. However, they also often display unfavorable properties such as higher cytotoxicity and undesired pharmacokinetic profile. Nonetheless, natural products-driven design can significantly contribute to the enrichment of the existing pool of biologically active substances with new unique pharmacophores of higher structural complexity. Within the framework of this programme, we have extensively studied the modifications of the 3,5-disubstituted butenolide lead structure (I) which resulted in a new class of highly antifungally active compounds (II)^{2,3}.



R₁ = aryl; R₂ = alkyl, subst.alkyl, aryl

As regards possible mechanism of action, the ease of the elimination process of (II) together with the absence of a relationship between chirality at C(5) and fungistatic activity suggest that structure (III) might be the active metabolite^{2,4}. Recent results have also shown that the active substance damages fungal cell membrane⁵. The

above-mentioned results support further development which is directed towards the preparation and screening of new 3-(halogenated phenyl)-5-alkylidene butenolides (IV, V) to confirm the proposed mechanism of action.



R_1 = halogenated phenyl; R_2 = alkyl; R_3 = alkyl, subst.phenyl;
 R_4 = alkyl, subst.alkyl,...

All synthesized compounds were tested against a panel of human pathogenic yeasts and filamentous fungi. MICs of certain derivatives, especially some of (IV), matched those of the parent structure (II).

- [1] Zapf S., Anke T., Sterner O. *Acta Chem. Scand.* 1995, 49, 233-234.
 [2] Pour M., Špulák M., Buchta V., Kubanová P., Vopršalová M., Wsól V., Fáková H., Koudelka P., Pourová H., Schiller R. *J. Med. Chem.* 2001, 44, 2701
 [3] Pour M., Špulák M., Balšánek V., Kuneš J. and Buchta V. *Bioorg. Med. Chem.* 2003, 11(13), 2843.
 [4] Nobilis M., Pour M., Šenel P. *Journal of Chromatography B* 2007, 853(1-2), 10.
 [5] Vale-Silva L. A., Buchta V., Vokurková D. and Pour M. *Bioorg Med. Chem. Lett.* 2006, 16, 2492.

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ADENOSINE A_1 AND A_{2A} RECEPTOR LIGANDS: SYNTHESIS, PHARMACOLOGY AND MOLECULAR MODELLING STUDIES

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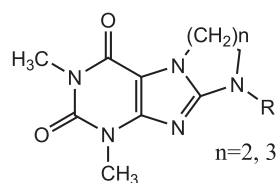
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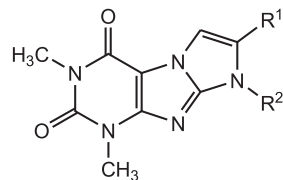
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The physiological effects of extracellular adenosine are mediated by four G protein-coupled receptors: A_1 , A_{2A} , A_{2B} and A_3 adenosine receptors (ARs). Selective A_1 AR antagonists have demonstrated promising therapeutic potential for the treatment of cognitive diseases, renal failure, Alzheimer's disease and cardiac failure. Adenosine A_{2A} receptor antagonists may be useful for the treatment of acute and chronic neurodegenerative disorders such as cerebral ischemia, Parkinson's and Huntington's diseases, as drugs controlling motor function and exhibiting neuroprotective properties.

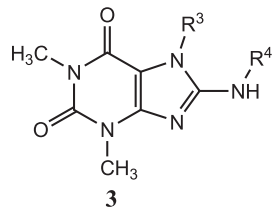
For adenosine A_1 and A_{2A} receptors xanthine derivatives have been designed as potential antagonists, synthesised and studied for their pharmacological activity. Tricyclic annelated xanthines exhibited high submicromolar affinity to the A_1 and A_{2A} ARs. The most active compounds contained a six or seven-membered annelated ring (1) [1-3]. To continue our investigation, xanthine ligands with a five-membered third ring (2) were synthesised and showed good nanomolar activity and selectivity. Moreover, derivatives with an open structure, e.g. 8-aminoxanthines (3), were obtained and displayed high submicromolar affinity and selectivity. X-ray structure analysis was performed for selected compounds.



1



2



3

To understand the selectivity of the obtained compounds and to design derivatives for further synthesis molecular modelling studies have been performed. The ligands were docked into the A_1 and A_{2A} ARs models [4] using CAChe 7.5 software with flex-flex docking module. Moreover, the spatial properties of the molecules were studied in order to explain the differences in the affinity of the isomeric compounds.

Acknowledgement

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- [1] A. Drabczynska, C.E. Müller, S.K. Lacher, B. Schumacher, J. Karolak-Wojciechowska, A. Nasal, P. Kawczak, O. Yuzlenko, E. Pekala, K. Kiec-Kononowicz. *Synthesis and biological activity of tricyclic aryl-imidazo-, pyrimido-, and diazepinopyrimidones*. Bioorg. Med. Chem. 2006, 14, 7258-7281.
- [2] A. Drabczynska, C.E. Müller, J. Karolak-Wojciechowska, B. Schumacher, A. Schiedel, O. Yuzlenko, K. Kiec-Kononowicz. *N9-Benzyl-substituted 1,3-dimethyl- and 1,3-dipropyl-pyrimido[2,1-f] purinediones: synthesis and structure-activity relationships at adenosine A₁ and A_{2A} receptors*. Bioorg. Med. Chem. 2007, 15, 5003-5017.
- [3] A. Drabczynska, C.E. Müller, A. Schiedel, B. Schumacher, J. Karolak-Wojciechowska, A. Fruszinski, W. Zobnina, O. Yuzlenko, K. Kiec-Kononowicz. *Phenylethyl-substituted pyrimido[2,1-f]purinediones and related compounds: structure-activity relationships as adenosine receptor ligands*. Bioorg. Med. Chem. 2007, 15, 6956-6974.
- [4] O. Yuzlenko, K. Kiec-Kononowicz. *Molecular modelling of A₁ and A_{2A} adenosine receptors: Comparison of rhodopsin- and α_2 -adrenergic-based homology models through the docking studies*. J. Comput. Chem. 2008, DOI: 10.1002/jcc.21001

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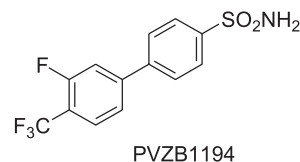
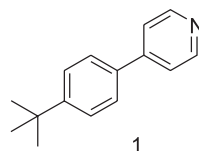
BIS(HTERO)ARYL DERIVATIVES AS UNIQUE KSP INHIBITOR

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Mitotic kinesins play essential roles for the formation and functioning of the mitotic spindle, chromosome segregation and transportation, mitotic checkpoint control and cytokinesis. Therefore, targeting mitotic kinesins is endeavoured in drug discovery field. Among mitotic kinesin inhibitors, a large number of kinesin spindle protein (KSP, also known as human Eg5) inhibitors have been reported and five inhibitors are advanced to clinical trials.

We had also identified compound 1 as a KSP inhibitor from our chemical library [1]. Structural modifications for both (hetero)aromatic rings provided several potent inhibitors. Among them, PVZB1194 displayed potent KSP ATPase inhibition ($IC_{50} = 0.12 \mu M$), antiproliferative activity ($IC_{50} = 5.5 \mu M$) and mitotic arrest ($IC_{50} = 9.4 \mu M$) with monoastral phenotype in HeLa cells. Interestingly, this molecule revealed a unique property, namely weak inhibition in the absence of microtubules and distinct KSP localization in mitosis from monastrol. Our observation indicates that the inhibitory mechanism may be possibly different from that by most of other KSP inhibitors. In this symposium, we will present design, synthesis, SAR and biological effects of PVZB1194 analogues.



- [1] J.-i. SAWADA *et al.*, Screening for mitotic kinesin KSP inhibitors: Implication of the microtubule binding regions of KSP motor domain as drug target. 18th EORTEC-NCI-AACR symposium on molecular targets and cancer therapeutics. (Prague) Poster 311, November 9, 2006.

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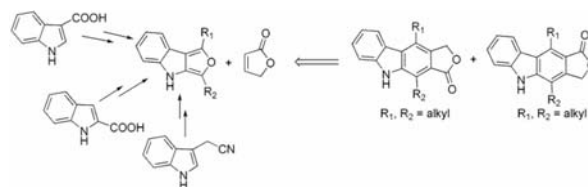
SYNTHESIS AND ANTITUMOR ACTIVITY OF NEW ELLIPTICINE DERIVATIVES CONTAINING A BUTIROLACTONE SUBUNIT

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Ellipticine and several of its derivatives isolated from *Apocyanaceae* plants are natural products with significant antitumor activity by different mechanisms of action. Ellipticine is a polycyclic molecule that exhibits cytotoxic activity against several cancer cells.¹⁻² They structure have been widely modified in order to obtain more efficacious, selective and less toxic new compounds.³

A new series of condensed isobenzofuro-indoles has been prepared and their potential antitumor activity was examined. These compounds have been obtained by Diels-Alder cycloaddition of the furo-indole diene with the 2(5H)furanone.



Different synthetic pathways for the preparation of the intermediate diene have been proposed. The most convenient synthesis starts from the indole-2-carboxylic acid requiring 6 reaction steps to afford the diene. It is clear from the experiments realized that indole derivatives are very sensitive and the synthesis laborious.

References

- [1] M. Stirová, C. A. Bieler, M. Wiessler, E. Frei. *Biochem. Pharmacol.* 2001, 62, 1675-1684.
- [2] E. Tian, T. H. Landowski, O. W. Stephens, S. Taccoby, B. Barlogie, J. D. Shaughnessy. *Mol. Cancer Ther.* 2008, 7, 500-509.
- [3] M. Romero, P. Renard, D.-H. Caignard, G. Atassi, J. Solans, M. D. Pujol. *J. Med. Chem.* 2007, 50, 294-307.

P536

SYNTHESIS AND ANTIMYCOBACTERIAL ACTIVITY OF NEW HYDRAZONOFORMAMIDE DERIVATIVES OF ISONIAZID

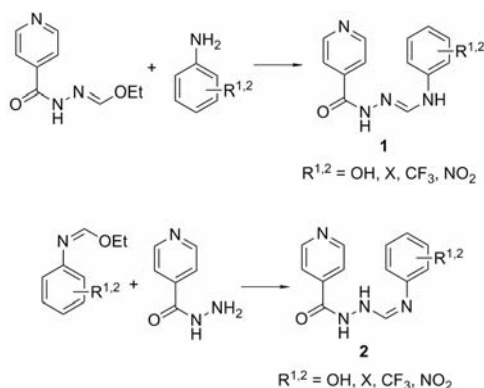
Eva Vavriková^a, Jarmila Vinsova^a, Slovenko Polanc^b, Juana Monreal Ferriz^a, Ales Imramovsky^a

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Mycobacterium tuberculosis (*M. tbc.*) is an etiological agent of tuberculosis (TB), one of the most dangerous infectious diseases in the world. Recently, it became the serious problem due to multi-drug-resistant TB and also dormant forms which are resistant to anti-tuberculosis drugs. Globally, 9.2 million new cases and 1.7 million deaths from TB occurred in 2006 [1]. Isoniazid is the most widely used anti-tuberculosis drugs with MIC 0.01 – 0.25 µg/ml against the most susceptible strain *M. tbc.* In the bacteria cells, INH is activated by catalase–peroxidase enzyme to a range of reactive species or radicals that attack multiple targets, e.g., mycolic acid synthesis (InhA, KasA), DNA damage, lipid peroxidation and NAD metabolism in cell. [2].

We have synthesized prodrug forms of isoniazid with disubstituted anilines connected by CH bridge [3]. Two different kinds of reactions were used. Activated INH gave hydrazones **1** and ethyl *N*-substituted phenylformimidate yielded derivatives **2**. The substituents were chosen on the basis of QSAR calculation [4].



The synthesis, general characteristics, physical and antimycobacterial activity evaluation against *Mycobacterium tuberculosis* and three non-tuberculosis strains will be presented.

The study was supported by MSM 0021620822 and GAUK 285/2006/B-CH/FaF.

References

- [1] Global tuberculosis control 2008 – surveillance, planning, financing: WHO report 2008
- [2] Shi R., Itagaki N., Sugawara I. Overview of anti-tuberculosis drugs and their resistance mechanismus. Mini-Reviews in Med. Chem. 7, 1177 (2007)
- [3] Imramovský A., Polanc S., Vinšová J., Kočevár M., Jampílek J., Recková Z., Kaustová J.: A new modification of anti-tubercular active molecules. Bioorg. Med. Chem. 15, 2551-2559 (2007).
- [4] Vinšová, J.; Imramovský, A.; Jampílek, J.; Monreal, J. F.; Doležal M.: Recent Advances on Isoniazide Derivatives. Anti-Infective Agents in Medicinal Chemistry 7(1), 1-20 (2008).

P537

SYNTHESIS, STABILITY AND ANTIMYCOBACTERIAL ACTIVITY EVALUATION OF ALIPHATIC CARBAMATES OF SALICYLANILIDES AS PRODRUG FORMS

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Salicylanilides have shown significant in vitro antimycobacterial activity against *Mycobacterium tuberculosis* and non tuberculosis strains *M. avium* and *M. kansasii* which are resistant to the first-line drug, isoniazid [1,2]. The presence of phenolic hydroxyls in salicylanilides seems to be necessary for the activity and is probably responsible for oxidative phosphorylation cleavage [3] but also adds irritative properties to the compounds. Our strategy in the design of potential antimycobacterial active agents has been mainly oriented towards the synthesis of the prodrug form of the most anti-tuberculosis active salicylanilides, by attaching different aliphatic isocyanates to form carbamates as it was described in literature for protection of other phenolic drugs [4].

The starting salicylanilides were prepared by the reaction of substituted salicylic acids with the appropriate anilines in chlorobenzene with PCl₃ in micro-wave reactor.

Corresponding carbamates were prepared by the treatment of the synthesized salicylanilide with equivalent of triethylamine and the appropriate isocyanate (Figure 1).

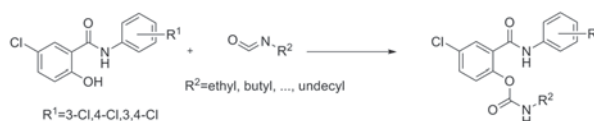


Figure 1. Synthesis of salicylanilide carbamates

Synthesis, stabilities, activities and toxicity of the series of the synthesized carbamates will be presented.

This work was financially supported by the Research project MSM 002162082 and FRVŠ 1079/2008/G6

References

- [1] Vinšová J., Imramovský A: *Ces. Slov. Farm.* **53**, 294 (2004).
- [2] Waissner K., Bureš O., Holý P., Kuneš J., et al.: *Arch. Pharm.* **336**, 53 (2003).
- [3] Terada H., Goto S., Yamamoto K., Takeuchi I., Hamada I., Miyake K., *Biochim. Biophys. Acta*, **936**, 504 (1988).
- [4] Hansen. J, Mørk. M, Bundgaard. H: *Int. J. Pharm.*, **81**, (2-3), 253-61, (1992)

P538

SYNTHESIS AND CYTOTOXICITY OF METAL 8-QUINOLINETHIOLATES

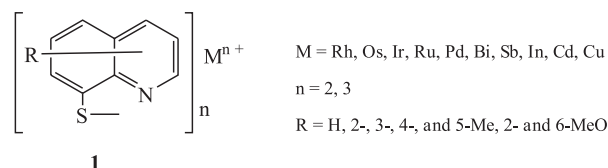
Edmunds Lukevics^a, Irina Shestakova^a, Daina Zaruma^b, Yanis Ashaks^b

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A series of metal 8-quinolinethiolates **1** has been prepared. Their cytotoxicity on tumour cell lines HT-1080 (human fibrosarcoma) and MG-22A (mouse hepatoma) has been investigated.

It has been found that the position of the substituent and nature of the metal essentially influenced the cytotoxicity and toxicity of the compounds **1**. The 6-methoxy derivatives of Rh, Os, Ir, Bi, Sb and In exhibited the highest cytotoxicity on tumour cells ($LC_{50} < 1 \mu\text{g ml}^{-1}$) but they were also highly toxic for normal fibroblasts of mice embryos NIH 3T3. The lowest cytotoxicity on all cells was observed for 2-substituted complexes. The best cytoselective toxicity was found for 5-methyl derivatives, e.g. the Ir complex had LC_{50} 5-7 $\mu\text{g ml}^{-1}$ for tumour cells and 220 $\mu\text{g ml}^{-1}$ for normal fibroblasts (LD_{50} 1785 mg kg^{-1}).



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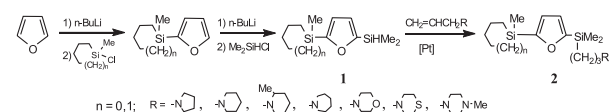
APPLICATION OF HYDROSILYLATION FOR THE PREPARATION OF HETEROCYCLIC ANTICANCER AMINES

Luba Ignatovich, Ilze Sleiksha, Irina Shestakova, Ilona Domracheva, and Edmunds Lukevics

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Highly cytotoxic amino derivatives of 2-(1-methylsilacyclohexyl/pentyl)-5-dimethyl-silylfuran (LC_{50} 1-3 $\mu\text{g/ml}$) have been prepared by hydrosilylation reaction of heterocyclic N-allyl amines with corresponding hydrosilane in the presence of platinum catalysts. The effects of the amines and the silacycle were examined to establish structure-activity relationships. The starting 2-(1-methylsilacycloalkyl)-5-dimethylsilylfurans (**1**, n = 0, 1) have been prepared from the furan by two consecutive organolithium syntheses. They have been used for the hydrosilylation of heterocyclic allyl amines in the presence of platinum catalysts.

The reaction afforded a series of amines **2** containing three different heterocycles in good yield. The cytotoxicity of amines **2** on tumour cells HT-1080, MG-22A and normal mouse fibroblasts 3T3 has been investigated *in vitro*. All studied compounds exhibited high cytotoxic activity (LC_{50} ~1-6 $\mu\text{g/ml}$). In morpholino and perhydroazepino series the silacyclohexyl derivatives were more active than analogous silacyclopentyl derivatives.



P540

THE USE OF NOVEL FLOW REACTOR OPERATING UNDER SUPERCRITICAL CONDITIONS

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Among the many advantages attributed to flow reactors, highly efficient heat exchange is one of the most important. The ability to heat reactions to high temperatures in seconds and accurately control the set temperature is essential for maintaining high reaction rates and selectivity. At ThalesNano, we have created a microfluidic reactor called the X-Cube FlashTM capable of heating and pressurizing reactions up to 350°C and 200 bar respectively in seconds what conditions are above the critical temperature and pressure points of many compounds.

The system can perform different homogeneous reactions from simple alkylation and esterification to Diels-Alder reactions. In addition reactions what can be carried out only just in the presence of catalysts under batch condition can be successfully performed without any catalysts needed in the X-Cube FlashTM eliminating the necessary purification/scavenging step and the presence of catalyst residue what is a key factor in pharmaceutical research.

The presentation will detail the results from these experiments with comparisons to conventional batch and microwave reactions. Flow reactors can improve upon the yield and reaction time generated through microwave and conventional heating and therefore to expand the X-Cube FlashTM can offer an efficient and practical solution for the medicinal chemical space.

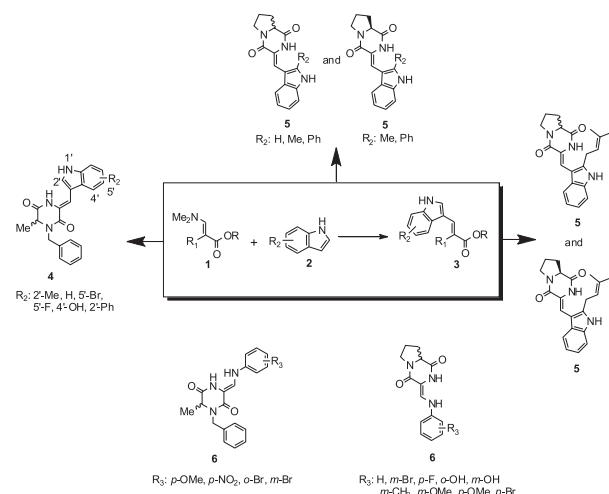
P541

DIMETHYLAMINOENAMINONES IN THE SYNTHESIS OF SOME CYCLODIPEPTIDE ALKALOID ANALOGUES

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3-Dimethylaminomethylidenepropenoates (dimethylaminoenaminones) **1** were already successfully used as versatile reagents in the synthesis of various heterocyclic systems and some natural product analogues.¹ Especially interesting is the coupling reaction of such enaminones **1** with 3-unsubstituted indole derivatives **2**, producing useful intermediates **3** in the synthesis of α,β -unsaturated tryptophan (Δ tryptophan) analogues, thus representing a good alternative for the Horner-Wadsworth-Emmons olefination. With this in mind we have prepared various analogues of natural cyclodipeptides consisting of Δ tryptophan such as dipodazine **4**² and tryprostatin B **5**³ analogues, and some unnatural cyclodipeptides with the arylamino moiety **6**.



References

- [1] B. Stanovnik, J. Svete, *Chem. Rev.* 2004, **104**, 2433-2480.
- [2] J. Waggener, D. Bevk, A. Meden, J. Svete, B. Stanovnik, *Helv. Chim. Acta* 2006, **89**, 240-248.
- [3] a) J. Waggener, U. Grošelj, A. Meden, J. Svete, B. Stanovnik, *Tetrahedron* 2008, **64**, 2801-2815, b) J. Waggener, J. Svete, B. Stanovnik, *Synthesis* 2008, DOI: 10.1055/s-2008-1072515.

