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P61

Quinolones as potential anti HIV-1 agents targeted at Tat/TAR recognition

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The interaction between Tat and TAR is an attractive target for the development of new antiviral agents against HIV-1. We have recently demonstrated that some aminoquinolones with antiviral activity are able to interact effectively with the provirus LTR in the region where the TAR structure is located, showing in vitro the ability to disrupt the complex with Tat. These encouraging results prompted us to analyse the activity of new quinolones designed rationally to bind the TAR bulge. These compounds are the first of a new series in which the modes of interaction with the nucleic acid and the protein/RNA complex differ significantly from those referring to fluoro- and aminoquinolones bearing the classical keto-carboxylic function. Indeed, the quinolone ring serves as a stacking moiety between base pairs of the nucleic acid and as a scaffold for a phenyl ring mono or o-disubstituted with basic chains, intended to bind electrostatically the phosphate backbone of TAR. The new quinolones are able to interfere with Tat-TAR complex depending on precise structural requirements, as demonstrated by electrophoresis mobility shift assay (EMSA); in our experimental conditions the most active compound showed a better activity than the lead aminoquinolone of the previous series, validating our new approach and prompting the study of new quinolone congeners to fully explore the structural properties of the active drugs and to optimize the pharmacokinetic parameters of these basic compounds.

P62

Antiviral activity of some (5-chloro-2-oxobenzothiazolin-3-yl) aceto/ propanohydrazide derivatives

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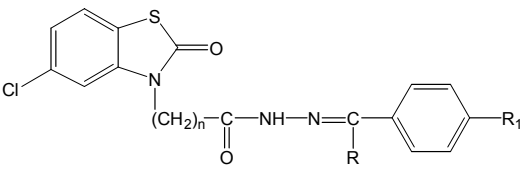
The structural and therapeutic diversity coupled with commercial viability of various molecules have fascinated organic and medicinal chemists for many years[1]. There has been considerable interest in the chemistry of 2-(3H)-benzothiazoles, which is a core structure in various synthetic pharmaceuticals displaying a broad spectrum of biological activity including antimicrobial, antifungal, antituberculosis, anticonvulsant, antiinflammatory and analgesic activities. At the same time, a considerable number of hydrazide/hydrazone derivatives have been reported to demonstrate antiviral activity.

Based on above findings, we synthesized eleven (5-chloro-2-oxobenzothiazolin-3-yl)aceto/propanohydrazide derivatives to test their antiviral activity against DNA and RNA viruses.

The antiviral activities as well as cytotoxicity were tested against *Herpes simplex* (HSV) as DNA virus and *Parainfluenza*-3 *virus* (PI-3) as RNA virus using Vero (African green monkey kidney) and MDBK (Madin-Darby Bovine Kidney) cell line cultures. Acyclovir (16-<0.25μg/ml) and Oseltamivir (32- <0.25μg/ml) were employed in the test as references. Synthesized compounds resulted in antiviral activity at 16-1μg/ml concentration range.



R: H, CH_3
R_1:H, Cl, CH_3, OCH_3

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Cancer

P63

Design and development of lantadenes as antitumor agents

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Lantana plant has encroached upon a large land area in India as well as other parts of the world and imposed a great threat to grazing animals and overall ecology. Eradication of this weed cannot be excepted in the near future by the use of conventional methods. However, this plant has been in use in folk medicine in different parts of the world. During the past few years, a number of chemical compounds have been reported from this plant and have been investigated for their pharmacological properties. Recently, the triterpeniods named lantadenes isolated from lantana leaves have been found to exhibit antitumor activities and therefore, studies for the development of lantadenes as potential antitumor agents will be rational way to utilize this biomass as a resource for drug discovery and development. A number of lantadenes **(Fig.1)**

are present in *Lantana*, majority of these are present only in very small amounts. They differ very little in their physicochemical properties and therefore, isolation of these potential compounds in sufficient quantities required for various studies presents a challenging job. These compounds can also be prepared by semi-synthetic procedures using 22β-hydroxyoleanonic acid, which can be obtained by hydrolysis of lantadenes. These compounds can be synthesized by extracting lantadenes and transforming them to 22β-hydroxyoleanonic acid by hydrolysis and utilize this compound as the starting material for the preparation of number of lantadenes by acylation of the hydroxy group present at 22- position. It is hoped that this strategy will result in the discovery of antitumor agents and utilize this obnoxious weed as a resource.

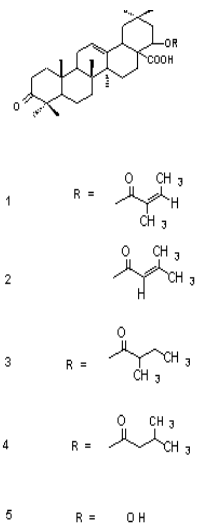

Fig. 1. Structures of various lantadenes present in Lantana plant.

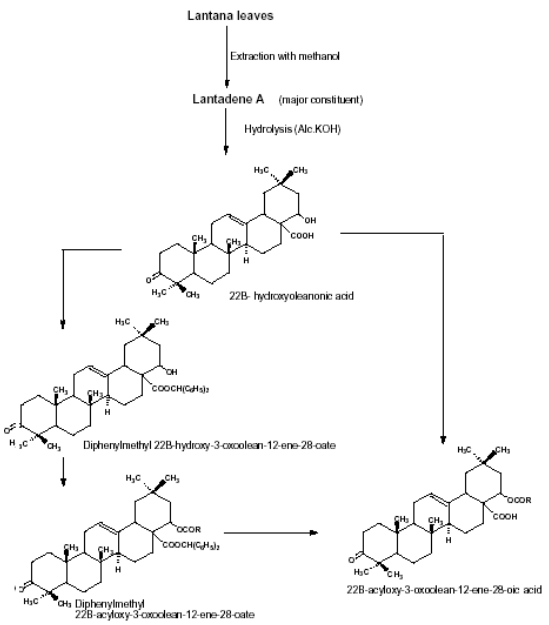

Fig. 2. Sequence of steps involved in the preparation of lantadenes

P64

In Vitro Cytotoxic Evaluation of New Triorganotin Derivatives of Hydroxy- Anthraquinones and Napththoquinones

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Objectives of the study: Completing our research[1,2,3] on the synthesis and biological evaluation of anthraquinonato- and naphthoquinonato triorgano-tin compounds, formulated as $R_3Sn(\mu\text{-}Q)SnR_3$ (R = Bu, Ph, Bz; Q = quinonato- ligand), we will present herein the results of the in vitro experiments conducted aiming to evaluate the potential cytotoxic effect of the tribenzyl-, triphenyl- and tributyl- tin analogues

Methodology: The compounds were synthesized by reacting the organotin hydroxide with the parent quinone and were thoroughly characterized by mass spectra data and multi-nuclear NMR mmeasurements. Herein, we have measured a percent (%) of cytotoxicity of the new compounds against five human tumor cell lines K562 (Chronic myelogenous leukemia), MCF-7 (Breast adenocarcinoma, estrogen receptor positive, ER+), HeLaS3 (Epitheloid carcinoma of cervix), PC3 (Prostate cancer), Hs 294T (Melanoma, metastatic to lymph node) and one non-tumor human cell line MRC5 (Lung foetal fibroblasts). The compounds have been tested using the SRB assay for 48h (NIH screening protocol).

Results- Conclusions: All the new organotin compounds were found to be very toxic not only against tumor cells but against normal MRC5 cells as well. Dose dependent response was found for all cell lines except PC3 -i.e. cytotoxicity of compounds increased with concentration, whereas in PC3 cell line the highest response was obtained with the smallest concentration. The same response was obtained in the second experiment with PC3 cells therefore we conclude that this is true response, not an experimental mistake. HeLa cells appeared slightly more sensitive to all compounds when compared to other cell lines. The naphthoquinonato complexes are found approximately 100 fold more potent than Doxorubicin against HeLa cells.

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[2] M. Bakola-Christianopoulou, P.D. Akrivos, V. Valla, C. Tsipis. Synthesis and spectroscopic study of anthraquinonato- and naphthoquinonato-bridged binuclear tribenzyl-tin compounds. ASCMC-Moscow 2004,International Symposium on Advances in Synthetic, Combinatorial and Medicinal

P64 Table 1: IC50 values of the New Triorganotin Derivatives of Hydroxy- Anthraquinones and Napththoquinones

	IC50, μM, 48h, SRB				
	K562	MCF 7	HeLa	Hs 294T	MRC 5
*Doxorubicin	0.82	1.19	5.08	29.85	0.32
$(Ph_3Sn)_2$-Naphthazarine	0.38	1.46	0.04	0.59	0.06
$(Bu_3Sn)_2$-(Naphthazarine)	>100	>100	0.05	24.58	9.76
Ph_3Sn-(Juglone)	0.09	2.28	0.06	0.58	0.0012
Bu_3Sn-(Juglone)	0.55	19.97	4.67	23.33	0.0004
Ph_3Sn-(Lawsone)	0.29	0.12	9.87	0.28	0.0082
$(Ph_3Sn)_2$-(Quinizarine)	0.83	0.08	0.21	0.4	0.03
$(Bu_3Sn)_2$-(Quinizarine)	>100	3.02	2.06	2.24	12.29
$(Ph_3Sn)_2$-Anthrarufin	5.66	0.02	1.42	0.45	0.039
$(Ph_3Sn)_2$-Leucoquinizarin	0.45	0.17	0.36	0.44	0.098
$(Bu_3Sn)_2$-Leucoquinizarin	18.77	1.09	1.41	0.29	1.96
(Ph_3Sn)-Alizarin	0.82	0.29	0.92	0.62	0.02

Chemistry. World Trade Center -Moscow, Russia - May 5-8, 2004. Book of abstracts: P199

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P65

Activating the transpltinum geometry using 4-piperidinopiperidine ligand

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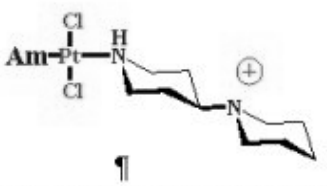
In continuation to our previous work on activation of trans platinum complexes using non-planar heterocyclic piperidine and piperazine ligands,[1, 2, 3] a series of *trans*-$PtCl_2$(Am)(4-Piperidino-piperidine)], (Am = NH_3, methyl amine, dimthyl amine, propyl amine, isopropyl amine, n-butyl amine and cyclohexyl amine) complexes have been synthesized, characterized, and some have been crystallized. The biological activities of the complexes (IC_{50}) have been assessed against three pairs of Cisplatin sensitive and resistant human ovarian cell lines. An update of the *in vitro* and *in vivo* activities vs. structural modification in the ligands Trans to the Pip-Pip, together with DNA and protein binding behaviors will be presented in the talk.



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Am:·NH_3,·MA,·DMA,·NPA,·IPA,·NBA,·CHA,·4-Pic

[1] Khazanov, E., Barenholz, Y., Gibson, D., Najajreh, Y. (2002) Novel apoptosis-inducing trans-platinum piperidine derivatives: synthesis and biological characterization. J. Med. Chem. 45(24), 5196-204.

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[3] Kasparkova, J., Novakova, O., Marini, V., Najajreh, Y., Gibson, D., Perez, J. M., Brabec, V. (2003) Activation of trans geometry in bifunctional mononuclear platinum complexes by a piperidine ligand. Mechanistic studies on antitumor action. J. Biol. Chem. 28, 278(48), 47516-25.

P66

New Perspectives in the Treatment of Glioblastoma: Enzastaurin, a Kinase Inhibitor with Dual Mode of Action

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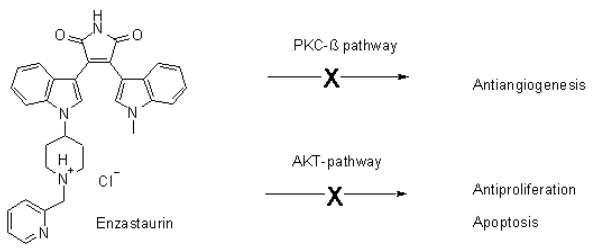
Enzastaurin, a ATP-competitive, selective PKC-ß inhibitor[1] showed in clinical studies (phase II) a number of progression free long term survivals in the treatment of glioblastoma, the most common and most malignant of primary brain tumors. Enzastaurin is a potent (IC_{50} = 6nM) and selective inhibitor of PKC-ß and exhibited a robust antiangiogenic effect in preclinical studies[2]. It is hypothesized that the vascularisation of the tumor is interrupted and thus an indirect antitumorigenic effect is achieved.

In a new preclinical study[3] it has been demonstrated that enzastaurin in addition exhibited a direct antiproliferative and atoptose inducing effect on tumor cells, This is explained by a suppression of the AKT signaling pathways.

Further clinical studies for the treatment of tumors are under way.



Scheme 1

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[2] Teicher, B. et al. unpublished results.

[3] Graff, J. R.; McNulty, A. M.; Hanna, K. R.; Konicek, B. W.; Lynch, R. L.; Bailey, S. N.; Banks, C.; Capen, A.; Goode, R.; Lewis, J. E.; Sams, L.; Huss, K. L.; Campbell, R. M.; Iversen, P. W.; Neubauer, B. L.; Brown, T. J.; Musib, L.; Geeganage, S.; Thornton, D. Cancer Research **2005**, *65(16),* 7462-7469.

P67

Synthesis of Ring-A Substituted Estrogens of Potential Therapeutic Activity

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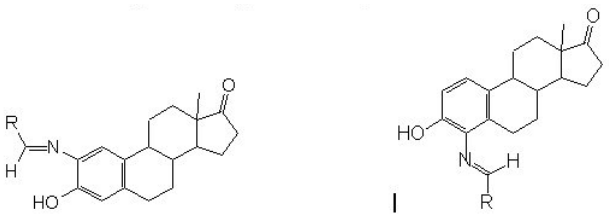
Despite the wide variety of structural modifications that have been realized in the evolution of steroidal antiestrogens [1], we felt that the role of C-2 and C-4 functionality of estrogens had not been fully elucidated. A strategy has been to couple the vectors, estrone or estradiol, with a potentially active side chain moiety.

In this regard, we report herein the synthesis of several nuclear modified estrone derivatives and we describe some of the biological properties of these new steroidal compounds both *in vivo* and *in vitro* as an exploration of the effect of the size of the substituent in the 2- and 4-positions of the steroidal skeleton on the biological activity of the compounds.



Preliminary results of the uterotrophic and antiuterotrophic screening tests, as assessed by the increase in uterine weight assay[2], indicated that in general, all tested compounds were less estrogenic than estradiol. Ring A substitution at position 2 and 4 with an imine moiety produced compounds that possessed significant reduced estrogenicity relative to estradiol (4%-8%). Results of co-administration of the test compounds along with estradiol indicated that a high order of blockade for estrogenic activity was displayed by some compounds (64-70% antiuterotrophic activity). The RBA assay is currently under investigation.

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P68

Synthesis and Antitumor Activity of New Gene-Targeted Alkylating Agents, N-Mustard Linked to DNA-Affinic 9-Anilinoacridines

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Sciences, Academia Sinica, Taipei 115, Taiwan. ****Department of Pharmacology, University of Medicine and Dentistry of New Jersey ¡V Robert Wood Johnson Medical School, Piscataway, NJ 08854, USA.

A series of new gene-targeted antitumor agents, 9-anilinoacridine derivatives bearing an alkylating N-mustard residue at the acridine chromophore, were synthesized for evaluating their antitumor activity. The N-mustard residue was linked to the C4 of the acridine ring with an O-ethylene (O-C2) or O-butylene (O-C4) spacer. These target compounds were prepared by the condensation of the C-4 N-mustard substituted 9-chloroacridines with various substituted 1,3-phenylene diamines. It was revealed the newly synthesized compounds exhibited significant cytotoxicity with IC_{50} values in a range of 3 to 20 nM in inhibiting various human tumor (such as human acute lymphoblastic leukemic cells CCRF-CEM, lung carcinoma A549, colon carcinoma HCT-116 and breast carcinoma MX-1) cell growth in vitro and did not exhibit cross-resistance against vinblastine-resistant (CCRF-CEM/VBL) or taxol-resistant (CCRF-CEM/taxol) cells. It also showed that the title compounds were DNA cross-inking agents. Of these agents, compounds **BO-0940** and **BO-0944** possessed potent antitumor activity in nude mice bearing the human breast carcinoma MX-1 xenograft: 96% and 77% tumor suppression were observed at doses of 10 and 2 mg/kg (Q2D¡Ñ3, intravenous injection), respectively, under optimal therapeutic conditions.

P69

Hydrolytically stable sulfur containing analogues of the tumor antigen GM3-lactone

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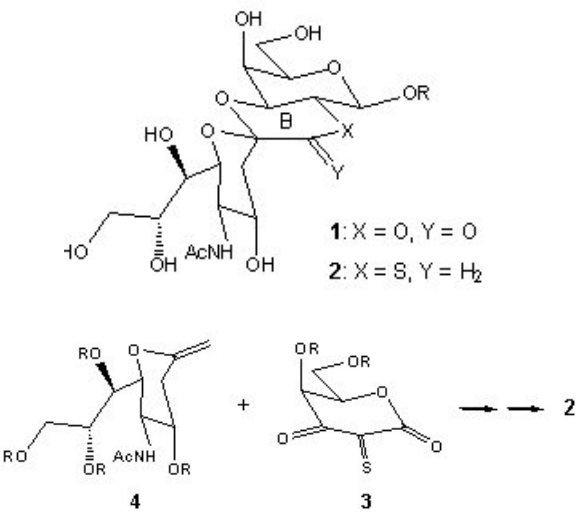
Studies on the reactivity of a monoclonal anti-melanoma antibody (M2590) with various cells having different GM3-lactone concentration (obtained in vivo by GM3-ganglioside) at their surface, have shown that it is a strong immunogen. However, lactones are unstable under physiological conditions, so that the equilibrium concentration of GM3-lactone at close-to-neutral pH is low, making it an immunogen of low efficiency. Since a high concentration of the immunogen is needed to induce a strong immune response, hydrolytically stable GM3-lactone analogues are welcome for having the immunogen available for immunization. Two interesting examples of such analogues have been reported [1].

We have designed a new analogue of GM3-lactone **1**, the sulfur containing compound **2**. Here we report on modeling studies performed to ascertain the correct analogy of **2** with **1** and, in particular, the conformational preferences of ring B which, in **1**, assumes a twisted-boat conformation. In the synthetic pathway to **2**, the key step consists in a hetero-Diels-Alder reaction between an a-thiono-β-keto-δ-lactone **3**, obtained from galactal, and a neuraminic acid analogue, **4**. Thus, modeling studies were also performed to make predictions on the stereochemical outcome of this reaction as a function of the structure of the addends in order to choose the suitable protecting groups and structural features which allow the correct configuration at the spiro-carbon atom of the intermediate leading to **2**.



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P70

Synthesis and cytotoxic activity of Platinum(II) and Platinum(IV) complexes with 2-hydroxymethylbenzimidazole ligands against MCF-7 and HeLa cell lines

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Cisplatin, and other platinum-based drugs such as carboplatin, and oxaliplatin, are used to treat testicular tumors as well as a variety of other human solid tumors, but most of these drugs used are intrinsically resistant and acquired resistance commonly develops during treatment [1].

The replacement of ammine groups can result in different structural and formational alterations in target DNA, which may affect the character of biological effects of the anologues [2]. It has been shown that increasing cytotoxicity of cisplatin analogues, in which NH_3 groups were replaced by more hydrophobic amine ligands, correlated with growing hydrophobicity of these analogues [3].

Some Pt(IV) complexes have shown potential as powerful anticancer drugs. It is widely believed that reduction to Pt(II) is essential for the anticancer activity of Pt(IV) complexes to be effected. The reduction potentials of diam(m)ine Pt(IV) complexes are dependent on the nature of the axial and equatorial ligands, but the axial ligands generally exert the stronger influence [4].

In the present study, as an extension of the investigation, on the probable antitumor activity of platinum complexes of benzimidazole ligands, to determine the effect of axial and equatorial ligand variation on the cytotoxic activities of the platinum complexes a series of Pt(II) and Pt(IV) complexes with 2-hydroxymethyl benzimidazoles as non-leaving amine ligand and chloro, iodo, hydroxo ligands as leaving groups were synthesized and evaluated for their *in vitro* cytotoxic activities on the human MCF-7 and HeLa cell lines.

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P71

Identification of a Terphenyl-Based Small Molecule That Induces Differentiation in Leukemia Cells

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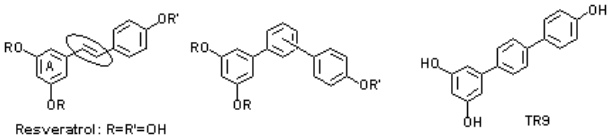
Because of our ongoing interest in finding new apoptosis-inducing agents, we are particularly intrigued by molecular scaffolds that allow the parallel synthesis of substituted derivatives. Actually, the parallel procedure is suitable for rapidly obtaining variously substituted analogs, particularly when structure-based design strategy is not applicable due to the lack of knowledge of a specific target. Thus, we recently synthesized a small library of resveratrol analogs: many derivatives were more active than resveratrol as apoptosis-inducing agents in HL60 leukemia cells, and some of them were active toward resistant HL60R cells [1].



Given these results, we aimed at increasing the structural diversity of the new molecules by synthesizing a second series of derivatives, which incorporate a phenyl ring as a bioisosteric substitution of the alkenyl bridge. Thus, through a Suzuky cross-coupling we obtained a small series of terphenyls that were tested on several leukemia cell lines. One of the new derivatives, **TR9**, behaved differently from the others, as it was able to block the cell cycle in G_0-G_1 phase and also to induce differentiation in acute myelogenous leukemia HL60 cells. Moreover, it was active on both multidrug resistance and Bcr-Abl expressing cells that were resistant to resveratrol.

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P72

Binding of the anti-cancer prodrug CB1954 to the activation enzyme NQO2 revealed by the crystal structure to their complex

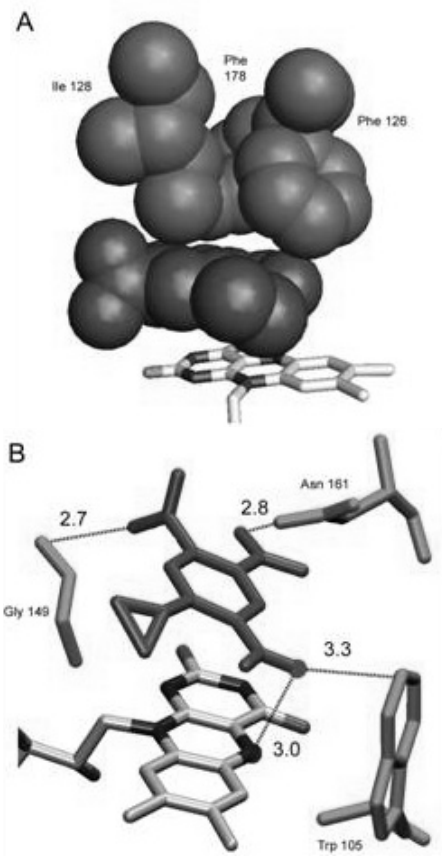
M. Abukhader*, J. Heap**, C. De Matteis*, B. Kellam*, S. Doughty*, N. Minton**, M. Paoli*

*School of Pharmacy and **The Institute of Infection, Immunity and Inflamation, Centre for Biomolecular Sciences, University of Nottingham, University Park, Nottingham, NG7 2RD, UK.

CB1954 is an important anti-cancer prodrug currently in clinical trials. In human tissue this prodrug undergoes reductive bioactivation by the enzyme NQO2 which is over-expressed in several cancer types. The prodrug CB1954 is converted from its non-cytotoxic form into a highly cytotoxic agent which then binds and causes damage to the DNA through covalent cross-links thus leading to the death of the cancer cell.