P542

PYRIDYL- AND PYRIMIDINYL-SUBSTITUTED PYRROLE, THIOPHENE AND FURAN DERIVATIVES AS CDC7 KINASE INHIBITORS

Alessandra Cirila, Marina Caldarelli, Antonella Ciavolella, Valter Croci, Antonella Ermoli, Barbara Forte, Antonella Isacchi, Maria Menichincheri, Antonio Molinari, Alessia Montagnoli, Enrico Pesenti, Antonio Pillan, Sonia Rainoldi, Corrado Santocanale, Alessandra Scolaro, Marco Tatò, Barbara Valsasina, Ermes Vanotti and Daniele Volpi

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The cell division cycle Cdc7 kinase is an essential protein that promotes DNA replication. Inhibition of this kinase causes p53 independent tumor cell death while normal cells are capable of surviving to Cdc7 inhibition for long periods of time.¹ For this reason pharmacological inhibition of Cdc7 can support novel strategy for the development of oncologic therapeutics. SAR studies led to a basic scaffold able to provide a good activity and selectivity. Expansion of this class identified potent available compounds with good antitumor activity *in vivo* at tolerated doses.

- [1] Montagnoli, A.; Tenca, P.; Sola, F.; Carpani, D.; Brotherton, D.; Albanese, C.; Santocanale, C., *Cancer Res.* 2004, **64**, 7110-7116.

P545

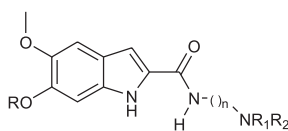
INDOLIC AND QUINOLINIC C2-CARBOXAMIDES: DESIGN, SYNTHESIS, CYTOTOXIC AND CYTOSTATIC POTENCY

Andrew Tsotinis^{1*}, Anna Klouvidaki¹, Gregory Sivolapenko², Efstathia Giannopoulou³, Katerina Kassela², Spiros Karathanos², Haralambos Kalofonos³, Christos Roussakis⁴

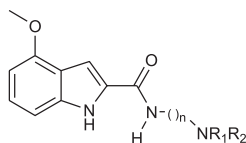
¹Faculty of Pharmacy, Department of Pharmaceutical Chemistry, University of Athens, Panepistimioupoli-Zografou, 157 71 Athens-Greece; ²University of Patras, School of Health Sciences, Department of Pharmacy, University Campus, Rion, Patras 26500, Greece; ³University of Patras, School of Health Sciences, Department of Medicine, University Campus, Rion, Patras 26500, Greece; ⁴ISOMER (Institut des substances et organismes de la mer), Laboratoire de Pharmacologie Marine, Faculté de Pharmacie, Nantes, France

Suitably substituted indoles and quinolines constitute the principal subunits of many linear and angular aromatic nuclei with chemotherapeutic properties [1]. Previously, we and others have published the synthesis of C5,C6-disubstituted 1*H*-indole-2-carboxamides (**I**), which by forming molecular complexes with DNA, inhibit its processing. As a result of this action, these probes display a noteworthy cytotoxicity in the NSCLC-N16-L16 carcinoma cell and have been identified as potential lead compounds for further development [2]. Based on the interesting cytotoxic profile of analogues (**I**) we present herein the design and synthesis of a new series of indolic (**II**) and quinolin-

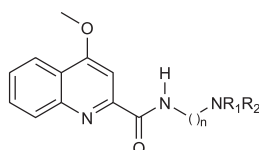
ic (III) C2-carboxamides, which constitute structurally related congeners of (I).



(I)



(II)



(III)

The synthesis of (II) involved treatment of commercially available 2-methoxybenzaldehyde with the methyl ester of azidoacetic acid to give 2-azido-3-(2-methoxy)propenoic methyl ester, which was cyclised in hot xylene to 4-methoxy-1*H*-indole-2-carboxylic acid methyl ester. The latter was saponified to the corresponding acid, which was converted to the desired compounds (II) by amidation with the appropriate amines in the presence of CDI in DMF. The new analogues (III) were prepared by amidation of 4-methoxy-2-quinolinecarboxylic acid with the requisite 1° amines in the presence of CDI in DMF. All of the new analogues were evaluated for *in vitro* cytotoxicity and cytostatic activity in the cancer cell lines Caco-2, DLD-1, HT-29, MCF-7, MDA-MB-468 and NSCLC-N16-L16 and found to have noteworthy potencies.

Acknowledgement

The Athens group is indebted to ELKE/UA for financial support via ELKE's Research Programme KA: 70/3/7836.

References

- [1] Sato, A.; McNulty, L.; Cox, K.; Kim, S.; Scott, A.; Daniell, K.; Summerville, K.; Price, C.; Hudson, S.; Kiakos, K.; Hartley, J. A.; Asao, T.; Lee, M. A Novel Class of *in Vivo* Active Anticancer Agents: Achiral *seco*-Amino- and *seco* Hydroxycyclopropylbenz[e]indolone (*seco*-CBI) Analogues of the Duocarmycins and CC-1065. *J. Med. Chem.*, 2005, 48, 3903-3918 and references cited therein.
- [2] Tsonitis, A.; Gerasimopoulou, M.; Vlachou, M.; Moreau, D.; Roussakis, C. C5,C6-Disubstituted 1*H*-indole-2-carboxamides: Synthesis and cytotoxic activity in the human non-small lung cancer cell line NSCLC-N16-L16. *Lett. Drug Des. Discov.*, 2006, 3, 14-16.

P546

THE ROLE OF MEDICINAL CHEMISTRY IN POST-GRADUATED INDUSTRIAL PHARMACY STUDIES ON THE EUROPEAN LEVEL

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Pharmacy, biology, chemistry and other life sciences graduated are potential employees for the pharmaceutical industry. But performed job market analyses show that they are often unprepared for such a challenge. Therefore a necessity arises for postgraduate studies in industrial pharmacy which appropriate curricula suitable for completion of a specific knowledge required in this field.

In order to fill this gap an attempts to organize an international project aimed at improving the quality and effectiveness of postgraduate studies in industrial pharmacy in European Union were undertaken. In this project, the Faculties of Pharmacy of the Jagiellonian University Medical College, Kraków, Poland, of the Universities of Montpellier, France, Barcelona, Spain and Hamburg, Germany and the Anpharm Pharmaceutical Joint-Stock Company, Warszawa, Poland are involved [1].

Medicinal Chemistry is an important part of the proposed curriculum of industrial pharmacy for them who are interested in working in the research and development units. The successful participant who completes this course shall be able to know: drug classification, target of drug action, methods of pharmacological screening, structure-activity relationships, physicochemical properties of drug, methods of search for new drugs, interpretation of basic pharmacological *in vivo* and *in vitro* tests, utilize programmes in structure-activity studies, find needed information for search for new drug studies.

In order to achieve the proposed goal of medicinal chemistry curriculum the following methods are involved: lectures, seminars, panel discussions, on-line courses, laboratories, workshops.

Acknowledgement

The study is supported by the Leonardo da Vinci project PL/06/B/F/PP/174049

References

- [1] <http://papaver.farmacja.cm-uj.krakow.pl/leonardo/>

P548

HERG POTASSIUM CHANNEL AND DRUG TRAPPING: BINARY QSAR AND INSIGHTS FROM PROPAFENONE DERIVATIVES

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^aEmerging Field Pharmacoinformatics, Department of Medicinal Chemistry; and ^bDepartment of Pharmacology, University of Vienna, Althanstrasse 14, 1090 Vienna, Austria

Acquired long QT syndrome causes severe cardiac side effects and represents a major problem in clinical studies of drug candidates. One of the reasons for development of arrhythmias related to long QT is inhibition of the human ether-a-go-go-related gene (HERG) potassium channel. Hence, there is increasing interest in computational models predicting HERG affinity in an early phase of the drug discovery and development process. However, recent studies on HERG functions showed that the HERG K⁺ channel is not only an antitarget (off-pharmacology) but also a drug target, especially in oncology and cardiology. The inner cavity of the HERG channel composes a promiscuous binding site and is capable of trapping diverse compounds in the closed channel state.

In this study we report a binary QSAR approach for classification of HERG trapped/non trapped compounds which combines ligand-based with structure-based approaches. Based on a dataset of 18 'trapped' and 'non trapped' HERG compounds, a binary QSAR model was generated using a set of parameters derived from ligand docking together with molecular descriptors calculated from the chemical structures using the software package MOE. Docking studies provided the calculated dock_pKi and an entropic term related to the number of ligand atoms immobilized during binding. In leave one out cross-validation runs, a total accuracy of 0.85-0.87 was obtained. Furthermore, docking runs provided insights into ligand binding modes at the open and closed states of the HERG channel which might explain differences in HERG potency and drug trapping.

Acknowledgments

Khac-Minh Thai is grateful for financial support provided by the ASEA-Uninet and the ÖAD. This work was supported by the University of Vienna under the framework of the PhD programme "Molecular Drug Targets".

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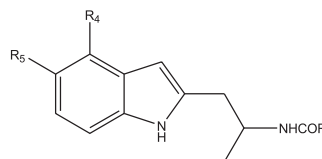
C-2- α C SUBSTITUTED INDOLE ALKYLAMIDES: DESIGN, SYNTHESIS, MELATONINERGIC ACTIVITY

Andrew Tsotinis^{1*}, Vasiliki Belekou¹, David Sugden²

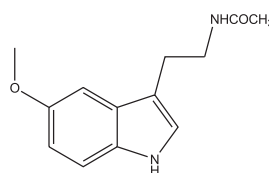
¹Faculty of Pharmacy, Department of Pharmaceutical Chemistry, University of Athens, Panepistimioupoli-Zografou, 157 71 Athens-Greece

²Division of Reproduction and Endocrinology, School of Biomedical and Health Sciences, King's College London, London Bridge, London SE1 1UL-UK

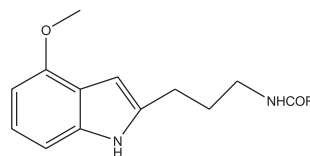
A series of new C2-substituted 5- and 4-methoxytryptamides (I), (II) and (IV) have been prepared and tested for their melatonineric activity. Conformational constraint at C-2- α C seems to decrease the population of the preferred conformation for agonism, as it renders the respective analogues of melatonin (III) receptor partial agonists [1-2].



(I): R₄=H; R₅=OMe; (II): R₄=OMe; R₅=H



(III)



(IV)

For the synthesis of analogues (I), commercially available 5-methoxyindole-2-carboxylic acid or its 4-methoxy congener (precursor of analogues (II) and (IV)) was converted by standard chemistry to the respective x-methoxyindole-2-carboxaldehyde. The latter by the sequence of the Henry (nitroethane and ammonium acetate), or Wittig reaction (analogues IV), reduction with lithium aluminum hydride and subsequent acylation with the appropriate acid anhydride or chloride led to the formation of the target molecules (I), (II) and (IV).

The melatonineric potency of the new analogues was assessed in a well-established, specific model of melatonin action, the pigment aggregation response of *Xenopus laevis* melanophores. Analogues (I) are agonists in this assay, whereas their C4-OMe congeners (II) are partial agonists and so are compounds (IV). Binding studies on all new analogues on the recombinant human MT₁ and MT₂ subtypes expressed in NIH 3T3 cells, are currently underway.

Acknowledgement

The Athens group is indebted to ELKE/UOA for financial support via ELKE's Research Programme KA: 70/3/7836.

References

- [1] Andrew Tsotinis, Margarita Vlachou, Andreas Eleutheriades, Effie Prinea, Darren Ebreo, Muy-Teck Teh and David Sugden, *Chem. Pharm. Bull.*, 2002, 50, 31-39.
- [2] A. Tsotinis, M. Vlachou, D. P. Papahatjis, T. Calogeropoulou, S. P. Nikas, P. J. Garratt, V. Piccio, S. Vonhoff, K. Davidson, M. T. Teh, D. Sugden, *J. Med. Chem.*, 2006, 49, 3509-3519.

P550

INVESTIGATION OF SYNTHESIS OF (2R,3S)-3-PHENYLISOSERINE HYDROCHLORIDE - SIDE CHAIN OF PACLITAXEL

Barbara Kakol, Magdalena Jezierska-Zieba, Michal Fedorynski, Jacek Cybulski,

Industrial Chemistry Research Institute, Rydygiera 8 St., 01-793 Warsaw, Poland

(2R,3S)-3-phenylisoserine hydrochloride is an α -amino- β -hydroxyacid, which constitutes part of greater molecule commonly known as *Paclitaxel*. *Paclitaxel* is a bioactive substance derived from *Taxus brevifolia* that grows off coasts of the Pacific Ocean in the USA. This compound shows high activity in treatment of the ovary cancer, the nipple and lungs. Isolation of this medicine from natural sources has not been applied in practice so far. Total synthesis of it was not realized till now. The best method of preparation of *Paclitaxel* seems to be the semi-synthesis method. This method consists in isolation of fragment of *Paclitaxel* molecule from needles of *Taxus baccata* diterpene, so called 10-deacetylbaccatine III, and its condensation with *N*-benzoyl-(2R,3S)-3-phenylisoserine.

The technology of (2R, 3S)-3-phenylisoserine hydrochloride production has been elaborated and implemented at the Industrial Chemistry Research Institute.

The six step process is realized without separation and purification of intermediate products. This results in significant simplification of the process and at the same time enables to obtain product of high quality and enantiomeric purity (*ee* 99%). A method of utilization of solid and liquid wastes formed during the synthesis of (2R,3S)-3-phenylisoserine hydrochloride has been developed.

The method and conditions for analysis of (2R, 3S)-3-phenylisoserine hydrochloride by high-performance liquid chromatography (HPLC) were elaborated.

We tested five analytical columns. The isocratic elution of separated compounds was carried out by mobile phase – methanol/sodium phosphate buffer 90:10 v/v, using constant flow rate 1,0 cm³/min. Chromatographic analysis was performed on a Merck LaChrom D-7000 Liquid Chromatograph (detector UV, λ =210nm).

The influence of solvent, pH and ionic strength of mobile phase on separation and peak symmetry was also studied.

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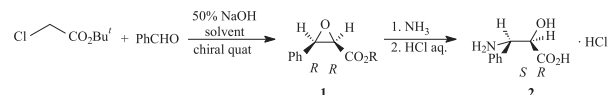
INVESTIGATION OF SYNTHESIS OF 3-PHENYLGLYCIDIC ACID ESTERS – A PACLITAXEL PRECURSORS

Magdalena Jezierska-Zieba, Michal Fedorynski, Barbara Kakol, Jacek Cybulski

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Esters of (2R,3R)-3-phenylglycidic acid **1** are important intermediates in the synthesis of *Paclitaxel* and *Diltiazem*. The Darzens reaction (condensation of α -halogenomethyl carbonyl compounds with aldehydes or ketones, carried out in the presence of a base) is one of the most powerful methods for the synthesis of these compounds.

Among many base/solvent systems, which can be used for this purpose, the most convenient seems to be concentrated aqueous solution of alkali metal hydroxide in the presence of quaternary ammonium salt (quat) as a catalyst (PTC – phase transfer catalysis¹).



Chiral Darzens condensations have been investigated using different substrates and PTC conditions. However, to our best knowledge high levels of stereoselectivity were obtained using α -halo ketones, α -halo sulfones and α -haloacetamides, but not α -haloacetic esters as substrates².

In order to obtain compound **1**, we studied Darzens reaction between alkyl chloroacetates and benzaldehyde carried out under PTC conditions in the presence of chiral quats.

We tested many chiral quats derived from cinchona alkaloids, the influence of the solvent, temperature and the kind of alkali metal hydroxide on the stereoselectivity of Darzens reaction between *t*-butyl chloroacetate and benzaldehyde carried out under PTC conditions.

References

- [1] For recent reviews see: E. V. Dehmlow, S. S. Dehmlow, *Phase-Transfer Catalysis*, 3rd Ed., Verlag Chemie, Weinheim, 1993; M. M?kosza, M. Fedory?ski, *Catal. Rev.*, 2003, 45, 321.
- [2] Recent review: T. Ooi, K. Maruoka, *Angew. Chem. Int. Ed.*, 2007, 46, 4222.

P552

VIRTUAL SCREENING FOR NEW INHIBITORS OF THE ENZYME ENOYL-ACP-REDUCTASE INHA OF MYCOBACTERIUM TUBERCULOSIS

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Mycobacterium tuberculosis is known to be a defensive fortress hard to be taken. One of the essentials of the bacterial defense system is the cell wall. Due to its structural properties rarely a compound is able to intrude: it is a bilayer of peptidoglycans, and also consists of multiple sheets of lipids and mycolic acids. Additionally, porins are present in the mycobacterial cell wall only in very small amounts insufficient to significantly facilitate small molecule transport [1].

Here, the focus is on the enzyme enoyl-ACP-reductase InhA. It is part of the fatty acid synthesis type II (FAS II) system and catalyzes the reduction of enoyl-acyl-[acyl-carrier-protein] derivatives of carbon chain length 4 to 16 using the essential cofactor NADH. When inhibited, unsaturated fatty acids accumulate, the formation of the cell wall is disturbed, the bacterial defense is weakened, and the organism eventually dies.

Computational methods have been applied in a step-wise fashion in order to search for new InhA inhibitors. Starting with a ligand-based approach, known inhibitors from the pyrrolidine carboxamide (PC) class [2] were chosen to define a first pharmacophore model. Screening a small part of the ZINC database validated the model by retrieving compounds similar to the PCs, and gave valuable hints with respect to new structurally desirable features. These results were taken into account in the subsequent target-based pharmacophore derivation. The binding site was subjected to a GRID analysis with several probes, and pharmacophore models were built with MOE based on this analysis and on the results of the ligand-based approach. For screening purposes a conformational database of the 'drug-like' subset of the ZINC database was created; special constraints were applied to take into account the pharmacokinetic requirements for *mycobacterial* bioavailability. Hits of the pharmacophore screening were visually inspected and the most suitable candidates selected for docking with the program AutoDock. To validate the scoring and ranking, known active compounds were included in the docking process. The most promising compounds were chosen for further studies and experimental testing of their inhibitory activity.

References

- [1] X. Hong, A. J. Hopfinger, *Biomacromolecules*, 2004, 5, 1052-1065
- [2] X. He, A. Alian, R. Stroud, P. Ortiz de Montellano, *J. Med. Chem.*, 2006, 49, 6308-6323

P553

SYNTHETIC PHOSPHATIDYLINOSITOL MANNOSIDE ANALOGUES: POTENT VACCINE ADJUVANTS

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It is now well recognized that effective vaccines against pathogens like *Mycobacterium tuberculosis*, HIV, HCV and *Plasmodium falciparum* require the activation of various components of the immune response. For example, induction of humoral and T cell responses are both likely to be important. Purified protein antigens require adjuvantation to convey adequate protection because they are generally poor immunogens. Adjuvants like monophosphoryl lipid A (MPL) and saponin extract QS21 are receiving considerable attention because they elicit potent cell-mediated immune reactions that contribute significantly to vaccine effectiveness.¹

This work describes the synthesis and adjuvant activity of various phosphatidylinositol mannoside (PIM) analogues. PIMs are mycobacterial cell wall components that are known to possess various immunomodulating activities. Key synthetic steps described will include selective glycosylation and phosphorylation reactions including the unexpected formation of cis-mannosides containing an O-2 directing group.^{2,3} The synthesis of a carbonyl containing PIM analogue will also be described.⁴ Specific PIM analogues were shown to be more potent *in vitro* and more effective *in vivo* than MPL.⁵

- [1] Vandepapeliere, P.; Horsmans, Y.; Moris, P.; Van Mechelen, M.; Janssens, M.; Koutsoukos, M.; Van Belle, P.; Clement, F.; Hanon, E.; Wettendorff, M.; Garcon, N.; Leroux-Roels, G. Vaccine Adjuvant Systems containing monophosphoryl lipid A and QS21 induce strong and persistent humoral and T cell responses against hepatitis B surface antigen in healthy adult volunteers, *Vaccine*, 2008, 26, 1375.
- [2] Ainge, G. D.; Parlane, N.; Denis, M.; Hayman, C. M.; Larsen, D. S.; Painter, G. F. Phosphatidylinositol Mannosides: Synthesis and Adjuvant Properties of Phosphatidylinositol di- and tetramannosides, *Bioorg. Med. Chem.* 2006, 14, 7615.
- [3] Ainge, G. D.; Parlane, N. A.; Denis, M.; Harer, A.; Hayman, C. M.; Larsen, D. S.; Painter, G. F. Phosphatidylinositol Mannoside Ether Analogues: Synthesis and Interleukin-12 (IL-12) Inducing Properties, *J. Org. Chem.* 2007, 72, 5291.
- [4] Painter, G. F.; Ainge, G. D.; Gibson, D.; Gulab, S. A. Analogues of Phosphatidylinositol Mannosides, *Unpublished Results*.

- [5] Parlane, N. A.; Denis, M.; Severn, W. B.; Skinner, M. A.; Painter, G. F.; La Flamme A. C.; Ainge G. D.; Buddle B. M.; Larsen, D. S. Purified and synthetic phosphatidylinositol mannosides are efficient mucosal adjuvants, *Immunological Investigations*, 2008, 37, 129.
- [6] Wedlock, N.; Denis, M.; Painter, G. F.; Ainge, G. D.; Vordermeier, M.; Hewinson, G.; Buddle, B. M. Protection against bovine tuberculosis induced by BCG is enhanced by co-administration of adjuvanted mycobacterial protein vaccine, but abrogated by co-administration of adjuvant alone, *Clinical and Vaccine Immunology*, accepted for publication March 2008.

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IDENTIFICATION OF NOVEL BRAIN PENETRANT PPAR γ PARTIAL AGONISTS

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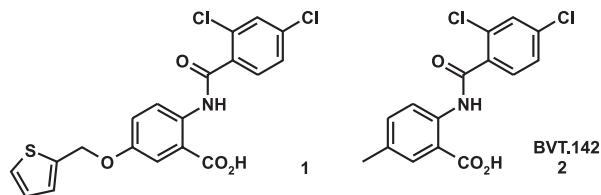
Neurosciences Centre of Excellence for Drug Discovery, GlaxoSmithKline, New Frontiers Science Park, Third Avenue, Harlow, UK, CM19 5AW

PPAR γ is one of a family of peroxisome proliferator activating receptors (PPAR). These ligand gated receptors are expressed in cells throughout the body and act as nuclear transcription factors that play a major role in cell metabolism (particularly lipid and glucose) and cell differentiation.¹ PPAR γ activation is known to increase insulin sensitivity and the PPAR γ agonists rosiglitazone and pioglitazone are marketed drugs for the treatment of type II diabetes.

Alzheimer's Disease (AD) is the most common cause of dementia, and is a devastating disease that is increasingly affecting ageing populations. The disease affects over 30 million people worldwide, with forecasts suggesting this will quadruple by the year 2047.²

Exploratory analyses from a recent clinical study in AD patients suggested that non-carriers of the apolipoprotein E (APOE) ϵ 4 gene exhibited cognitive and functional improvement in response to rosiglitazone treatment when compared to placebo.³

It has been demonstrated that PPAR γ is expressed in the human central nervous system (CNS).⁴ We were interested in understanding the role of PPAR γ in the CNS and in identifying novel series of PPAR γ partial agonists with good brain penetration which would allow greater access to the target within the CNS. As such, we instigated some work around the Biovitrum compound **1** an analogue of the PPAR α/γ agonist BVT.142 (**2**).⁵ The carboxylic acid moiety is known to be generally restrictive when attempting to gain access to the CNS and we were interested in identifying replacements for this group that retained PPAR γ activity but would allow increased brain penetration.



This poster will describe the work towards this goal of identifying non-acids within this series that maintained PPAR γ activity and had increased access into the CNS.

- [1] Clarke et. al., *Am. J. Clin. Nutrition*, 1999, 70(4), 566-571.
- [2] Brookmeyer et. al., *Am. J. Public Health*, 1998, 88, 1337.
- [3] Risner et.al., *Pharmacogenomics Journal*, 2006, 6(4), 246-254.
- [4] Sastre et. al., *Proc. Natl. Acad. Sci. U. S. A.* (2006), 103(2), 443-8.
- [5] Thor et.al., *Biorg. Med. Chem. Lett.*, 2002, 12, 3565.

P555

GRIND-2. A NEW GENERATION OF ALIGNMENT-INDEPENDENT MOLECULAR DESCRIPTORS FOR DRUG DISCOVERY.

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The GRIND-INdependent Descriptors (GRIND) (**1**) are 3D molecular descriptors based on Molecular Interaction Fields (MIF) which do not require the structural alignment of the compounds. GRIND were originally developed for 3D QSAR applications but since their publication they have been applied at diverse stages of drug discovery; from library design to virtual screening. Even if these applications were mostly successful, the use of GRIND in practice has shown some drawbacks and limitations. In this communication we report the development of a new generation of molecular descriptors (GRIND-2), based on the same principles of GRIND but much improved in some key aspects, like the discretization of the MIF and the encoding algorithm.

With respect to the MIF discretization, the GRIND-2 use the novel AMANDA algorithm (**2**) to extract biologically relevant hot-spots from any MIF. This new method produces more relevant results, requires no human intervention and is much faster than the equivalent algorithm implemented in GRIND. The novel AMANDA algorithm has been subjected to intensive validation on large series of crystallographic complexes, and on series of drug-like compounds, demonstrating significant improvements in all the aforementioned aspects.

With respect to the encoding, the Maximum Auto and Cross Correlations (MACC) implemented in GRIND suffered from some problems, like with the low consistency

of the results obtained for series of structurally related compounds (3). In order to address this problem a new algorithm called Consistently Large Auto and Cross Correlation (CLACC) has been developed in our group. The so obtained descriptors are much easier to interpret and lack property-confusion effects which could have a detrimental effect on the predictive ability of GRIND models with the additional advantage of providing a field-based alignment of the structures in the series.

In this communication we report the GRIND-2 method for the first time together with some application examples.

References

- [1] Pastor M, Cruciani G, McLay I, Pickett S, Clementi S. GRIND-Independent descriptors (GRIND): a novel class of alignment-independent three-dimensional molecular descriptors. *J Med Chem* 2000; 43:3233-43.
- [2] Duran A, Martinez G, Pastor M. Development and validation of AMANDA, a new algorithm for selecting highly relevant regions in molecular interaction fields. Submitted
- [3] Pastor M. In *Molecular Interaction Fields, Applications in Drug Discovery and ADME Prediction*. Ed. Cruciani G. Wiley-VCH, Weinheim, 2006, pp.117-43.

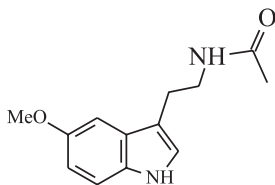
P556

SIMPLE APPROACH TO SYNTHESIS OF 2-SUBSTITUTED MELATONIN ANALOGS

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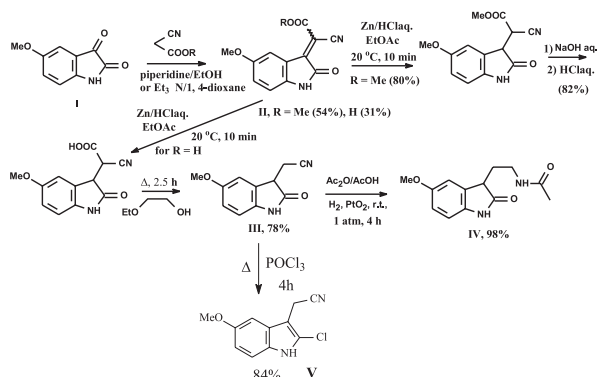
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Melatonin (3-(2-acetylaminoethyl)-5-methoxyindol) is an endogenous hormone with a wide spectrum of action, but its medicinal use is restricted due to its low selectivity.



Recent computational and empirical researches have shown that some modified in 2nd position melatonin analogs have significantly improved selectivity, especially the binding selectivity to the melatonin M₂-type receptor compared to other types. The substitution of 2nd position is usually carried out before the indole ring assembling. However, it is not always possible. To provide an easy way to 2-substituted melatonin we have elaborated and tested the following scheme.

We have also shown that compounds IV and V can be easily transformed to various 2-substituted melatonin derivatives – a potential melatonin receptor agonists.



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MONOAMINE OXIDASE (MAO) ENZYME INHIBITION BY INDAZOLES AND β -CARBOLINES.

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Monoamine oxidase (MAO) is a mitochondrial flavoenzyme occurring as two isozymes MAO-A and MAO-B, exhibiting different substrate and inhibitor selectivities, and involved in the brain and peripheral oxidative catabolism of neurotransmitters and biogenic amines, including dopamine, serotonin, norepinephrine, tyramine and tryptamine, as well as in the metabolic bioactivation of neurotoxic amines such as MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) neurotoxin. MAO is a known target for antidepressant and neuroprotective drugs. Simple β -carboline derivatives are bioactive compounds and reversible inhibitors of MAO isozymes, and some of these compounds have been detected and isolated from various sources of natural products, such as plants, foods and tobacco smoke, exhibiting MAO inhibition (1,2). In this work, we have studied the MAO inhibitory properties of several indazole compounds, including nitroindazole derivatives such as 7-nitroindazole, and of some simple β -carboline derivatives previously found in natural products. Enzymatic reaction was accomplished with kynuramine and MPTP as substrates whereas a HPLC-(MS) chromatographic method was used for enzymatic analysis. The results showed that simple β -carboline and nitroindazole derivatives exhibit inhibitory properties over MAO enzymes.

[1] T. Herraiz, C. Chaparro. *Biochem. Biophys. Res. Commun.* 326, 378, 2005.

[2] T. Herraiz, C. Chaparro. *Life Sci.* 78, 795, 2006.

P559

BRAZILIAN PROPOLIS AS A POTENTIAL SOURCE OF NEW LEAD COMPOUNDS: BIOCHEMICAL MECHANISMS OF ACTION AND IDENTIFICATION OF ARTEPILLIN C IN ACTIVE EXTRACTS

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Propolis is a resinous material prepared by *Apis mellifera* bees from trees exsudates, wax and saliva, used in bee hives to avoid microbial contamination of the eggs and to embalm the corpses of dead invasors. Brazilian propolis is well known for the pharmacological activities displayed, such as antimicrobial, antioxidant and antitumoral activities. The major components of Brazilian propolis are phenolic acids derived from benzoic and cinnamic acids, what differ it from European propolis, which are richer in flavonoids¹.

Despite the large popular use of ethanolic propolis extracts for throat infections and the many scientific experiments published in order to prove their pharmacological activities, the molecular mechanisms of action of propolis components are still unclear. In order to explore this question further, aiming to identify new lead compounds, we have developed experiments for determining the effects of an ethanolic propolis extract from the city of Guarapari (Brazil) on mutant *S. cerevisiae* strains lacking some antioxidant defenses, as well as a methodology for the separation of propolis components based on their pK_a values followed by GC/MS analysis¹.

We studied the antioxidant properties of propolis during oxidative stress caused by menadione (15mM/1h) and hydrogen peroxide (2mM/1h). Tolerance against oxidative stress showed that treatment with 25µg/mL propolis extract increased tolerance to oxidative stress in all strains tested. However, cells harboring deficiency on Sod1 presented reduced capacity to acquire tolerance against menadione stress. In fact, propolis treatment was responsible to increase 60% of Sod1 activity on wild type cells, which is not observed in Sod1 deficient cells. It has also been analyzed the protective role of propolis in lipid peroxidation. Cells exposed to oxidative conditions presented high levels of lipid oxidation; however, after treatment with propolis we observed a significant decrease on lipid peroxidation only in hydrogen peroxide stress. These results suggest that antioxidant properties of propolis might be related with reducing the lipid peroxidation levels and Sod1 expression as well. Capture of free radicals by flavonoids and phenolic compounds could not be excluded.

The fractionation of the propolis extract showed a high antioxidant activity for fractions richer in strong and medi-

um force acids. The GC/MS analysis showed that the major components of these fractions were artepillin C (3,5-diprenyl-4-coumaric acid) and its nor-prenyl analogue². Since their absolute concentrations are low, we have developed a new synthetic methodology³ for obtaining higher amounts of these compounds, as well as for the synthesis of new analogues, in order to obtain SAR data related to the antioxidant activity.

These results show that Brazilian propolis is a potential source of new lead compounds for treatment of degenerative disorders associated with oxidative stress.

Acknowledgements

CAPES, CNPq, FAPERJ

- [1] Santos, Bastos, E.M.A., Uzeda, M., Carvalho, M.A.R., Farias, L.M., Moreira, E.S.A., Braga, F.C. 2002 *J. Ethnopharmacol.* 80: 1-7.
- [2] Souza, R.M., Souza, M.C., Patitucci, M.L., Silva, J.F.M. 2007 *Z. Naturforsch. C* 62: 801-807.
- [3] Estrada, G.O.D., Souza, A.L.F., Silva, J.F.M., Antunes, O.A.C. 2008 *Catalysis Comm.* 9: 1734-1738.

P560

REVISITING THE PHARMACOLOGICAL ACTIVITY OF ARYLETHANOLAMINES: DESIGN OF NEW DERIVATIVES

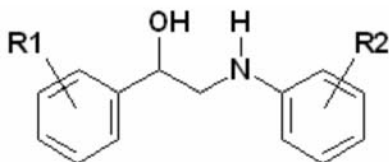
Joaquim Fernando M. Silva¹, Biank Tomaz Gonçalves¹, Pierre Mothé Esteves³, Lucas Villas-Boas Hoelz¹, Luana Braga Pontes², Marcio Galindo Kiuchi², Roberto Takashi Sudo², Gisele Zapata-Sudo²

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Cardiovascular diseases are the major cause of deaths all over the world, and hypertension is the most prevalent of all of them¹. Arylethanolamines were the first compounds that displayed antihypertensive activity clinically employed, due to its ability to block beta1-adrenergic receptors. This class of compounds was substituted by the aryloxypropanolamines, such as propranolol and atenolol, which displayed a higher activity². Since then, arylethanolamines were relegated to a second plan in antihypertensive medicinal chemistry research.

Previous works on the SAR of arylethanolamines described a lower activity for arylethanolamines and aryloxypropanolamines bearing a N-aryl moiety, but in non-classical antihypertensive propanolamines this moiety enhanced the pharmacological activity. In order to explore this controversy and due to the high prevalence of hypertension in Brazil, we synthesized six

arylethanolamines bearing a further aromatic ring attached to the nitrogen atom and evaluated their antihypertensive activity on isolated rat papilar muscle, as well as in normal and spontaneously hypertensive rats.



The compounds showed moderate to high activity when compared to propranolol in the rat papilar muscle assay. *In vivo* experiments showed that one of these compounds has an antihypertensive activity due to α_1 and β -adrenergic receptors blockage, leading to a rapid on-set and prolonged activity.

A conformational analysis of these compounds were performed on a two-step methodology, using PM3 force field followed by DFT calculations in order to obtain the minimal energy conformation of neutral and charged species. Our compounds showed a conformational arrangement of the hydroxyl moiety that differs from some classical β blockers. We also compared the energy of complexation of these compounds with formate ion as a model for the interaction of β adrenergic blockers with an Asp residue in the β_1 -adrenergic receptor. The results showed an enhanced affinity of our compounds, and the spatial orientation of the hydroxyl group seems to display an important role on these results.

The current results show that arylethanolamines bearing N-aryl moieties are potent adrenergic blockers, displaying *in vitro* and *in vivo* antihypertensive activities, showing that older SAR studies failed to describe the role of these moieties for adrenergic receptor affinity of arylethanolamines.

Acknowledgements

CAPES, CNPq, FAPERJ

- [1] American Heart Association. Heart and stroke update 2004 (www.americanheart.org)
- [2] Main, B.G. β -Adrenergic receptor *in* Hansch, C. Comprehensive medicinal chemistry: the rational design, mechanistic study & therapeutic application of chemical compounds. V.1, Oxford, Pergamon press. Pharmaceutical Division, Alderly Park UK, 1990, 187-227

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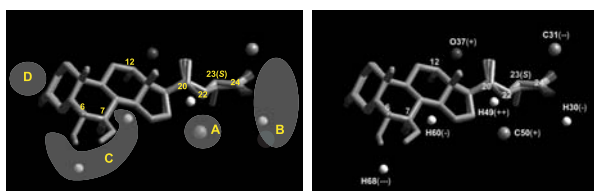
MOLECULAR FIELD ANALYSIS AND QUANTITATIVE STRUCTURE-ACTIVITY STUDIES (MFA 3D-QSAR) UNVEIL NOVEL FEATURES OF BILE ACID RECOGNITION AT TGR5 RECEPTOR

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Until 1999, the scientific interest in bile acids (BAs) was centred on their role as solubilising agents of cholesterol, fatty acids and liposoluble vitamins. In 1999, it was discovered that one member of the metabolic nuclear receptors, the Farnesoid X Receptor (FXR), could be endogenously activated by BAs.¹ This concept was suddenly strengthened with the finding that an orphan metabotropic receptor, TGR5 (GPBAR1 or M-BAR), was specifically activated by BAs.² In view of these observations, it is now widely accepted that BAs are signalling molecules of hormonal pathways endowed with pleiotropic activities. The main features of BA pathways are the presence of genomic and non genomic actions that are respectively mediated by the activation of the intracellular FXR receptor, and the membrane TGR5 receptor. In order to investigate the physiological and pathological implications of genomic and non genomic functions of BAs, selective and potent modulators of FXR and TGR5 are sought. In this framework, we have recently reported the synthesis and the biological evaluation of a series of naturally-occurring and semisynthetic BA derivatives as TGR5 agonists.³ The results were useful to define the first structure-activity relationships of TGR5 agonists and to unveil that at least four diverse pockets constitute the binding site of TGR5.

In this communication, we report a molecular interaction field analysis (MFA) and a 3D-QSAR study aimed at assessing the quantitative contribution of the four regions to the modulation of the receptor. The results show a good predictive model and indicate a statistically relevant degree of correlation between the TGR5 activity and the molecular interaction fields produced by discrete positions of the BA scaffold. These information will be fruitful to design new potent and selective modulators of the non genomic actions of BAs.



References

- [1] a) Wang, H. B.; Chen, J.; Hollister, K.; Sowers, L. C.; Forman, B. M. *Mol. Cell* 1999, 3, 543-553. b) Parks, D. J.; Blanchard, S. G.; Bledsoe, R. K.; Chandra, G.; Consler, T. G. *et al. Science* 1999, 284, 1365-1368. c) Makishima, M.; Okamoto, A. Y.; Repa, J. J.; Tu, H.; Learned, R. M. *et al. Science* 1999, 284, 1362-1365.
- [2] a) Maruyama, T.; Miyamoto, Y.; Nakamura, T.; Tamai, Y.; Okada, H. *et al. Biochem Bioph Res Co* 2002, 298, 714-719. b) Kawamata, Y.; Fujii, R.; Hosoya, M.; Harada, M.; Yoshida, H. *et al. J Biol Chem* 2003, 278, 9435-9440.
- [3] a) Pellicciari, R.; Sato, H.; Gioiello, A. *et al. J. Med. Chem.* 2007, 50, 4265-4268. b) Sato, H.; Macchiarulo, A.; Thomas, C.; Gioiello, A.; Une, M.; Hofmann, A. F.; Saladin, R.; Schoonjans, K.; Pellicciari, R.; Auwerx, J. *J. Med. Chem.* 2008, 51, 1831-1841.

P566

FRAGMENT BASED DESIGN, SCAFFOLD HOPPING AND QSAR: FROM PROMISCUOUS LIGANDS TO SELECTIVE NON-IMIDAZOLE HISTAMINE H₄ INVERSE AGONISTS WITH ANTI-INFLAMMATORY ACTIVITY *IN-VIVO*

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The human histamine H₄-receptor (H₄R) is postulated to play a role in a variety of inflammatory conditions such as atopic asthma, allergic rhinitis and rheumatoid arthritis.^{1,2} Starting from a flexible alignment model of the known H₄R antagonist JNJ7777120³ and H₄R agonist clozapine⁴, a series of fragments was designed with moderate affinity for the H₄R. From these fragments, a quinoxaline heterocycle was decorated with a variety of substituents leading to high H₄R affinity. A scaffold hopping approach was then attempted to decorate a second fragment based on the quinazoline heterocycle. Guided by QSAR the quinazoline heterocycle was further optimized and found to be a promising new scaffold for further development into compounds for putative clinical applications. The compounds found in this study bind the H₄R in the single-digit nanomolar range and display a range of activities at the H₄R of rat, mouse and human ranging from agonism to antagonism and inverse agonism. *In-vivo* administration of these compounds to rats revealed them to have potent anti-inflammatory effects.

[1] Oda, T., *et al. J. Biol. Chem.* 2000, 275 :36781-36786

[2] Lim, H., *et al. Curr. Top. Med. Chem.* 2006, 6:1365-1373

[3] Jablonowski, J., *et al. J. Med. Chem.* 2003, 46:3957-3960

[4] Smits, R., *et al. J. Med. Chem.* 2006 49, 4512-4516.

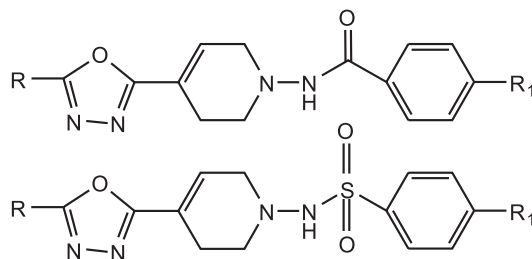
P567

SYNTHESIS OF SUBSTITUTED 1,3,4-OXADIAZOL-2-YL TETRAHYDROPYRIDINES AS ANTI-CANCER AGENTS

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Tetrahydropyridine (THP) ring moieties have attracted synthetic interests for being essential structures in many medicinal agents. In addition, 1,3,4-oxadiazoles and their derivatives were reported to possess potential antimicrobial, antifungal, anti-inflammatory and anti-cancer agents. The anti-inflammatory and anti-cancer activities of compounds consisting of reduced pyridine ring systems were investigated in our laboratory. Our earlier research indicated that the pharmacological activities of the THP derivatives depended on the nature of the substituents on THP ring system. It was postulated that incorporation of the THP moiety might enhance the biological activity of 1,3,4-oxadiazole derivatives. Thus, the design and synthesis of novel series of 1,3,4-oxadiazoles containing carbonyl/sulfonyl tetrahydropyridine moieties with potential as anti-cancer and anti-inflammatory activities were initiated. In the current investigation, we synthesized many analogs maintaining the 1,3,4-oxadiazol-2-yl benzoyl/benzenesulfonyl amino-1,2,3,6 tetrahydropyridine analog having modifications on both the oxadiazole and phenyl rings. We incorporated substituents on the rings that could affect the molecule's electron density, lipophilicity and steric configurations.