The CB1954-NQO2 complex was resolved at 2.0Å resolution. The binding of the prodrug is dominated by hydrophobic contacts which are made with the "roof" of the binding site, formed by one isoleucine and two phenylalanine side chains. In addition to an extensive π-stacking interactions, which are established between the prodrug and the isoalloxazine ring of the FAD cofactor. Meanwhile, the specific orientation of the ligand is determined by two key polar contacts.

The detailed view of the molecular recognition between CB1954 and NQO2 provides a platform for structure-guided design of new drug and/or prodrug candidates for NQO2.



P73

Indanesulfonamides as carbonic anhydrase inhibitors. Towards structure-based design of selective inhibitors of the tumor-associated isozyme CA IX

A. Thiry*, M. Ledecq*, A. Cecchi**, J. Dogné*, J. Wouters*, C. Supuran**, B. Masereel*

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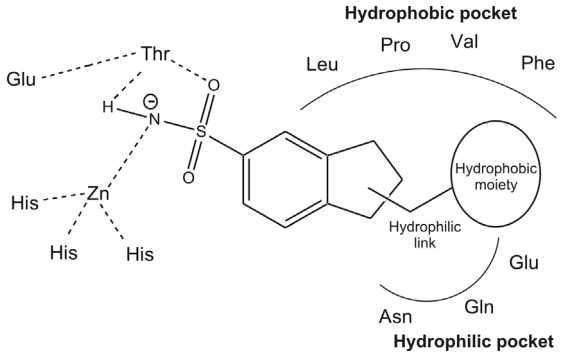
The human carbonic anhydrase isozyme IX (hCA IX) becomes an interesting pharmacological target due to its overexpression in cancer and its absence in normal tissue. hCA IX is clearly implicated in the acidification of the extracellular pH and contributes to the survival and the growth of tumor cells [1].

The indanesulfonamide template was first described as a potent inhibitor against hCA I and hCA II [2]. On the basis of these preliminary results, we designed indanesulfonamides based on the recently described “tail approach” [3]. A general pharmacophore has been drawn from the analysis of CAs active site and from the structure of inhibitors described in the literature. Different hydrophobic side chains, which target the hydrophobic pocket, were incorporated in the indanesulfonamide scaffold with an amide linker to interact with the hydrophilic part of the active site.



The inhibitory activities of indanesulfonamides were evaluated against the hCA IX and against two other biologically relevant isozymes (hCA I and II). In order to establish preliminary hypothesis for the design of new selective CA IX inhibitors, we conducted molecular modeling studies. We describe here the first hCA IX model built by homology with another CA isozyme previously crystallized. Docking studies were performed to explore the binding mode of our indanesulfonamide derivatives.

[1] Robertson N. et al.: Cancer Res. 64 (2004) 6160-5.

[2] Chazalette C. et al.: Bioorg Med Chem Lett. 14 (2004) 5781-5786.

[3] Supuran C.T. et al. Med. Res. Rev. 23 (2003) 146-189.

P74

Diketo hexenoic acid (DKHA) derivatives: the first class of selective non-nucleoside inhibitors of mammalian terminal deoxynucleotidyl transferases endowed with cytotoxic effect against leukemic cells

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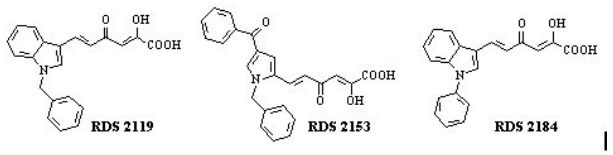
Mammalian terminal deoxyribonucleotidyl transferase (TDT) catalyzes the non-template-directed polymerization of deoxyribonucleoside triphosphates and has a key role in V(D)J recombination during lymphocyte and repertoire development. Over 90% of leukemic cells in acute lymphocytic leukemia and approximately 30% of leukemic cells in the chronic myelogenous leukemia crisis show elevated TDT activity. This finding is connected to a poor prognosis and response to chemotherapy and reduced survival time. DNA polymerase lambda, homolog to TDT1 can synthesise DNA in a template-independent fashion. DNA polymerase lambda might be involved in the nonhomologous end joning (NHEJ) recombinational repair pathway of DNA double strand breaks. During a random screening on various polymerases we found that some aryl diketo hexenoic acids (DKHAs) (RDS 2119, RDS

2153, RDS 2184), previously synthesized by us as inhibitors of HIV-1 integrase and HCV RNA-dependent RNA polymerase, showed interesting activity against mammalian terminal deoxyribonucleotidyl transferases.2(Figure)



Novel derivatives related to the above lead compounds will be shown and preliminary SAR studies will be discussed.

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[1] Hübscher U, Maga G, Spadari S. Eucaryotic DNA Polymerases. Ann Rev Biochem 2002;71:133-163.

[2] Locatelli GA, Di Santo R, Crespan E, Costi R, Roux A, Hübscher U, Shevelev I, Blanca G, Villani G, Spadari S, Maga G. Diketo Hexenoic Acid Derivatives Are Novel Selective Non-Nucleoside Inhibitors of Mammalian Terminal Deoxynucleotidyl Transferases, with Potent Cytotoxic Effect against Leukemic Cells

P75

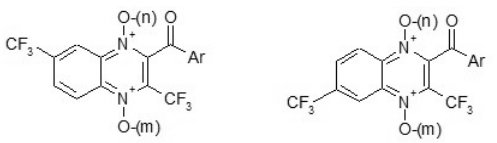
Synthesis and biological evaluation of new 3,6(7)-bis-trifluoromethyl-2-arylcarbonylquinoxaline 1,4-di-N-oxide derivatives

A. Marin, B. Solano, A. Burguete, E. Vicente, R. Villar, S. Perez, I. Aldana, A. Monge

Universidad de Navarra

As a continuation of our research of 3-trifluoromethylquinoxaline 1,4-di-*N*-oxides derivates as anticancer agents [1], a new series have been synthesized introducing another trifluoromethyl group in 6 and 7 position. Having both 6 and 7 positional isomers separetly, the present study analizes the relationship between these sustitutions and activity.

Selectives reductions of these compounds were also carried out with the aim of study the importance of these *N*-oxides in the activity.



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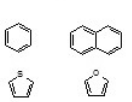


Synthesized compounds are being evaluated at the National Cancer Institute (NCI, Bethesda, USA) againts a 3-cell line panel [2], consisting of MCF7 (breast), NCI-H460 (lung), y SF-268 (CNS). These active compounds were then tested in the full panel of 60 human tumor cell lines derived from 9 cancer cell types [3].

Acknowledgement: This work has been carried out thanks to the financial support of the FIS project (1051005, October 2005) and we want to express our gratitude to the National Cancer Institute (NCI, Bethesta, USA) for the evaluation of the anticancer activity.

[1] Zarranz B, Jaso A, Aldana I, Monge A. Synthesis and anticancer activity evaluation of new 2-alkylcarbonyl and 2-benzoyl-3-trifluoromethyl-quinoxaline 1,4-di-*N*-oxide derivatives. *Bioorg Med Chem* 2004;12:3711-3721.

[2] Boyd MR. *Principles and Practice of Oncology*. 1989;1-12.

[3] A. Monks, D. Scudiero, P. Skehaan, R. Shoemaker, K. Paull, D. Vistica, C. Hose, J. Langley, P. Cronise, A. Vaigro-Wolff, M. Gray-Goodrich, H. Campbell, J. Mayo and M.R. Boyd. Feasibility of high-flux anticancer drug screen using a divers panel of cultured human tumor cell lines. *J Natl Cancer Inst* 1991;83(11),757-766.

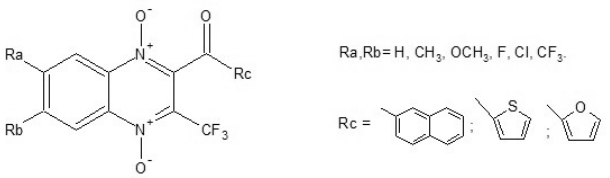
P76

Synthesis and biological evaluation of new 3-trifluoromethyl-2-arylcarbonylquinoxaline 1,4-di-N-oxide derivatives

B. Solano, A. Marin, A. Burguete, E. Vicente, R. Villar, S. Perez, I. Aldana, A. Monge

Universidad de Navarra

Cancer is an altered system of growth that originates within the biosystem of a patient. From now until the year 2020, the number of people affected by cancer will reach 20 million. As a continuation of our research [1] and with the aim of obtaining new anticancer agents, we have synthesized new series of 3-trifluoromethyl-2-arylcarbonylquinoxaline 1,4-di-*N*- oxide derivatives with the general structure:



All of the compounds have shown *in vitro* antitumoral activity against three cell lines [2] consisting of MCF7 (breast), NCI-H460 (lung) and SF-268 (CNS). These active compounds were then evaluated in the full panel of 60 human tumor cell lines derived from nine cancer cell types [3]. Finally, the most active compounds were selected for the *in vivo* hollow fiber assays.

Acknowledgement: This work has been carried out thanks to the financial support of the FIS project (1051005, October 2005) and we want to express our gratitude to the National Cancer Institute (NCI, Bethesta, USA) for the evaluation of the anticancer activity.

[1] Zarranz B, Jaso A, Aldana I, Monge A. Synthesis and anticancer activity evaluation of new 2-alkylcarbonyl and 2-benzoyl-3-trifluoromethyl-quinoxaline 1,4-di-N-oxide derivatives. *Bioorg Med Chem* 2004;12:3711-3721.

[2] Boyd MR. *Principles and Practice of Oncology*. 1989;1-12.

[3] A. Monks, D. Scudiero, P. Skehaan, R. Shoemaker, K. Paull, D. Vistica, C. Hose, J. Langley, P. Cronise, A. Vaigro-Wolff, M. Gray-Goodrich, H. Campbell, J. Mayo and M.R. Boyd. Feasibility of high-flux anticancer drug screen using a divers panel of cultured human tumor cell lines. *J Natl Cancer Inst* 1991;83(11),757-766.

P77

Sulfonamide and Sulfonyl derivatives as potential agents anti-cancer

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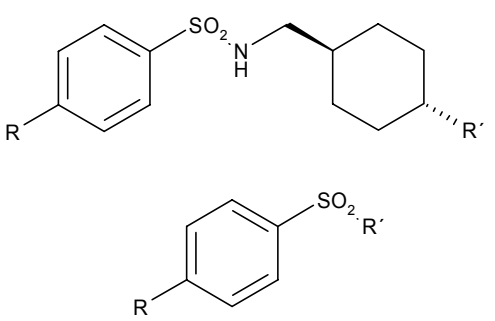
Cancer is to major to killer disease throughout human history, associate to high costs in the health programs, with a high level of mortality and morbidity. The compounds derived from sulfonamide have exhibited an ample diversity of pharmacologics activities, interestingly presented an inhibiting activity of the growth in tumor cells, being this type of attractive molecules for the development of new agents wit h therapeutic potential [1, 2].

In the present study a series of compounds derived from sulfonamide and sulfonyl was synthesized, which were evaluated by the National Cancer Institute of the U.S.A.. The Divison of Cancer Treatment and Diagnostic (DCTD) of the NCI uses as primary test of unique dose a panel *in vitro* of three cellular lines MCF7, NCI-H460 and SF-268, later applies a consistent model of 60 tumor cellular lines.



The synthesized compounds: *trans-N*-[4-(4-chlorophenylaminomethyl)cyclohexylmethyl]biphenyl-4-sulfonamide, *trans-N*-[4-(3,4-dimethoxyphenyl-4-aminocarbonyl)cyclohexylmethyl]bencene sulfonamide and *N´*-[4-(trifluoromethyl)benzoyl]-1-(biphenyl-4-ylsulfonyl)piperidine-4-carbohydrazide present a reduction equal or smaller growth to 32% in cellular lines of lung cancer and ovary. These compounds can be considered for the development of potent anticancer drugs.

[1] Soma S, Srikanth K, Suchandra B, Bikash D, Shovanlal G and Tarun J. *N*-Substituted-2-(substituted benzenesulphonyl) glutamines as antitumor agents. Part II: Synthesis, biological activity and QSAR study. *Bioorganic & Medicinal Chemistry*. 2004; 12 (6): 1413-1423.

[2] Srinivasa N, Reddy M, Cosenza S, Gumireddy K, Bell SC, Premkumar E, Ramana MV. Synthesis of new coumarin 3-(*N*-aryl) sulfonamides and their anticancer activity. *Bioorganic & Medicinal Chemistry Letters 2004; 14 (15): 4093-4097.*

P78

Development of chalcones as potential anticancer agents

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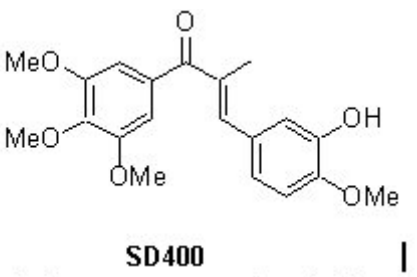
*University of Salford. **Moffitt Cancer Center ***Emory University.

We have identified a phenylbutenone (IC_{50} K562 = 60 μM) from the Chinese mint *Scutellaria barbata*. [1] Structure-activity relationship (SAR) studies led to the discovery of chalcone **SD400** (IC_{50} K562 = 0.21 nM). [2-4] *In vitro* biological studies allowed us to elucidate its mode of action: the drug interacts with tubulin, a protein that is essential for cell division and cell shape, at the colchicine-binding site and inhibits assembly into microtubules. [5,6]



SD400

In 2004, Ravelli published the structure of tubulin:colchicine, giving a much needed insight into the protein's structure and function. This structure helped us gain an understanding of how chalcone SD400 interacts with tubulin. [7] This understanding has allowed us to design a new generation of chalcones which are powerful inhibitors of tubulin assembly. Pharmacokinetic studies have allowed us to optimise the drug-like propeties of these agents which are now ready to enter clinical trials.

P79

Structure-activity Relationship of Olomoucine II Derivatives as CDK Inhibitors

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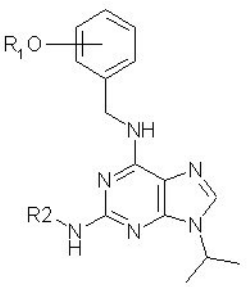
Laboratory of Growth Regulators, Palacky University Institute of Experimental Botany, Academy of Sciences of the Czech Republic, Šlechtitelů 11, 783 71 Olomouc-Holice, Czech republic

Based on our previous experience with synthesis of purines, novel 2,6,9-trisubstituted purines derived from the olomoucine II lead structure (1) were prepared and assayed for the ability to inhibit CDK1/cyclin B kinase. Most of the newly synthesized compounds display about 10 times higher inhibitory activity than roscovitine, potent and specific CDK1 inhibitor.

These compounds were also tested for their cytotoxicity and were found to be more potent *in vitro* against tumour cells than purvalanol A, the most active CDK inhibitor. Selected compounds may provide an effective therapy for cancer or other proliferative CDK-dependent diseases.

Key Words: CDK inhibitors; Olomoucine II; Purvalanol A; Cell cycle regulation; Antiproliferative effect



R1 = hydrogen, methyl

R2 = aminoalkyl, hydroxyalkyl

[1] Kryštof, V.; Lenobel, R.; Havlíèek, L.; Kuzma, M.; Strnad, M.; Bioorganic & Medicinal Chemistry Letters 12 (2002) 3283-3286

P80

Synthesis and biological evaluation of negative adrenomedullin modulators as antiangiogenic and anticancer drugs candidates

V. Roldós*, J. Zapico*, J. Rodríguez*, S. Martín*, M. García*, M. Julián*, R. Martínez**, A. Martínez**, B. De Pascual-Teresa*, A. Ramos*

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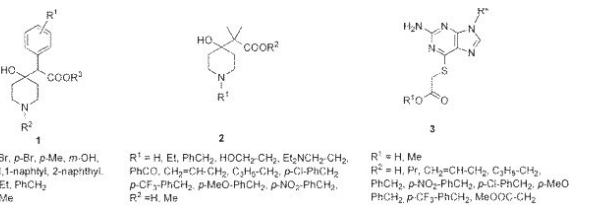
Adrenomedullin (AM) is a peptide hormone implicated in the pathophysiology of several diseases such as hypertension, cancer, diabetes, and renal disorders, that has become an interesting new target for the development of drugs [1]. In a recent high-throughput screening (HTS) study, the negative modulators **1a** ($R^1 = R^3 = H$, $R^2 = Et$) and **3a** ($R^1 = H$, $R^2 = Pr$), among others, have been identified [2]. Compound **1a** showed an interesting antiproliferative activity against a breast cancer cell line (T47D), demonstrating that AM could be used as a new target for the development of antitumor drugs. In this work, two new series of structurally related negative modulators have been synthesized, and their affinity towards AM has been determined and compared with the lead compounds.



R^1 = *m*-Br, *p*-Br, *p*-Me, *m*-OH, 2-thienyl, 1-naphthyl, 2-naphthyl.
R^2 = H, Et, $PhCH_2$
R^3 = H, Me

R^1 = H, Et, $PhCH_2$, $HOCH_2$-CH_2, Et_2NCH_2-CH_2, PhCO, CH_2=CH-CH_2, C_3H_5-CH_2, *p*-Cl-$PhCH_2$, *p*-CF_3-$PhCH_2$, *p*-MeO-$PhCH_2$, *p*-NO_2-$PhCH_2$,
R^2 =H, Me

R^1 = H, Me
R^2 = H, Pr, CH_2=CH-CH_2, C_3H_5-CH_2, $PhCH_2$, *p*-NO_2-$PhCH_2$, *p*-Cl-$PhCH_2$, *p*-MeO $PhCH_2$, *p*-CF_3-$PhCH_2$, MeOOC-CH_2

We have tested the synthesized compounds for their cytotoxic activity against T47D. AM has also been described as an angiogenic factor, therefore negative modulators could be useful as antiangiogenic agents [1]. Thus, their efficacy as antiangiogenic drugs is currently being tested and we already have some very promising preliminary results with some of our small molecules.

[1] Julian, M., et al., Eur J Med Chem, 2005; 40:737-750.

[2] Martinez, A., et al., Endocrinology, 2004; 145:3858-3865.

P81

Micronucleus formation and cytotoxic activity of some metal complexes in cultured human lymphocytes

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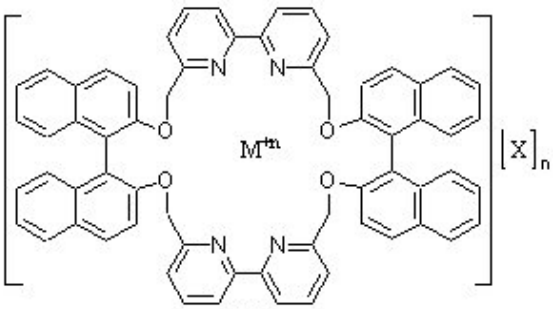
In this research, metal-ion controlled synthesis of some complexes in the presence of Zn(II), Cd(II), Hg(II), Pb(II) were studied. ±2,2'-dihydroxy-1,1'-binaphthyl in hot anhydrous DMF was treated with K_2CO_3 slowly. The solution was gently boiled and 6,6'-*bis*(bromomethyl)-2,2'-bipyridine in DMF added during 30 min. Gently reflux was maintained for 2 h and solvent was distilled from the mixture which was then poured into water. Creamy solid was filtred off washed with dilute aqueous NaOH solution and water, and dried. The appropriate metal-ion salt and ligand were dissolved or suspanded in methanol. The reaction mixture was refluxes for 2 h. and filtreted hot. The complexes were collected and dried. The structure of the compounds were elucidated by IR, ^{1}H-NMR, MASS and Elemental Analyses values.

In this study, genotoxicity of some metal complexes have been investigated by cytokinesis-blocked micronucleus (CBMN) assay in cultured human lymphocytes. CBMN technique is widely used for the assessment of genotoxic activities such as chromosome breakage, loss, rearrangement and also cytotoxic properties such as cell division inhibition, necrosis, apoptosis of chemical compounds. Blood cultures have been set up from two healthy donors and treatment has been done with different concentrations. Future results will provide information on whether these substances have cytotoxic and genetic damage capacity in human.



$[ML^{M}](X)_n$

M= Cu^{2+}, Cd^{2+}, Pb^{2+}.

X= NO_3^-, ClO_4^-. n=1, 2.

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[3] C. M. Che, J. S. Huang, Coord. Chem. Rev., 2003; 242: 97-113.

[4] Fenech, M., Mutation Research, 2000; 455: 81-95.

[5] Abou-Eisha, A., Marcos, R., Creus, A., Mutation Research, 2004; 564: 51-56.

[6] Nersesyan A, Perrone E, Roggieri P, Bolognesi C., Chemotherapy, 2003;49(3): 132-137.

[7] Fimognari,C., Berti, F., Iori, R., Cantelli-Forti, G., Hrelia, P., Mutation Research, 2005; 582: 1-10.

P82

Carbohydrate derivatives as source of biological activity compounds

J. Vega-Perez, M. Vega-Holm, E. Blanco, J. Candela-Lena, F. Alcudia, F. Iglesias-Guerra

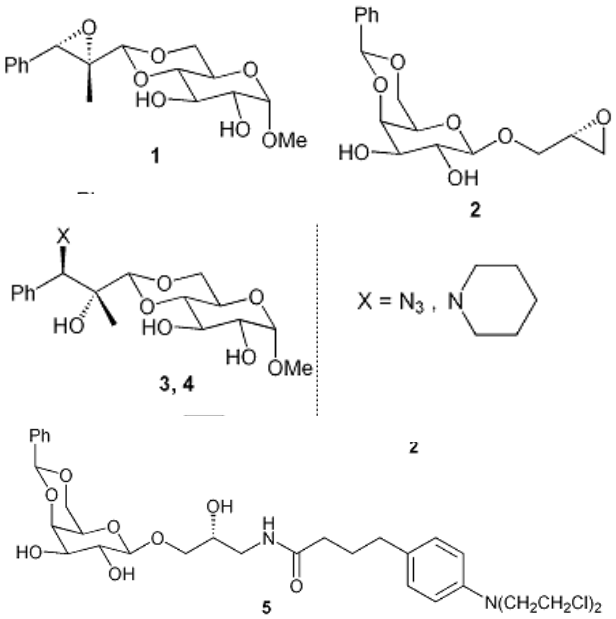
Universidad de Sevilla, Facultad de Farmacia, Dpto Química Orgánica y Farmacéutica

Carbohydrates are often used as chiral templates in stereoselective transformations as well as biological carriers of compounds with therapeutic activity. Our research interest is focused on these two targets.

On one hand the study of the stereochemistry of the epoxidation reaction of different alkenyl sugar derivatives [1]. These oxiranes are interesting in themselves due to they are reactive compounds which can act as alkylating agents **1**, **2**.

On the other hand oxiranes are suitable compounds to be transformed in others. It is well known the importance of isoserina derivatives as a part of natural products with biological activity such as anticancer- agents, immune response modifiers, HIV-1 protease inhibitors. In this sense we have obtained isoserina analogue fragments **3**, **4** joined to carbohydrate derivatives. Alkylating agents are important compounds used in antineoplastic therapies [2]. We have prepared chlorambucil carbohydrate derivatives employing oxiranes above mentioned as starting material **5**.

We want to notice the interest, in terms of hydrophilic-lipophilic balance, transport and localization of the drug, of the fact that than oxirane function as the fragment with biological activity are joined to a sugar moiety.