R, R₁ = Alkyl, Aryl, Halogen, Alkoxy, Aminoalkyl, Cyano

The starting material was 4-(5-methyl/phenyl-1,3,4-oxadiazol-2-yl)pyridine obtained by the reaction of isonicotinic acid hydrazide and triethyl orthoacetate/orthobenzoate which were heated under reflux for 24 h. *O*-mesitylene sulfonyl hydroxyl amine (MSH) was used to prepare the *N*-amino salt as an aminating agent. Mesitylene sulfonyl chloride was added with stirring to a solution of ethylacetohydroxymate and triethylamine in dimethylformamide at 0° C. Hydrolysis of this compound with the mixture of *p*-dioxane-70% perchloric acid and

allowing them to react for 45 min gave a white solid of MSH. 4-(5-methyl/phenyl-1,3,4-oxadiazol-2-yl)pyridine was reacted with MSH in dichloromethane to produce 1-amino-4-(5-methyl/phenyl-1,3,4-oxadiazol-2-yl)pyridine mesitylenesulfonate. Reaction of the amino salt with substituted benzoyl chlorides/ benzenesulfonyl chlorides in anhydrous tetrahydrofuran containing triethylamine gave stable benzoyl/benzenesulfonyl imino ylides. This was followed by reduction with sodium borohydride in absolute ethanol, which furnished the target compounds 1-(substituted benzoyl/benzenesulfonylamino)-4-(5-methyl/phenyl-1,3,4-oxadiazol-2-yl)-1,2,3,6-tetrahydropyridine. These compounds were evaluated for their anti-inflammatory activity using cyclooxygenase-1 (COX-1) and COX-2 inhibition assays (indomethacin as a reference control) and *in vitro* activity on MCF-7 breast cancer cell lines. The cytotoxicity activities of the compounds were tested using tomaxifen as a control. All the compounds tested showed inhibition of COX-1 and COX-2, almost nonselectively. The 4-methylbenzenesulfonyl amino THP analog containing the 5-phenyl-1,3,4-oxadiazol-2-yl substituent showed the highest COX-2 preference (COX-1/COX-2 = 2.11). This compound also exhibited the most potent cytotoxicity with an IC₅₀ value of 22.02 µg/mL.

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SUPPRESSION OF LIPOPOLYSACCHARIDE(LPS)-MEDIATED INFLAMMATORY RESPONSES BY PELUBIPIROFEN, 2-[4-(OXOCYCLOHEXYLIDENEMETHYL)-PHENYL] PROPIONIC ACID, THROUGH INHIBITION OF NF-κB SIGNALING IN MACROPHAGE CELLS.

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Inflammatory mediators such as prostaglandins (PGs), nitric oxide (NO) and cytokines play vital roles in pathogenesis of bacterial lipopolysaccharide (LPS)-induced inflammation. In this study, we determined anti-inflammatory activity of pelubiprofen, 2-[4-(Oxocyclohexylidenemethyl)phenyl] propionic acid, by diminishing LPS-induced PGE₂ and NO production in murine macrophage RAW 264.7 cells and mouse peritoneal macrophage cells. Potent reduction of LPS-induced PGE₂ production was induced by down-regulation of cyclooxygenase-2 (COX-2) and prostaglandin E₂ synthase expression. In particular, COX-2 activity was also remarkably declined by the pelubiprofen. Moreover, reduction on inducible nitric oxide

(iNOS) protein and mRNA expression by pelubiprofen was correlated with the decrease of LPS-induced NO production. In addition, pelubiprofen reduced cytokine production such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and interleukin-6 (IL-6). To clarify the mechanism involved, modulation of nuclear factor-kappa B (NF-κB) pathway was examined. Pelubiprofen inhibited the DNA binding and transcriptional activity of NF-κB. This effect was accompanied with parallel reduction of NF-κB translocation to the nucleus through the suppression of inhibitory kappa Bα (IκBα) phosphorylation and degradation. Moreover, Pelubiprofen was found to inhibit the IκB kinaseβ (IKKβ) activity and phosphorylation of extracellular-signal-related kinase1/2 (ERK1/2). Thus, pelubiprofen exhibit its anti-inflammatory properties in activated macrophage cells via inhibitory effects on NF-κB signaling. Taken together, these findings provide a molecular basis for anti-inflammatory properties ascribed to pelubiprofen.

P569

TARGETING KINASES WITH IRREVERSIBLE INHIBITORS TO OVERCOME DRUG RESISTANCE MUTATIONS

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Kinases are a class of ubiquitous enzymes that regulate protein function by phosphorylation. The receptor tyrosine kinase Epidermal Growth Factor Receptor (EGFR) plays an important role in cell proliferation and differentiation. EGFR is found to be deregulated in many human cancer types and is therefore an important drug target. Erlotinib (Tarceva®) and Gefitinib (Iressa®) are potent reversible ATP competitive EGFR inhibitors and have been released by the FDA in 2003 for the targeted therapy of Non-Small Cell Lung Cancer (NSCLC). Patients treated with these drugs initially show a good response. However, long-term treatment often results in drug resistance and relapse due to a single point-mutation (T790M) at the so called "gatekeeper" position. The introduced methionine residue prevents reversible inhibitors such as Erlotinib and Gefitinib from binding due to steric hindrance and loss of an essential hydrogen bonding interaction. Interestingly, irreversible inhibitors that covalently bind to the ATP binding pocket of EGFR, have shown to overcome this drug resistance in EGFR-T790M. At least four irreversible EGFR inhibitors are currently in phase II/III clinical trials. One of our aims is to understand the mechanism by which irreversible inhibitors overcome drug resistance in EGFR-T790M mutant variants. In order to characterize covalent inhibitors equipped with different electrophiles, kinetic studies and protein ligand complexes will be presented to demonstrate the binding mode and binding mechanism of irreversible inhibitors to drug resistant kinases.

[1] Michalczyk et al. (2008) Bioorg Med Chem.

[2] Blair et al. (2007) Nature Chem Biol.

[3] Kobayashi et al. (2005) Cancer Cell

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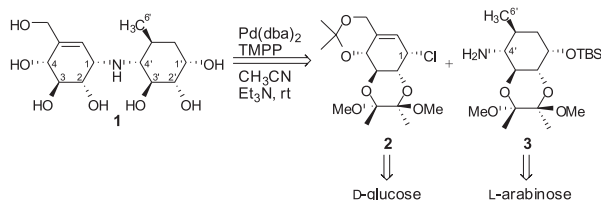
PSEUDO-ACARVIOSIN: SYNTHESIS AND BIOLOGICAL EVALUATION AS A NEW CLASS OF ANTI-DIABETIC AGENTS

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A novel pseudo-1,4'-*N*-linked disaccharide, pseudo-acarviosin **1**, was constructed via a key palladium-catalyzed coupling reaction¹ of pseudo-glycosyl chloride **2** (prepared from D-glucose via a novel direct intramolecular aldol addition in 12 steps) and pseudo-4-amino-4,6-dideoxy- α -D-glucose **3** (prepared from L-arabinose via an unusual *trans*-fused isoxazolidine-selective intramolecular nitrone-alkene cycloaddition² in 11 steps). This acid stable and water-soluble **1** with half the molecular weight of acarbose has been shown to be a potent inhibitor of sucrase and glucoamylase.



References

[1] Shing, T. K. M.; Kwong, C. S. K.; Cheung, A. W. C.; Kok, S. H.-L.; Yu, Z.; Li, J.; Cheng, C. H. K. *J. Am. Chem. Soc.* 2004, 126, 15990-15992 and references cited therein.

[2] Shing, T. K. M.; Wong, A. W. F.; Ikeno, T.; Yamada, T. *J. Org. Chem.* 2006, 71, 3253-3263.

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2,6,8-TRISUBSTITUTED 3-HYDROXYCHROMONE DERIVATIVES AS USEFUL PROBES FOR FLUORESCENCE MICROSCOPY IN LIVING CELLS

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Development of new, unique, intrinsic fluorophores for cellular studies is of significant importance in the field of chemical biology. Such compounds can provide information on a broad range of molecular processes and properties, including binding interactions, DNA sequencing, conformation changes and cellular substructures¹. The interest in 3-hydroxychromone derivatives as cellular probes has increased in recent years due to their characteristic fluorescence properties²⁻³. In addition, chromones have also been defined as privileged structures in drug discovery⁴⁻⁵.

The aim of the study was to synthesise a series of diverse 2,6,8-trisubstituted 3-hydroxychromones (Figure 1), characterise their fluorescence properties and explore their potential use as cellular probes in living cells. The project involved challenging chemistry, fluorescence measurements and multiphoton fluorescence microscopy on HeLa-cells. The results showed that fluorescent 3-hydroxychromone derivatives exhibit high quantum yield, high molar extinction coefficients and excellent cellular permeability properties. Our results demonstrate that these compounds can be used for microscopy studies in living cells.

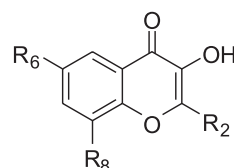


Figure 1. 2,6,8-Trisubstituted 3-hydroxychromone

References

[1] Lakowicz, J.R. *Principles of Fluorescence Spectroscopy*, 3rd ed; Springer-Verlag New York Inc: USA, 2006

[2] Guharay, J; Sengupta, B; Sengupta, P.K. *Proteins*, 2001, 43, 75-81

[3] Klymchenko, A.S; Duportal, G.; Ozturk, t.; Pivovarenko, V.G.; Merly, Y.; Demchenko, A.P. *Chem Biol*, 2002, 9, 1199-1208

[4] Evans B. E et al. *J. Med. Chem.* 1988, 31, 2235

[5] Horton D.A.; Bourne G.T.; Smythe M.L. *Chem. Rev.* 2003, 103, 893-930

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ORIGINAL CLOZAPINE-LIKE COMPOUNDS

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From a database of approximately 1500 new compounds, we found purines (1) and dihydropurines (2) tridimensionally superimposable to clozapine by virtual screening (Fig. 1). By binding experiments with [³H]-ketanserin at human serotonin 5-HT_{2A} receptors transfected in CHO cells, we observed that some of these compounds have a good affinity for these receptors, showing purines higher affinity than dihydropurines. This was an interesting finding because there are not similar compounds described in the literature with affinity for serotonin 5-HT_{2A} receptors. We report here novel scaffolds for developing new 5-HT_{2A} receptor antagonists as a first step for antipsychotic drug-discovery processes.

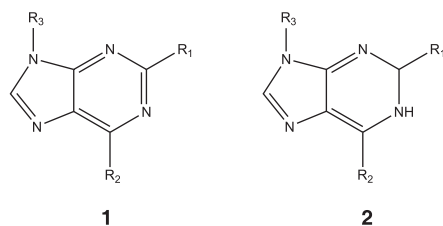


Fig. 1 – Purines (1) and dihydropurines (2) substituted with different chemical groups (R₁, R₂ and R₃).

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SYNTHESIS AND ANTITUBERCULAR ACTIVITY OF (Z)-2-(NITROIMIDAZOLEMETHYLENE)-2(3H)-BENZOFURANONE DERIVATIVES

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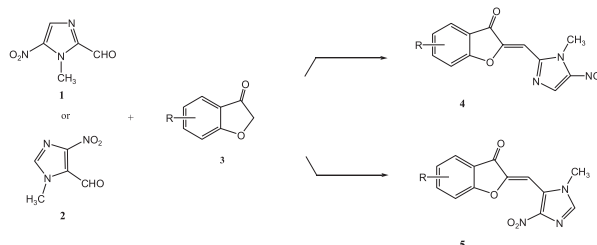
The recent emergence of outbreaks of multidrug resistant tuberculosis (MDR-TB) pose a serious threat to the treatment of the disease. Tuberculosis (TB) has undoubtedly increased in prevalence in most countries due to human immunodeficiency virus (HIV) epidemic. In response to these alarming statistics and trends, WHO declared TB to be a global public health emergency. Therefore, there is a necessity of searching for and synthesizing new active compounds with less side-effects.

The use of nitroheterocyclic drugs (such as 5-nitroimidazoles) as antibacterial, antiprotozoal, and anti-cancer agents is well-established [1]. Furthermore, a series of (Z)-2-benzylidene-6,7-di-hydroxy-3(2H)-benzofuranones have shown antibacterial activity by inhibiting the choris-

mate synthase, a key enzyme in the shikimic acid pathway which is essential for the synthesis of aromatic amino acids in bacteria [2].

The title compounds were prepared according to the scheme shown below. 3(2H)-Benzofuranones **3** are key intermediates for the production of the desired compounds. Condensation of latter **3** with corresponding carbaldehyde **1** in acetic acid, in the presence of catalytic amount of sulfuric acid afforded compound **4**, while title compound **5** was prepared by a different method in acetic anhydride in the presence of anhydrous sodium acetate [3].

All the synthesized compounds were screened against *M. tuberculosis* M₃₇Rv and shown excellent activity in comparison with rifampicine as the reference drug.



References

- [1] Edwards, D. I. *J. Antimicrob. Chemother.* 1993, **31**, 9.
- [2] M. G. Thomas, C. Lawson, N. M. Allanson, B. W. Leslie, J. R. Bottomley, A. McBride, O. A. Olusanya, *Bioorg. Med. Chem. Lett.* 2003, **13**, 423.
- [3] N. Hadj-esfandiari, L. Navidpour, H. Shadnia, M. Amini, N. Samadi, M. A. Faramarzi, A. Shafiee, *Bioorg. Med. Chem. Lett.* 2007, **17**, 6354.

P574

IDENTIFICATION OF POTENTIAL LIGAND BINDING SITES AND VIRTUAL SCREENING OF LIGANDS FOR ZRF1 BIOMARKER IN CANCER THERAPY

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Head and neck squamous cell carcinoma (HNSCC) is one of the most common tumors, afflicting more than 500,000 patients annually and regarded as a major cause of morbidity and mortality worldwide. HNSCC studies revealed a tumor-specific high level of expression of ZRF1, identifying ZRF1 as a potential biomarker for early detection of HNSCC. ZRF1 has a N-terminal J domain which includes a DnaJ motif, showing homology to Zuotin (a putative Z-DNA binding protein in yeast) and a C-terminal two-repeats Myb domain which are also found in the DNA binding domains of several transcription factors such as the c-Myb oncoprotein. Search for a ligand targeting the Z-DNA binding site of ZRF1 could contribute to anti-

cancer drug discovery. With this objective, we have built a model for ZRF1 DnaJ domain using a homology modeling approach. Quality of the model was assessed by stereochemical, residue packing and environment criteria. In order to predict favorable binding sites on ZRF1 DnaJ domain we have used two distinct methodologies: the hydrophobic binding free energy-based (Q-SITE Finder) approach and the Molecular Interaction Field studies. The consensus results have indicated two putative ligand binding sites, which have been used as a filter to virtual screening experiments. In this context, different databases of drug-like compounds were screened using a flexible docking approach. Novel potential anti-cancer candidates have been selected for biological activity assays.

P575

STRUCTURAL AND CONFORMATIONAL ASPECTS AFFECTING THE MOLECULAR RECOGNITION OF SUBSTRATES AND INHIBITORS BY INDOLEAMINE-2,3-DIOXYGENASE (IDO)

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Indoleamine 2,3-dioxygenase (IDO) is a heme-containing enzyme that catalyzes the first and rate-limiting step of the Kynurenine pathway along the major route of Tryptophan (L-Trp) catabolism. The reaction consists in the oxidative cleavage of the pyrrole ring of L-Trp and the production of N-formyl kynurenine. The observation that the expression of IDO is induced by IFN γ and the discovery of the involvement of the enzyme in protecting the fetus from maternal immunity, have evidenced a role for IDO in the control of the immune response. Furthermore, the involvement of IDO in the mechanisms of immune tolerance has led to associate the early observations of elevated expression of the enzyme in various human cancers to the participation of IDO in the tumor immuno-editing process. Results from preclinical studies of small molecule inhibitors of the enzyme have shown the feasibility to block the IDO mediated tumor immuno-editing process and to enhance the efficacy of current chemotherapeutic agents. These data support the notion that IDO is a novel therapeutic target for the development of new cancer drugs.¹

In this communication, we report our efforts to investigate substrate recognition and enhancer binding at IDO. Docking experiments of L-Trp into the crystal structure of the ferric form of IDO, in particular, have evidenced that the substrate correctly binds the enzyme if a dioxygen moiety is already docked into the sixth coordination site of the iron-heme. According to the proposed binding mode, the indolic ring of the substrate is packed with a flexible loop (residues 260-265) through hydrophobic interactions. Furthermore, the results have allowed to identify a putative binding site for the enhancers and to the con-

struct three different complexes of the enzyme: IDO-Fe(III)/superoxide (complex A), IDO-Fe(III)/superoxide/L-Trp (complex B), IDO Fe(III)/superoxide/L-Trp/3-indole-ethanol (complex C). These complexes have been used as input structures to perform coarse graining simulations in order to explore the conformational transitions of IDO in response to substrate and enhancer binding. The results have evidenced key structural and conformational elements involved in substrate recognition. In particular, different states of the enzyme are observed in the final conformations of complexes A-C that involve a diverse conformation of two key structural elements: an electrostatic gate composed by Arg231 and the 7-propionate moiety of heme, and the region 355-385. Each of these elements may adopt a closed and open conformation. These results have risen the hypothesis that the movements of the electrostatic gate and region 355-385 control the shift between the inactive and active states of the enzyme by regulating the access of substrates into the catalytic site and the exit of products. Moreover, docking studies of inhibitors have been performed in different states and conformations of IDO. Then, linear regression analyses (LRA) have been used to correlate the activity data and the binding energy of the inhibitors in order to identify the state and conformation of the enzyme that better recognized the chemical class of the inhibitor. These information are currently used to address the design of novel selective and potent IDO inhibitors and to set up structure-based virtual screening protocols.

- [1] Muller, A. J.; Malachowski, W. P.; Prendergast, G. C. Indoleamine 2,3-dioxygenase in cancer: targeting pathological immune tolerance with small-molecule inhibitors. *Expert Opin Ther Targets* 2005, 9, 831-49.

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ASSAYING THE EFFECTS OF BETULINS ON *CHLAMYDIA PNEUMONIAE* BY TIME-RESOLVED FLUOROMETRIC IMMUNOASSAY

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Chlamydia pneumoniae is a remarkable, world wide pathogen and removal of this intracellular parasite is difficult. Normally it has a two stage developmental cycle with an infectious elementary body (EB) stage and a dividing reticulate body (RB) stage. It has also a third possible developmental stage in chronic infection, aberrant Chlamydia (AB), which can be seen in presence of some growth limiting factor such as penicillin (1). These different stages alone make *C. pneumoniae* a difficult target for drug or vaccination development. Another considerable aspect in treating *C. pneumoniae* infection is its ability to infect multiple tissue types like epithelial, myocardial and cells of nervous system (2, 3). Acute *C. pneumoniae*

infection is associated with upper respiratory infections, but the chronic infection is associated with asthma and atherosclerosis (4, 5). Recently the chronic infection has been linked also to Alzheimer's disease (6). These factors suggest that *C. pneumoniae* generates heavy economical losses and human suffering, and should thus be under intensive research.

Good progress has been achieved in *C. pneumoniae* research, and this has lead to discovery of new, potential inhibitory substances, but breakthrough is still missing (7, 8). A selected group of 20 betulin derivatives synthesized by us were tested against *C. pneumoniae* with time-resolved fluorometric immunoassay (9). Many of these betulin derivatives (10) proved to be highly active in concentrations as low as 1 μ M. The structure-activity relationship will be discussed.

References

- [1] Matsumoto A, Manire GP: Electron microscopic observations on the effects of penicillin on the morphology of Chlamydia psittaci. *J Bacteriol* 101:278-285, 1970.
- [2] Quinn TC, Gaydos CA: In vitro infection and pathogenesis of Chlamydia pneumoniae in endovascular cells. *Am Heart J* 138:S507-S511, 1999.
- [3] Balin B, Gérard H, Arking E, Appelt D, Branigan P, Abrams J, Whittum-Hudson J, Hudson A: Identification and localization of Chlamydia pneumoniae in the Alzheimer's brain. *Med Microbiol Immunol* 187:23-42, 1998.
- [4] Hahn DL, Peeling RW, Dillon E, McDonald R, Saikku P: Serological markers for Chlamydia pneumoniae in asthma. *Ann Allergy Asthma Immunol* 84:227-233, 2000.
- [5] Saikku P, Leinonen M, Mattila K, Ekman M-R, Nieminen MS, Mäkelä PH, Huttunen JK, Valtonen V: Serological evidence of an association of a novel chlamydia TWAR, with chronic coronary heart disease and acute myocardial infarction. *Lancet* 2:983-986, 1988.
- [6] Gérard HC, Dreses-Werringloer U, Wildt KS, Deka S, Oszust C, Balin BJ, Frey WH, Bordayo EZ, Whittum-Hudson JA, Hudson AP: Chlamydia (Chlamydia) pneumoniae in the Alzheimer's brain. *Immunol Med Microbiol* 48:355-366, 2006.
- [7] Alvesalo J, Vuorela H, Tammela P, Leinonen M, Saikku P, Vuorela P: Inhibitory effect of dietary phenolic compounds on Chlamydia pneumoniae in cell cultures. *Biochem Pharmacol* 71:735-741, 2006.
- [8] Alvesalo JK, Siiskonen A, Vainio MJ, Tammela PSM, Vuorela PM: Similarity based virtual screening: a tool for targeted library design. *J Med Chem* 49:2353-2356, 2006.
- [9] Tammela P, Alvesalo J, Riihimäki L, Airanne S, Leinonen M, Hurskainen P, Enkvist K, Vuorela P: Development and validation of time-resolved fluorometric immunoassay for screening of antichlamydial activity using a genus-specific europium-conjugated antibody. *Anal Biochem* 333:39-48, 2004.
- [10] Alakurtti Sami, Mäkelä Taru, Koskimies Salme, Yli-Kauhaluoma Jari: Pharmacological properties of the ubiquitous natural product betulin. *Eur J Pharm Sci* 29:1-16, 2006.

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FORMULATION OF REACTION PATHWAYS FOR THE PREDICTION OF MAMMALIAN XENOBIOTIC METABOLISM

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A fundamental requirement of an expert system for the prediction of drug metabolism is a dictionary of biotransformations representing the known phase I and phase II conversion processes in mammals. There is also a growing interest within the pharmaceutical industry in metabolic intermediates formed during these processes. This is due largely to an increased awareness of their potential implication in toxicity, including adverse idiosyncratic drug reactions, and mechanism-based enzyme deactivation (suicide inhibition).

This poster will describe an approach whereby intermediate metabolites can be introduced into biotransformation schemes where the mechanism is not well characterised and the intermediate metabolites could, potentially, be controversial. Such generalised reaction schemes are built using a combination of knowledge from the scientific literature and an understanding of the underlying principles of enzyme action and organic chemistry.

It will be demonstrated how proposed pathways deal with: 1) accounting for all of the known facts regarding example sequences even though these facts are incomplete, 2) being consistent with the structure-metabolism relationships for these biotransformations, and 3) accounting for all atoms within these biotransformations even when all metabolites may not be fully characterised.

The mechanistic hypotheses will be presented for three examples: 1) the partial oxidative ring degradation of piperazines, 2) the oxidative fragmentation of β -carbinolamines as seen in some β -adrenergic agonists and antagonists, and 3) the sequential oxidation of simple imidazole compounds, rationalising the loss of aromaticity for these cases.

P578

COMPUTATIONAL ANALYSIS OF A₃ ADENOSINE RECEPTOR AGONISTS AND ANTAGONISTS USING TOPOMER COMFA AND FLEXIBLE DOCKING

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Adenosine receptors (ARs) belong to the superfamily of G-protein-coupled receptors (GPCRs), and they consist of four subtypes: A₁, A_{2A}, A_{2B}, and A₃. ARs are ubiquitously expressed but differentially distributed throughout the human body. Among them, A₃ receptor is the most recently identified adenosine receptor and is

becoming an important target for drug discovery. A₃AR agonists are known for the treatment of myocardial and cerebral ischemia and cancer, and A₃AR antagonists are being investigated as anti-asthma, anti-glaucoma, and anti-inflammatory agents.

Most of A₃AR agonists have been derived by modifying adenosine itself. So far, reported potent and selective A₃AR antagonists are xanthine derivatives and, recently, nucleoside A₃AR antagonists were also discovered. In order to analyze the differences between the nucleoside A₃AR agonists and antagonists, we performed 3D-quantitative structure activity relationship (QSAR) studies using Topomer CoMFA.

Compared with the traditional CoMFA, which depends on the alignment and conformer selection of the input ligands, Topomer CoMFA automates the creation of models by merging the the CoMFA and topomer technologies. A series of input ligands are individually broken into fragments at the central acyclic single bonds, while removing any core fragment structurally common to the entire series. Then, standard topomer 3D models are automatically constructed for each fragment, and a set of steric and electrostatic fields is generated for each set of topomers.

In addition to Topomer CoMFA analysis, we carried out docking studies using an A₃AR homology model and compared the docking conformations with the Topomer CoMFA contour maps. The results suggest directions to optimize A₃AR agonists and antagonists, and the details will be presented at the meeting.

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FLAVONOIDS AS POTENTIAL ANTIHYPERGLYCEMICS: AN IN SILICO STUDY OF THEIR ALPHA-AMYLASE INHIBITORY MODE

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Flavonoids are polyphenol compounds, widely distributed in plants and possessing various interesting therapeutics properties including antiatherosclerotic, antioxidative, and anti-inflammatory effects[1]. Potential anti-diabetic properties of some flavonoids have also been investigated, with the assessment of their inhibitory effect on alpha-glucosidase and alpha-amylase[2-4]. As a representative flavonoid, flavone structure is shown in Fig.1. In this study, the binding mode of flavonoids to alpha-amylases was investigated *in silico*. A selection of flavonoids: apigenin, baicalein, luteolin (flavones), myricetin, fisetin (flavonols), genistein (isoflavone), and naringenin (flavanone) ligands were docked into porcine pancreatic alpha-amylase (PPA), human salivary alpha-amylase (HSA), and human pancreatic alpha-amylase (HPA) with the use of the Autodock program. Two putative binding sites were found on these enzymes while using the blind docking mode, assessing the whole struc-

ture of the enzymes for possible interaction pockets (Fig.2). The first one (site 1) is part of the active site of the enzyme, while the second one (site 2) is structurally similar to a maltotriose binding site found on the crystal structure of a chimeric bacterial alpha-amylase and postulated to play a role in locating the enzyme on the starch granule [5]. The possibility of a ligand binding to site 2 could not be discarded and should be checked with experimental techniques. However, further studies were focused on site 1, based on the fact that a higher number of protein-ligand interactions could occur in this site, which forms also a deeper pocket. Experimental data exist for the extent of inhibition of PPA with these ligands [4], but not for the human enzyme; however, HPA structure was used further on, because the amino acids located in the vicinity of docked ligands (5 A°) were identical in PPA and HPA. It was found that a recurring low-energy docking pose occurs in the subsites -2 and -1 of the active site, including mainly a stacking hydrophobic interaction of the A and C rings with W59. The B ring may have similar interactions with Y62 and when hydroxyl groups are present, further h-bond interactions occur with the catalytic residues situated between subsites -1 and +1 (E 233, D300 and D197). This binding mode correlates with experimental results, for example explaining the lower inhibition of naringenin by its inability to interact with W59 as well as the ligands possessing two phenyl rings. These results could be of use in the proposal and design of new ligands for this target.

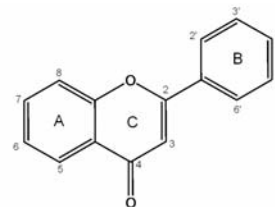


Fig. 1

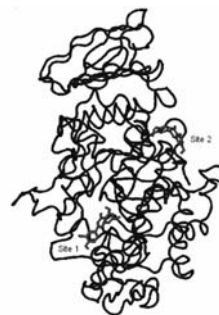


Fig. 2

- [1] R.J. Nijveldt, E. van Nood, D.E.C. van Hoorn, P.G. Boelens, K. van Norren and P.A.M. van Leeuwen. *Am J. Clin. Nutr.* 2001, 74:418–25.
- [2] I. Funke and M. F. Melzig. *Pharmazie* 2005, 60: 796–797.
- [3] J.-S. Kim, C.-S. Kwon and K. Ho Son. *Biosci., Biotechnol. Biochem.* 2000, 64:2458-2461.

[4] K. Tadera, Y. Minami, K. Takamatsu and T. Matsuoka. *J.Nutr.Sci.Vitaminol.* 2006,52:149-153.

[5] A.M. Brzozowski, D.M. Lawson, J.P. Turkenburg, H. Bisgaard-Frantzen, A. Svendsen, T.V. Borchert, Z. Dauter, K.S. Wilson and G.J. Davies. *Biochemistry.* 2000, 39: 9099-9107

P580

KINETIC AND J-RESOLVED STATISTICAL TOTAL CORRELATION NMR (STOCSY) APPROACHES TO STRUCTURAL INFORMATION RECOVERY IN COMPLEX REACTING MIXTURES: APPLICATION TO ACYL GLUCURONIDES

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We demonstrate here a new statistical spectroscopic approach for recovering structural information on unstable intermediates formed in reaction mixtures. We exemplify this approach with respect to the internal acyl migration reactions of 1- β -O-acyl glucuronides (AGs), which rearrange at neutral or slightly alkaline pH over a period of minutes to hours to yield a series of positional glucuronide ring isomers and α/β anomers, in the order, 1- β (starting material) 2- β , 2- α , 1- α , 3- β , 3- α , and 4- β , 4- α , together with the aglycone and glucuronic acid hydrolysis products. Multiple sequential 800 MHz cryoprobe ¹H NMR spectra (1D and 2D J-resolved, JRES) were collected on a 5.1 mM solution of a synthetic model drug glucuronide, 1- β -O-acyl (S)-mono- α -methyl phenylacetic glucuronide (MPG) in a 0.1 M sodium phosphate buffer in D₂O at pD 7.4 over 18 h to monitor the reaction which leads to the formation of the eight positional isomers and hydrolysis products. As the reaction proceeds and new isomers form, the NMR signal intensities vary accordingly allowing the application of a novel Kinetic variant on Statistical Total Correlation Spectroscopy (K-STOCSY) method to recover the connectivities between proton signals on the same reacting molecule based on their intensity co-variance through time. We performed K-STOCSY analysis on both the standard 1D NMR spectra and the skyline projected singlets of the ¹H-¹H JRES NMR spectra through time, i.e. the K-JRES-STOCSY experimental variant, which increases the effective spectral dispersion and is ideally suited for the analysis of heavily overlapped spin systems. High statistical correlations were observed between mutarotated α and β anomers of individual positional isomers, as well as directly acyl migrated products and anti-correlation observed between signals from com-

pounds that were being depleted as others increased e.g. between the 1- β and 2- α /2- β isomers. This statistical kinetic approach enabled the recovery of structural connectivity information on all isomers allowing unequivocal resonance assignment and this approach to spectroscopic information recovery has wider potential uses in the study of reactions that occur on the second-to-minute time-scale in conditions where multiple sequential NMR spectra can be collected. JRES-STOCSY is also of general use as a method for recovering spectroscopic information in highly overlapped NMR spin systems or complex mixture analysis.

P581

HPLC-MS AND ¹H NMR SPECTROSCOPIC STUDIES ON THE TRANSACYLATION KINETICS OF A SET OF STRUCTURALLY SIMILAR ARYLACETIC ACYL GLUCURONIDES

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Carboxylic-acid containing drugs are often metabolised to 1- β -O-acyl glucuronides (AGs). These can undergo an internal chemical rearrangement, and the resulting reactive positional isomers can bind to endogenous proteins, with clear potential for adverse effects. A number of carboxylic-acid containing drugs have been withdrawn from the market due to hypersensitivity reactions. These drugs form fast degrading 1- β -O AGs and exhibit protein binding, thus a tentative link between the degradation rate of a drug and its reactivity has been formed. A number of structurally similar AGs have been synthesised here for this study, including ibufenac (withdrawn from the market due to hypersensitivity reactions), R- and S-ibuprofen, α -dimethyl ibuprofen, phenylacetic acid, R- and S- α -methyl phenylacetic acid, and α -dimethyl phenylacetic acid. Each AG was incubated at 37°C in pH 7.4 buffer and human plasma. Aliquots were taken at specific time-points and analysed using ¹H NMR spectroscopy and HPLC-MS. The degradation rates (a combination of transacylation and hydrolysis) were calculated by measuring the decrease in the 1- β anomer over time. The results show that phenylacetic acid AG has the fastest degradation rate, whilst increased methylation at the α -carbon slowed the degradation rate significantly. A similar trend was seen with the ibuprofen series. Whilst the ibuprofen compounds are similar in structure to the phenylacetic acids, they differ by the presence of an isobutyl arm, and have much slower degradation rates.

The reason for the longer degradation rates remains unknown at this stage and requires further investigation, but steric hindrance is thought to play a large role, with increased methyl substitution at the α -carbon affecting the ability of the carbonyl carbon to undergo nucleophilic attack and thus acyl migration. These findings show that there is a direct relationship between the structure of a drug and its propensity to undergo transacylation and hydrolysis reactions, this is of importance in deriving quantitative structure-reactivity relationships.

P584

DESIGNING IN DRUG SPACE: A STRATEGY USING MOLECULAR BUDGETS TO DRIVE 7TM LIGAND DESIGN

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The concept that 'drug space' was defined by certain physicochemical descriptors, although now well established, was originally highlighted by Lipinski¹ as an area deserving greater attention of the Medicinal Chemist.

In a simplistic view of drug space descriptors (polar surface area, lipophilicity, Mwt etc) these global physicochemical properties will approximate to the sum of the properties of the individual components, the atoms. Thus each atom carries a value in terms of drug space descriptors. Consequently, the Medicinal Chemist can readily define a molecular budget that is available for them to spend in building, designing or optimising templates in drug space. In reality, small combinations of atoms introduce subtleties that may increase or decrease the descriptor values compared to the sum of the component atoms, thus the molecular budget is refined to incorporate these subtleties.

In this poster we describe the successful design of bioavailable 7TM ligands using the concept of molecular budgets to guide ligand design and optimisation in real time.

[1] C.A. Lipinski, Drug Discov. Today Technol. 1, 337-341 (2004). Lipinski C.A. *et al.* Adv. Drug Deliv. Rev. 23, 3, (1997)

P585

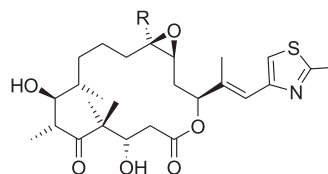
SYNTHESIS AND STRUCTURE-ACTIVITY RELATIONSHIPS OF C12-C13-OXAZOLINE DERIVATIVES OF EPOTHILONE A

Bernhard Pfeiffer, Kurt Hauenstein, Philipp Merz, Jürg Gertsch, Karl-Heinz Altmann*

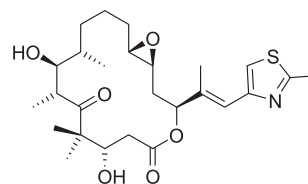
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Epothilones (Epo's) (**1**) are natural products isolated from the myxobacterial strain *Sorangium cellulosum* that exhibit potent *in vitro* and *in vivo* antiproliferative activity. The inhibition of cancer cell growth by epothilones is based on the stabilization of cellular microtubules, *i.e.* they exhibit a "taxol-like" mechanism of action. The widespread interest in the chemistry and biology of these macrolide-based microtubule-stabilizing agents has resulted in an advanced understanding of the structure-activity relationships for epothilones based on the synthesis and semi-synthesis of numerous analogues. At least seven of these have entered clinical evaluation as potential anticancer drugs and one of them has obtained FDA approval last year for the treatment of breast cancer [1].

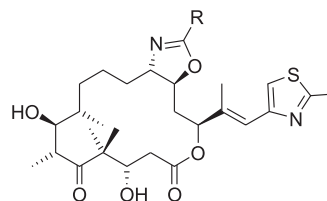
Our own SAR work on epothilones has shown that *trans*-epothilone A (**2**) exhibits antiproliferative activity in a similar concentration-range as natural epothilone A (**1a**) itself [2]. As a further extension of our previous work on *trans*-epoxide and *trans*-cyclopropane-based analogs of Epo A we now present the semi-synthesis and biological evaluation of a new series of epothilone derivatives that are characterized by the presence of a 2-substituted *trans*-fused C12-C13-oxazoline ring (**3**). Intriguingly, some of these derivatives show antiproliferative activities and tubulin-polymerizing potencies that are comparable with those of the parent compound Epo A. A clear structure-activity relationship can be delineated for these analogs with respect to the nature of the 2-substituent on the oxazoline ring. Possible interaction modes of these new epothilone derivatives with tubulin have been investigated by molecular-modeling.



(**1a**) R=H Epothilone A
(**1b**) R=Me Epothilone B



(**2**) *trans*-Epothilone



(**3**) R = aryl, alkyl

References

- [1] K.-H. Altmann, B. Pfeiffer *et al.*, *ChemMedChem* 2007, 2, 396-423.
 [2] K.-H. Altmann *et al.*, *Helv. Chim. Acta.* 2002, 85, 4086-4110

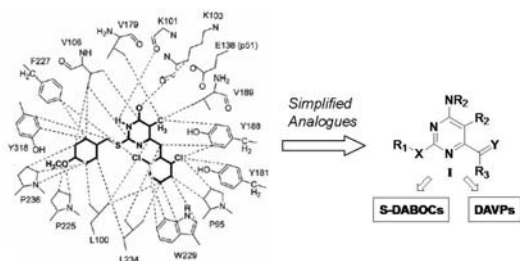
P586

A MULTIDISCIPLINARY APPROACH FOR THE IDENTIFICATION OF NOVEL HIV-1 NON-NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITORS: S-DABOCs AND DAVPS

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In the fight against AIDS, first and second generation non-nucleoside reverse transcriptase inhibitors (NNRTIs) are now established as part of highly active antiretroviral therapy (HAART) for treating HIV infection due to the less severe adverse effects associated with NNRTIs administration in comparison to therapies based on other anti-HIV-1 agents. However, because of the rapid selection of mutants, further new drugs with activity against the clinically relevant drug-resistant viruses are needed.



In this context, 3,4-dihydro-2-alkoxy-6-benzyl-4-oxypyrimidines (DABOs) have been the object of many studies aimed at identifying novel analogues endowed with potent inhibitory activity towards HIV-1 wild type and especially drug-resistant mutants. Accordingly, based on the encouraging results obtained from the biological screening of our internal collection of S-DABO derivatives, we started with the systematic functionalization of the pyrimidine scaffold to identify the minimal required structural features for RT inhibition.^{1,2} We report here how the combination of synthetic, biological, and molecular modeling studies led to the identification of two novel and promising classes of NNRTIs, namely 1) S-DABO cytosine analogues (S-DABOCs) possessing interesting activity towards NNRTIs resistant mutants and 2) 4-

dimethylamino- 6-vinylpyrimidines (DAVPs) representing the first NNRTIs competing with the nucleotide substrate after binding to the non-nucleoside inhibitors binding pocket (NNIBP) of the free enzyme.^{3,4}

- [1] Radi, M.; Petricci, E.; Maga, G.; Corelli, F.; Botta, M. Parallel Solution-Phase Synthesis of 4-Dialkylamino-2-methylsulfonyl-6-vinylpyrimidines. *J. Comb. Chem.* 2005, 7, 117-122.
 [2] Radi, M.; Contemori, L.; Castagnolo, D.; Spinosa, R.; Este, J. A.; Botta, M. A versatile route to C-6 arylmethyl functionalized S-DABO and related analogues. *Org. Lett.* 2007, 9, 3157-3160.
 [3] Maga, G.; Radi, M.; Zanolli, S.; Manetti, F.; Cancio, R.; Hübscher, U.; Spadari, S.; Falciani, C.; Terrazas, M.; Vilarrasa, J. and Botta, M. Discovery of Non-Nucleoside Inhibitors of HIV-1 Reverse Transcriptase (RT) Competing with the Nucleotide Substrate *Angew. Chem. Int. Ed.* 2007, 46, 1810-1813.
 [4] Radi, M.; *et. al.*, Multidisciplinary Approach for the Identification of Novel HIV-1 Non-Nucleoside Reverse Transcriptase Inhibitors: S-DABOCs and DAVPs. *ChemMedChem*, 2008, 3, 573-593.

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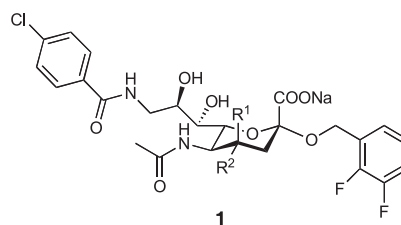
THERMODYNAMIC AND KINETIC BINDING PROPERTIES OF MAG-ANTAGONISTS

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The injured adult mammalian central nervous system is an inhibitory environment for axon regeneration due to specific inhibitory proteins. One of these neurite outgrowth inhibitors [1] is the myelin-associated glycoprotein (MAG) [2]. It belongs to the siglec family (sialic-acid binding immunoglobulin-like lectin). In previous studies, we identified high affinity antagonists [3, 4]. However, they showed unsatisfactory kinetic binding properties, i.e. fast off-rates and therefore short residence times.

We therefore investigated the influence of various modifications on the insufficient kinetic properties of these MAG antagonists. For this purpose, different substitutions were introduced to the 4 position of the neuraminic acid derivative **1**.



The thermodynamic and kinetic properties of the new antagonists were analyzed by surface plasmon resonance studies.

References

- [1] Crocker, R. H., *Curr. Opin. Struct. Biol.* 2002, 12, 609.
- [2] Quarles, R. H., *J. Neurochem.* 2007, 100, 1431.
- [3] Shelke, S., Gao, G-P., Mesch, S., G  thje, H., Kelm, S., Schwardt, O., Ernst, B., *Bioorg. Med. Chem.* 2007, 15, 4951.
- [4] Method for the identification of new leads for drug candidates, PCT/WO2007/105094 A1, priority: March 14, 2006.

P588

DISCOVERY OF BNC105P, A CLINICAL CANDIDATE FOR THE TREATMENT OF CANCER: A CASE STUDY IN THE APPLICATION OF THE DIVERSITY-ORIENTED SYNTHESIS TECHNOLOGY, MULTICORE®.

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Drug-discovery efforts invariably involve the identification of a start-point molecule (library screening hit, in silico screening hit or literature compound) that exhibits some affinity for a target or some relevant pharmacology, but which must undergo substantial optimisation in order to represent a viable therapeutic. Poor control over the properties of the start-point molecule and/or the lack of a tractable synthesis process, are often key limitations to such programs. MultiCore® is a diversity-oriented synthesis tool that allows fluid movement, synthetically, across a range of both similar and dissimilar scaffolds that exhibit a drug-like bias. This tool provides for novel strategies for optimisation, where-by the pharmacophoric features of a compromised start-point molecule can be "transplanted" onto a range of different, drug-like scaffolds and more effectively optimised.

Our discovery of BNC105P, a clinical candidate for the treatment of cancer, serves as a case study for the practical application of MultiCore®. Using the natural product, combrestatin A4 as a start-point compound, we have applied MultiCore® to the identification of new and improved agents on patently distinct scaffolds. In particular, BNC105P, exhibits 20 fold greater potency, 100 fold greater selectivity and a 20 fold greater therapeutic index compared to CA4.

P589

QSAR ANALYSIS OF CELECOXIB DERIVATIVES

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With cancer-related fatalities being the second leading cause of death in the US, understanding the activity of effective chemotherapeutic agents is critical to addressing prostate and other cancers. Celecoxib, an FDA approved drug for the treatment of colon tumors, has been

used successfully as a lead compound in the development of anti-proliferative agents. Expanding the utility of this molecule, Zhu *et al.* created a library of celecoxib derivatives that diminished cell proliferation by inhibiting PDK1 activity in PC-3 cells at low micromolar concentrations. The versatility of their most successful analog, OSU-03013, has been documented for a number of tumors. By evaluating the structure activity of this library of molecules we were able to create a QSAR model for predicting the anti-proliferative activity of structurally similar molecules. Several novel molecules were developed whose experimental activity demonstrate the success of the model. The development of the model and the synthesis and biological activity of the molecules will be presented.