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P83

Search for second generation drugs based on the antitumor anthracycline antibiotic carminomycin active against MDR-tumor cells

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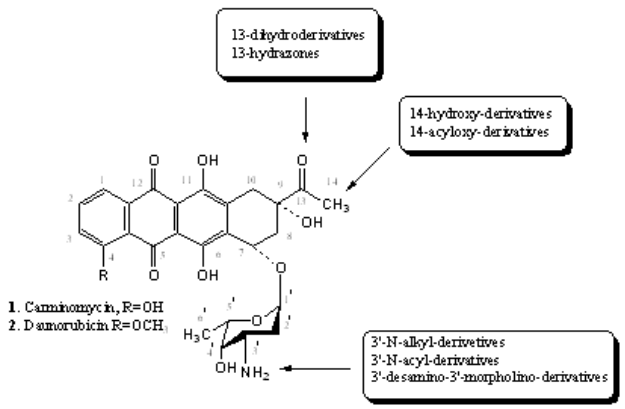
Anthracycline antibiotics are among the most effective chemotherapy agents currently in use for cancer treatment; however irreversible cardiac damage is a major dose-limiting toxicity, restricting life-time cumulative dose. Besides, the therapeutic activity of many drugs of this class is low if tumor cells express multidrug resistance (MDR) mediated by a decreased drug accumulation. Carminomycin (**1**) actually is natural anthracycline antibiotic, originally developed in the . Carminomycin (**1**) proved to be active for the treatment of metastatic breast cancer, soft tissue sarcomas, acute lymphoblastic or myeloblastic leukemias. It was shown that the degree of cardiotoxicity, gastrointestinal toxicity, as well as alopecia, for carminomycin are milder then those encountered with doxorubicin. Carminomycin is well absorbed after oral or subcutaneous administration and is effective against MDR-tumor cells of different types. Recently novel method of synthesis of carminomycin (**1**) starting from daunorubicin (**2**) has been developed.

With the aim of decreasing toxic side effects (especially cardiotoxicity) and also of finding drugs effective in treatment tumors resistant to existing cytostatics, investigations have been carried out on the synthesis of different analogues of carminomycin (**1**). The main directions of chemical modification were developed and the series of new analogues were obtained: 1) 3'N-acyl-derivatives [1], 2) 3'-desamino-3'-morpholinoderivatives, 3) 14-O-hemiadipates and –pimelates [1,2] and 4) 3'-N-alkylderivatives with hydrophilic moieties [3]. Antitumor activity of novel anthracycline derivatives was investigated on different tumor cell lines, including MDR sublines and in *in vivo* experiments. Carminomycin derivatives proved to be perspective agents in these experiments because they retained activity of carminomycin to overcome MDR of tumor cells of different types.



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P84

Development of New Cytotoxic Cytokinin Nucleosides

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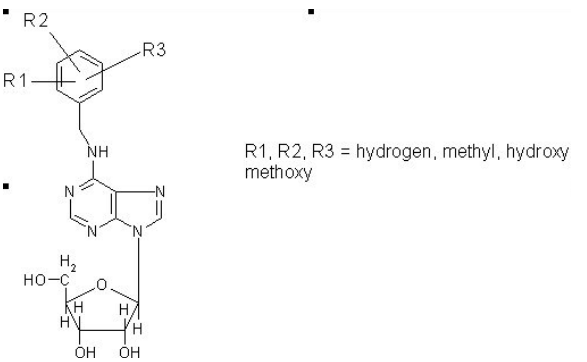
Cytokinins are group of plant growth regulatory substances, derived from adenine and substituted at the N^6-position with an isoprenoid or aromatic side-chain. A range of 6-benzylaminopurines naturally occur in plants and exhibit high biological activity. Others have been synthesized and have shown a promising anti-cancer activity *in-vitro*. In our laboratory, group of N^6-benzyladenosines, di- and trisubstituted on the benzyl ring, have been recently prepared, characterized and tested in different plant and animal systems.

To study the most promising compounds of this group in more details, rapid and efficient method for their purification and quantification in biological samples was developed. The method is based on immunoaffinity chromatography and liguid chromatography-mass spectrometry. Using this analytical approach, a pharmacokinetic study on per oral bioavailability was performed on Balb/c mice.

Key Words: N^6-benzyladenosines, cytotoxicity, immunoextraction, LC-MS, bioavailability



P85

Discovery and optimisation of novel bioavailable phthalazinone inhibitors of poly(ADP-ribose)polymerase

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KuDOS Pharmaceuticals Ltd

PARP-1 activation is an immediate cellular response to metabolic, chemical or ionising-radiation induced damage. Studies have shown that inhibition of PARP-1 activity enhances the effects of certain classes of chemotherapeutics as well as ionising-radiation indicating the potential for PARP-1 inhibition as a combination therapy in oncology. Moreover, recent studies[1] have shown that in certain genetic backgrounds tumour cells are extremely sensitive to the effects of PARP-1 inhibition without the need for the presence of a cytotoxic agent. Therefore inhibitors of PARP-1 activity could be of significant utility in the oncology setting.

From high throughput screening and early SAR studies 4-benzyl-*2H*- phthalazinones were identified as a potential starting point for synthetic chemistry to identify novel PARP-1 inhibitors. We showed that further structural elaboration around the *meta* position of the pendant benzyl moiety improved potency[2]. Further development of this key pharmacophore has lead to the discovery of a number of enzymatic and cellularly potent PARP-1 inhibitors

This poster describes the optimisation of the 4-benzyl-*2H*-phthalazinone series with particular emphasis on refinement of pharmacokinetic parameters. Compounds arising from this optimisation campaign show potential use as orally available PARP-1 inhibitors in oncology either in combination with existing cytotoxics or as a monotherapy in target tumour types.

P86

Synthesis of spacer-linked symmetrical disaccharides analogues

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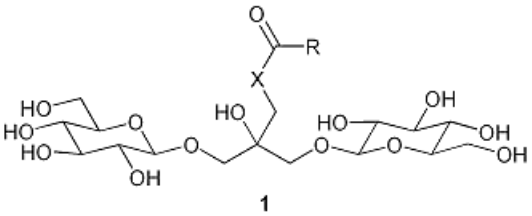
Carbohydrates play essential roles in biological and pharmacological process. In the last years we have come developing a line of investigation focused on the preparation of compounds with potential anticancer activity from amino sugars [1]. Alkylating agents are pioneering drugs in the treatment of malignant metastases, but their selectivity for neoplastic tissues is generally low. It is noteworthy that in a strategy to obtain new highly selective drugs against carcinoma cells in rapid growth, which have a great demand for primary metabolites such as carbohydrates, the latter are used as antitumour agent carrier [2]. In previous works we have reported the synthesis of different chlorambucil derivatives of amino sugars and we have studied the cytotoxic and cytostatic activity of some of them against several types of cell lines [3].

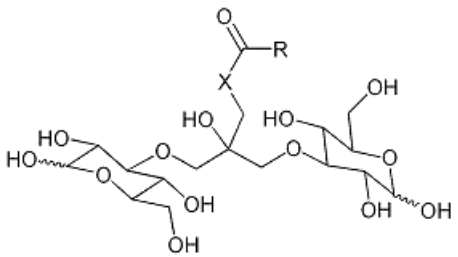
In the present communication we describe the synthesis of disaccharides analogues in which two glucose units are linked through a modified glycerol fragment. The chlorambucil (widely used clinically in the treatment of different types of cancer) was joined to the glycerol moiety by an ester or an amide function (**1** and **2**).



1

X = O, NH

R = $(CH_2)_3$–C$_6$H$_4$–$N(CH_2CH_2Cl)_2$



2

[1] Iglesias-Guerra F, Candela JI, Blanco E, Alcudia F, Vega-Pérez JM. Alkylating Agents from Sugars: Synthesis of Chlorambucil Derivatives Carried by Chiral Glycosyl Glycerols Derived from D-Glucosamine. Chirality 2002;14:199-203.

[2] Halmos T, Santarromana M, Antonakis K, Scherman D. Synthesis of glucose-chlorambucil derivatives and their recognition by the human GLUT1 glucose transporter. Eur J Pharmacol 1996;318:477-484.

[3] Iglesias-Guerra F, Candela JI, Bautista J, Alcudia F, Vega-Pérez JM. Alkylating Agents from sugars. Alkyl hexopyranoside derivatives as carrier systems for chlorambucil. Carbohydr Res 1999;316:71-84.

P87

6-Substituted Pyrrolo[3,4-c]pyrazoles: An Improved Class of CDK2 Inhibitors

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We have recently reported on a new class of CDK2/cyclin A inhibitors based on a bicyclic tetrahydropyrrolo[3,4-c] pyrazole scaffold [1]. The introduction of small alkyl or cycloalkyl groups in position 6 of the bicyclic pyrrolo-pyrazole scaffold allowed to vary substitutions at the other two diversity points thus generating compounds with improved biochemical and cellular characteristics. Optimisation of the physical properties and pharmacokinetic profile led to a compound which exhibited good efficacy *in vivo* on A2780 human ovarian carcinoma.

[1] P. Pevarello, D. Fancelli, A. Vulpetti, R Amici, M. Villa, V. Pittalà, P. Vianello, A. Cameron, M. Ciomei, C. Mercurio, J. R. Bischoff, F. Roletto, M. Varasi, M. G. Brasca, *Bioorg. Med. Chem. Lett*. **2006**, 16, 1084-1090.

P88

Anti-tumor-promoting activity of tibolone and its metabolites

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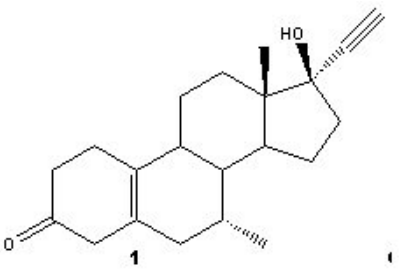
Cancer chemoprevention is dedicated to identifying agents with potential preventive roles in cancer. It consists of administration of synthetic or natural compounds to halt or inhibit the onset of cancer and is based on the conventional multistage carcinogenesis model. According to this model, tumor promotion is a long and reversible stage that could be efficiently suppressed [1]. A wide variety of compounds, with very different structures, are known to have anti-tumor-promoting activity. Among them steroids exhibit remarkable inhibitory effect on chemical carcinogenesis [2], and widely prescribed steroids, such as some progestins, are also potentially useful for the treatment of some hormone-dependent cancer diseases [3].



1

To evaluate their anti-tumor-promoting activity, the progestin tibolone (1) and its metabolites, prepared through chemoenzymatic procedures [4], have been submitted to the short term in vitro assay for the inhibition of Epstein-Barr virus early antigen (EBV-EA) activation induced by the tumor promoter 12-O-tetradecanoylphorbol-13-acetate (TPA). All the compounds showed high inhibitory activity, and tibolone and its 3-a-hydroxy metabolite, when tested in the in vivo two-stage carcinogenesis test, exhibited also inhibitory effects on mouse skin tumor promotion.[1] Murakami A, Ohigashi H, Koshimizu K. Chemoprevention: insights into biological mechanisms and promising food factors. Food Rev Int 1999; 15: 335-395.[2] Konoshima T, Takasaki M. Anti-tumor-promoting activities (cancer chemopreventive activities) of natural products. Kyoto Pharmaceutical University, Kyoto, Japan. Studies in Natural Products Chemistry 2000; 24 (Bioactive Natural Products - Part E), 215-267.[3] Pasqualini JR, Paris J, Sitruk-Ware R, Chetrite G, Botella JJ. Progestins and Breast Cancer. Steroid Biochem Molec Biol 1998; 65: 225-235.[4] Ferraboschi P, Colombo D, Reza-Elahi S. A pratical chemoenzymatic approach to the synthesis of 3-hydroxy metabolites of tibolone. Tetrahedron-Asymmetry 2002; 13: 2583-2586.

P89

Synthesis of some 2,4-di- and 2,3,4-trisubstituted benzimidazo[1,2-a]pyrimidines and evaluation of their cytotoxicities toward F2408 and 5RP7 cells

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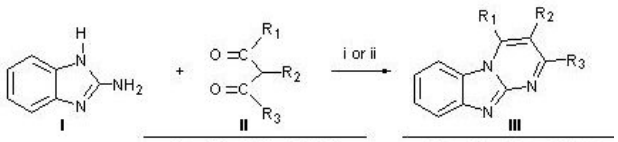
There is an evidence that the fused imidazole compounds were antidepresant, anticonvulsant as physycoactive agent, *invitro* AMPA and NMDA receptor antagonist, antiviral and cytotoxic, mutagenic, antitumor, antihelmintic, antiinflammatory, hypoglysemic and platelet aggregation ihibitory.

Our interest in the derivatives of imidazo[1,2-a]pyrimidine with potential biologically activity, prompted us to design new derivatives which could prove active on neoplasm inhibition. Herein, we are focused on the synthesis of benzimidazo[1,2-a]pyrimidines. The title compounds (**1-10**) were prepared by condensation of 2-amino-benzimidazole (I) and dicarboxylated esters or ?,?-unsaturated dione (II) in PhMe and MeOH or in PPA, respectively, by the one-pot cyclocondensation reactions. Complete structural assignments of 2,4-di- and 2,3,4-trisubstituted benzimidazo[1,2-a]pyrimidine derivatives were established by ^{1}H-NMR, ^{13}C-NMR and IR, EI-MS spectroscopic investigations and x-ray crystallographic data.

The cytotoxic activities of title compounds (**1-10**) against 5RP7 and F2408 cell lines were performed by MTT assay. Some compounds showed a dose- and time-dependent cytotoxic effects onto both cell lines.

This work was partly supported by the “Anadolu University Scientific Research Projects Foundation”, AÜBAP, Eskişehir-Türkiye (Project # 990321)



R_1	R_2	R_3	Comp.
BnO	H	Me	IIa
EtO	H	Ph	IIb
EtO	H	n-Pr	IIc
EtO	Ph	Me	IId
EtO	Me	Me	IIe
Ph	H	Me	IIf
Ph	H	Ph	IIg
Me	H	Me	IIh
Me	Me	Me	IIj
Me	Et	Me	IIk

R_1	R_2	R_3	Comp.
OH	H	Me	**1**
OH	H	Ph	**2**
OH	H	n-Pr	**3**
OH	Ph	Me	**4**
OH	Me	Me	**5**
Ph	H	Me	**6**
Ph	H	Ph	**7**
Me	H	Me	**8**
Me	Me	Me	**9**
Me	Et	Me	**10**

i; refluxed at boiling point of in mix. solvent of PhMe + MeOH or EtOH for 10-40 hrs, (Method A)(-H_2O, -EtOH or -BnOH); ii;heated at 110-120°C in PPA for 30min, (Method B)(-H_2O)

P90

Effect of the levan chemical derivatives on tumor cells proliferation and morphology

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The polyfructan levan had antitumor activity which is mostly explained by macrophage activation. However there are also some reports about the direct action of this polyfructan on some tumor cells lines [1]. The aim of present study is to test the cytotoxicity of fructose polymer levan from Z.mobilis and two its chemically modified form (levan oxidized-reduced form with opened fructose ring and levans sulfate) as well as dextran sulfate on mouse breast cancer cells (4T1 cell line) in vitro . The MTT and LDH tests were performed after incubation of 4T1 cells with different concentrations (up to 1 mg/ml) of mentioned above polysaccharides. The MTT tests show inhibition of the mitochondrial activity of 4T1 cell by all tested polysaccharides and this inhibition correlated with polysaccharide concentration. The visual cell investigation by microscope however did not indicate significant difference between control and cells incubated with levan and levan oxidized-reduced form, while in the presence of levan sulfate and dextran sulfate significant changes in cells morphology occur. The tumor cells cultivated in medium containing sulfated polysaccharides formed small spherical shape aggregates. This effect was observed at concentration of levan and dextran sulfate starting from the 5 μg/ml. LDH tests were done to evaluate cell proliferation and membrane integrity in the presence of mentioned polysaccharides. Results from LDH tests showed that there was not observed significant influence of unmodified levan and levan oxidized-reduced form on 4T1 cell proliferation, while the levan sulfate , as well as, dextran sulfate inhibited 4T1 cell growth and increased cell membrane damage.

[1] Sang-Ho Yoo , Eun Ju Yoon , Jaeho Chac, Hyeon Gyu Lee (2004) Antitumor activity of levan polysaccharides from selected microorganisms. International Journal of Biological Macromolecules 34: 37–41

P91

Fructans as anticancer agents - stimulators of natural killer cells' action

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Background. Natural killer (NK)-cells are the main components of innate immune defense against development of tumors: NK show direct cytolytic activity against malignant cells, act through cytokine production, and also play a principal role in linking innate and adaptive immunity - a significant correlation between NK-cells and T-cells activity has been proven. It is reported that mice with a low function of NK-cells are more susceptible to carcinogen-induced cancers; a surgically mediated decrease in NK-cells activity has been implicated as the major contributing factor, associated with a considerable increase in metastasis. The negative consequences, related to NK-cells immunosuppression, can be decreased by nutritional optimization of the immune status. Our aim was to investigate the natural fructans influence on innate anticancer immunity. Method. In a period of 3 months, the influence of chicory and Jerusalem artichoke inulins on male rats of Wistar line NK-cells; count and activity was examined. Results. In comparison with a control group having no natural fructans additives in the feeds, the number of NK-cells in blood for both inulins were increased up to 195.5-198.2 %. The activity of NK-cells manifested itself in activation of lysozyme, being the instrument of NK-cells citotoxicity, and by recruitment of an adaptive immunity response - the number of T-cells in blood raised to 170.0 %. Fructans had stimulated the increment of relative mass coefficient of thymus conjointly with maturing, proliferation and differation of T-lymphocytes. The increment of T-cells; specific activity - activation of macrophages and B-cells, and modulation of production of T-helper cells cytokines INF-gamma; and TNF-alpha; under influence of the fructans had been established. Conclusions. Natural fructans can induce antitumor effect through activation of NK-cells; due to a diversity of their antitumorous action, the plant inulins can be recommended for permanent dietary use to improve the immune status of cancer patients.

P92

Synthesis of an Anticancer Vaccine Candidate; Double C-Glycoside Analog of sTn

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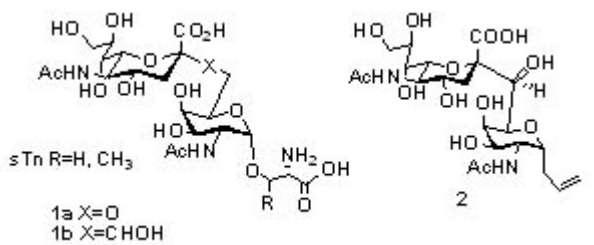
The development of vaccines against carbohydrates is of crucial importance in the fields of therapeutic glycobiology and immunology. A significant portion of the anti-tumor response against cancers involves carbohydrate tumor antigens. Microorganisms often express carbohydrate antigens and the immune response of the host to these antigens is an important mechanism of defense. Neuraminic acids are biologically important, since they occupy the terminal position of the glycans on macromolecules outside cells and cell membranes and are involved in recognition, cell-interactions, neuronal transmission, ion transport, reproduction, differentiation, epitope masking and protection. Cell surfaces interact with receptors, hormones, enzymes toxins and viruses and other pathogens that use Neu5Ac to localize on the surface of cells they infect. Neuraminic acid *C*-glycosides might be useful in preparing immunogens for active immunization against neuraminic acid containing glycoconjugates in the design and preparation of anti-viral, anti-bacterial and anti-cancer vaccines.



Sialyl-Tn, (sTn **1a**) is found on the HIV envelope glycoprotein gp120 and in tumor-associated antigens present in the glycoproteins on the surface of cancer cells, including those associated with carcinomas of the breast, prostate, pancreas, colon, ovary, lung, and stomach. The sTn antigen is well known as a prognostic indicator, and has proven to be an effective target for therapy of cancers. Conjugate vaccines of sTn-KLH (Keyhole limpet hemocyanin carrier-protein) showed remarkable immunogenicity, resulting in the production of both IgM and IgG type antibodies. The sTn *C*-glycoside **1b** has been synthesized and its KLH-conjugate is currently under biological evaluation.

An sTn double *C*-glycoside analog **2** was designed and synthesized to reduce the number of synthetic steps required to synthesize, to facilitate its conjugation to KLH; and to further increase biological half-life and enhance immunological response.

Studies are underway to conjugate the sTn double *C*-glycoside hapten **2** to KLH carrier protein for biological evaluation as a vaccine.

P93

Synthesis and Cytotoxic Properties of Novel Derivatives of Noscapine

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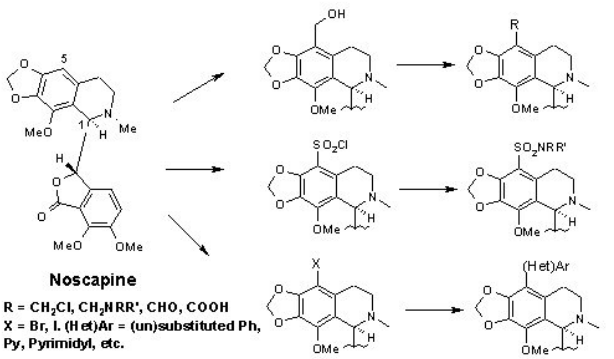
Noscapine (alkaloid isolated from *Papaver somniferum L. Papaveraceae*) has been long used as an oral cough suppressant with no known toxic side effects. Recent reports indicate a possibility for a novel therapeutic use of this old drug. It was shown that noscapine exhibit an anti-cancer activities toward both *Taxol*- and *Vincristine*-resistant cancerous cell lines. These findings along with its oral bioavailability and blood-brain barrier permeability attracted a great deal of attention in this past decade. Noscapine was also reported to inhibit hypoxia-inducible factor-1 (HIF-1) pathway, which plays important role in proliferative diseases.

In this work, we have synthesized a series of new noscapine analogs and evaluated their cytotoxic and cell cycle arresting activity in tumour cell lines.



Our main synthetic focus was to introduce a diverse set of substituents into the 5-position of the tetrahydroisoquinoline ring. Semisynthetic molecules include Br, I, CH_2OH, CH_2Cl, CH_2NRR', CHO, COOH, COMe, SO_2Cl, SO_2NRR', Ar and Hetaryl. The derivatives were synthesized with high yields (>60%) and had *de* 100% related to the natural product stereochemistry. A biological activity of the resultant compounds was profiled using a panel of human tumor cell lines, DLD-1 (colorectal adenocarcinoma), DU-145 (metastatic hormone-refractive prostate cancer), T-47D (metastatic breast cancer), and Jurkat T cells (leukaemia). The derivatives were shown to have different levels of cell specificity profiles in the cytotoxicity assays. Two synthetic analogues were also shown to induce apoptosis in Jurkat T cells and possess an anti-

mitotic activity as evident from the data obtained on a panel of the human cancer cells.

P94

Anticancer activity of an acid-sensitive doxorubicin conjugate with lactosaminated albumin in rat hepatocellular carcinoma

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Doxorubicin (DOXO) was coupled to lactosaminated human albumin (L-HSA) through an acid-sensitive hydrazone linker in order to enhance the drug concentrations in well differentiated hepatocellular carcinomas (HCCs), which express the asialoglycoprotein receptor (ASGP-R) and can accumulate L-HSA through this receptor. In rats with diethyl nitrosamine induced HCCs, L-HSA coupled DOXO exerted marked anticancer activity, whereas the free drug was completely ineffective [1]. In recent experiments, we unexpectedly found that L-HSA-DOXO enhanced the drug concentrations in all rat HCCs, including the poorly differentiated forms of the tumor, not expressing the ASGP-R. The $AUCs_{(0\text{-}24h)}$ of drug concentrations in the well differentiated forms of HCC were approximately 3-fold higher in rats treated with L-HSA-DOXO compared to those treated with free DOXO and 2-fold higher in the moderately and poorly differentiated forms of HCCs. Moreover, in animals injected with the conjugate, the ratios between the AUCs of the drug concentrations in the tumor and those in heart and intestine (target organs of DOXO toxicity) were 6-10 times higher than the same ratios calculated in free DOXO injected animals.

In conclusion, our latest results give an insight into the role of active and passive tumor targeting and suggest that L-HSA-DOXO might be usefully administered to treat all the forms of HCCs, including the poorly differentiated ones that represent the majority of the cancer advanced lesions for which an effective systemic chemotherapy is actively sought.

[1] Fiume L, et al. J Hepatol 2005; 43: 645-652.

P95

Synthesis and Structure Elucidation of Several Triazene and Diazene Derivatives

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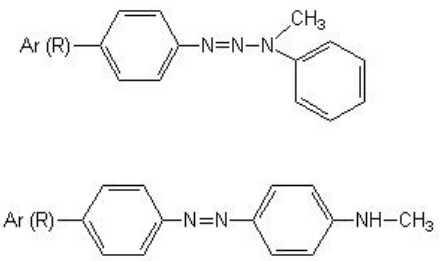
As the incidence and already high mortality rates of malignant melanoma have been steadily inreasing in recent decades. Numerous chemotherapeutic agents have shown activity in the treatment of metastatic malignant melanoma, such as dacarbazine (dimethyl triazene imidazole carboxamide). Dacarbazine and berenil, which possess a triazene group, are the first compounds used in clinical practise. One of the most prevalent uses of triazenes is in the development of anticancer molecules. Daidone and co-workers have recently reported antiproliferative activity of several triazene derivatives obtained from diazonium salts [1]. In view of the above considerations, we planned the present study to further investigate this subject. With the aim to obtain active anticancer compounds, we synthesized a series of triazene derivatives, in which diazonium salts of aromatic primary amines were treated with N-methyl aniline. The reactions between 4-aminobenzoic acid, sulphaguanidin, sulphadiazine and N-methyl aniline did not produced the expected triazene compounds but instead a high yield of diazenes. The structures of eight new synthesized compounds were confirmed by elemental analysis (C, H, N, S) and UV, IR, ^{1}H–NMR. All synthesized compounds are also evaluated for their anticancer potency.



[1] Daidone G, Raffa D, Maggio B, Raimondi MV, Plescia F, Schillaci D Synthesis and antiproliferative activity of triazenoindazoles and triazenopyrazoles : a comparative study. Eur. J. Med. Chem. 2004; 39 : 219-224

P96

Synthesis and antitumor characterization of pyrazolic analogues of the marine pyrroloquinoline alkaloids: wakayin and tsitsikammamines

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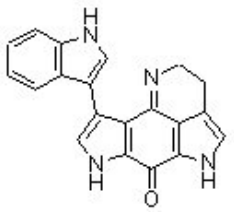
A series of aza-analogues of the marine alkaloids wakayin **1** and tsitsikammamines A (**2**) and B (**3**) have been synthesized. The strategy used was based on [3+2] cycloaddition reactions involving 3-ethylamine-indole-4,7-dione and different diazo-reagents.



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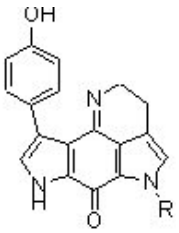

2 R = H
3 R = Me

All the compounds were evaluated in vitro for antiproliferative activity against five distinct cancer cell lines and for their inhibitory effect on topoisomerase isoenzymes I and II.

Some of the compounds inhibited the topoisomerase I and/or II catalyzed relaxation of supercoiled DNA at a concentration comparable to the drugs camptothecin and etoposide. Only a few of them exhibited cytotoxic activity with IC50 values in the micromolar range.

P97

Synthesis of potential cytostatically active 6-Amino-11,12-dihydrobenzo[c]phenanthridine derivatives using Stilbenes and substituted o-Methylbenzonitriles as reactants

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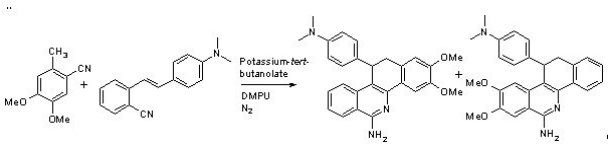
Benzo[c]phenanthridines are well-known for their broad spectrum of pharmacological activities including antimicrobiological and cytotoxic effects. In particular the antitumoral activity both of natural and synthetic benzo[c]phenanthridine derivatives has been reported for several members of this class of compounds [1].The most important natural compound is fagaronine which can be isolated from the radix of fagara zanthoxyloides only in poor yields. Therefore, an easy access to benzo[c]phenanthridines is an interesting goal to obtain larger quantities and a greater variety of this cytostatic substance class.



In previous studies we developed such an efficient synthesis for 11-substituted 6-amino-11,12-dihydrobenzo[c]phenanthridines and their dehydro derivatives [2]. The reaction mechanism which was postulated for this synthetic method involves a stilbene as an intermediate [3]. In order to confirm the reaction mechanism and to obtain new benzo[c]phenanthridine derivatives with a variety of exocyclic substituents in various positions stilbenes were used as starting material. With this approach several new benzo[c]phenanthridines have been synthesized and the reaction mechanism outlined in [3] could be confirmed . In accordance to our expectations we found that each synthesis yields two different benzo[c]phenanthridine derivatives. The ratios of the resulting isomers were then determined by high performance liquid chromatography.

[1] Simeon P, Rios J, Villar A. Pharmacological activities of benzophenanthridine and phenanthrene alkaloids. Pharmazie 1989;44:593-597.

[2] Kock I, Heber D, Weide M, Wolschendorf U, Clement B. Synthesis and biological evaluation of 11-substituted 6-aminobenzo[*c*]phenanthridine derivatives, a new class of antitumor agents. J Med Chem 2005;48:2772-2777.

[3] Clement B, Weide M, Wolschendorf U, Kock I. A two-step synthesis of cytostatically active benzo[c]phenanthridine derivatives. Angew Chem Int Ed Engl 2005;44:635-638.

P98

Receptor based design, synthesis and evaluation of a library of new histone deacetilase inhibitors

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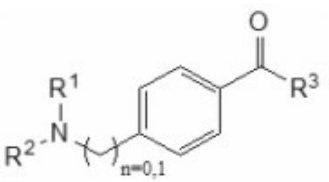
Modification of the acetylation state of the e-amino group of specific lysine residues of histones plays a crucial role in the regulation of the transcriptional process and, thus, it is central to cell proliferation, differentiation and apoptosis[1]. Two families of enzymes, histone acetyl transferases (HATs) and histone deacetylases (HDACs), are involved in the regulation of this equilibrium. Recent studies show that inhibition of HDACs causes arrest of cell growth and induces cell differentiation. As a consequence, there is an increasing interest on the development of new HDAC inhibitors as potential anticancer drugs.



1

Using recently reported structural data on different isoforms of HDACs[2,3], we have conducted docking stud-

ies to design a library of potential inhibitor compounds based on scaffold **1**. These compounds have been synthesised using solid and liquid phase methodologies, and their antiHDAC activity has been evaluated.

[1] Pennisi, E. Opening the way to gene activity. Science 1997;275:155-157.

[2] Somoza, J.R. Structural snapshots of human HDAC8 provide insights into the class I histone deacetylases. Structure 2004;12:1325-1334.

[3] Wang, D-F., Helquist, P., Wiech, N.L., Wiest, O. Toward selective histone deacetylase inhibitor design: Homology modeling, docking studies, and molecular dynamics simulations of human class I histone deacetylases. J. Med. Chem. 2005; 48:6936-6947.

P99

Development of supramolecular nanocarriers of doxorubicin for tumor-specific drug delivery

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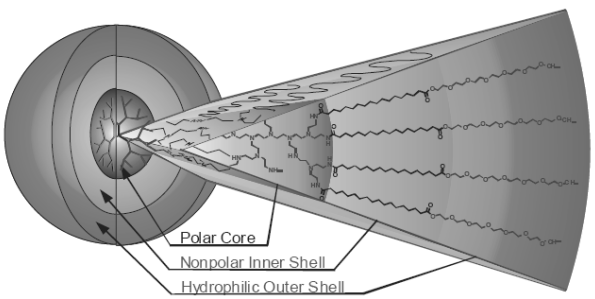
Tumor-specific drug delivery with nanocarriers based on dendritic polymers has received increasing attention in the last few years [1,2]. In the past, we have developed multishell polymers with a hyperbranched poly(ethylene imine) (PEI) core, an aliphatic inner shell, and a PEG outer shell varying in the size of the PEI core, the chain length of both hydrophobic inner shell and PEG outer shell as well as the degree of functionalization [3]:



Model studies with several dyes revealed that they were complexed and formed supramolecular aggregates of approximately 100 nm in diameter as shown by cryo-TEM and dynamic light scattering. In the present work, we could show that the anticancer drug doxorubicin formed similar complexes with several multishell polymers in organic solvents as well as under aqueous conditions. High loading ratios of 4:1 to 9:1 (molecules of the drug per molecule polymer) were obtained when the degree of functionalization of the core was >70 %. The aggregates with doxorubicin were readily isolated by size-exclusion chromatography and showed good stability in physiological buffer and blood plasma.

[1] Haag R, Kratz F. Polymer therapeutics: concepts and applications. Angew. Chem., Int. Ed. 2006;45:1198-215.

[2] Ambade AV, Savariar EN, Thayumanavan S. Dendrimeric micelles for controlled drug release and targeted delivery. Mol Pharm 2005;2:264-272.

[3] R. Haag, M. Radowski, Multishell Nanotransporters, 2004, German Patent Application DE 10 2004 039 875

P100

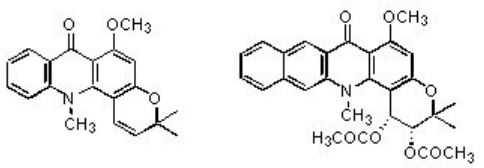
Synthesis and Cytotoxic and Antitumoral Activity of 1,2-Dihydroxy-1,2-dihydrobenzo[a]acronycine diesters

T. Nguyen*, C. Sittisombut*, S. Boutefnouchet*, M. Lallemand*, S. Michel*, M. Koch*, F. Tillequin*, M. David-Cordonnier**, B. Pfeiffer***, L. Kraus-Berthier***, S. Leonce***, A. Pierré***

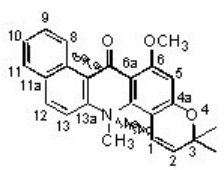
*Laboratoire de Pharmacognosie de l'Université Paris 5, UMR CNRS N° 8638,Faculté de Pharmacie, 4 Av. de l'Observatoire, F-75006 Paris. **INSERM U814, Institut de Recherches sur le Cancer de Lille et Université de Lille II, Place de Verdun F-59045 Lille. ***Institut de Recherches Servier, Cancer Research Division, 125 Chemin de Ronde, F-78290 Croissy sur Seine

The natural pyranoacridone acronycine (**1**), first isolated from *Acronychia baueri* Schott (Rutaceae) in 1948, was subsequently shown to exhibit a broad spectrum of activity against numerous experimental models of solid tumors. Nevertheless, its moderate potency and very low solubility in aqueous solvents severely hampered subsequent clinical trials. Significant improvements in terms of potency were obtained with modified derivatives exemplified by *cis*-1,2-dihydroxy-1,2-dihydrobenzo[*b*]acronycine diesters. A representative of this series, **2**, currently entering phase II clinical trials under the code S23906-1, displayed a particularly broad antitumor spectrum [1].

In a continuation of our studies[2] we describe here the synthesis and the biological properties of 6-methoxy-3,3,14-trimethyl-3,14-dihydro-7*H*-benzo[*a*]pyrano[3,2-*h*]acridin-7-one (**3**) and of related *cis*-1,2-dihydro-1,2-diol esters and diesters. The aim of the present work is to determine the influence of the mode of fusion of the additional aromatic ring onto the natural acronycine tetracyclic core on DNA-alkylation, and cytotoxic and antitumor properties.



1 **2** S23906-1



3

[1] Costes, N.; Le Deit, H.; Michel, S., Tillequin, F.; Koch, M.; Pfeiffer, B.; Renard, P.; Léonce, S.; Guilbaud, N.; Kraus-Berthier, L.; Pierré, A.; Atassi, Gh. Synthesis and cytotoxic and antitumor activity of benzo[*b*]pyrano[3,2-*h*]acridin-7-one analogues of acronycine. *J. Med. Chem.* **2000**, *43*, 2395-2402.

[2] Michel, S.; Gaslonde, T.; Tillequin, F. Benzo[*b*]acronycine derivatives: a novel class of antitumor agents. *Eur. J. Med. Chem.* **2004**, *39*, 649-655.

P101

Maturation of autotaxin (NPP2), a secreted metastasis-enhancing lysophospholipase D

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KULeuven Division of Biochemistry* and **Department for Human Genetics / Flanders Interuniversity Institute for Biotechnology, Faculty of Medicine, K.U.Leuven, B-3000 Leuven, Belgium

NPP2, also known as autotaxin, is a secreted 125-kDa glycoprotein that stimulates the motility and the proliferation of normal as well as tumour cells. The expression of NPP2 is increased in various malignancies like breast and lung cancer, and this upregulation correlates with the invasiveness of the cancer cells. The effects of NPP2 can be attributed to its ability to hydrolyse lysophosphatidylcholine, resulting in the release of the signalling molecule lysophosphatidic acid (LPA). The latter is known to activate multiple pathways via interaction with a range of G-protein coupled receptors.

The extracellular localization of NPP2 and its role in the metastasis of cancer cells make it an attractive therapeutic target. In this context, we have investigated the maturation pathway of this protein, including its mechanism of secretion and the role of N-linked glycosylation. We have found that NPP2 is secreted via the classical secretory pathway and that a N-terminal fragment that was believed to function as a transmembrane domain actually functions as a signal peptide. In addition, the N-terminus of NPP2 needs further trimming by furin to obtain fully active enzyme [1]. Furthermore we found that NPP2 is N-glycosylated and that the enzyme is completely inactivated by the removal of one specific glycan chain.

[1] Jansen S.,Stefan C., Creemers J.W.M., Waelkens E., Van Eynde A., Stalmans W. and Bollen M. Proteolytic maturation and activation of autotaxin (NPP2), a secreted metastasis-enhancing lysophospholipase D. J Cell Sci, 2005, 118, 3081-3089.

P102

Aurones as chemopreventive agents via the induction of NAD(P)H: quinone acceptor oxidoreductase

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Coordinate induction of phase 2 enzymes in mammalian cells including the NAD(P)H: quinone acceptor oxidoreductase or quinone reductase 1 (QR1) is an important defense mechanism against toxic and carcinogenic effects of xenobiotics [1, 2]. In a search for new chemoprotective QR1 inducers, we focused on the aurones (2-benzylidene-benzofuran-3(2H)-ones), a subclass of flavonoids with wide biological potentials. A series of aurones were synthesized and screened in murine Hepa1c1c hepatoma cells [3] for an initial structure-activity survey. Certain hydroxylated aurones were especially potent inducers (*CD = 1-5 μM). Surprisingly, unsubstituted Ring B compound of subseries 1 (CD = 1.3 μM) was among the potent inducers identified whereas removal of all substituents on Ring A (subseries 3) diminished activity. Loss of the entire Ring B greatly reduced inductive activity indicating the importance of a Micheal reaction acceptor element. A particular halogenated aurone was identified as the most potent QR1 inducer in this investigation.

Subseries 1: R1 = OCH_3
Subseries 2: R1 = OH
Subseries 3: R1 = H
R2 = Various substituents including hydroxyls, methoxyls, and halogens.

[1] Talalay P, De Long MJ, Prochaska HJ (1988) Identification of a common chemical signal regulating the induction of enzymes that protect against chemical carcinogenesis. *Proc. Natl. Acad. Sci. U.S.A.* **85**: 8261.

[2] Dinkova-Kostova AT, Talalay P (2000) Persuasive evidence that quinine reductase type 1 (DT diaphorase) protects cells against the toxicity of electrophiles and reactive forms of oxygen. *Free Rad. Biol. Med.* **29**: 231.

[3] Prochaska HJ,

P103

Synthesis and cytotoxic evaluation of benzophenantridine- and pyrimidobenzoisoquinolin-quinones

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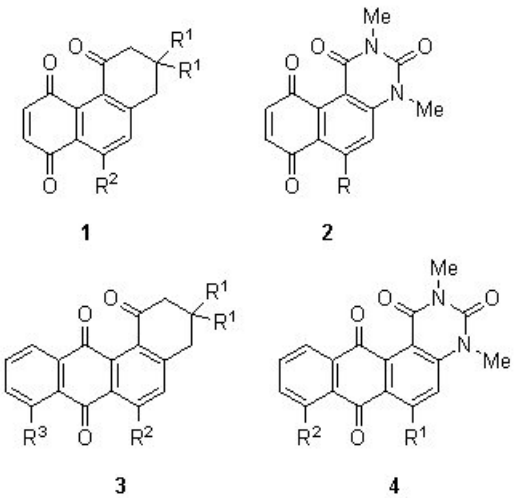
The replacement of nitrogen atoms by the CH groups in anticancer drugs (*i.e.* ametantrone and 11-deoxydaunomycin) that provide antitumor active analogues,

has shown to be an effective strategy to design new potential antitumor compounds.[1,2] These *N*-heterocyclic aromatic congeners could potentially retain the same planar shape of the drug chromophore necessary for the molecular recognition of the host. Furthermore, the basic and electron-withdrawing properties of the *N*-heterocycles seem to improve the affinity for the biological target and/or redox cycling mechanism. As part of a program aimed to the synthesis of carbo- and heterocyclic quinones we became interested in the synthesis and cytotoxic evaluation of aza-congeners of the angucyclinone skeleton. Herein we report our initial results. Phenanthridine- and pyrimido[4,5-*g*]isoquinolinquinones **1** (R^1 = H, Me; R^2 = H, Me) and **2** (R = H, Me), prepared in a simple operation from acylhydroquinones and cyclic enaminones, were employed as precursors of angular tetracyclic quinones **3** (R^1 = R^2 = H, Me; R^3 = H, OH) and **4** (R^1 = H, Me; R^2 = H, OH) through regiocontrolled cycloaddition with 1-trimethylsyliloxybuta-1,3-diene.

The new compounds showed *in vitro* cytotoxic activity against fibroblast and four tumor cell lines. The results indicate pronounced antitumor activity for some of the tested compounds compared with carbocyclic analogues.



1 **2**

3 **4**

[1] Krapcho A P, Petra ME, Getahun Z, Landi JJ, Stallman J, Polsenberg JF, Gallagher CE, Maresch MJ, Hacker MP; *6,9-Bis[aminoalkyl)amino]benzog]isoquinoline-5,10-diones. A novel class of chromophore-modified antitumor anthracen-9,10-diones: Synthesis and antitumor evaluation.* J. Med. Chem. 1994, 37: 828-837.

[2] Kita Y, Kirihara M, Fujii Y, Okunaka R, Akai S, Maeda H, Tamura Y, Shimooka K, Ohishi H, Ishida T. *Synthetic Anthracyclines: Total synthesos of D-ring pyridine and pyrazine analogues of 11-deoxydaunomycin.* Chem. Pharm. Bull. Jpn. 1991, 39: 857-864.

This work was supported by FONDECYT (GRANT Nº 1060591).

P104

Estrogenic Action of Substituted Pyrroles

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A variety of nonsteroidal compounds with hetetocyclic core like furans, pyrazoles and imidazoles might act as potent ligands for the estrogen receptor (ER). In this study, we prepared alkyl and aryl-substituted pyrroles and assessed their behavior as ER ligands. To investigate the influence of the hydrophilic character and the potency to function we synthesized two types of compounds with different ability to form hydrogen bonds to amino acids in the ligand binding domain of the ER. We found practical and facile synthetic procedures to obtain the two pyrrole types of different substitution patterns. In order to analyze the estrogenic potency the pyrroles were evaluated in the luciferase assay using the ERá-positive MCF-7 2a cell line. In further experiments we analyzed the possibility of the new pyrroles to down-regulate the ERá in MCF-7 cells by using westernblot mehod. The cytotoxic potency of the pyrroles was evaluated on the same cell line. The stability of the compounds under test conditions was very high. After incubation at 37 °C for 48 h no degradation products were detected in the HPLC-chromatograms.

P105

Synthesis and anti-proliferative activity of 17b-heterocycle functionalised estra-1,3,5(10)-triene derivatives

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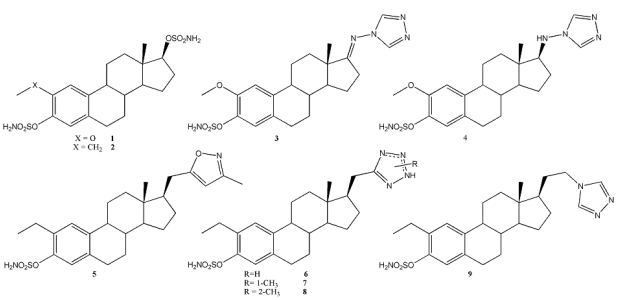
[*]Department of Pharmacy and Pharmacology & Sterix Ltd., University of Bath, Claverton Down, Bath, BA 27AY, UK. [**]Institut de Recherche Henri Beaufour, 91966 Les Ulis Cedex, France. [***]Endocrinology and Metabolic Medicine, Imperial College, St. Mary's Hospital, London, W 21NY, UK.

The estradiol *bis*-sulfamate derivatives 2-MeOE2bisMATE (**1**) and 2-EtE2bisMATE (**2**) exhibit potent anti-proliferative effects against human breast (MCF-7) [1, 2] and prostate (DU145) cancer cells. SAR studies have revealed that H-bonding effects around C-17 of suitably 2-substituted estradiol 3-*O*-sulfamates are key to high anti-proliferative activity.



In this study we explored replacement of the 17-*O*-sulfamate group with various heterocycles (triazole (**3**), (**4**) and (**9**), oxazole (**5**), tetrazole (**6**)-(**8**)) linked to position C-17 through various linkers with the aim of discovering novel anti-proliferative agents. These compounds were synthesised from 3-*O*-benzyl-2-ethylestrone in 6 to 8 chemical steps and were evaluated alongside their corresponding phenol for activity against the proliferation of DU145 cells. As observed in previous work all the phenolic precursors were found to be inactive (GI_{50}>100 μM) highlighting the importance of the 3-*O*-sulfamate group. The series of tetrazoles (**6**)-(**8**) exhibited interesting SAR, thus, whilst the non-substituted compound (**6**) proved inactive, the 1-methyltetrazole (**7**) (GI_{50}=0.57 μM) showed promising activity and was 3 times more active than the 2-methyltetrazole compound (**8**) (GI_{50}=1.64 μM). Compound (**5**) (GI_{50}=1.83 μM) proved equipotent to the closely related tetrazole (**8**) whereas (**9**) (GI_{50}=4.52 μM), in which a longer alkyl linker between C-17 and the heterocyclic unit is present, proved 20 fold less potent than (**2**). This study shows that potent anti-proliferative activity can be retained by substitution of the *O*-sulfamoyl group of (**2**) by a heterocycle (1-methyltetrazole **7**) and that the nature, position and steric environment of the H-bond acceptor within the heterocycle strongly impact on the potency of these novel compounds.

[1] Newman SP et al. Int J Cancer 2004; 109: 533-540

[2] Leese, MP et al. J Med Chem 2005**;** *48*: 5243-5256

P106

Methoxylated Stilbenes as Inducers of NAD(P)H: Quinone Reductase Type 1

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Quinone reductase 1 (QR1) is an important enzyme involved in protecting cells against electophilic and oxidative assault. Its activity is commonly monitored as a marker for protective enzyme induction and as a target for the development of novel chemopreventive agents [1]. Resveratrol had been reported to possess modest QR1 activity [2]. In an attempt to improve the QR1 induction activity of resveratrol, several derivatives of resveratrol (Series I-III) were synthesized and evaluated for inductive activity on Hepa1c1c hepatoma cells [3].