P590

BAK-BCL-X_L INTERACTION AND REGULATION OF APOPTOSIS : A NEW SYNTHETIC ALPHA-HELIX MIMETICS BASED OF THE BIPHENYL 4,4'-DICARBOXAMIDE SCAFFOLD

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The apoptosis is one of the most important mechanism used by cells to regulate the homeostasis of the organism. Moreover the programmatic cell-death is a great natural weapon against some pathologies characterized by an uncontrolled cell growing, such as cancer. Several proteins exhibit an important role in the regulation of the apoptosis, and in particular the protein family of Bcl-2¹. This group of proteins is made up of both anti-apoptotic and pro-apoptotic factors and the interactions between them determine the equilibrium in that cell activity. More important is the consideration that in tumor cell several anti-apoptotic factors are overexpressed². Therefore the inhibition of their activity, through the interaction with these proteins, would be an interesting target in anti-cancer drug design. The alpha-helix, being the most common secondary structure present in the proteins, corresponds often to the exposed surface in the protein-protein interaction. To the date several synthetic alpha-helix mimetics have been reported in the literature, most of them able to be almost perfectly overlapped to small alpha-helix sections. Hamilton and co. have showed different scaffolds that mimic small alpha-helix, and in particular the *i*, *i*+3 or *i*+4, *i*+7 residues³. Herein we report a new class of mimetics, based on the biphenyl 4, 4' dicarboxamide scaffold (**3**), which can simulate, compared with the previous examples, an extra aminoacidic residue, the *i*+11 (Fig.1).

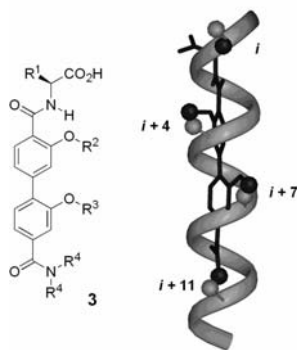


Fig. 1

A library of compounds, characterized by different lateral substitutions, have been synthesized and tested as inhibitors of Bak-Bcl-x_L interaction, both members of the Bcl-2 proteins family⁴. Some of these molecules exhibit a good activity, showing a low micromolar K_i value. The inhibition has been checked through a Fluorescence Polarization Competition Assay^{5,6}, using Bcl-x_L and a fluorescent labeled Bak peptide, displaced *in situ* by biphenyl compounds. Computational studies suggest that the alpha-helix mimetic fits with a good approximation into the hydrophobic Bak cleft of the surface of Bcl-x_L.

References

- [1] Alexandrina Burlacu, *J. Cell. Mol. Med.* Vol 7, No 3, 2003 pp. 249-257
- [2] Fisher, D. E., Apoptosis in Cancer-Therapy - Crossing the Threshold. *Cell* 1994, 78, (4), 539-542.
- [3] (a) O. Kutzki, H.S. Park, J.T. Ernst, B.P. Orner, H. Yin, and A.D. Hamilton*, *JACS*, 2002, 124, 11838-11839; (b) J.T. Ernst, J. Becerril, H. Soon Park, H. Yin and A.D. Hamilton*, *Angew. Chem. Int. Ed.* 2003, 42, No. 5; (c) H. Yin, G.-i. Lee, K.A. Sedey, J. M. Rodriguez, H.-G. Wang, S. M. Sebt and A.D. Hamilton, *JACS*, 2005, 127, 5463-5468.
- [4] M. Sattler, H. Liang, D. Nettlesheim, R. P. Meadows, J. E. Harlan, M. Eberstadt H. S. Yoon, S. B. Shuker, B. S. Chang, A. J. Minn, C. B. Thompson, S. W. Fesik* *Science*, 1997, 275, 983.
- [5] Haichao Zhang,* Paul Nimmer, Saul H. Rosenberg, Shi-Chung Ng, and Mary Joseph, *Analytical Biochemistry* 307 (2002) 70-75
- [6] Kutzki, O.; Park, H. S.; Ernst, J. T.; Orner, B. P.; Yin, H.; Hamilton, A. D., Development of a potent Bcl-x(L) antagonist based on alpha-helix mimicry. *Journal of the American Chemical Society* 2002, 124, (40), 11838-11839.

P591

STUDY OF ASYMMETRIC SYNTHESSES ON β -CARBOLINES

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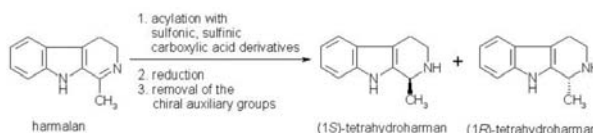
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β -Carbolines are widespread compounds considering the flora, many of them having important physiological effect, being therefore often investigated for pharmacological effects. Many 1-substituted tetrahydro- β -carbolines are known in optically active form as well, and several syntheses of them were published in recent years.

Asymmetric synthesis of 1-substituted β -carbolines was attempted, using the easily available 1-methyl-3,4-dihydro- β -carboline (harmalan) as model compound. Acylation of the latter by various chiral sulfonic and sulfinic acid derivatives as well as by carboxylic acids and chlorides, resulted in substitution at the N-2 position or at the methyl group of the harmalan. The new C1 asymmetry center was formed by reduction of the resulting optically active dihydro intermediate. After removal of the chiral auxiliary groups, the enantiomeric excess of the 1*R*- and 1*S*-tetrahydroharman products was studied.



Beside the effect of the chiral auxiliary groups, influence of the applied solvent, reagent and catalyst was also examined. For the separation of the intermediate diastereomers, new HPLC and GC methods were developed.

References

- [1] L. Hazai, M. Milen, P. Kolonits, A. Dobó, Cs. Szántay: Preferred Carbon vs. Nitrogen Sulfonylation on β -carbolines; *Synthetic Communications*, 31(6), 121-129 (2001)
- [2] J. Fekete, M. Milen, L. Hazai, L. Poppe, Cs. Szántay, A. Kettrup, I. Gebefügi: Comparative Study on Separation of Diastereomers by HPLC; *Chromatographia*, 57, 147-153 (2003)
- [3] M. Milen, L. Hazai, P. Kolonits, Á. Gömöry, Cs. Szántay, J. Fekete: Preparation and Separation of 1-Methyl-1,2,3,4-tetrahydro- β -carboline Enantiomers by HPLC Using New Derivatization Reagent; *Journal of Liquid Chromatography & Related Technologies*, 27(18), 2921-2933 (2004)
- [4] M. Milen, L. Hazai, P. Kolonits, Gy. Kalas, L. Szabó, Á. Gömöry, Cs. Szántay: Studies on Stereoselective Approaches to β -carboline Derivatives; *Central European Journal of Chemistry*, 3(1), 118-136 (2005)

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NEW SYNTHESIS OF NEW PHTHALIDES

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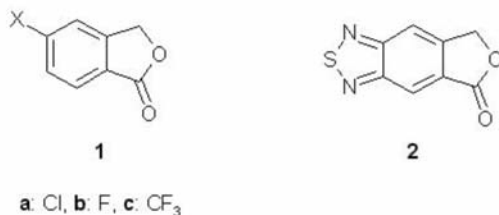
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Phthalides (3H-isobenzofuran-1-ones) are useful intermediates in the synthesis of various heterocycles widely used in medicinal chemistry. The methods described for the synthesis of phthalides **1a-c** are cumbersome and give the products in low yield^[1,2,3].

New efficient synthesis has been elaborated for phthalides **1a-c** by using directed *ortho* metallation methodology^[4,5,6]. The synthesis of the new phthalide **2** will be also presented.



References

- [1] Irvine, Robert W.; Kinloch, Stephen A.; McCormick, Alison S.; Russell, Richard A.; Warrenner, Ronald N. *Tetrahedron* 1988, 44; 4591-4604.
- [2] Warrenner, R. N.; Liu, L.; Russell, R. A. *Tetrahedron* 1998, 54, 7485-7496.
- [3] Abaev, V. T.; Dmitriev, A. S.; Gutnov, A. V.; Podelyakin, S. A.; Butin, A. V. *J. Heterocycl. Chem.* 2006, 43, 1195-1204.
- [4] Beak, P.; Brown, R. A. *J. Org. Chem.* 1982, 47, 34-46.
- [5] Beecham Group PLC.; US4918083.
- [6] Gschwend, H. W.; Hamdan, A. *J. Org. Chem.* 1975, 40, 2008-2009.

P593

NEW AND EFFICIENT PHOSPHINIC ACIDS SYNTHESIS

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Organophosphorus compounds are important substrates in the study of biochemical processes, and compounds of tetracoordinate pentavalent phosphorus are widely used as biologically active compounds. Mimics of amino acids in which the carboxylic function is replaced

by phosphorus analogues have attracted particular interest. Among these phosphorus functions, phosphinic acid moiety is an excellent mimic of the tetrahedral transition state of amid bond hydrolysis and is more stable than phosphonic or phosphonamidic isosteres. Thus, phosphinic compounds occupy an important place and reveal diverse and interesting biological and biochemical properties: phosphinic peptides have been reported to be potent inhibitors of several matrixins (MMPs),^[1] aminoalkylphosphonic acids are widely studied^[2,3] as antibacterial agents, enzyme inhibitors, haptens for catalytic antibodies, or anti HIV agents, ...

The development of methods for the preparation of phosphinic acids is so important and currently attracting growing interest. The most commonly employed methods to prepare H-phosphinates suffer from several limitations: large excess of reagents, difficulties to avoid formation of symmetrically disubstituted phosphinates, handling difficulties of some starting materials.

In connection with various projects requiring the synthesis of functionalized H-phosphinates, we became aware of the relative lack of convenient and general methods to access these intermediates. We thus initiated researches to develop novel methods for P-C bond formation based on the uses of a protected phosphinic acid originally described by E. Keith Baylis, easier and safer to handle. We will present here our new methodology using inexpensive starting materials and enabling the obtaining of phosphinic acids R'P(O)OHR'' with R' and R'' varying (fig 1).

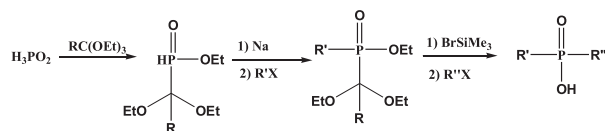


Figure 1: new phosphinic acids synthesis

- [1] M. Matziari, F. Beau, P. Cuniasse, V. Dive, A. Yiotakis, *J. Med. Chem* 2004, 47, 325.
- [2] E. A. Boyd, A. C. Regan, K. James, *Tetrahedron Letters* 1994, 35, 4223.
- [3] A. Ryglowski, P. Kafarski, *Tetrahedron* 1996, 52, 10685.

P594

IDENTIFICATION AND DEVELOPMENT OF [5-CHLORO-2-OXO-6-(2-SUBSTITUE PHENYL-1,3-THIAZOL-4-YL)-3-SUBSTITUE BENZOXAZOLE DERIVATIVES AS INHIBITOR OF COX

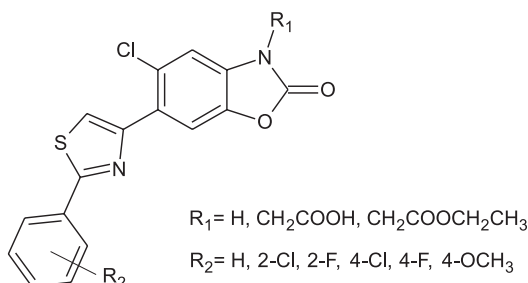
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The principal pharmacological effects of nonsteroidal anti-inflammatory drugs (NSAIDs) are due to their ability to inhibit prostaglandin synthesis by blocking cyclooxyge-

nase (COX) activities¹. COX enzyme catalyzes the initial step of arachidonic acid metabolism and prostaglandin production. The discovery of two COX isoenzymes; COX-1 and COX-2, and the detection of their various functions and regulations, have initiated the search for new and putatively more selective inhibitors of prostaglandin biosynthesis. Specifically, selective COX-2 inhibitors (coxibs) were developed in order to improve anti-inflammatory and analgesic activities and to reduce side effects in the gastrointestinal tract². By the discovery of potential cardiovascular risks of coxibs, new strategies were started to be searched in inflammation therapy. Selective COX-2 inhibition, accompanying suppression of TXA₂ a product of COX's pathway, have been investigated as a new methodology in inflammation therapy³.

Our ongoing studies on the derivatives of 5-chloro-2-oxo-3H-benzoxazole (chlorzoxazone) prompted us to design new compounds which have both TXA₂ synthase inhibitory activity and COX-2 inhibitory activity. The activities of the compounds were evaluated by enzyme immunoassay method on COX isoforms.



[1] Zhihua Sui, Jihua Guan, Michael P. Ferro, Kathy McCoy, Michael P. Wachter, William V. Murray, Monica Singer, Michele Steber, Dave M. Ritchie and Dennis C. Argentieri. "1,3-Diarylcycloalkanopyrazoles and Diphenyl Hydrazides as Selective Inhibitors of Cyclooxygenase-2". *Bioorganic&Medicinal Chemistry Letters*. 2000; 10: 601-604

[2] J. Meyer-Kirchraht and K. Schrör. "Cyclooxygenase-2 Inhibition and Side-effects of Non-steroidal Anti-inflammatory Drugs in the Gastrointestinal Tract". *Current Medicinal Chemistry*. 2000; 7: 1121-1129

[3] Egan K. M., Wang M., Lucitt M. B., Zulas A. M., Pure E., et al. "Cyclooxygenases, Thromboxane, and Atherosclerosis. Plaque Destabilization by Cyclooxygenase-2 Inhibition Combined With Thromboxane Receptor Antagonism". *Circulation*. 2005; 111: 334-342.

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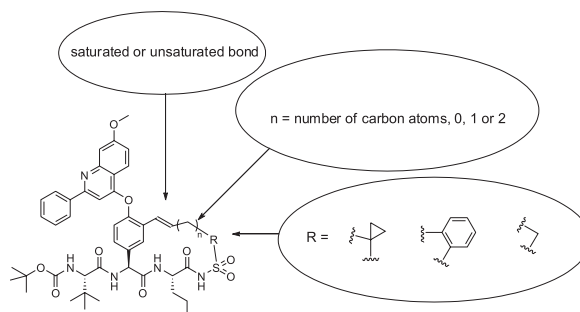
NEW MACROCYCLIC P2 PHENYLGLYCINE-BASED INHIBITORS TARGETING HEPATITIS C VIRUS NS3 PROTEASE

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The NS3 protease from hepatitis C virus (HCV) is a well studied drug target. A problem with inhibitors targeting the NS3 protease is that they are often of peptidic character and prone to degradation by proteases. Macrocyclisation is a way to diminish proteolysis and can often improve pharmacokinetic properties.

A series of P2 phenylglycine-containing macrocyclic HCV NS3 protease inhibitors, with ring-closure spanning between P2 and P1', incorporating acyl sulfonamides with different sulfonamide substituents and linker lengths have been synthesized with a ring-closing metathesis reaction and biochemically evaluated.



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DESIGN AND SYNTHESIS OF 2(1H)-PYRAZINONE-BASED HEPATITIS C VIRUS NS3 PROTEASE INHIBITORS

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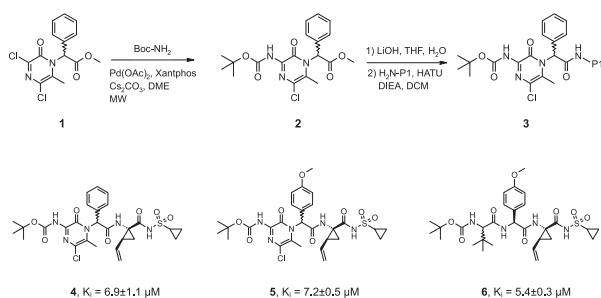
We have previously reported the discovery of phenylglycine (Phg) as a promising new P2 residue in tripeptide Hepatitis C virus (HCV) NS3 protease inhibitors¹. Aiming at inhibitors with decreased peptide character, we decided to synthesize a series of inhibitors combining 2(1H)-pyrazinones in the P3 position with Phg in the P2. 2(1H)-pyrazinones are known β -strand conformation inducers, and since the natural substrates of proteases generally adopt extended conformation prior to binding, incorporation of a β -strand inducer would in theory be beneficial also for inhibitor binding.

With a fast microwave-heated method for the synthesis of the pyrazinone core developed in our labs, the P2-P3 building block **1** can be synthesized in 20 minutes compared to several hours or days using conventional

heating. Further substitution of the pyrazinone is easily achieved in the 3-position: the 3-chloro atom is very reactive towards nucleophiles while the 5-chloro is not, which makes the substitution selective. There are numerous literature examples of such substitutions. By using a Buchwald-type of Pd-catalyzed coupling, we have been able to introduce even weak nucleophiles such as Boc-NH₂ in the 3-position, resulting in compound **2**. Ester hydrolysis followed by standard peptide coupling in solution furnishes the final tripeptide mimic **3**.

Using this strategy, we have synthesized inhibitors **4** and **5**. As 1:1 mixtures of L- and D-Phe, they are equipotent with the tripeptide reference **6**¹, but **4** and **5** have a significantly decreased peptide character.

Further investigations concerning the nucleophile and the P1 group is underway.



[1] P. Örtqvist et al, *Bioorg. Med. Chem.*, 2007, 15, 1448-1474.

P597

THE DISCOVERY OF SMALL PEPTIDES AS POTENT LIGANDS OF THE SPECIFIC BINDING SITE FOR SUBSTANCE P (1-7)

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Substance P (SP), which is a neurotransmitter and neuromodulator at the G-protein coupled NK1 receptor, plays a well known role in pain transmission [1]. The N-terminal fragment of SP, the heptapeptide SP₁₋₇ (H-Arg-Pro-Lys-Pro-Gln-Gln-Phe-OH), is a bioactive metabolite that has been shown to oppose several effects of SP [2]. Specific binding sites for SP₁₋₇ that differ from the NK1 receptor have been identified in both mouse and rat [3, 4]. In the aspiration to characterise the binding site for SP₁₋₇ and for studies aiming to reveal the role of the heptapeptide in complex animal models, drug-like low molecular weight SP₁₋₇ mimetics as research tools are highly desirable. Recently, we reported a detailed structure-activity relationship (SAR) study of the heptapeptide, which demonstrated that C-terminal amidation of SP₁₋₇ and of all truncated analogues potentiate the binding affinity [5].

In this work, similar SAR studies have been performed on the endogenous ?-receptor agonist, endomorphin-2 (EM-2, H-Tyr-Pro-Phe-Phe-NH₂), which has been shown to interact with the SP₁₋₇ binding site with only a 10-fold reduction in affinity compared to SP₁₋₇ [4]. The SAR studies of EM-2 resulted in the discovery of a very potent small peptide. With this peptide as a starting point a series of compounds was synthesized with the intention to explore the steric, hydrophobic and polar requirements of the binding site. A subset of the compound series was prepared using the focused hierarchical design of experiments (FHDoe) strategy for amino acid selection [6].

References

- [1] Zubrzycka, M., Janecka, A. *Endocr Regul.* 34, 195 (2000)
- [2] Hallberg, M., Nyberg, F. *Curr Protein Pept Sci.* 4, 31 (2003)
- [3] Igwe, O J. et al. *J Neurosci.* 10, 3653 (1990)
- [4] Botros, M. et al. *Peptides* 27, 753 (2006)
- [5] Fransson, R. et al. *Neuropeptides* 42, 31 (2008)
- [6] Muthas, D. et al. *J. Chemometrics* 21, 486 (2007)

P598

SYNTHESIS AND BIOLOGICAL ACTIVITY OF NEW [3-ARYLMETHYLIDENYL]INDOLIN-2-ONES AS INHIBITORS OF KINASES

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Cyclin-dependent kinases (CDKs) constitute a family of protein kinases targeted for the treatment of major human diseases as cancer, several viral infections and neurodegenerative diseases.¹ Recent studies have shown that the inhibition of kinases has become an interesting target in the pharmaceutical industry.²

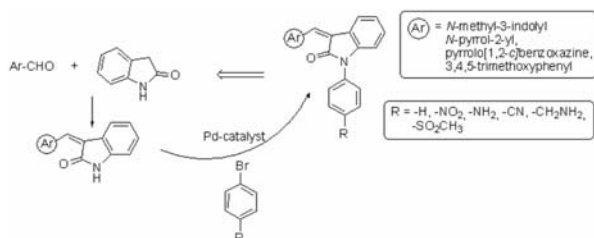
The aim of the present work was to synthesize new 3-substituted indolin-2-ones and study the *in vitro* anticancer activity and also the direct inhibition of kinases evaluating the kinases selectivity.

We synthesized these new indolin-2-ones with the aim of improving the antitumor activity and selectivity of this new scaffold possessing *N*-aryl substituent. In general, reported indolin-2-ones have alkyl (-CH₃) or unsubstituted (-H) the *N*-indolic atom.³

N-Aryl-[3-arylmethyliden]indolin-2-ones were prepared by condensing the indolin-2-one (oxindole) with the corresponding aldehyde in the presence of base, according the classic Knoevenagel condensation.

Finally, the *N*-arylation of the indolin-2-one moiety was accomplished by coupling of the corresponding bromoaryl and the 3-arylmethyliden]indolin-2-ones and the optimal combination of Palladium-catalyst, ligand

(BINAP) and base. The 3-substituted indolin-2-ones may exist as either the *Z* or *E* isomer depending on the characteristics of the *C*-3 and *N*-1 substituents. The two isomer compounds have been distinguished by NMR-techniques.



References

- [1] P. M. Fisher. *Curr. Med. Chem.* 2004, 11, 1563.
- [2] H. Weinmann, R. Metternich. *ChemBioChem.* 2005, 6, 455.
- [3] L. Sung, N. Tran, F. Tang, H. App, P. Hirth, G. McMahon, C. Tang. *J. Med. Chem.* 1998, 41, 2588.
- [4] M. Romero, Y. Harrak; J. Basset, L. Ginet, P. Constans, M: D. Pujol. *Tetrahedron* 2006, 62, 9010.