The most active compound was found in series II, where R = 2 –OCH_3 (**1**). This compound induced QR1 activity by twofold at 12 μM. Only the trans isomer was active and conversion to 2-OH diminished activity. Inclusion of another *o*-methoxy group on ring A to give E-4',2,6-trimethoxystilbene retained activity. Activity was also observed in the symmetrical E-2,2'-dimethoxystilbene. Interestingly, these compounds failed to demonstrate QR1 inducing properties when screened on a mutant cell line (Hepa1c1c7bp'c1) which lacked CYP1A1. Thus, **1** may be a bifunctional QR1 inducer, with activity dependent on metabolic conversion by CYP1A1.

[1] Dinkova-Kostova AT, Talalay P (2000) Persuasive evidence that quinine reductase type 1 (DT diaphorase) protects cells against the toxicity of electrophiles and reactive forms of oxygen. *Free Rad. Biol. Med.* **29**: 231.

[2] Heo YH, Kim S, Park JE, Jeong LS, Lee SK (2001) Induction of quinone reductase activity by stilbene analogs in mouse hepa1c1c7 cells. *Arch Pharm Res.* **24:** 597.

[3] Prochaska HJ, , Talalay P. (1992) Rapid detection of inducers of enzymes that protect against carcinogens. *Proc. Natl. Acad. Sci. U.S.A.* **89**: 2394.

P107

Antiproliferative Basic Chalcones with P-Glycoprotein Modulating Properties

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Methoxylated chalcones bearing N-methylpiperidinyl substituents on Ring A have been found to inhibit the growth of human tumour cells at IC_{50} values of < 5 μM [1]. Further synthetic efforts focused on the inclusion of other basic moieties at various positions on the chalcone template. These moieties include N-ethylpiperidine, N-methylpiperazine, pyridine and 4-(N-piperidinyl)piperidine. The basic moieties were introduced into either ring A or ring B of the chalcone. Some examples are shown in Figure 1:

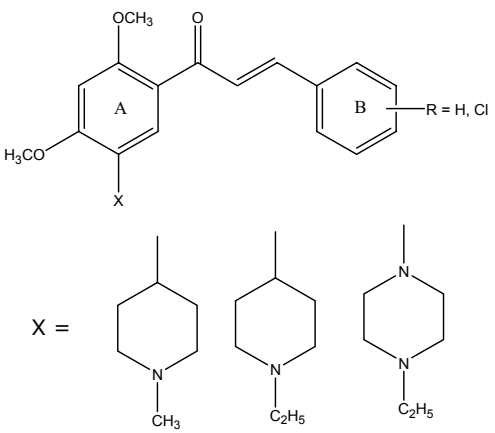

Figure 1: Representative basic chalcones investigated for antiproliferative and P-glycoprotein modulating activity.

Many of these compounds were found to have submicromolar IC_{50} values against HCT116 tumour cells. The interaction of these compounds with the efflux protein P-

glycoprotein was investigated by monitoring the accumulation of calcein AM in wild type and mutant MDCK cells that overexpress P-glycoprotein [2]. It was found that the basic chalcones increased the accumulation of calcein AM in the mutant cell line, indicating that these compounds were inhibitors of the efflux protein. Chalcones without the basic functionality were found to be less effective in modulating the activity of P-glycoprotein. The combination of two properties – antiproliferative and P-glycoprotein inhibitory activity – serve to emphasize the potential of basic chalcones as novel bifunctional agents for cytostatic therapies.

[1] Liu XL, Go ML (2006). Antiproliferative properties of piperidinylchalcones. *Bioorg. Med. Chem.* **14:** 153.

[2] Hamada A, Miyano H, Watanabe H, Saito H. (2003) Interaction of imatinib mesilate with human P-glycoprotein. *J. Pharmacol. Exp. Ther.* **307:**824.

P108

Nitric oxide releasing derivatives of [2-chloroethyl)ureido]benzoic acid esters as potential antineoplastic agents

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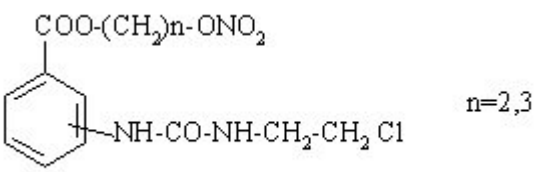
Cancer is a malignant disease responsible for high level of mortality and morbidity. Numerous chemotherapeutic agents have been developed but chemotherapeutic agents often fail when drug resistant tumour cells become predominant. It has recently been reported that N-phenyl-N'-(2-chloroethyl) urea forms a covalent link with tubulin cystein residue [1], and NO also has a cytotoxic effect on tumour cells [2]. We describe here the synthesis and growth inhibition activity of [(2-chloroethyl)ureido]benzoic acid ethyl and propylnitroxy esters. First, [(2-chloroethyl)ureido]benzoic acids were prepared from the corresponding o, m, or p-amino benzoic acids by treatment with 2-chloroethylisocyanate and K_2CO_3 in ethanol[3]. The ester derivatives of these acids were then prepared by the reaction of nitrooxyalcohols DCC and DMAP in acetonitrile[4].



The synthesized compounds were screened for their cytotoxic effects on HEK 293 and 3 t_3 (mouse fibroblast), HeLa (cervical cancer), Raji (Burkitt Lenfoma) and Hep-3B(hepatocellular carcinoma) cells. To find effects of anti-cancer and cytotoxic properties, the CellTiter 96® aqueous non-radioactive cell proliferation assay is applied. The target compounds showed anticancer activity.

[1] Moreau, E, Fortin S., Desjardins M., Rousseau L. C., Petitclere, E.,Gaudreault C. Optimized N- phenyl –N'-(chloroethyl) ureas as potential antineoplastic agents. Bioorganics & Medicinal Chemistry 2005; 13/24, 6703-6712.

[2] Lechner M., Lirk P., Rieder J., Inducible nitric oxide synthase in tumor biology: The two sides of the same coin, Review, Seminars in cancer Biology 2005; 15, 277-289.

[3] Akgun H., Hollstein U., Hurwitz L., Synthesis of some substituted quinazolinedions as potential inhibitors of smooth muscle contraction, J. of Pharmaceutical Sciences, 1988;9/77 735-739.

[4] Weber C., Homann A., Fricke, U., LehmannJ., No donors part ([1]: Synthesis and vasodilating activities of substituted benzylnitrates compared to cyclohexylmethylnitrate and GTN. Eu. J. of Med. Chem. 2003; 38, 581-586.

P109

The New C-5 Substituted Uracil Derivatives of 4,5-didehydro-5,6-dideoxy-L-Ascorbic Acid: Synthesis, Antitumoral and Antiviral Evaluations

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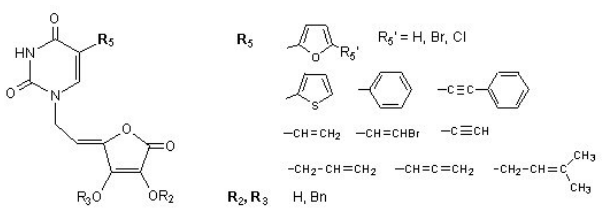
We have found in our previous studies that some pyrimidine and purine derivatives of 4,5-didehydro-5,6-dideoxy-L-ascorbic acid possess pronounced cytostatic activities against some malignant human tumor cell lines [1]. In this connection we have synthesized a series of novel C-5 pyrimidine derivatives of 2,3-di-*O*-benzyl-4,5-didehydro-5,6-dideoxy-L-ascorbic acid (Figure) by Stille reaction of the 5-iodouracil derivative of L-ascorbic acid with unsaturated stannanes.



The novel compunds were evaluated for their cytostatic activities against malignant human tumor cell lines: pancreatic carcinoma, cervical carcinoma, breast carci-

noma, laryngeal carcinoma, colon carcinoma, human T-lymphocytes, as well as human fibroblast cells. Inhibitory activity evaluations of those compounds against herpes simplex virus type 1 and 2, vaccinia virus, varicella-zoster virus, vesicular stomatitis virus, Coxsackie virus B4, respiratory syncytial virus, parainfluenza virus, reovirus-1, sindbis virus and Punta Toro virus were also performed. The results of antitumoral and antiviral evaluations will be presented.

[1] (a) T. Gazivoda *et al*, *Carbohydrate Res.* 2006 (*341*) 433; (b) T. Gazivoda *et al*, *Bioorg. Med. Chem.* 2005 (*13*) 131; (c) S. Raić-Malić *et al*, *J. Med. Chem.* 2000 (*43*) 4806; (d) S. Raić-Malić *et al, J. Med. Chem.* 1999 (*42*) 267

P110

Synthesis and biological evaluation of indolic analogues of combretastatin A4 as potential antivascular agents

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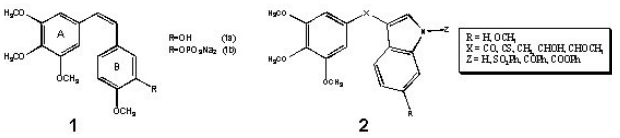
Combretastatin A-4 (CA-4) (**1a**), a stilbene derivative, is a potent vascular disrupting agent (VDA) with the structural requirement of a *cis*-configuration to maintain a molecular geometry and a correct orientation of both phenyl groups. A water soluble derivative, the combretastatin A-4 phosphate prodrug (CA-4P) (**1b**), is currently under clinical trials (phase II) [1].

The two-carbon linker of our parent compound **2** was maintained between the 3,4,5-trimethoxyphenyl ring and the phenyl part of the 3-indolyl moiety. A series of indolic analogues of CA-4 was synthesized by means of an efficient strategy. To determine the optimal distance and angle between the two rings, indolylmethane derivatives with various substituted bridges (X= CO; CS; CH_2; CHOH; $CHOCH_3$) have been prepared. Several compounds were identified as potent inhibitors of tubulin polymerization and also displayed cytotoxic activities on B16 melanoma cells at a nanomolar level. Both activities were well correlated with the ability to induce morphological changes of EA·hy 926 endothelial cells [2].

In conclusion, the *cis*-stilbene skeleton of CA-4 could conveniently be replaced by the 3-aroylindolic moiety, this latter could be considered as a mimic of CA-4 B-ring. Thus, any isomerization leading to inactive *trans* compounds is avoided.



1 2

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P111

Inhibitor Activities of Uracil-Mannich Derivatives on Mammalian Type I DNA Topoisomerase I

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DNA topoisomerases are nuclear enzymes that control and modify the topological states of DNA by catalyzing the concerted breaking and rejoining of DNA strands [1]. Topoisomerase inhibitors as a class of pharmacological agents have the potential to exhibit selective antibacterial, antifungal, antihelmintic, antiviral activity. Topoisomerase inhibitors have also anticancer activitiy. In this study, [(dimethylamino)methyl]pyrimidine-2,4(1*H,*3*H*)-dion **(A2)** and 5-(piperazinomethyl)pyrimidine-2,4(1*H,*3*H*)-dion **(A3)** compounds (Uracil-Mannich derivatives) which include potent alkylating [3] and antimetabolite [4] agents were synthesized via traditional Mannich reaction and evaluated for their mammalian type I DNA topoisomerase inhibitory activity, via *in vitro* supercoil relaxation assays. Among the compounds 5-(piperazinomethyl)pyrimidine-2,4(1H,3H)dion **(A3)** was found the most potent topoisomerase I inhibitor activity.

Key Words: *uracil, mannich bases,* alkylating agents, antimetabolite *type I DNA topoisomerase, plasmid supercoil relaxation assays,*

[1] Sun, Q., Gatto , B., Yu, C., Liu, A., Liu, F.L., LaVoie, J.E.. (1995)., *J.Med.Chem., 38: 3638-3444* .

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P112

Effects of Aldoxime-Ether Derivatives in Plasmid Supercoil Relaxation Activity of Mammalian DNA Topoisomerase I

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DNA topoisomerases have been shown to be important molecular targets for a broad range of therapeutics such as antimicrobials, antiparasitics and antitumor agents as many drugs have been shown to convert these important nuclear enzymes into DNA-breaking nucleases, resulting in efficient cell killing [Wang 196, Goldman et al, 1997]. In this study, dichloro, phtalimido substituted pyridine-aldoxime-ether derivatives; namely1-(Propyl)-3-[([(2,6-dichlorophenyl)methoxy]imino)methyl]pyridinium bromide (**1**), 1-(Propyl)-3-[([phtalimidomethoxy]imino)-methyl]pyridinium bromide (**2**)**,** 3-[([phtalimidomethoxy]-imino)methyl]pyridine (**3**) 4-[([phtalimidomethoxy]imino)-methyl]pyridinium bromide (**4**) were synthesized [Kapkova et al, 2006] and evaluated for their interference on mammalian type I DNA topoisomerase activity, via *in vitro* supercoil relaxation assays. Camptothecin, a known poision of eukaryotic topoisomerase I, was used as reference compound throughout the assays. Our results showed that the compounds, we synthesized, exhibited partial interference on the enzyme's activity with an implication of the significance of the molecular weight of the compound.

Key Words: *oxime-ether,* Williamson ether synthesis, *type I DNA topoisomerase, plasmid supercoil relaxation assays,*

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P113

Secondary Metabolites and Derivatives from Maytenus cuzcoina as potential cancer chemoprevention agents

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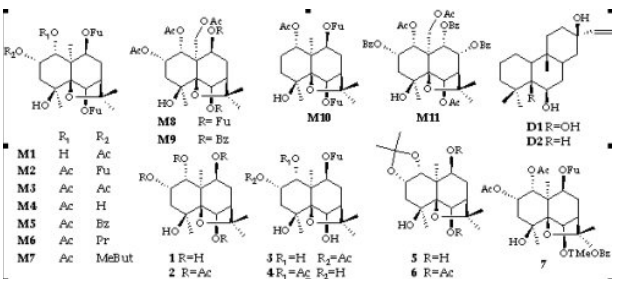
Cancer prevention is now one of the most urgent projects in public health. Inhibition of the tumor promoting stage in the multistage of carcinogenesis has been regarded as the most promising method for cancer prevention. In the search for cancer chemopreventive agents, the inhibitory effects on Epstein-Barr virus early antigen (EBV-EA) induction by the tumor promoter, 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA), have been an effective bioassay system for the primary screening of inhibitors of tumor promotion [1]. Several classes of natural products such as terpenoids, in particular diterpenes and sesquiterpenes, have previously been reported as antitumor-promoters [2].

With this background and as part of an intensive study of the bioactive metabolites from species of Celastraceae family, in this communication we report on the isolation of two new diterpenes with a rosane skeleton (D1 and D2) and two new dihidro-β-agarofuran sesquiterpenes (S1 and S11), from *Maytenus cuzcoina*, and the preparation of eight derivatives (1-8). These compounds were tested for their inhibitory effects on Epstein-Barr virus early antigen, induced by TPA in Raji Cells, and the structural-activity relationship studies were established.



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P114

Application of [3+2] Cycloadditions to the Synthesis of Novel Inhibitors ofHistone Deacetylases with marked in vitro and *in vivo* antitumoral activity

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Histone modifications are epigenetic events commonly found in human tumours. One of the most promising groups of epigenetic drugs are histone deacetylase inhibitors (HDACIs).

The design criteria are based on the electronic and steric features of the active site, the ca. 11Å channel that connects the active site and the outside, and the shallow pockets on the surface of these enzymes. The resulting candidate molecules were synthesized by means of a convergent and regiospecific procedure based on [3+2] cycloadditions between azomethine ylides and alkenes. The synthetic approach is versatile and allows the generation of molecular libraries. Moreover, the resulting compounds fulfill the Lipinski rules and have satisfactory *in silico* ADME features.

These compounds induced a significant increase in acetylation state of both histones H3 and H4 in a dose-dependent manner. These compounds showed a potent citotoxic effect in vitro with IC50 values very similar to obtained with other known HDACIs. These results were related with the induction of apoptosis and cell cycle arrest. Moreover, these compounds inhibited the growth of *in vivo* tumour xenografts in nude mice models, with no apparent toxicity. Docking simulations have been carried out in order to understand the origins of the biological activities observed.

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Cardiovascular Drugs

P115

Design, synthesis, physicochemical and biological evaluation of new antiatherogenic agents

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Atherosclerosis and related complications, heart attack, stroke and peripheral vascular diseases represent the prevalent cause of morbidity and mortality in industrialized countries. Atherogenesis is actually considered as a chronic inflammatory disease with potentially acute complications such as plaque rupture and thrombosis, that involves a complex sequence of events associating endothelial dysfunction, transendothelial migration of mononuclear cells, lipid accumulation, smooth muscle cell proliferation, leading finally to atheroma plaque formation.

Antioxidants suppress formation of active oxygen species by reducing hydroperoxides and hydrogen peroxide, by sequestering metal ions, scavenging active free radicals and/or clearing damage. A large number of antioxidants are phenolic compounds.

Our aim is to develop a class of new molecules **sharing double properties,** antioxidant (able to inhibit oxidative stress, LDL oxidation by vascular cells and their proatherogenic consequences), and carbonyl scavenger (able to scavenge carbonyl compounds, thus protein dysfunction induced by derivatization, and their physiopathological consequences).

We have identified best hits (patent) concerning monomers of cinnamic acid derivatives and their phosphorylated analogues. New compounds based on the dimeric forms of cinnamic acids (and phosphorylated analogues) have been elaborated. Reaction pathways leading to homodimers or heterodimers have been investigated and compounds elaborated. New hydrazone derivatives were synthesized

Compounds synthesized are studied chemically as antioxidants (DPPH scavengers) and their biological activity evaluated through *in vitro* testing.

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P116

Discovering new scaffolds to build ACAT inhibitors

A. Gelain*, N. D'Onofrio*, D. Barlocco*, B. Kwon**, T. Jeong**, K. Im**, L. Legnani***, L. Toma***

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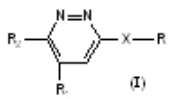
Acyl-CoA:cholesterol acyl transferase (ACAT) is present in the endoplasmatic reticulum of a variety of cells in two isoforms, namely ACAT-1 and ACAT-2. By esterification of cholesterol with activated fatty acids, ACAT regulates both cholesterol absorption and low density lipoprotein (LDL) assembly. ACAT inhibitors have been

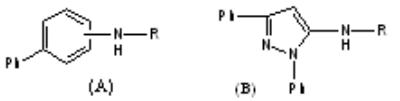
regarded as possible antiatherosclerotic agents and suggested as potentially useful in the treatment of Alzheimer's disease [1].

In our previous studies [2,3] on pyridazine derivatives of general formula (I), we identified several new ACAT inhibitors. To investigate the importance of the pyridazine ring as a pharmacophoric moiety, we have now synthesized two new series of compounds, in which the heterocycle was replaced by a phenyl (A) or a pyrazole moiety (B), respectively. The data concerning their inhibitory activity and selectivity towards hACAT-1 and hACAT-2, as well as the results of molecular modeling studies will be reported in this presentation.



(I)

R_1, R_2 = H, Ph, aryl
R= alkyl, aryl, substituted-ureidyl-alkyl
X= S, SO, SO_2, NH, O



(A) (B)

R = alkyl, acyl

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P117

Synthesis of 4-aryl-2-methyl-5-oxo-1, 4, 5, 6, 7, 8-hexahydro quinoline 3-carboxylate derivatives

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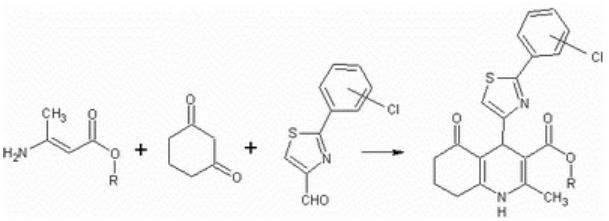
Keywords : Synthesis, Calcium channels, 1,4-dihydropyridine

4-Aryl-1, 4-dihydropyridine-3, 5-dicarboxylic diesters have shown to be indispensable for the treatment of cardiovascular diseases. Some of these compounds are potent calcium channel blockers, and some others have agonistic activity on calcium channels. A new series of this type were designed and synthesized with agonistic activity on calcium channels for the treatment of congestive heart failure.

They may also be useful in the case of resistance to chemotherapeutic cancer treatment (Multiple Drug Resistance). Condensation of 1 with 2 and 3 in ethanol yielded several derivatives of 4. Yields were between 40% - 92%. The pharmaceutical effects are under evaluation.



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P118

3,4-Diamino Piperidines as Factor Xa Inhibitors

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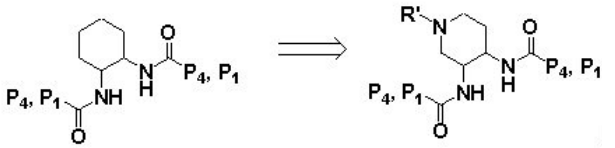
Blood coagulation factor Xa (fXa) is a trypsin-like serine protease which cleaves prothrombin to thrombin leading to blood clot formation within the blood coagulation cascade. Inhibitors of fXa exert an anticoagulant effect by directly decreasing the generation of thrombin. Because of its important role in blood coagulation, fXa has emerged as an important target for development of new antithrombotic agents. We have been interested in the development of novel, orally bioavailable fXa inhibitors and have described several chemotypes with a variety of different P1 moieties, P4 moieties and central templates. In this presentation, we would like to report our findings on the synthesis and SAR of a series of 3, 4-diaminopiperidines. The use of a piperidine cycle as the central template for the presentation of the side chains into the S1 and S4 pockets was shown to be satisfactory. In addition, the presence of the nitrogen in the central core allowed structural modifications to fine tune the physical-chemical properties of the molecules. Various P1 and P4 groups were tolerated at both the 3- and 4-position of the piperidine ring. However, compounds with the P1 moeity at the 3-position were more active than the corresponding analogues with P1 at the 4-posi-

tion with the most potent compounds having subnanomolar factor Xa inhibitory activity.



P119

Synthesis and Anticoagulant Actions of Sulphated Flavonoids

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Anticoagulant and antithrombotic activities are among the most widely studied properties of sulphated macromolecules. Considering some problems associated to clinical use of heparin and derivatives which include risk of bleeding and short intravenous half-life [1], an emerging field of research is the development of sulphated esters of flavonoids [2,3].

Diosmin (**1**) and hesperidin (**2**) are well-known venotropic agents [4]. In order to associate an anticoagulant action sulphated derivatives of **1** and **2** were prepared. To establish structure-activity relationships the respectively aglycons diosmetin (**3**) and hesperitin (**4**) were also submitted to sulphatation (Fig. 1).

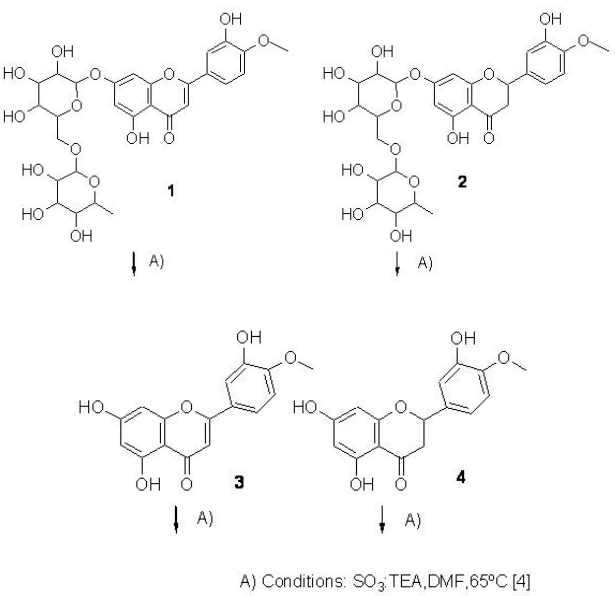

Fig. 1. Flavonoids submitted to sulphatation.

The four sulphated flavonoids obtained, diosmin and hesperidin 2'',2''',3'',3''',4'',4'''-*O*-hexasulphates and diosmetin and hesperidin ,7-*O*-dissulphates were characterized by IV, ^{1}H and NMR, COSY, HSQC, HMBC and EM-FAB.

In the present work, we report that the sulphated derivatives of diosmin (**1**) and hesperidin (**2**) showed an important *in vitro* anticoagulant action, evidenced by the increase of APTT and PT times, similar to the known anticoagulant hirudin.