P599

ETHIONAMIDE BOOSTERS: A NEW STRATEGY TO FIGHT MULTI-DRUG RESISTANT TUBERCULOSIS

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With approximately 9 million people developing active tuberculosis (TB) every year and 1.7 million deaths annually, TB is far from under control. Human immunodeficiency virus (HIV) infection dramatically increases the risk of developing active tuberculosis and is driving the TB epidemic in Africa.¹ The increasing spread of multidrug-resistant TB (MDR-TB) and the increasing emergence of extensively drug-resistant (XDR) TB, urge the use of second-line drugs, such as ethionamide. These drugs are less effective, more toxic and are used for a longer period of treatment.

Ethionamide is a prodrug that requires activation by the mycobacterial monooxygenase EthA to be effective. Recently, the team of one of our collaborators (A. Baulard) showed that the limited efficacy of ethionamide is due to a bacterial transcriptional regulator called EthR.²

In this paper, we show that compounds that inhibit EthR increase the sensitivity of the *M. tuberculosis* to ethionamide.

Our goal is to boost ethionamide activation in patients to lower the dose of ethionamide and minimize side effects, making the therapeutic regimen more efficient, improving compliance and reducing the occurrence of resistant strains of mycobacteria.

By using multidisciplinary approaches, high-throughput screening, radiocrystallography, rational design and Surface Plasmon Resonance (SPR), our group has discovered ligands that increase efficacy of ethionamide by at least 25-fold *in vitro*, and 3-fold *in vivo*.

We report here the identification, optimization and activities of EthR inhibitors while keeping physicochemical and pharmacokinetic properties, in a drug-like range.

References

- [1] Global tuberculosis control: surveillance, planning, financing: WHO report 2008, (WHO/HTM/TB/2008.393).
- [2] EthR, a repressor of the TetR/CamR family implicated in ethionamide resistance in mycobacteria, octamerizes cooperatively on its operator, Engohang-Ndong J, Baillat D, Aumercier M, Bellefontaine F, Besra GS, Loch C, Baulard AR, *Molecular Microbiology* 51 (1), 175-188, 2004.

P600

MOLECULAR DYNAMICS AS A MEANS TO DESIGNING PET TRACERS FOR HSV1-TK-BASED GENE THERAPY

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Therapeutically efficient nucleoside analogues against Herpes Simplex Virus 1 are selectively activated via its Thymidine Kinase (HSV1-TK). Further metabolized to triphosphates, they inhibit viral replication by blocking the viral DNA-polymerase. Being trapped inside the cell due to their negative charges, they are suitable for Positron Emission Tomography (PET) to monitor HSV1-TK activity *in situ*. Here, we apply molecular dynamics to the design of PET tracers for HSV1-TK-based gene therapy. With this method, we assess the stability of HSV1-TK regions allowing binding of experimentally determined high affinity substrates and spot more flexible regions where substitutions could be beneficial. To this end, 3 different high resolution crystal structures of HSV1-TK in complex with their respective substrates have been used. We present the protocol setup as well as the method validation combining AMBER 9.0 [1] and NAMD 2.6 [2], including a 50 ps minimization-equilibration and a 5 ns trajectory simulation. To evaluate docking poses of substrate analogues previously generated with Gold 3.1.1 [3],

the above-mentioned protocol has been applied and the results have been compared to the reference simulations. Subsequently, first substitutions possibly improving the binding affinity are pointed out.

- [1] DA Case, TE Cheatham, T Darden et al. The Amber biomolecular simulation programs. *J Comput Chem*. 2005, 26, 1668.
- [2] JC Phillips, R Braun, W Wang et al. Scalable molecular dynamics with NAMD. *J Comput Chem*. 2005, 26, 1781.
- [3] G Jones, P Willett, RC Glen. Molecular recognition of receptor sites using a genetic algorithm with a description of desolvation. *J Mol Biol*. 1995, 245, 43.

P602

EMERALD: AN INTEGRATED IN HOUSE TOOL FOR COMBINATORIAL LIBRARY DESIGN

Isabelle Ortmans[§], Bénédicte Lallemand[§], Daniel Smaragd[§] and Florence Moureau[§]

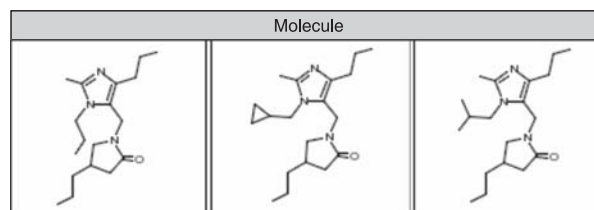
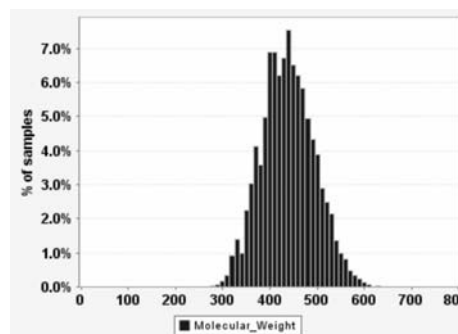
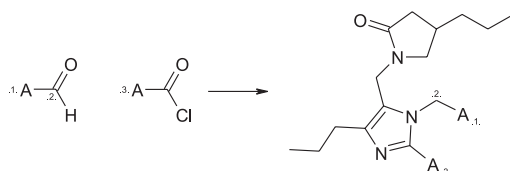
UCB Pharma SA, Chemin du Foriest - B-1420 - Braine-l'Alleud, Belgium; [§]Chemistry, CADD; [§]Chemistry, Combinatorial Chemistry; [§]Therapeutic Projects Informatics

The emergence of high-throughput technologies for the synthesis and screening of large numbers of compounds has opened up new horizons in the area of drug development. In this respect the impact of combinatorial chemistry in drug discovery is largely determined by both the optimization of the synthetic scheme and the design of libraries, i.e. the reagent selection process.

Library design is a multi-objective process since the targeted chemical space, either focused or large, has to be explored efficiently while simultaneously ensuring "drug-like" properties of the compounds and considering synthetic requirements. With millions of virtual end products, reagent selection is intractable by hand and requires computer-aided selection tools.

Reagent selection is usually a time consuming task which requires many iteration cycles between synthetic and computational chemists. To optimize this fundamental process, a tool integrating all library design steps (EMERALD, Enumeration of Molecules by Exploration of Reagents Applied to Library Design) has been developed in house in order to make combinatorial library design facilities accessible to all chemists. The software offers various functionalities such as reagent filtering, library enumeration and library profiling.

EMERALD has already been successfully applied to the design of libraries in a number of therapeutic projects, with significantly reduced time delays associated with the design process.



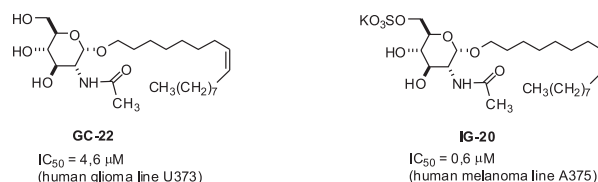
P603

EFFECT OF NOVEL ANTITUMOURAL GLYCOSIDES ON GANGLIOSIDE CONTENT IN GLIOMA AND MELANOMA CELLS

Isabel García-Álvarez,^a Juan Antonio Martínez,^b Ernesto Doncel-Pérez,^{a,c} Manuel Nieto-Sampedro,^{a,c} Meritxell Egado-Gabás,^d Josefina Casas,^d and Alfonso Fernández-Mayoralas^b

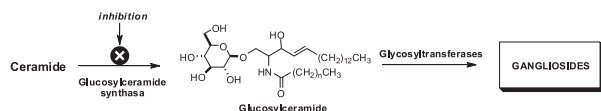
^aHospital Nacional de Paraplégicos, SESCAM, Finca la Peraleda s/n, 45071 Toledo, Spain. ^bInstituto de Química Orgánica General, CSIC, Juan de la Cierva 3, 28006 Madrid, Spain. ^cInstituto Cajal, CSIC, Avda. Doctor Arce 37, 28002 Madrid, Spain. ^dRUBAM Instituto de Investigaciones Químicas y Ambientales de Barcelona, CSIC, Jordi Girona 18, 08034 Barcelona, Spain.

We have recently described^[1] the synthesis and biological activity of *N*-acylglucosamine derivatives with the ability to inhibit the division of human glioma and melanoma cells in culture. Glycosides **GC-22** and **IG-20** inhibited the division of glioma and melanoma cells exhibiting IC₅₀ values in the range of micromolar concentration or below.



The mechanism of action of the glycosides has been studied under the hypothesis they could act altering ganglioside biosynthesis. These molecules have been shown to have crucial regulatory roles in tumor onset and progression.^[2] The effect of the antitumoural glycosides on the gangliosides expressed by C6 glioma and A375 melanoma cell lines was studied by HPLC/MS, as well as

on the ceramide content which was analyzed by TLC. The results showed that **GC-22** reduced ganglioside synthesis in both cell lines and increased the amount of ceramide. Considering the biosynthetic pathway of gangliosides, **GC-22** could be operating by inhibiting glucosylceramide synthase, the enzyme catalyzing the initial step in the synthesis. In order to check this hypothesis we have evaluated the activity of glucosylceramide synthase in the presence of **GC-22** and **IG-20**.



Acknowledgments

Financial support by the Servicio de Salud de Castilla la Mancha Community (SESCAM), MEC (CTQ2007-67403/BQU), and CSIC (PIF, 200580F0062) is greatly appreciated.

[1] I. García-Álvarez, G. Corrales, E. Doncel-Pérez, A. Muñoz, M. Nieto-Sampedro, A. Fernández-Mayoralas. *J. Med. Chem.* 2007, 50, 363-373.

[2] S. Birklé, G. Zeng, L. Gao, R. K. Yu, J. Aubry. *Biochimie.* 2003, 85, 455-463.

P604

DETECTION OF METABOLITE CHANGES IN CULTURED C6 GLIOMA CELLS WITH OLEYL GLYCOSIDES BY ¹H MAS NMR

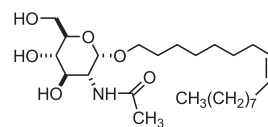
Isabel García-Álvarez,^a Leoncio Garrido,^b Ernesto Doncel-Pérez,^{a,c} Manuel Nieto-Sampedro,^{a,c} and Alfonso Fernández-Mayoralas.^d

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We have previously studied the effect of antitumoural glycosides on ganglioside formation in glioma and melanoma cells. One of the glycosides, **GC-22**, which inhibited the division of cultured C6 glioma cells ($IC_{50}=1.3 \mu M$),¹ produced a reduction in the total ganglioside synthesis, as well as a moderate rise in the ceramide content (see Fernández-Mayoralas *et al.* abstract).

Here, we report the detection of changes in other metabolites in cultured C6 glioma cells in presence of oleyl glycosides using high-resolution magic angle spinning ¹H NMR. This technique has become a powerful tool in the study of biological process; changes in the spectral pattern of tumours are being used in an attempt to detect or predict the progression of human brain tumors,² and it allows to obtain the spectra of the whole cells with high spectral resolution.

Experimental results showed a rise in choline-containing metabolites when C6 cells were treated with **GC-22** (Figure 1), suggesting the induction of apoptosis by this glycoside as a possible mechanism to explain the observed growth cell inhibition. This hypothesis was corroborated by DNA analysis, which showed DNA fragmentation in C6 cells cultured with **GC-22**.



GC-22

$IC_{50} = 1,3 \mu M$
(rat glioma line C6)

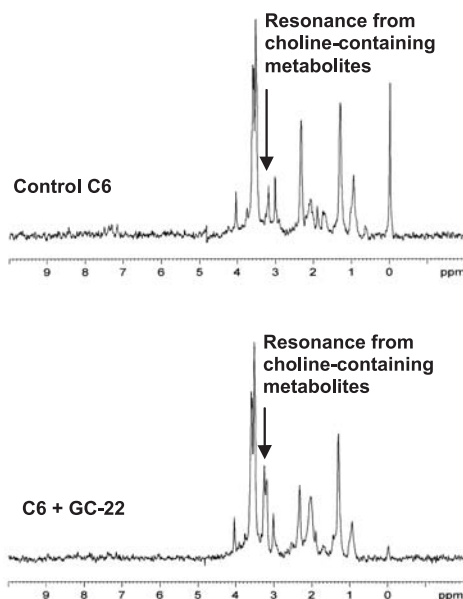


Figure 1. ¹H MAS NMR spectra of C6 (control) and C6 cultured with **GC-22** (10 μM).

Acknowledgments

Financial support by the Servicio de Salud de Castilla la Mancha Community (SESCAM), CSIC (PIF, 200580F0062) and MEC (CTQ2007-67403/BQU) is greatly appreciated.

[1] García-Álvarez, I.; Corrales, G.; Doncel-Pérez, E.; Muñoz, A.; Nieto-Sampedro, M.; Fernández-Mayoralas, A. *J. Med. Chem.* 2007, 50, 363-373.

[2] Howe, F. A.; Opstad, K.S. *NMR Biomed.* 2003, 16, 123-131.

P605

SYNTHESIS, X-RAY STRUCTURE AND BIOLOGICAL ACTIVITIES OF NOVEL 1-[(IMIDAZOLIDIN-2-YL)IMINO]AZOLES*

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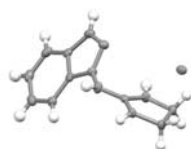
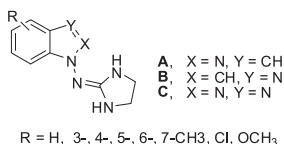
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Continuing our previous studies on novel imidazoline compounds¹⁻³ with selective affinity to α_2 -adrenoceptor or imidazoline I₁/I₂ receptor, we have prepared several new series of indazole (**A**), benzimidazole (**B**) and benzotriazole (**C**) derivatives containing (imidazolidin-2-yl)imino moiety at position 1. Structures of all iminoimidazolidine derivatives obtained were confirmed by spectroscopic data (¹H- and ¹³C-NMR) and selected compounds were subjected to the single crystal X-ray structure analysis.

The *in vitro* assays showed very low or no affinity of benzimidazoles (**B**) and benzotriazoles (**C**) for α_2 -adrenoceptors, imidazoline I₁ and imidazoline I₂ binding sites imidazoline I₂. On the other hand, indazole derivatives (**A**) were found to be inactive at imidazoline I₂ receptors ($K_i > 100.000$). The most interesting compound in this series, 1-[(imidazolidin-2-yl)imino]indazole (*marsanidine*), exhibited a high affinity for α_2 -adrenoceptors ($K_i = 14$ nM) and a very high α_2/I_1 selectivity ratio = 3879.

The *in vivo* cardiovascular properties of indazole derivatives (**A**) were evaluated after intravenous infusion in anaesthetized male Wistar rats. The highest hypotensive activity was found for 1-[(imidazolidin-2-yl)imino]-7-methylindazole (Δ MAP = - 43.5 mmHg, ED₅₀ = 0.6 μ g/kg), which also exhibited a good affinity for α_2 -adrenoceptors ($K_i = 53.5$ nM) and moderate affinity for imidazoline I₁ receptors ($K_i = 387$ nM).

The above results suggest that *marsanidine*, a selective α_2 -agonist, may find medicinal uses in anaesthesia as analgesic, sedative, anxiolytic, hemodynamic-stabilizing and organ-protective agent, while its 7-methyl analogue is a good candidate for further development as a centrally acting antihypertensive drug.



References:

- [1] Sączewski F., Kornicka A., Rybczynska A., Hudson A.L., Miao S.S., Gdaniec M., Boblewski K., Lehmann A. 1-[(Imidazolidin-2-yl)imino]indazole.— Highly α_2/I_1 selective agonist: synthesis, X-ray structure and biological activity; *J. Med. Chem.* 2008 - in press.
- [2] Sączewski F., Hudson A.L., Tyacke R.J., Nutt D.J., Man J., Tabin P., Sączewski J. *Eur. J. Pharm. Sci.* 2003, 20, 201-208.
- [3] Sączewski F., Tabin P., Tyacke R.J., Maconie A., Sączewski J., Kornicka A., Nutt D.J., Hudson A.L. *Bioorg. Med. Chem.* 2006, 14, 6679-6685.

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P606

¹H NMR METABOLOMIC APPROACH OF THE LASER-INDUCED CHOROIDAL NEOVASCULARIZATION IN MICE

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Age-related Macular Degeneration (AMD) is a main cause of vision loss in the western world among people older than 50. Ninety per-cent of vision loss due to AMD results from the exudative form, which is characterized by choroidal neovascularization (CNV), defined as newly formed blood vessels arising from choriocapillaries. Age-related changes inducing pathologic neovascularization are incompletely understood. However, vascular endothelial growth factor (VEGF)-A, a diffusible growth factor promoting angiogenesis (the sprouting of endothelial cells from preexisting vasculature) and vascular permeability, has emerged as a potent angiogenic factor involved in CNV formation. A successful application of anti-VEGF therapy in the clinic is obviously a turning point in AMD treatment. Nevertheless, despite such important advances, critical issues remain to be addressed. To better understand the etiology of this pathology, we have used and improved a model of laser-induced murine choroidal neovascularization (LIM-CNV). Until now, metabolic changes in patients with AMD are deeply unknown. In consequence, we decided to use metabolomic approach in the LIM-CNV experimental model.

Metabolomics is a relatively new branch of « omics » technologies focused on the analysis and measurement of endogenous metabolites. These experiments provide a challenge to improve the knowledge of physiological sta-

tus related to metabolome and in general to functional genomics.

For this purpose, sera from induced and non-induced mice have been collected and the metabolic profiles of these samples were determined by ¹H NMR. The spectra were then analyzed by statistical discriminant methodologies (PCA, ICA, PLS-DA).

This approach allows the differentiation of the laser-induced mice from the control group. Moreover, several discriminating spectral zones have been identified leading to the emergence of different putative biomarkers. These first interesting results open new way in the study of the CNV metabolism.

P607

THIOFLAVIN T DERIVATIVES AS MARKERS FOR AMYLOID-BETA FIBRILS: TOWARD THE STRUCTURAL OPTIMIZATION OF LIGAND DESIGN FOR HIGH AFFINITY

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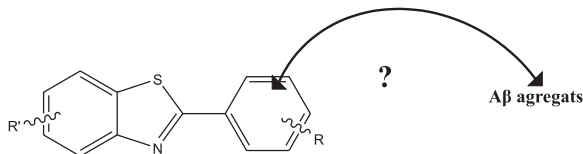
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Alzheimer's disease is a common neurological disease of chronic dementia, memory loss and cognitive impairment. Central to the neuropathology of AD are the senile plaques (SPs) and the neurofibrillar tangles (NFTs), depositions composed of β -amyloid peptide ($A\beta$) and tau protein, respectively. Aggregated $A\beta$ senile plaque has a beta-sheet secondary structure and is arranged as fibril. Formation and accumulation of aggregates of $A\beta$ peptide in the brain are critical in the development and progression of AD; in which oxidative stress represent a field of current intensive studies. There is a great interest in molecules able to bind specifically to aggregates. Such markers of amyloid fibrils would allow their early detection and specificity would allow to distinguish $A\beta$ fibrils from other amyloids deposits. This is of importance from basic research up to clinical application. In particular, such molecules give access to molecular imaging techniques (MRI, SPECT, PET.....) for early diagnostic of this disease. Three main categories of TEP ligands of AD-associated aggregates are currently under investigation ThioflavinT (ThT), styrylbenzene (SB) and aminophenyl derivatives.

ThT has been used for several decades to stain amyloids such as $A\beta$. ThT is not specific for amyloids of $A\beta$ as it reacts with many types of amyloids. Yet not much is known about the structure/function relationships explaining the nature of the interaction marker/ $A\beta$ aggregate and most improvement of binding affinity seemed to arise from empirical studies. The reported insights are scarce. A better knowledge of the binding sites of markers derived from ligands as ThT would allow a more rational ligand

design. In order to address this question and to get deeper insight the nature of the interaction between uncharged ThT derivatives, we synthesized some derivatives and compared their binding affinity to amyloid fibrils, which allow us to propose some important feature regarding the marker/ $A\beta$ fibrils interaction.



Reference

- [1] Nordberg, Lancet Neurology 2004, 3, 519.
- [2] R. Tycko, Protein Pept Lett 2006, 13, 229.
- [3] M. P. Mattson, Nature 2004, 430, 631.
- [4] D. J. Selkoe, Science 2002, 298, 789.
- [5] C. Talmard, L. Guilloueu, Y. Coppel, H. Mazarguil, P. Faller, ChemBiochem 2007, 8, 163
- [6] R. Leuma Yona, S. Mazères, P. Faller, E. Gras, ChemMedChem, 2008, 3, 63.

P608

ENCODED SELF-ASSEMBLING CHEMICAL LIBRARIES, A NOVEL TOOL IN DRUG DISCOVERY

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The de novo discovery and development of selective drugs for the multitude of protein targets originating from functional genomic research remains a challenging task. Display technologies (such as phage display (1, 2) and ribosome display(3)) can be used for the isolation of polypeptides (e.g. antibodies) against virtually any given target. The success of these technologies relies mainly on the physical linkage between phenotype and genotype which allows both the production of libraries of very large size and the identification of binding library members from a simple affinity-based panning experiment.

A similar "selection/amplification" approach for the discovery of low-molecular weight compounds capable of specific binding to protein targets of choice has so far been lacking. In principle, large collections of chemical compounds, individually coupled to unique oligonucleotides, allow similar affinity capture procedures for small molecular entities, followed by PCR-based amplification and identification of preferred binding specificities (for a review, see (4)). Such DNA-encoded chemical libraries may be con-

structured as single-pharmacophore libraries [in which suitable chemical moieties are added in a stepwise fashion to molecular scaffolds; (5-7)] or as dual-pharmacophore chemical libraries [also termed Encoded Self-Assembling Chemical (ESAC) libraries (8)]. The latter methodology relies on the combinatorial self-assembly of sublibraries (consisting of chemical moieties which are displayed on oligonucleotides) and is particularly suited for the production of large libraries for de novo lead discovery as well as for the maturation of binding affinity and specificity, starting from low-affinity lead compounds.

Here, we discuss the use of ESAC technology for the de novo isolation and affinity maturation of potent inhibitors of MMP-3 and trypsin (9, 10), and the discovery of portable human serum albumin binders, which are now in clinical development (11).

References

- [1] Smith, G. P. (1985) Filamentous fusion phage: novel expression vectors that display cloned antigens on the virion surface. *Science* 228, 1315-1317.
- [2] Clackson, T., Hoogenboom, H. R., Griffiths, A. D., and Winter, G. (1991) Making antibody fragments using phage display libraries. *Nature* 352, 624-628.
- [3] Hanes, J., and Pluckthun, A. (1997) In vitro selection and evolution of functional proteins by using ribosome display. *Proc Natl Acad Sci U S A* 94, 4937-4942.
- [4] Scheuermann, J., Dumelin, C. E., Melkko, S., and Neri, D. (2006) DNA-encoded chemical libraries. *J Biotechnol* 126, 568-581.
- [5] Brenner, S., and Lerner, R. A. (1992) Encoded combinatorial chemistry. *Proc Natl Acad Sci U S A* 89, 5381-5383.
- [6] Halpin, D. R., and Harbury, P. B. (2004) DNA display II. Genetic manipulation of combinatorial chemistry libraries for small-molecule evolution. *PLoS Biol* 2, 1022-1030.
- [7] Gartner, Z. J., Tse, B. N., Grubina, R., Doyon, J. B., Snyder, T. M., and Liu, D. R. (2004) DNA-templated organic synthesis and selection of a library of macrocycles. *Science* 305, 1601-1605.
- [8] Melkko, S., Scheuermann, J., Dumelin, C. E., and Neri, D. (2004) Encoded self-assembling chemical libraries. *Nat Biotechnol* 22, 568-574.
- [9] Melkko, S., Zhang, Y., Dumelin, C. E., Scheuermann, J., and Neri, D. (2007) Isolation of High-Affinity Trypsin Inhibitors from a DNA-Encoded Chemical Library. *Angew Chem Int Ed Engl* 46, 4671-4674.
- [10] Scheuermann, J., Dumelin, C. E., Melkko, S., Zhang, Y., Mannocci, L., Jaggi, M., Sobek, J., and Neri, D. (2008) DNA-encoded chemical libraries for the discovery of MMP-3 inhibitors. *Bioconjug Chem* 19, 778-785.
- [11] Dumelin, C. E., Trussel, S., Buller, F., Trachsel, E., Bootz, F., Zhang, Y., Mannocci, L., Beck, S. C., Drumea-Mirancea, M., Seeliger, M. W., Baltes, C., Muggler, T., Kranz, F., Rudin, M., Melkko, S., Scheuermann, J., and Neri, D. (2008) A portable albumin binder from a DNA-encoded chemical library. *Angew Chem Int Ed Engl* 47, 3196-3201.

P613

SYNTHESIS AND DERIVATISATION OF 4''-O-AMINO-AVERMECTIN B_{1a}

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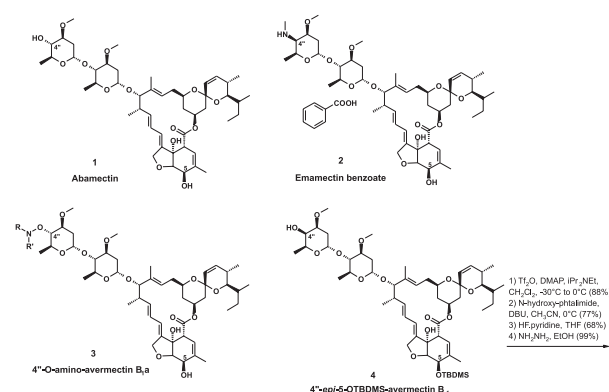
Syngenta Crop Protection Münchwilen AG, Research Chemistry Optimisation WST-820.2.15, Schaffhauserstrasse, CH-4332 Stein AG, Switzerland.
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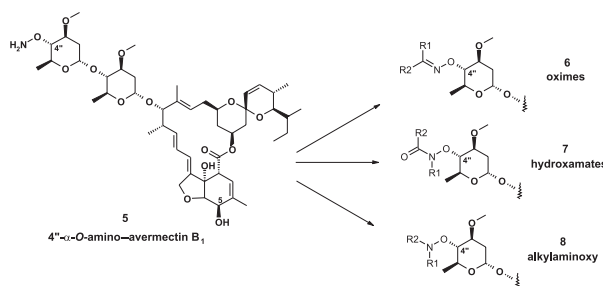
Abamectin **1** (Vertimec®) and Emamectin benzoate **2** (Proclaim®) represent two members of the Avermectin class of macrocyclic lactones, which have been successfully introduced in the crop protection market. Abamectin **1**, a natural product isolated by fermentation from *Streptomyces avermitilis*, consists of a mixture avermectin B_{1a} and avermectin B_{1b} introduced to the market in 1985 as an acaricide and insecticide by Merck Sharp & Dohme Agvet (now marketed by Syngenta AG). Emamectin benzoate **2** is a semi-synthetic insecticide that controls all agronomically important Lepidoptera in vegetables and cotton. It was introduced to the market by Novartis (now Syngenta Crop Protection AG) in 1997 [1,2].

In the course of these studies, we became interested in the synthesis of 4''-O-amino-avermectins **3**, which might combine insecticidal properties of Abamectin and Emamectin.

Thus, 4''-epi-5-OTBDMS-avermectin B1 **4** was converted into its a-triflate, which was displaced by N-hydroxyphthalimide to afford 4''-O-amino-avermectin B₁ **5** after cleavage of the silyl and the phthalimide protecting groups. This novel intermediate was transformed into various derivatives, including oximes **6**, hydroxamates **7** and alkylaminox derivatives **8**.

In the poster, the studies towards efficient synthetic routes to these novel avermectin analogues will be discussed. In addition, the attractive insecticidal activity of this novel class of avermectins will be presented.





References

- [1] *The pesticide manual: a world compendium, 13th edition*, Tomlin, C.D.S. (Ed.); British Crop Protection Council, 2003.
- [2] T. Pittnera, *Chloride Channel Activators/New Natural Products (Avermectins and Milbemycins)*, in: *Modern Crop Protection Compounds, Volume 3*, W. Krämer, U. Schirmer (Eds.), WILEY-VCH, Weinheim, pp. 1069-1088, 2007.

P614

ANTI-ASTHMATIC DRUGS IN PLASMA/SERUM – A REAL ANALYTICAL CHALLENGE

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Topical application of anti-asthmatic drugs is the main therapy nowadays. As the applied (inhaled) amount of drug is as low as 10 – 500 micrograms, determination in plasma/serum is extremely difficult. The concentration levels are usually between 1 – 1000 pg/mL plasma or serum. HPLC-tandem-MS is the only analytical technique which is applicable for this task.

Sample preparation is also a challenge as the drugs have different lipophilic and hydrophilic behaviour. The drugs are for inflammation (steroid structure) and bronchodilatation (often amphoteric).

The determination of the following drugs will be discussed:

- (Inflammation) Ciclesonide and active metabolite/ Fluticasone Propionate/Budesonide.
- (Bronchodilatation) Formoterol/Salbutamol (=Albuterol)/Salmeterol

P615

PYRAZOLE-BASED CATHEPSIN S INHIBITORS WITH ARYLALKYNES AS P1 BINDING ELEMENTS

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Cathepsin S (CatS), a cysteine protease of the papain family, is found in the lysosome of certain antigen-presenting cells. Through proteolytic degradation of the invariant chain (Ii), a chaperone protein for major histocompatibility complex class II molecules (MHCII), CatS

plays a key role in facilitating antigen presentation to CD4⁺ T-cells. Inhibition of CatS activity impedes the removal of Ii from MHCII molecules, and may help to regulate immune hyperresponsiveness by attenuating antigen presentation.

A co-crystal structure of a compound from our original lead series of noncovalent pyrazole-based cathepsin S inhibitors bound to a Cys25Ser mutant of cathepsin S helped to elucidate the binding mode of these molecules and facilitated the design of a new class of potent, selective, and bioavailable arylalkyne analogs. The design strategy, SAR, and binding mode of this series will be presented.

P617

THE IMPACT OF BIOMARKERS IN MEDICINAL CHEMISTRY

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Nowadays, an increasingly educated population is demanding more and more information about the specific forms of diseases and how they might be arrested or cured with new therapies custom-designed for according to individual clinical status.

While medicinal chemistry has relied traditionally on the availability of target information or the appropriate animal or cellular model of disease, biomarker information may provide the opportunity to generate new drug discovery hypotheses faster and in a more selective way.

Historically, medicinal chemistry has benefited from other scientific and technological disciplines in order to evolve. Examples include QSAR and computer-aided drug design (IT), combinatorial chemistry and high-throughput screening (robotics), among others. Biomarkers would add biological sciences to these already consolidated synergies.

Furthermore, the commercial success of agents such as imatinib and trastuzumab has alerted the pharmaceutical industry as to the potential value of “biomarker-guided” drugs. In addition, new evidence continues to emerge showing how the early institution of biomarker-based assays can more reliably predict both efficacy and toxicity in the preclinical and early clinical phases of drug development. This can allow for both improved success rates in the selection of compounds to move forward and cost savings associated with earlier cessation of the evaluation of novel compounds destined to fail.

With this in mind, it becomes clear that the creation of a repository of disease-specific biomarkers that includes their biological basis and their target identification and validation would be a significant asset for all those involved in the discovery of better and safer drugs.

A review of the efforts in biomarkers research and how medicinal chemistry can benefit from these advances will be outlined during the presentation.

P618

SYNTHESIS AND HYPOCHLOROUS ACID SCAVENGING ACTIVITY OF INDOLE DERIVATIVES

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Natural products incorporating an indole moiety correspond to a broad chemical family ubiquitously found in microorganisms, plants and animals. This type of compounds is mainly related to the tryptophan metabolism. The most important members of the family are the plant hormone, indole-3-acetic acid, and the animal hormone, melatonin. An important characteristic of indole-containing compounds is that they may be useful as chemical preventive agents against inflammation and oxidative stress related diseases, like cancer and neurodegenerative diseases.^{1,2} Considering the possible contribution of reactive oxygen species and reactive nitrogen species for these ailments, the development of novel antioxidant indole derivatives is an emerging area, with a wide range of therapeutic applicability.

In order to study the structure-activity relationship an indole based library was synthesised and the antioxidant activity of the obtained compounds was evaluated by studying their scavenging activity against hypochlorous acid (HOCl). The HOCl scavenging assay was performed by using a fluorescent methodology, based on the HOCl-induced oxidation of dihydrorhodamine 123 to rhodamine 123, in a microplate reader.

The novel indole derivatives synthesised and tested in the present study were shown to be of reassuring importance for the development of new antioxidant drugs. Indeed, all except one of the tested compounds demonstrated activity against HOCl in a concentration-dependent manner, at concentrations similar to those required for the effectiveness of the well known indole antioxidants tryptophan and tryptamine.

[1] E. Fernandes, D. Costa, S.A. Toste, J.L.F.C. Lima, S. Reis. *Free Rad. Biol. Med.* 37 (2004) 1895.

[2] S. Stolc. *Life Sci.* 65 (1999) 1943.

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P619

SYNTHESIS OF NEW 3,4-BENZO(CYCLOALKYL) COUMARINS AS MAO INHIBITORS

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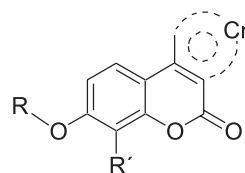
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Coumarins are a large family of compounds of natural and synthetic origin that shown numerous biological activities¹. We studied recently this structural moiety with special attention to their antioxidative, anticoagulant, enzymatic inhibition and anticancer proprieties. Respect to the monoamine oxidase inhibition, our findings revealed that MAO-A and MAO-B affinity and selectivity can be efficiently modulated by appropriate substitutions in the coumarin ring, in particular in the 3/4 and 7 positions².

With the aim to find out the structural features for the MAO inhibitory activity and selectivity, in the present communication we report the design, synthesis and biological study of a new series coumarin derivatives with a cycloalkene or benzene ring condensed in the 3,4 position. The substituents in this new scaffold have been chosen taking in account their lipophilic, steric and/or electronic properties.



The preliminary results of pharmacological study using recombinant human MAO-A and MAO-B isoenzymes indicated that most of the synthesized compounds are powerful and selective MAO inhibitors.

References

- [1] -Borges, F.; Roleira, F.; Milhazes, N.; Santana, L.; Uriarte, E. *Current Medicinal Chemistry*, 2005, 12, 887-916.
- [2] Santana, L.; Uriarte, E.; González-Díaz, H.; Zagotto, G.; Soto-Otero, R.; E. Méndez-Álvarez, E. *Journal of Medicinal Chemistry*, 2006, 49, 1149-1156.

ADDITIONAL ABSTRACTS*

P003

IN VIVO HEPATOPROTECTIVE EFFECTS OF CUPRESSUS SEMPIVERENS L. AND JUNIPERUS PHOENICEA, TRADITIONAL MEDICINAL PLANTS, AGAINST EXPERIMENTALLY INDUCED LIVER INJURY IN RATS

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The *in vivo* hepatoprotective effects of methanolic extracts of *Cupressus sempiverens* L. and *Juniperus phoenicea*, traditional medicinal plants, against experimentally induced liver injury in rats were evident from serum biochemical parameters as total protein, albumin, urea, creatinine, total lactate dehydrogenase activity, LDH isoenzymes and liver histopathological analysis. The data obtained demonstrated that rats injected with a single toxic dose of CCL₄ and sacrificed after 24 hours induced remarkable disturbances in the levels of all tested parameters. However, rats injected with the toxic agent and left for one and a half month to self recover showed moderate improvements in the serum biochemical parameters and hepatic histopathological changes induced by CCL₄. On the other hand, treatment with both extracts ameliorated the levels of the disturbed biochemical parameters and histopathological changes, *Cupressus sempiverens* revealing a more remarkable effect. It could be concluded that the two extracts under study possess potent activities against hepatotoxicity compared to hepatic self recovery and may thus be helpful in treatment and safe recovery of liver disorder.

Keywords:

Cupressus sempiverens, *Juniperus phoenicea*, CCL₄, hepatotoxicity, histopathological, protein, lactate dehydrogenase activity, LDH isoenzymes.

P141

DEVELOPMENT AND VALIDATION OF A RAPID DERIVATIVE SPECTROPHOTOMETRIC METHOD FOR DETERMINATION OF TROPICAMIDE IN EYE DROPS

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Tropicamide is an antimuscarinic agent with short duration of mydriatic and cycloplegic effect. Tropicamide is used for refractive examinations and is available as 0.5

or 1% (w/v) ophthalmic solution containing excipients and preservative for optimal eye tolerance and activity. Since tropicamide use is increasing, it is very much essential to develop simple and suitable analytical method with sufficient sensitivity and selectivity for its determination in dosage forms for routine quality control analysis. Non-aqueous titration method is reported in EP, USP and BP for determination of tropicamide in raw material (EP 2002; USP 2006; BP 2005). Spectrophotometric method for determination of tropicamide in eye drops is also reported in USP and BP after extraction of the active compound into chloroform and back extraction into dilute sulfuric acid. All of these methods are time-consuming and relatively complicated for routine analysis. Direct UV-visible spectrophotometric method is not suitable for determination of tropicamide in eye drops due to the spectral overlapping of tropicamide and other ingredients in dosage form. Derivative spectrophotometry provides a greater selectivity than common spectrophotometry and offers a powerful approach for resolution of band overlapping in quantitative analysis of multi-component mixtures without prior chemical or physical separation.

In this study a zero-crossing third and fourth derivative spectrophotometric method was described for determination of tropicamide in eye drops. The measurements were carried out at wavelengths of 263.8 and 255.4 nm for third-derivative and fourth-derivative respectively. Under the optimized conditions, the linearity of the calibration curves were validated by the high values of correlation coefficients ($r^2 > 0.999$) in the range of 10-100 µg/ml for tropicamide in the presence of excipients. The within-day and between-day precision and accuracy were not more than 1.4%. The limit of determination was 10 µg/ml for tropicamide. The method was successfully applied for determination of tropicamide in eye drops without any interference from excipients or need to prior separation before analysis.

P521

[L-C₅H₁₀NO₂]₄[SiW₁₂O₄₀].4H₂O AS A NEW ORGANIC-INORGANIC HYBRID POLYOXOMETALLATE

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Polyoxometallates are large crystalline discrete molecular structures composed of metal cations bridged by oxide anions, providing high electron affinity. These complexes are attractive as they are very stable, readily prepared and well characterized spectroscopically. Current interest for derivatized polyoxometallates originates from the fundamental interest of modeling catalysis by metal oxides as well as from potential interesting applications in different fields such as bifunctional catalysis and antiviral

*Late submissions that are not in the printed abstract book.

and antitumoral chemotherapy. During the past twenty years, it has been well established that size, shape, and charge density of many polyoxoanions and their counter ions strongly influence the suspected pharmaceutical properties of these inorganic materials.[1, 2]

Recently, increasing attention has been devoted to the biological properties originated from polyoxometallates with an organic group anchored to their backbone. These compounds are interesting precursors for hybrid organic-inorganic materials which could provide a variety of interesting properties such as anti-viral, antineoplastic, catalytic and anti-carcinogenic properties. Amino acids are the basic units of many biological molecules and their incorporation into large polyatomic structures may illuminate key interactions of great importance in bioinorganic chemistry.[3, 4]

In the frame of this strategy, we were interested in the synthesis of a new derivative with potentially promising biological activity. We report herein a simple route for the synthesis of $[L-C_5H_{10}NO_2]_4[SiW_{12}O_{40}] \cdot 4H_2O$ as a new organic-inorganic hybrid material which is stable at room temperature. Findings showed that $SiW_{12}O_{40}$ polyoxoanion fragment acted as a molecular acceptor and attracted nitrogen and oxygen atoms of the organic donor, L-proline.

It is believed that the polyoxometallate moieties were arranged in good order to form large holes in the structural backbone. These cavities were occupied by the prolinium cations and water molecules. Synthesis of this new compound thanks to high acidic characteristic of heteropolyacids as stable anions which interact with organic species, L-proline as cationic salt. The unique properties of these organic-inorganic hybrid materials like stability in liquid and solid phases, stability in ambient temperature, and their biological and catalytic properties, make them a wonderful choice in pharmacology, medical science and catalysis reactions.

References:

- [1] M. T. Pope, "Heteropoly and Isopoly Oxometallates", Springer, NewYork, 1983.
- [2] M. T. Pope and A. Muller, "Polyoxometallate: From platonic solids to antiretroviral activity", Kluwer Academic, Dordrecht, 1994.
- [3] L. C.W. Baker and D. C. Glick, Chem. Rev. 98, 3 (1998).
- [4] D. C. Crans, M. Mahroof-Tahir, and O. Anderson, Inorg. Chem. 33, 5586 (1994).

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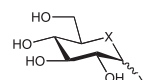
TOWARDS THE SYNTHESIS OF SELENIUM CONTAINING CARBOHYDRATES

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Glycosidase enzymes play important roles in the biochemical processing of molecules containing carbohydrates, they are responsible for the hydrolysis of polysaccharides into monomers or cleavage of bonds between carbohydrates and aglycons. Inhibition of these enzymes *in vivo* has the potential to control certain cellular functions and glycosidase inhibitors therefore have been shown to exhibit promising chemotherapeutic applications against diabetes,¹ cancer,² and viral infection including HIV.³

Examples include 5-deoxy-5-thio-D-glucose (1) which has been shown to be a potent inhibitor of cellular D-glucose transport and also selectively toxic to hypoxic tumour cells,⁴ nojirimycin (2), deoxynojirimycin (3) and the butylated analogue (4) which exhibit antibacterial activity and have been proposed as chemotherapeutic agents to treat HIV.⁵ Selenium analogues of some current glycosidase inhibitors have previously been shown to be more potent than their sulfur equivalents⁶ and analogues of these important compounds (5a-c) therefore offer an exciting prospect in terms of their synthesis and applications.



- 1: X = S; Y = OH
- 2: X = NH; Y = OH
- 3: X = NH; Y = H
- 4: X = NBu; Y = H
- 5a: X = Se; Y = H
- 5b: X = Se; Y = OH
- 5c: X = Se⁺R⁻; Y = H

- [1] Leonhardt, W.; Hanefield, M; Fischer, S.; Schulze, J, *J. Clin. Invest.* 1994, 24, 45-49
- [2] Dennis, J.W.; Koch, K.; Beckner, D.J, *Natl. Cancer Inst.*, 1989, 81, 1028
- [3] Karlsson, G.B.; Butters, T.D.; Dwek, R.A.; Platt, F.M. *J. Biol. Chem.* 1993, 268, 570-576
- [4] Whistler, R.L.; Lake, W.C.; *Biochem. J.*, 1970, 130, 919
- [5] Schewe, T., *Gen. Pharmacol.*, 1995, 26, 1153

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GENERAL METHODS FOR FLASH CHROMATOGRAPHY USING DISPOSABLE COLUMNS

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As technology has evolved available guidelines for normal-phase flash chromatography have become less relevant. Years of experience performing such chromatography with disposable columns have been condensed into simple suggestions useful for translating TLC results into either isocratic- or gradient-flash chromatography. The described studies should provide researchers with adequate information to reduce the use of silica, solvent, time, and money.