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Acknowledgments: FCT (I&D 226/94), FEDER, POCTI for financial support

P120

Selective Inhibitors of CYP11B2 for the Treatment of Congestive Heart Failure

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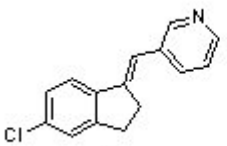
Recently we proposed inhibition of aldosterone synthase (CYP11B2) as a novel strategy for the treatment of congestive heart failure and myocardial fibrosis. Aldosterone synthase is the key enzyme of mineralocorticoid biosynthesis. Two recent studies (Rales and Ephesus) showed that aldosterone antagonists (spironolactone, eplerenone) reduce mortality in heart failure patients and in patients after myocardial infarction. But as a correlation between the use of aldosterone antagonists and hyperkalemia associated mortality was observed, the blockade of aldosterone formation by non-steroidal inhibitors of CYP11B2 is to be preferred. We expect these to be as effective as the aldosterone antagonists but to have less side effects on the endocrine system than steroidal compounds.

Because of the high similarity of CYP11B2 with CYP11B1 (93% sequence homology), which must not be effected, the development of selective inhibitors of CYP11B2 is extremely challenging. Molecular modelling approaches and rational drug design strategies led to the development of two different classes of highly selective and potent non-steroidal inhibitors of CYP11B2.



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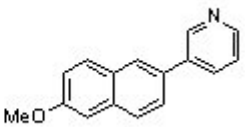

II

Compounds **I** and **II** show IC_{50} values of 26 nM and 6 nM and selectivity factors in respect of CYP11B1 of 57 and 263. The biological activity of these compounds regarding inhibition of different other human CYP enzymes like CYP17, CYP19 and hepatic CYPs underlines their high selectivity. Pharmacokinetic data and examinations for peroral absorption using Caco-2 monolayers are very promising. Both examples show $t_{1/2}$ values from 4 to 5 h and are highly bioavailable.

P121

Benzyl ethers, a new class of thyromimetics

E. Koch, A. Garcia-Collazo, A. Cheng, E. Kallin, J. Hallberg, J. Löfstedt, M. Rahimi-Gahdim, N. Garg, T. Eriksson, M. Bengtsson, A. BäCkbro Saeidi, M. FäRnegåRdh, T. Hansson, J. Malm

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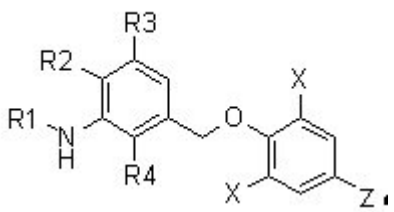
Hyperlipidemia is a predisposing risk factor for development of coronary heart disease that is the leading cause of morbidity and mortality in the developed world. Endogenous thyroid hormones are potent lipid lowering agents [1] but cannot be used therapeutically in patients with hyperlipidemia, mainly due to cardiovascular toxicity. There exist two subtypes of thyroid hormone receptors (TR), α and β, unequally distributed in the body. As TRα is most abundant in the heart and most effects of thyroid hormones on the heart are mediated through TRα, a reasonable strategy is the development of TR agonists that either are tissue selective or that interact selectively with TRβ [2].

We have prepared a new class of thyroidmimetics, a series of benzyl ethers. The results of a reporter cell assay employing CHOK1-cells (Chinese Hamster Ovary cells) stably transfected with hThRα 1 or hThRβ1 and an alkaline phosphate reporter-gene downstream of a thyroid response element (TRAFα and TRAFβ) reveals that the ligands from the series ranges from partial antagonists to full agonists. The SAR as well as some structural findings will be discussed.



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P122

Discovery of KR-33028, a novel Na+/H+ exchanger isoform-1(NHE-1) inhibitor as a cardioprotective agent

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Since the excessive activation of Na+/H+ exchanger isoform-1 (NHE-1) has been known to play an important role in the progression of ischemia/reperfusion injury, many efforts have been devoted to develop a potent and selective NHE-1 inhibitor as cardioprotective drug. From our efforts to find a novel NHE-1 inhibitor based on bicyclic template, we found that (benzo[b]thiophene-2-carbonyl)guanidines with 4-substituent including halogen, nitrile, nitro, alkyl, and aryl groups showed good NHE-1 inhibitory activity which was well translated into the cardioprotective efficacy. In isolated rat ischemic heart model, the 4-cyano compound (KR-33028) significantly improved the recovery of cardiac contractile function(63% LVDP), diminished the contracture(20 mmHg LVEDP), and reduced the damage of myocyte(13 IU/g LDH), compared with the vehicle group(13% LVDP, 55 mmHg LVEDP, and 28 IU/g LDH). In addition, KR-33028 excellently limited the infarct size in the in vivo myocardial infarction rat(38% IS/AAR vs 59% of vehicle) and beagle dog model. Furthermore, KR-33028 showed a good pharmacokinetic and safety profile. This study suggests the possibility that KR-33028 can be developed as a cardioprotective agent against ischemia/reperfusion injury.

P123

Blocked dihydropterins: potential drugs for cardiovascular disease

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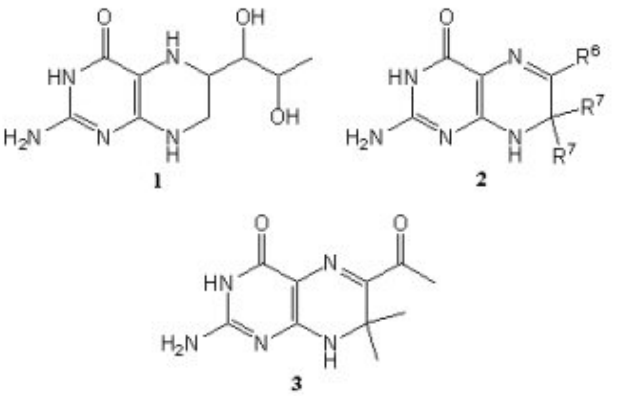
Several disease states have been identified in which there is a deficiency of the naturally occurring cofactor for NOS, tetrahydrobiopterin (**1**) [1,2]. There is evidence that supplementation with **1** has clinical efficacy, but its high polarity makes it unsuitable for oral administration. Moreover, tetrahydro compounds such as **1** have limited stability to oxidation. 7,7-Dialkyl-7,8-dihydropterins with a variety of C6 substituents (**2**) are attractive alternatives to **1** because the lipophilicity can be modulated by choice of both C7 and C6 substituents [3]. Since the crystal structures for all three NOS isoforms are known, the possibility also exists for achieving isoform selectivity. In the case of disease targets such as pulmonary hypertension, diabetes and atherosclerosis, the target enzyme is endothelial nitric oxide synthase (eNOS). By screening a library of compounds of general structure **2** synthesised at Strathclyde, we have showed that 6-acetyl-7,7-dimethyl-7,8-dihydropterin (**3**) is capable of acting as a stimulator of nitric oxide production in cells and tissues, where there is a deficiency of tetrahydrobiopterin, and *in vivo* in mice in a model of pulmonary hypertension. Data will be presented and possible mechanisms of action discussed.



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P124

Direct thrombin inhibitors built on the azaphenylalanine scaffold

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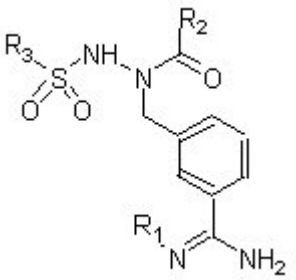
The central role in coagulation processes makes thrombin an important target for therapeutic agents designed for thrombus prevention. In earlier studies we identified a novel series of noncovalent, low molecular weight thrombin inhibitors built on the conformationally restricted azaphenylalanine scaffold that incorporate a benzamidine or weakly basic benzamidoxime element at the P1 position.[1,2]



Benzamidine based compounds and their benzamidoxime analogs, for which we propose *in vivo* reduction, were evaluated as inhibitors of three serine proteases in the standard chromogenic assay. We have studied the antithrombotic potential of compounds in two models of venous thrombosis in rat.[3]

[1] Zega A, Mlinšek G, Šepic P, Golič-Grdadolnik S, Šolmajer T, Tschopp T, Steiner B Kikelj D, Urleb U. Design and structure-activity relationship of thrombin inhibitors with an azaphenylalanine scaffold: potency and selectivity enhancements via P2 optimization. Bioorg Med Chem 2001; 9: 2745-2755.

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P125

Novel class of antithrombotic compounds with dual function possessing thrombin inhibitory and fibrinogen receptor antagonistic activities

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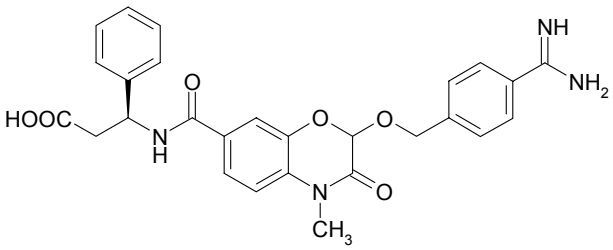
Rational design of ligands that act on specific multiple targets is being increasingly recognized as a challenging

approach in medicinal chemistry [1]. Recently, we have discovered 1,4-benzoxazin-3(4H)-one derivative 1 possessing both thrombin inhibitory and fibrinogen receptor antagonistic activities [2]. Combination of anticoagulant and antiaggregatory activity in the same molecular entity is a promising approach in the search for novel antithrombotic agents.



1

Optimization of the lead compound 1 was performed in the benzoxazinone core as well as in P1 and P3 parts of the molecule in order to achieve better affinity for binding to fibrinogen receptor and more potent thrombin inhibition. The results of this strategy directed towards improved antithrombotic compounds with dual action will be presented.

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P126

1-[3-(4-Aryl-piperazin-1-yl)-2-hydroxy]-3,3-diphenyl-pyrrolidin-2-one derivatives as potential alpha 1 adrenoceptor antagonist

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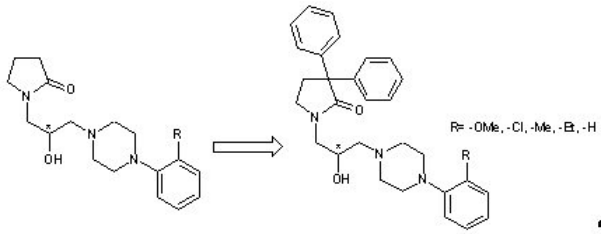
*Department of Physicochemical Drug Analysis, Faculty of Pharmacy Jagiellonian University Medical College. **Department of Pharmacodynamics, Faculty of Pharmacy, Jagiellonian University Medical College, Kraków, Poland

The α1 adrenergic receptors (α1-ARs) belong to a family of G-protein coupled seven-transmembrane helix receptors, which are mainly involved in the cardiovascular and central nervous system. Ligands acting as antagonist at the α1-ARs subtypes have been used in the treatment of a variety of diseases including hypertension, and prostatic hypertrophy by relaxation of vascular smooth muscles containing a high concentration of α1-AR [1]. However, α1-adrenoceptors antagonists belong to a various chemical groups, the arylpiperazines represent the most studied one.



We have previously reported that various 1-[3-(4-arylpiperazin-1-yl)-2-hydroxy]-propyl-pyrrolidin-2-one derivatives are a1-AR antagonist (pKi a1 6.14 – 7.13) and posses strong antihypertensive and antiarrhythmic activities [2]. To improve the α1-AR antagonistic activity displayed by these arylpiperazine compounds, it was decided to modify their structure by introduction of two phenyl rings into 3rd position of pyrrolidin-2-one. Taking into consideration that at the 2nd position of propyl chain is an asymmetric carbon atom affords to synthesized enantiomers of the title compounds were undertaken. The obtained compounds were tested in vitro for their α1- and α2- AR binding affinity.

The study was supported by the Ministry of Science and Education grant 2P05F 024 29

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P127

Synthesis and pharmacological profile of benzanilide derivatives

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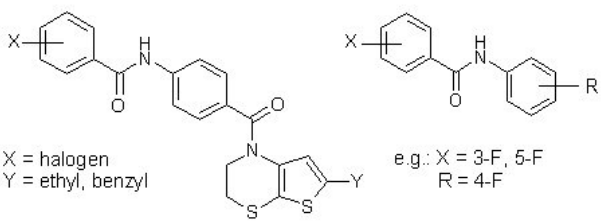
On the basis of a previous publication [1] we synthesized new benzanilides by simplification of nonpeptide vasopressin antagonists with the new scaffold 2,3-dihydrothieno[2,3-b][1,4]thiazine substituted with various 4-(benzoylamino)benzoyl moieties.



The effect of compound 1, 2 and 3 in the concentrations between 1 and 100 µmol/l on smooth muscle preparations (aorta, arteria pulmonalis and terminal ileum) of the guinea pig was investigated. Compound 1 (100 µmol/l) caused a decrease of contraction force (fc) of aortic rings of 37,9 ± 3,7 % (n=6) precontracted by 90 mmol/l KCl, compound 2 (100 µmol/l) had no effect on aortic rings (-3,4 ± 4,2 %, n=8) and compound 3 (100 µmol/l) reduced fc of aortic rings to 49,1 ± 6,0 % (n=6). Similar experiments as on aortic rings were carried out using arteria pulmonalis rings. Compound 1 (100 µmol/l) showed a relaxing effect of 21,3 ± 4,1 % (n=7), compound 2 (100 µmol/l) of 19,3 ± 4,9 % (n=8) and compound 3 (100 µmol/l) of 18,3 ± 6,0 % (n=6). The relaxing effect of the three compounds was also studied on terminal ilea. Compound 1 (100 µmol/l) reduced fc to 91,0 ± 5,2 % (n=4), compound 2 (100 µmol/l) to 34,4 ± 1,2 % (n=5) and compound 3 (100 µmol/l) to 70,5 ± 3,8 % (n=5). A new method for measuring the endothelial nitric oxide release was used to elucidate the mechanism of action. Further derivatives showed even more potent effects.

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P128

The quantitative determination of valsartan in human plasma by HPLC/FLD system

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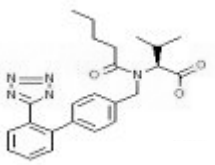
Valsartan is a nonpeptide, orally active antihypertensive, as a specific angiotensin II (A II) antagonist acting on the AT1 reseptor subtype. It is N-(1-oxopentyl)-N-[[2'-(1H-tetrazol-5-yl) [1,1'-biphenyl]-4-yl]methyl]-L-valine with an empirical formula C24H29N5O3 and a molecular weight of 435,5 [1].



Valsartan

The analytical method which is developed originally and validated in our laboratory, was used to evaluate the bioequivalency of two different brand name valsartan products.

After collecting the blood samples from 24 healthy male volunteers according to clinical trial protocol, the plasmas were separated and the concentration of valsartan were determined using HPLC/FLD system after liquid/liquid extraction.

Results of analysis showed that the retention time of valsartan and candesartan (internal standard) was 9.8 and 6.1 minutes respectively. Absolute and relative recovery was 78.31% and 91.70% respectively. The calibration ranges were 50 – 15000 ng/mL; LOQ was 50 ng/mL. After five days validation process, coefficient correlation was 0.99949 – 0.99987. In quality control samples, with-in-batch and batch-to batch accuracy ranges were 95.26 – 111.46% and 98.38 – 107.96% respectively; precision ranges were 0.90 – 5.35% and 2.36 – 6.92% respectively. In calibration standard samples, batch-to batch accuracy ranges were 90.52 – 106.39%; batch-to batch precision ranges were 1.75 – 5.14%.

Our whole study was conducted according to FDA regulations about bioanalytical method validation process [2].

In summary, our data indicate that our analytical method for plasma valsartan determination is simple, rapid and sensitive for bioequivalence studies.

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Chemical Genomics

P129

Identification of peptides in proteomics supported by predictions of HPLC retention by means of quantitative structure-retention relationships

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To quantitatively characterize the structure of a peptide and to predict its gradient retention time at given HPLC conditions three structural descriptors were proponed [1,2]: i) logarithm of the sum of retention times of the amino acids composing the peptide, ii) logarithm of the Van der Waals volume of the peptide, and iii) the logarithm of the peptide's calculated n-octanol-water partition coefficient. The first descriptor was obtained from empirical data for 20 natural amino acids, determined in a given HPLC system. Two other descriptors were calculated from the peptides' structural formulas using molecular modeling methods. A structurally diversified series of 98 peptides was employed and it was proved that predicted gradient retention times on several chromatographic systems were in a good agreement with the experimental data. The QSRR equations, derived for given systems operated at variable HPLC conditions allowed predicting peptide retention in that system. It was suggested that

matching the experimental HPLC retention to the theoretically predicted one for a presumed peptide, improvement of the original protein identification in proteomics could be obtained. In conjunction with MS data, prediction of the retention time for a given peptide can be used to improve the confidence of peptide identifications and to increase the number of correctly identified peptides.

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[2] Bączek T, Wiczling P, Marsza[33] MP, Vander Heyden Y, Kaliszan R, Prediction of peptides retention at different HPLC conditions from multiple linear regression models. J. Proteome Res. 2005;4:555-563.

P130

Selective Inhibitors of Human Lipid Kinase

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UCSF, USA

Our laboratory focuses on the development of novel chemically based tools to decipher signal transduction pathways on a genome-wide scale. We have developed a method for producing small molecules that are specific for any protein kinase of interest in a signaling cascade by combining protein design with chemical synthesis. These highly specific inhibitors of individual kinases have revealed a number of new principles of signal transduction that have complemented genetic and biochemical studies of cell signaling. Examples where new pathways and new functions can be revealed by small molecule inhibitors of protein kinases will be highlighted. A second area of interest in our laboratory is the tracing of direct kinase substrates. We have designed and synthesized unnatural ATP analogs which are substrates of our engineered kinases but are poorly accepted as substrates of wild-type kinases. This specific nucleotide substrate of any kinase of interest allows for the radiolabelling of the direct substrates of a wide variety of protein kinases including both serine/threonine and tyrosine kinases. New methods for the isolation and identification of low abundance substrates of kinases from cells will be discussed. Once a phosphoprotein substrate of a kinase is identified, the specific phosphorylation site is often difficult to identify using traditional tryptic peptide phosphorylation site mapping. Using a novel strategy based on the design of tailor made proteases which specifically cleave proteins after sites of phosphorylation, we have developed a rapid means to map protein phosphorylation patterns. Finally, a potential link between the unnatural ligands of engineered kinases and a set of plant hormones, the cytokinins, will be discussed in the context of a custom designed database created for the genome wide analysis of protein kinase catalytic domains.

Computer Assisted Drug Discovery

P131

Combining approach of 3D QSAR and docking studies to homology models of benzodiazepines binding sites of GABAA receptors: application to selectivity

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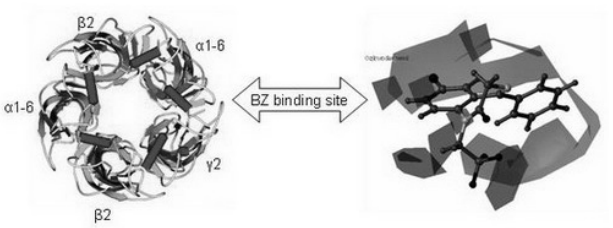
$GABA_A$ receptors present major inhibitor receptors in CNS. There are two main binding sites in these receptors: GABA binding site and binding site for benzodiazepines (BZ), major antidepressant and hypnotic agents for almost 40 years. Research in BZ space reveals several subtypes of BZ binding site (BZBS) depending on α and γ subunits of $GABA_A$ receptor and showing different pharmacological profile. Currently we can distinguish $\alpha1\beta2\gamma2$, $\alpha2/3\beta2\gamma2$, $\alpha5\beta2\gamma2$, $\alpha4/6\beta2\gamma2$ BZBS subtypes of $GABA_A$ receptor. Present work combines 3D QSAR (CoMFA, CoMSIA) and docking studies with homology models of subtypes of BZBS to reveal selectivity modes of action.

We have built homology models of $GABA_A$ receptors with composition $\alpha1\text{-}6\beta2\gamma2$ (varying only α subunit), all available mutational data were taken into account. Most active BZ-like compounds were docked into the models. 3D QSAR of BZ-like ligands of various selectivity profile were build displaying good to excellent statistical parameters (q^2=0.65-0.9). CoMFA and CoMSIA maps gave additional factor for homology model improvement in case of binding site uncertainties. The obtained 3D QSAR maps were joined with ligand-receptor complex and have shown excellent correlation in a mutual way. On the basis of above research modifications to existing ligands and new selective ligands were proposed.



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P132

Active Site Pressurization: A New Tool in the Structure-Based Design of Selective Cyclin-Dependent Kinase Inhibitors

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Cyclin-dependent kinases (CDKs) are essential in the intracellular control of the cell division cycle, apoptosis, multiple functions in the nervous system, transcription and differentiation. CDKs and their regulators are often deregulated in various diseases and pharmacological inhibitors of CDKs are currently being evaluated for therapeutic use against cancer, neurodegenerative disorders (e.g. Alzheimer's disease, amylotrophic lateral sclerosis and stroke) amongst many other diseases. The actual selectivity of most known CDK inhibitors, and thus the underlying mechanism of their cellular effects, is poorly understood.

All the small molecule CDK inhibitors described to date target the highly homologous ATP-binding pocket of the catalytic site of the kinase. Current structure-based design methodologies do not adequately take into account protein flexibility, instead often relying on static crystal structures. We present an application of a new methodology, Active Site Pressurization (ASP) which is used to examine the intrinsic flexibility of the ATP-binding pocket. ASP is a molecular dynamics based method in which Lennard-Jones particles are "pumped" into the recognition site, causing the protein to deform in an unbiased and energetically most favourable manner. The enlarged cavity indicates how structurally diverse ligands could potentially interact with in recognition site in a way that static crystal structure can not reveal. In this poster the effects of induced protein conformational change resulting from ASP on various CDKs will be examined.

P133

Synthesis and in silico study of 4-(aryl methyl -5-imidazolyl)-1,4-dihydro pyridines as calcium channel blockers

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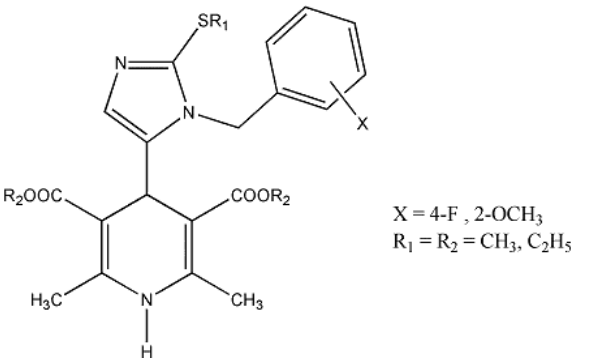
Department of Medicinal Chemistry, Pharmacy Faculty, Mashaad University of Medical Sciences, P. O. Box 91775-1365, Mashhad, Iran

The influx of extracellular Ca^{2+} through L-type potential dependent calcium channels is responsible for regulation of many physiological functions, including smooth and cardiac muscle contractions. The discovery that 1,4-dihydropiridine class of calcium channel antagonists inhibits this calcium influx ,represented a major therapeutic advance in the treatment of cardiovascular diseases. The dihydropydidine class of compounds, of which nifedipine is the prototype, has been the subject of many structure and activity relationship studies[1]. Also recently a model for interaction of nifedipine with calcium channel has ben developed [2]. In this project the o-nitrophenyl group at position 4 of of nifedipne was replaced with 2-alkylthio -1-arylmethyl imidazolyl substitute. Interaction of novel dihydropyridines with the model was studied using AutoDock 3.0



Comparison of docking energies and Ki values showed that all compounds should be effective and compound **5f** with k_i = 1.69 x 10^{-6}M and Dock energy = -12.95 kcal/mol should be the most active one. For nifedipine Ki and Dock energy was found to be 6.76 x 10^{-5}M and -8.49 kcal/mol respectively.

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P134

Molecular Informatics as an Enabling In Silico Technology Platform for Drug Discovery

J. Edgar

Novartis Institutes for BioMedical Research

The molecular informatics platform, as implemented today in the Molecular and Library Informatics (MLI) Technology Program at the Novartis Institutes for BioMedical Research (NIBR) Discovery Technologies, will be presented. The mission of the MLI program is primarily defined to contribute to the selection of screening hit and lead compounds using *in silico* methods. The MLI technology program aims to provide an integrated pipeline of computational methods for high-throughput *in silico* screening combining specific cheminformatics, bioinformatics, docking and 3D pharmacophore applications.

The four core activities of the group include: 1) Molecular diversity management; 2*) In silico* screening using HTD (High-throughput Docking) and 3D pharmacophore searching; 3) Integrated analysis of HTS (High-throughput Screening) and profiling data; and 4) Database management and software engineering in the field of *in silico* screening. The contribution of these activities to the drug discovery process will be summarized together with novel trends in the field.

P135

Developement of a docking strategy for the generation of kinase-focused libraries

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Cerep, 19 avenue du Québec, Villebon sur Yvette, 91951 Courtaboeuf cedex, FRANCE

Kinase enzymes play a crucial role in signal transduction and cellular proliferation and differentiation, and much effort is being done to generate chemical libraries of kinase ligands for high throughout screening, preferably on structure-based computational approaches[1].

We present here a docking and scoring strategy for the synthesis of kinase-focused libraries. To develop this approach, we have performed a systematic docking of two libraries in kinase crystal structures. The libraries tested are BioPrint (a "drug-like" database including more than 2500 drugs or drug related compounds) and a kinase-oriented library of 1440 compounds based around original pyrimidine scaffolds. We have also docked 123 well known kinase ligands described in publications and patents.

Kinase crystal 3D structures corresponding to ABL, EGFR and CDK2 were obtained from the Protein Data Bank. During docking, the protein remains rigid while the ligand is flexible allowing different conformations to be docked. Six scoring functions implemented in LigandFit have been calculated for each pose of each compound. The cut-offs were chosen according to the scores of the known inhibitors.

This strategy has been validated using the scoring of the known kinase ligands compared with BioPrint scoring, the kinase ligands being among the 5-7% best ranked compounds. When predicting the kinase activity of the compounds in the two libraries, approximately 86% of compounds from the kinase library passed the scoring filters for at least one kinase (for only 13% of compounds of BioPrint). Moreover, 8% of compounds from the kinase library passed the filters of the three kinases (for 0.6% of the BioPrint compounds), thus allowing to identify promiscuous scaffolds for kinase activity.

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P136

Comparative and pharmacophore model for deacetylase SIRT1

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Sirtuins are NAD-dependent histone deacetylases, which cleave the acetyl-group from acetylated proteins, such as histones but also the acetyl groups from several transcription factors and in this way can change their activities [1]. Of all seven mammalian SIRTs, the human sirtuin SIRT1 has been the most extensively studied [2].

We have built up a three-dimensional comparison model of the SIRT1 protein catalytic core (domain area from residues 244 to 498 of the full length SIRT1) in order to assist in the investigation of active site-ligand interactions and in the design of novel SIRT1 inhibitors [3]. In this study we also propose the binding-mode of recently reported set of indole-based inhibitors of SIRT1 [4]. The site of interaction and the ligand conformation were predicted by the use of molecular docking techniques. To distinguish between active and inactive compounds, a post docking filter based on H-bond network was constructed. Docking results were used to investigate the pharmacophore and to identify a filter for database mining.

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P137

Can adrenomedullin positive modulators act as matrix metalloproteinase-2 inhibitors?

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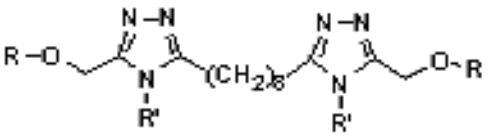
Adrenomedullin (AM) is a peptide hormone that plays a critical role in several diseases such as diabetes, hyper-

tension and cancer. Thus, it constitutes a novel and promising target for the design of new drugs.[1],[2]

A HTS carried out at NCI, detected some AM positive modulators (**1** and structurally related compounds) that showed an interesting hypotensive activity, [3] and were recently subjected to 3D-QSAR studies. [4]

These compounds are able to bind AM and increase the production of AMPc resulting from the interaction of AM with its receptor (AMR) by a mechanism not described so far, but these compounds alone can not interact with AMR. Furthermore, there is no direct correlation between affinity and production of the second messenger.[3]



1: R = phenyl; R' = phenylamino

These experimental evidences lead us to propose that these compounds may be increasing AM biodisponibility. It has also been shown that AM is specifically degraded by matrix metalloproteinase type 2 (MMP-2).5 Therefore, we have postulated that positive modulators could act as inhibitors of MMP-2.

A theoretical study carried out with computer aidded drug design techniques demonstrates that, from a structural point of view, all these compounds are able to bind MMP-2 active site and form stable complexes. These theoretical studies will be followed by experimental evaluation of the capacity of MMP-2 to digest gelatin gel in presence and absence of these compounds, following a protocol recently reported by one of us. [5]

Compound **1** bound to MMP2's active site.

The finding of this family of unexplored therapeutical agents could lead to new drugs useful for the treatment of a wide range of diseases such as hypertension and cancer.

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P138

Genotoxic activity and QSARs Studies on Some Benzoxazoles and Benzimidazoles

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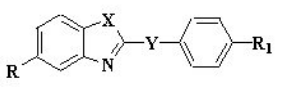
Genotoxic drugs are chemotherapy agents that affect nucleic acids and alter their function. These drugs may directly bind to DNA or they may indirectly lead to DNA damage by affecting enzymes involved in DNA replication [1]. The genotoxic chemotherapy drugs affect both normal and cancerous cells so that genotoxicity of these drugs belongs to their most serious side effects due to the possibility of inducing secondary malignancies. Although genotoxicity is a useful term whose precise definition is elusive and DNA damage plays an important role in most mechanisms underlying genotoxicity [2].

The *Bacillus subtilis* rec-assay has been specially developed to detect genotoxicity in chemicals having the rational based on the relative difference of survival of a DNA repair combination proficient strains and its deficient strain, which is interpreted as genotoxicity [3].

In this study, the DNA-damaging capacity of previously synthesized microbiologically active various benzoxazoles and benzimidazoles [4-7] given in Figure 1 were tested by using *B. subtilis* rec-assay to detect their genotoxic activities. Additionally, quantitative structure-activity relationships (QSARs) analyses was performed in order to determine the lead optimization by using the Hansch analysis method. The analysis was carried out on 22 analogues of which 16 were used in the training set and the rest considered for the test set. Physicochemical and indicator parameters were used in QSAR studies and the activity contributions for the ring system or substituent effects were determined from the correlation equation described by the results obtained from the computer assisted step-wise regression procedure.



X = O, NH
Y = —, CH_2, C_2H_4, CH_2O
R = H, Cl, CH_3, NO_2, NH_2
R_1 = H, Cl, Br, F, CH_3, NO_2, NH_2, $NHCH_3$

The best equation obtained from the QSAR analysis is given below;

log1/C = -0.22 (±0.07)Σ_{LUMO} -0.25 (±0.11)I_{YCH2} -0.21 (±0.19)σ_R +0.19 (±0.08)log P +2.17 (±0.23)

n=16, R^2= 0.927, s= 0.074, *F*= 16.737, Q^2= 0.733, s-PRESS= 0.102

The QSAR results reveal that a benzyl group on the second position of fused ring system and the energy of LUMO decreased the genotoxic activity while logP increased the activity. Besides, attaching an electrondonating group on position R enchanced the genotoxic activity.

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P139

Model of 15-hydroxyprostaglandin dehydrogenase (15-PGDH) enzyme, docking, and optimal chemical structure in thiazolidine-dione series for 15-PGDH inhibitors

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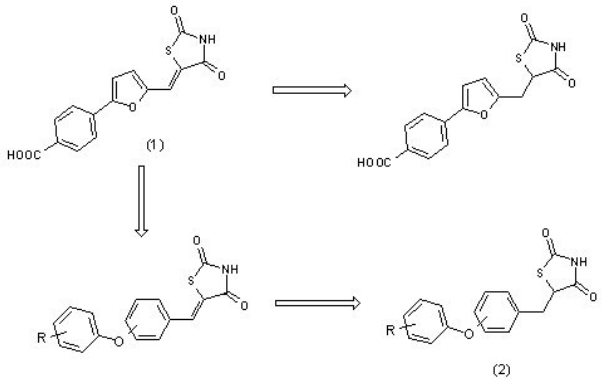
The important role of prostaglandins in hair growth in mammalian species has been emphasized by several studies. PGF2α was shown to stimulate human eyelash and murine hair growth [1] and Viprostol, a PGE_2 analog, was reported to stimulate human hair growth [2]. Prostaglandins are physiologically oxidized by the enzyme 15-hydroxyprostaglandin dehydrogenase (15-PGDH) to a ketone derivative which is devoid of activity on Prostanoid receptors (either EP or FP). In order to improve the natural prostaglandin life-time and thus its concentration in body tissue, we focussed on 15-hydroxyprostaglandin dehydrogenase inhibitors [3].

We identified and synthesized the thiazolidine dione (1) [4] as a new 15-PGDH inhibitor based on virtual screening via a pharmacophoric approach. The present study aimed to build a model of the 15-hydroxyprostaglandin dehydrogenase to design new inhibitors. Indeed, chemical alterations were made in order to reach optimal physico-chemical properties of this series [5]. A new class of thiazolidine dione derivatives (2) was synthesized via an aromatic nucleophilic substitution, a Knoevenagel condensation and a reduction successively. Molecular modelling, chemical synthesis and biological evaluation will be presented.



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P140

Molecular Dynamics Free Energy Perturbation (FEP) Calculations of R-, S- Rolipram Affinities for the PDE4B Enzyme

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Selective inhibitors of the PDE4 form the largest group of the inhibitors for any PDE family and have been studied as anti-inflammatory drugs targeting asthma and chronic obstructive pulmonary disease. In this work we have investigated quantitatively the inhibition of PDE4B by the R- and S- Rolipram configurations using the molecular dynamics Free Energy Perturbation (FEP) method. Relative affinities of R- and S- Rolipram have been calculated considering the receptor with two cations (MG^{+2}, and ZN^{+2}) in the binding site, (holoenzyme), with only 1 cation (ZN^{+2}) and in the lack of them (apoenzyme). Experimental results from biochemical studies are controversial and theorethical results are valuable to shed some light in order to resolve this issue. In all the studied situa-

tions, the R-Rolipram presented observable differences in binding affinities than the S- configuration, ranging from 15-fold to 3-fold for the holo and apo enzymes, respectively. The affinity for the enzyme containing only the ZN^{+2} ion is twice the value for the apoenzyme. However, analysis of the separation between the cations suggests that a hydroxyl group OH-, helps in maintaining the stability within the binding site and the ions separation distance is more in accordance with crystallographic data. Considering this simulation condition the ratio between the equilibrium constants between the R- and S- configurations was 34, favoring the former one.

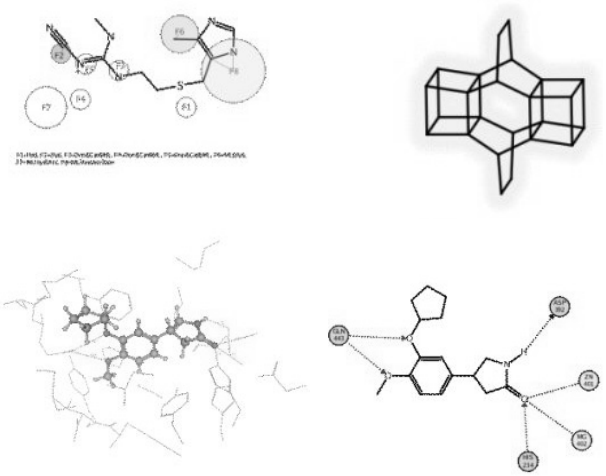
P141

An Algorithm for 2D Depiction

A. Clark, W. Altenhofen

CCG

MOE includes a comprehensive algorithm for depiction of 2D coordinates of chemical structures. The approach makes use of sampling of discrete aesthetic layout constraints in order to produce publication-quality diagrams with hitherto unachieved reliability and robustness. The algorithm is discussed and application examples are given for small molecules and protein-ligand interactions.



1RO6 A HYDROLASE inhibitor

P142

3D-QSAR study of erythromycin derivatives

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Erythromycin antibiotic was discovered in 1952 in culture broths of actinomycete *Streptomyces erythreus*, a microorganism isolated from a soil sample. Erythromycin and its derivatives bind to 50S bacterial ribosome and inhibit the elongation step of protein biosynthesis [1].

We previously reported a docking study of erythromycin A and analogues with the 50S ribosomal subunit of *Deinococcus radiodurans* [2] that provides view as to how these analogues interact with ribosomal RNA. In this abstract, we report a 3D-QSAR analysis of erythromycin analogues in order to seek new insights into the relationship between the structural information and the inhibitory potency. The probable binding conformations of erythromycin analogues were determined using a flexible docking approach. Another conformer sampling approach that using conformational analysis technique was also used. The predictive ability of the models was validated using a set of compounds that were not included in the training set. Mapping the 3D-QSAR models to the active site of 50S bacterial ribosome provides new insight into the interactions of ligand-ribosome complex. Models generated are also useful to the prediction of activities of new erythromycin derivatives.

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Drug Metabolism

P143

Formation of dimethylfumarate or methylhydrogenfumarate adducts with intarcellular glutathione ex vivo

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The mode of action of fumaric acid esters (FAE`s), effective in the treatment of psoriasis, is not fully understood.

For ex vivo pharmakokinetics of FAE`s the formation of glutathione (GSH) adducts might play some role.

Dimethylfumarate (DMF) was shown to deplete intracellular GSH ex vivo which is thought to be due to formation of an adduct between GSH and DMF.

To date, there are no data available about the effect of methylhydrogenfumarate (MHF) on GSH.

To quantify adducts of GSH and DMF (dimethyl-2-(S-glutathionyl)-succinate; GS-DMS) or of GSH and MHF (methyl-2-(S-glutathionyl)-hydrogen succinate; GS-MHS) a HPLC- MS method was developed using an ion trap mass spectrometer with ESI positive mode.

Primary human fibroblasts or HaCaT keratinocytes were incubated with DMF or MHF for different time points. In cell culture supernatants GS-DMS and GS-MHS,

respectively, could be detected for the first time. The results show a stronger depletion of intracellular GSH when cells were incubated with DMF than with MHF. In addition, a higher amount of GS-DMS as compared to GS-MHS could be detected.

It is concluded that the ability of DMF and MHF to decrease intracellular GSH can be correlated with the formation of the respective adducts.

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P144

N-Hydroxy-valdecoxib monohydrate: an active metabolite and/or a pro-drug of valdecoxib?

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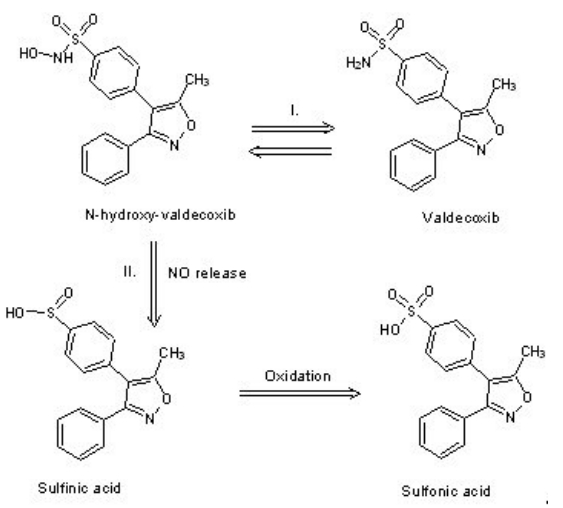
75 new analogues of valdecoxib have been prepared and their pharmacological properties have been studied *in vitro* an *in vivo*.

N-hydroxy-valdecoxib has been found to be the most effective compound among the examined analogues. *N*-hydroxy-valdecoxib formed a stable monohydrate [1] but the anhydrous form decomposed to the sulfinic acid via a free radical reaction.



N-hydroxy-valdecoxib has a remarkable pharmacological profile. It is more active than valdecoxib in the incapacitance test and in the Randall-Selitto model (inflammatory pain). It has also shown long lasting effects in the carrageenan induced paw edema test.

However, it is known from the literature [2] that *N*-hydroxy-valdecoxib is a metabolite of valdecoxib, our preliminary in vivo studies in rats indicate that *N*-hydroxy-valdecoxib forms an equilibrum with valdecoxib, and it may be an active metabolite.

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Drug Screening Technologies

P145

A bimodal probe for luminescence and magnetic resonance imaging

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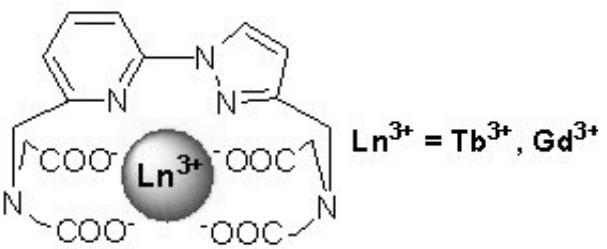
In recent years, two imaging techniques have emerged that have a great impact upon medical science: nuclear magnetic resonance imaging (MRI) and fluorescence microscopy imaging. Recent reports indicate that the use of probes with dual detection properties (fluorescence and MRI) offers the unique opportunity to directly correlate in vitro and in vivo imaging studies [1] [2]. Therefore, we investigated bimodal probes based on lanthanide organocomplexes which can be used in these two imaging techniques, depending on the physical properties of the lanthanide ion. The complexes of the Gd(III) ion are useful as contrast agents in MRI technique. The long emission lifetimes (ms range) of Eu(III) and Tb(III) ions allows fluorescence time-gated measurements without interferences with autofluorescence from biological molecules and tissues.

In this communication, we report on the study of a new polyaminocarboxylate ligand derived from *N,C*-pyrazolylpyridine and its Tb(III) and Gd(III) corresponding complexes.



In this work, we demonstrate that (1) the Tb(III)/Gd(III) complexes present a reasonable physiological stability in aqueous solutions, a key point when biological applications are concerned, (2) The Tb(III) chelate is strongly fluorescent in the visible domain having remarkable lifetime and quantum yield at room temperature, (3) the proton relaxivity of the Gd(III) chelate was found comparable to those of the clinically used Gd-DTPA and Gd-DOTA (magnevist® and dotarem®).

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P146

2,4-Methanoproline gomologues as building blocks for drug design

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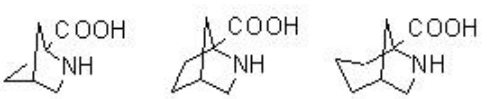
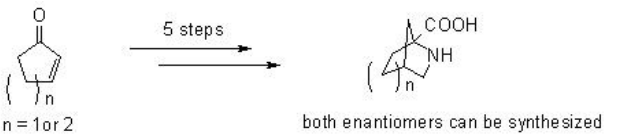
Conformationally restricted molecules often exhibit more efficient interactions with the corresponding biological targets than the flexible analogues. For example, many peptidomimetics reported to date contain rigid fragments; this can be achieved by incorporation of conformationally restricted amino acid residues. Libraries of conformationally constrained or rigid amino acids, designed to vary torsion angles in peptidomimetics in a systematic manner, are of particular interest. Such libraries can be composed of either isomers or homologues of amino acids. While the fixed torsion angles in the common rigid fragments are different within the set of compounds in such a library, other properties (steric bulkiness, lipophilicity, etc.) are similar. Therefore, the libraries could be very effective to study the structure-property relationships.

We wish to report the design and synthesis of a mini-library, which consists of three amino acids – bicyclic proline analogues. One of the compounds in the library is known 2-azabicyclo[2.1.1]hexane-1-carboxylic acid (trivial name 2,4-methanoproline) ***1***, which was found in *Atheleia herbert smithii* in 1980. Several syntheses of this compound have been reported since then. Two other amino acids, the methanoproline homologues ***2*** and ***3***, are novel.



1 **2** **3**

Analysis of molecular models showed that compounds ***1***–***3*** meet all the requirements for the libraries discussed above. The synthesis of compounds ***2*** and ***3*** was performed starting from the commercially available 2-cycloalken-1-ones in five steps. Both enantiomers of these amino acids were obtained.



P147

Selectivity-based compound screening by parallel analysis of a multi-protein panel using Biacore A100

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Candidate drug compounds are commonly screened by inhibition assays that reflect their affinity for the target protein. Binding selectivity is also a critical property, however, and assays providing both affinity and selectivity data at a relatively early stage may greatly facilitate the drug discovery process. Small molecules with good affinity and selectivity properties for the targeted subunit of a heterotrimeric protein were identified using a new protein interaction array system, BiacoreÒ A100 (Biacore AB). 1280 compounds were first assayed for binding to one subunit and the full-length protein. 60 compounds were then re-analyzed against a more extensive protein panel. Parallel analysis against full-length isomers, wild-type and mutant subunits and unrelated control proteins provided comprehensive affinity and selectivity information enabling well-informed selection of candidates. These findings could not have been obtained by conventional single-target analysis, demonstrating the value of a multi-protein panel approach.

P148

Use of RP-HPTLC systems for determination of lipophilicity of 3,5-dioxo-4-azatricyclo[5.2.2.02,6]-undecanes – 5-HT1A antagonists.

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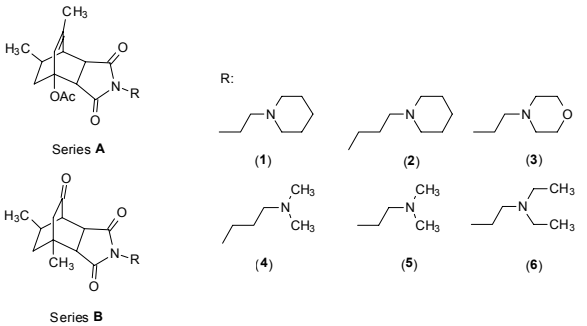
The lipophilicity of twelve 3,5-Dioxo-4-azatricyclo[5.2.2.02,6]undecanes – potential serotonergic 5-HT_{1A} receptors antagonists was determined by means of the reversed-phase RP-18W and RP-18 thin-layer chromatography. The use of the linear (Soczewinski-Wachtmeister) and square (Schoenmaker) equation for R_{MW} calculation was evaluated.



Due to the rather poor correlation between linear and square R_{MW} values, despite very good correlation coefficients for independent measurements for each solvent systems the results of the RP-18 measurements and use of the linear equation for the R_{MW} calculation were found to be the most reliable. Their reliability was also confirmed by the best values of F and *s*. The standardization (for six standards of known lipophilicity – log*P*) allowed calculation of the experimental lipophilicity ($\log P_{EXP}$) for compounds investigated.

P149

Fragment-based Lead Discovery by NMR: Novel modulators of PDZ domains

M. Schade

Combinature Biopharm AG , Berlin

NMR has advanced to a powerful fragment screening technology due to its unique resolution of binding sites and high sensitivity for loose protein fragment interactions [1]. Here we show the successful identification of novel, chemically diverse fragment hits for a demanding protein-protein-interaction target, namely the PDZ domain of human AF6 [2]. We demonstrate how the NMR-derived binding sites and the low complexity chemistry of fragments allowed for rapid synthetic optimization of one hit class to yield a 291 Da ligand that inhibits the protein-protein interaction in vitro. For this ligand we determined the 3D complex structure by NMR (Fig. 1) revealing the formation of a hydrophobic sub-pocket when the fragment ligand is bound. This unexpected induced-fit interaction through the rearrangement of the amino acid side chains of L25 and M23 opens up the door for the development of small molecule modulators of the large family of PDZ domains, several of which are involved in human disease mechanisms, such as cancer, pain and CNS disorders.

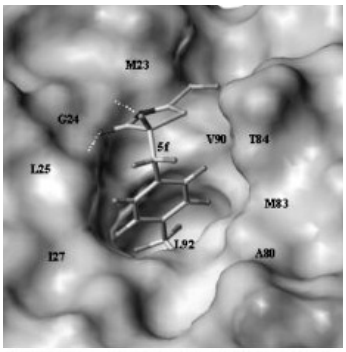


Fig. 1. 3D complex structure between the AF6 PDZ domain (light grey surface) and the fragment ligand (by atom coloring). The trifluoromethyl-phenyl moiety binds into a hydrophobic pocket, while the 2-mercapto-thiazolidinon moiety mediates H bond interactions (dashed lines) with the protein backbone amides of residues G24 and L25.

[1] M. Schade, H. Oschkinat. NMR fragment screening: Tackling protein-protein interaction targets. Curr. Opin. Drug Discov. Devel. (2005) 8, 365-373.

[2] M. Joshi, C. Vargas, K. Moelling, P. Boisguerin, A. Diehl, G. Krause, P. Schmieder, V. Hagen, M. Schade, H. Oschkinat. Discovery of Low-Molecular-Weight Ligands for the AF6 PDZ Domain. Angew. Chem. Int. Ed. (2006) in press.

P150

Serine proteases – from fragments to leads

G. Chessari*, C. Abell**, O. Callaghan*, M. Congreve*, N. Howard**, S. Howard*, M. Frederickson*, H. Jhoti*, C. Murray*, L. Seavers*, R. Van Montfort*, M. Vinkovic*

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At Astex, libraries of low molecular weight molecules (or 'fragments') have been designed and then screened using NMR and/or protein-ligand x-ray crystallography against a broad range of therapeutically relevant drug targets. This screening process (or PyramidTM) [1] has identified fragment hits for each target studied. Here we report the PyramidTM approach applied to two serine proteases: thrombin and urokinase. Fragment libraries were designed to avoid any well-precedented, strongly basic functionality. Screening hits included smaller fragments that bind to the S1 pocket and also a novel ligand, which binds exclusively to the S2-S4 thrombin pocket [2]. Approaches for optimization of these fragments will be described. Highly potent leads have been discovered either through the "growing-out" from fragments into adjacent pockets or through fragment linking. The computational tools and techniques used in the whole process will be presented in detail.

[1] Hartshorn MJ, Murray CW, Cleasby A, Frederickson M, Tickle IJ, Jhoti H. Fragment-based lead discovery using x-ray crystallography. J Med Chem 2005;48:403-413.

[2] Howard N, Abell C, Blakemore W, Chessari G, Congreve M, Howard S, Jhoti H, Murray CW, Seavers LCA, van Montfort RLM. J Med Chem 2006;49: 1346-1355.

P151

Enantioselective recognition of a three-way DNA junction by metallo-supramolecular cylinders

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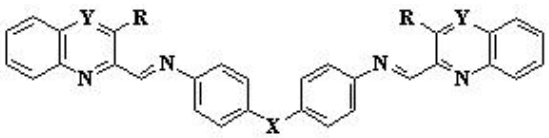
A metallo-supramolecular cylinder, whose size and shape is comparable to a protein zinc finger, has proven to fit into the major groove of B-DNA [1]. Because of its bimetallic nature, it has a high positive charge that should enhance its binding to the negatively charged DNA. More recently, a completely new mode of DNA recognition has been found. An atomic resolution X-ray crystal structure of a three-way DNA junction complexed to a cylinder shows that it fits perfectly into the central trigonal hydrophobic cavity of the DNA junction [2].

Based on these findings, we are studying chiral bis-iron helicates for their binding capacity to hexanucleotides in dilute solution by using uv-vis spectroscopy as well as circular dichroism polarimetry techniques. We are investigating how concentration and temperature may influence the stability of the helicate-oligo complex. Also, new ligands (such as the one shown in the picture) for self-assembly of helicates, with iron or copper nuclei, are being synthesised.



$X = CH_2, S, SO_2, O \quad Y = CH, N \quad R = H, CN$

[1] Hannon MJ, Moreno V, Prieto MJ, Moldrheim E, Sletten E, Meistermann I, Isaac CJ, Sanders KJ, Rodger A. Intramolecular DNA coiling mediated by a metallo-supramolecular cylinder. Angew Chem 2001;113(5):904-908.

[2] Oleksi A, Blanco AG, Boer R, Usón I, Aymamí J, Rodger A, Hannon MJ, Coll M. Molecular recognition of a three-way DNA junction by a metallosupramolecular helicate. Angew Chem Int Ed 2006;45:1227-1231.Chem Int Ed 2006;45:1227-1231.

P152

Synthesis and some pharmacological properties of 3-(2-methyl-furan-3-yl)-4-substituted-Δ2-1,2,4-triazoline-5-thiones

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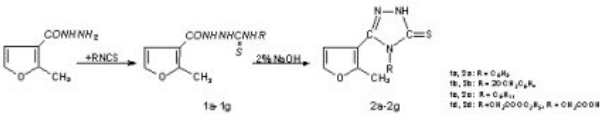
1,2,4-Triazole derivatives exhibit wide spectrum of pharmacological activity. One of the methods to synthesize these compounds is cyclization of their thiosemicarbazide derivatives in alkaline medium. This method was applied to synthesis new 1,2,4-triazole with furan system as show in the Scheme:



Compounds 2a–2c were investigated pharmacologically for their properties in mice. The experiments were carried out on male Albino-Swiss (20-24g). The compounds were administered intraperitoneally (ip) as suspension in 1% Tween 80 at a constant volume of 0.1 mL/10g body weight of mice. The compounds were administered in doses equivalent to 0.1; 0.05; 0.025; 0.0125; 0.0062 or 0.00312 of LD50. Control animals received the some volume of solvent. Each experimental groups consisted of eight animals. The permission for the animal tests and experiments has been given by the Ethical Board of the Medical University in Lublin.

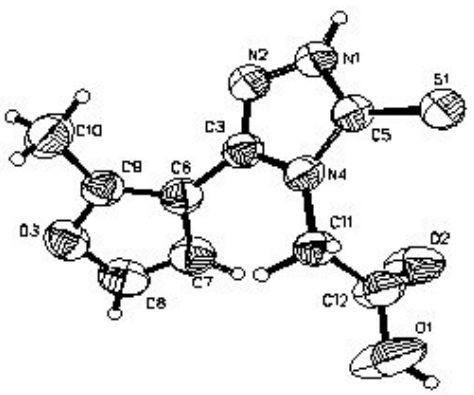


Fig. 1. **2d**: space group $P2_1/c$, a = 6.466(1), b = 14.845(3), c = 11.004(2) Å, β = 94.43(3)°.

None of the compounds were found to show neurotoxic activity because in the dose of 0.1 LD50 they did not effect the motor coordination of mice in the “chimney” test. Compounds 2a–2c in the doses of 0.05 and 0.1 of LD50 significantly prolonged the time of sleep induced by thiopental. Of the three examined compounds, only 2b in the dose of 0.1 of LD50 decreased (by about 25% vs control group) the tonic pentetrazol-induced seizures as well as mortality of mice. All compounds showed only weak antidepressive action; they were active only in the dose of

0.1 of LD50. Strong antinociceptive properties of 2a–2c were observed in a wide range of doses. In the "remaining" test all the compounds were inactive.

Molecular structure proposed for investigated 1,2,4-triazoles was confirmed by X-ray structure analysis of 2d (Fig.1).

P153

Synthesis and Biological Evaluation of Various Fenamic Acids

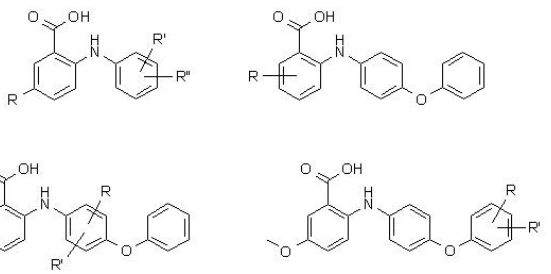
W. Ulf, F. Ingela, J. Stig, W. Lisbeth

Active Biotech Research AB

By using the Ullmann-Goldberg reaction on a series of substituted 2-halobenzoic acids several libraries of functionally varied fenamic acid derivatives have been synthesised in order to map the active site of dihydroorotate dehydrogenase (DHODH). In an enzyme-based assay several molecular features for optimal binding between ligand and enzyme could be evaluated and confirmed.

The anthranilic part of the ligand turned out to be very sensitive towards modifications. Activity decreased drastically with substitutions other than in the 5-position and this position also showed limited possibilities for variations. For example, going from methoxy to ethoxy rendered in a threefold decrease in affinity. Also, carboxylic acid O- or amino N- methylated derivatives were more or less inactive.

On the other hand, the other part of the system was more tolerable towards substitutions. The farther from the anthranilic system, the more variations could be made with conserved biological activity, indicating that this part of the ligand is situated at the outer region of the active site where flexibility of the enzyme framework is more pronounced.



P154

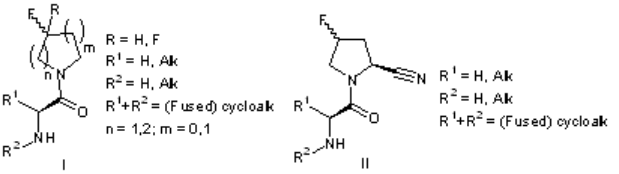
Synthesis and biological properties of fluorine-containing DPPIV inhibitors

S. Belyakov, W. Li, E. Oliver, D. Ferraris, B. Thomas !V, K. Wozniak, C. Rojas, S. Lautar, D. Kalvin

Department of Research, MGI Pharma, Baltimore, MD 21236 U.S.A.

Fluorine-containing inhibitors (FCI) of DPPIV possess enhanced oral bioavailability and substantial in vitro potency [1,2]. The latter is due to either increased hydrophobic interaction between DPPIV and FCI or conformational changes of FCI's fluorine-containing ring in the P1 pocket [2].

In our search for DPP IV inhibitors as novel CNS drugs, we examined two series of FCI of general formula (I) and (II).



Synthesis and SAR data for both series are discussed in this poster.

Based on in vitro screening and SAR analysis, selected FCI were evaluated ex vivo in plasma and brain homogenates to determine level of DPP-IV inhibition. While having comparable to des-fluoro analogs levels of activity in plasma, FCI displayed higher levels of brain activity.

Incidents of both acute and chronic toxicity were registered in rats upon IV administration of 2-cyano FCI (type II) at high doses. Sedation was a factor for several in vivo experiments involving these FCI at elevated (>100 mg/kg) doses. Such toxicity was not observed for des-fluoro analogs or for des-cyano FCI (type I).

[1] Hulin B, Cabral S, Lopaze MG, Van Volkenburg MA, Andrews KM, Parker JC. New fluorinated pyrrolidine and azetidine amides as DPPIV inhibitors. Bioorg Med Chem Lett 2005; 15:4770-4773.

[2] Fukushima H, Hiratate A, Takahashi M, Saito M, Munetomo E, Kitano K, Saito H, Takaoka Y, Yamamoto K. Synthesis and structure–activity relationships of potent 3- or 4-substituted-2-cyanopyrrolidine DPPIV inhibitors. Bioorg Med Chem 2004; 12:6053-6061

P155

Adamantane building blocks determine activity against pox viruses

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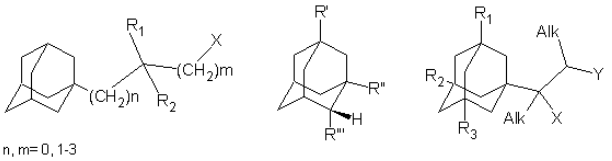
*Samara State Technical University, 443100 Samara, Russia. **State Research Center for Virology and Biotechnology Vektor, 630559 Kol'tsovo, Novosibirskaya Oblast, Russia

We have developed methods of synthesis of cage hydrophobic building blocks, having wide range of varying of steric characteristics, lipophility and different space bridge lengths between cage fragment and functional groups. Using unsaturated compounds, substituted alicyclic ketones, oxathiolanimines and nitrosochlorides we have synthesized wide series of mono-, di- and polyfunctional derivatives of adamantane, which are very promising building blocks for medicinal chemistry.



n, m= 0, 1-3

R_1,R_2,R_3= H, Alk, Ar

R'= H, Hal, OH

R''= OR, NR_1R_2

R'''= H, Hal, Alk, Bz

X,Y= COOH, NH2, OH, CN, NHCOOR, NHCOSR, NHCOR, $NHCONH_2$, NR_1R_2

We have investigated inhibitory action of the array of synthesized compounds against different orthopoxviruses (variola, monkeypox, cowpox, mousepox). On the basis of the research lead compounds have been discovered and are investigated on further stages. The predominant factor of antiviral activity against orthopoxviruses is the availability of the cage structural element and sufficiently high lipophility (logP < 5). Influence of different parameters of molecular structure on the degree of antiviral activity and possibility of directed modification of nucleoside analodues antiviral drugs by cage building blocks are discusses.

P156

Control of mitochondrial-dependent apoptosis by small molecules that act as inhibitors of the apoptosome

G. Malet*, M. Vicent*, G. Sanclimens**, A. Messeguer**, E. PéRez-Payá*

* Dpt. Of Medicinal Chemistry. Centro de Investigación Príncipe Felipe. E-46013 València, Spain. ** Department of Biological Organic Chemistry, IIQAB (CSIC). J. Girona 18, 08034 Barcelona, Spain.

The apoptosome is a holoenzyme multiprotein complex formed by cytochrome c-activated Apaf-1 (apoptotic protease-activating factor), dATP and procaspase-9 that link mitochondria disfunction with activation of the effector caspases. Consequently, this complex is an attractive target for the development of apoptotic modulators. In the present study the identification of compounds that inhibit the apoptosome-mediated activation of procaspase-9 from the screening of a diversity-oriented chemical library [1] is reported. The active compounds rescued from the library were chemically optimized to obtain conformationally restricted peptidomimetics by using solid-phase methodolgy combined with microwave assisted synthesis. These new derivatives bind to both recombinant and human endogenous Apaf-1 in a cytochrome c-non competitive mechanism that inhibits the recruitment of procaspase-9 by the apoptosome. In addition, they decrease the apoptotic phenotype in mitochondrial-mediated models of cellular apoptosis [2]. Further chemical modulation of these active molecules to improve their bioavailability will be also presented.

[1] Masip I, Cortés N, Abad MJ, Guardiola M, Pérez-Payá E, Ferragut J, Ferrer-Montiel A, Messeguer A. Design and synthesis of an optimised positional scanning library of peptoids: identification of novel multidrug resistance reversal agents. Bioorg. Med. Chem. 2005; 13: 1929-1936.

[2] Malet G, Martín A, Orzáez M, Vicent MJ, Masip I, Sanclimens G, Mingarro I, Ferrer-Montiel A., Messeguer A, Fearnhead HO, Pérez-Payá E. Small molecule inhibitors of Apaf-1-related caspase-3/-9 activation that control mitochondrial-dependent apoptosis. Cell Death Differ. 2006; 000.

P157

Properties of a [1,2,3]triazolo[5',1':6,1]pyrido[2,3-d]pyrimidine system as chemosensor for metal ions, anions and amino acids

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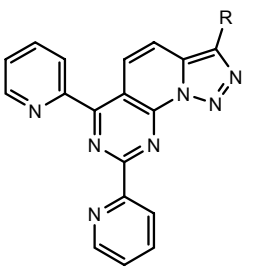
University of Valencia. *Department of Organic Chemistry, Faculty of Pharmacy, Valencia University, Avda. Vicente Andrés Estellés s/n, 46100 Burjassot (Valencia), Spain. **Molecular Science Institute, Department of Inorganic Chemistry, Faculty of Chemistry, Valencia University, C. Doctor Moliner nº 57, 46100 Burjassot (Valencia), Spain

The development of new chemosensor molecules is a very interesting field of research, particularly for sensing biologically or environmental relevant substrates. Anion recognition, detection and quantification is of great actual upsurge. We describe here the characteristics of a new [1,2,3]triazolo[5',1':6,1]pyrido[2,3-d]pyrimidine system for the direct colorimetric and fluorimetric sensing of metal ions, anions and amino acids.



1 R= CH_3
2 R= Phenyl
3 R= 2-Thienyl

Recently we have synthetized 3-methyl-6,8-di(2-pyridil)-[1,2,3]triazolo[5',1':6,1]pyrido[2,3-d]pyrimidine 1 by reaction of 7-lithio-3-methyl-[1,2,3]triazolo[1,5-a]pyridine with 2-cyanopyridine and identified by X-Ray diffraction analysis.[1] Compound 1 presents a very intense fluorescence emission at 464 nm and behaves as an interesting PCT chemosensor for metal ions (Cu+2, Zn+2), anionic species (NO3-, NO2-, H2PO4-, SO42-, Cl-) and amino acids (glutamic acid, aspartic acid, phenylalanine).[2]

The effects of replacing the methyl group at the 3-position by electron-donor groups (2 R= phenyl and 3 R= 2-thienyl) have been explored. Compounds 2 (505 nm) and 3 (540 nm) show red-shifted emission bands with

respect to 1. The presence of the phenyl and tiophenyl substituents activates the Internal Charge Transfer mechanism favouring the performance of the Zn2+ complexes as anion and amino acids chemosensors.

[1] Abarca B, Aucejo R, Ballesteros R, Chadlaoui M, García-España E, Ramirez de Arellano C. X- Ray characterization of 3-methyl-6,8-di(2-pyridil)-[1,2,3]triazolo [5',1':6,1]pyrido[2,3-d]pyrimidine. Arkivoc, (xiv), 71-75.

[2] Chadlaoui M, Estudios en triazoloazinas. Síntesis de ligandos polinitrogenados. PhD Thesis, Valencia University, March 2005.

P158

Somatostatin receptor agonists with anti-angiogenic activity: potential application in eye diseases

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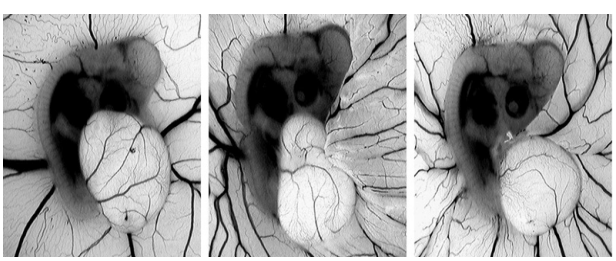
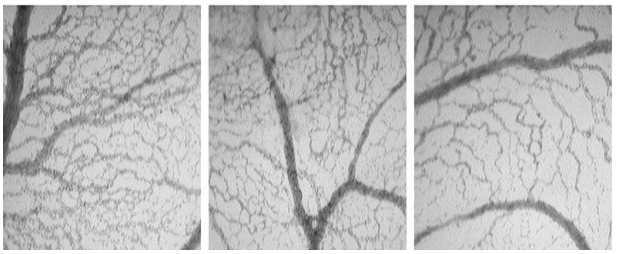
Somatostatin receptor (SSTR) agonists present one class of molecules that can inhibit angiogenesis. Octreotide ® is one such agonist with demonstrated anti-angiogenic activity that has found applications in cancer therapy and is in phase 3 clinical trials for treating diabetic retinopathy.

Alchemia has produced a library of proprietary anti-angiogenic small molecules (NCEs), using its drug discovery platform. The library was screened for affinity to SSTR subtypes and selected compounds showed efficacy in a number of in vitro and in vivo models of angiogenesis, including the murine corneal micropocket model. We are currently assessing candidate compounds for potential development as novel drugs for the treatment of ocular diseases. Early preclinical, safety and toxicology profiles have demonstrated minimal off-target pharmacology, and the pharmacokinetic profiles of the lead molecules showed good ADME characteristics. A lead candidate, ACL16907, has been selected from the panel of leads and produced on a kilogram scale, demonstrating the scalability of the generated compounds. The results of the anti-angiogenesis screens of our compound library and the potential use of anti-angiogenic compounds in the treatment of eye diseases such as age-related macular degeneration and diabetic retinopathy will be discussed.

Changes in CAM vessels following treatment with Alchemia lead compound





P159

Design, synthesis and in vitro activity of peptiomimetic inhibitors of myd88 dimerization

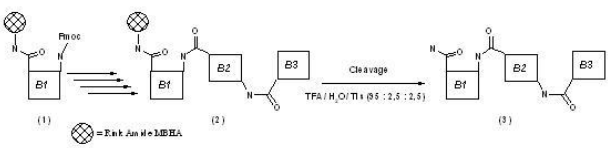
A. Ciacci*, G. Basile**, V. Bombardi**, N. Fantò*, G. Gallo*, V. Ruggiero*, M. Semproni*, D. Vignola*, M. Zibella**, P. Carminati*

*sigma tau. **tecnogen

Dimerization of myeloid differentiation factor 88 (MyD88) plays a crucial role in the signalling pathways triggered by interleukin (IL)-1 and Toll-like receptors in several steps of innate host defense [1]. In a previous paper, we demonstrated that an epta-peptide, derived from the Toll/IL-1 receptor (TIR) domain of MyD88 (Ac-RDVLPGT-NH2), was effective in inhibiting its homodimerization [2]. In this work, we describe the design and synthesis of a peptidomimetic library starting from a series of three building blocks. A peptidomimetic library targeting this protein-protein interaction was realized using statistical molecular design. A non-peptide scaffold was used, the three positions being varied. Selection was performed in the molecular space, with previous protein structure knowledge included in the design procedure. This resulted in a heavily reduced library, consisting of about 80 molecules, prepared by solid-phase synthesis.

The ability of the peptidomimetics to inhibit protein-protein interaction was assessed by yeast 2-Hyb experiments. Dimerization of MyD88, inside the S.cerevisiae nucleus, leads to activation of reporter genes responsible for growth in absence of particular nutrients. Molecules, positively selected in 2-Hyb, were further assayed in a mammalian cell system to assess their ability to inhibit NF-kB activation, a downstream transcription factor in MyD88 signalling, which elicits production of essential effector molecules for immune and inflammatory responses.

Interesting lead compounds were selected to further synthesize proper homologous series.



[1] Akira S, Takeda K. Toll-like receptor signalling. Nat Rev Immunol 2004;4:499–511.

[2] a) Loiarro M, Sette C, Gallo G, Ciacci A, Fantò N, Mastroianni D, Carminati P, Ruggiero V. Peptide-mediated

Interference of TIR Domain Dimerization in MyD88 Inhibits Interleukin-1-dependent Activation of NF-êB. J Biol Chem 2005;280: 15809-15814; b) PCT/EP/2005/056847.

P160

Development of a fluorescent microplate cell based assay for the rapid evaluation of human thromboxane A2 receptor modulators acting on alpha and beta isoforms

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*Natural and Synthetic Drugs Research Centre, Department of Pharmacy, Laboratory of Medicinal Chemistry, University of Liège, 1, Av. de l'Hôpital, B-4000 Liège, Belgium. **Department of Pharmacy, University of Namur, FUNDP, 61 rue de Bruxelles, B-5000 Namur, Belgium. ***School of Biomolecular & Biomedical Science, Conway Institute for Biomolecular and Biomedical Research, University College Dublin, Ireland.

Thromboxane A2 (TXA2) is an arachidonic acid metabolite implicated in pathologies such as stroke, myocardial infarction and atherosclerosis. Consequently, the design of TXA2 receptor (TP) antagonists remains of great interest in cardiovascular medicine. The action of TXA2 is mediated by a specific G-protein coupled receptor of which two alternative spliced isoforms, TPα and TPβ, have been described. The exact role of these two isoforms is not clearly understood. However, recent studies have described their implications in vascular physiology and pathology. Nevertheless, compounds though to act on TP receptors are routinely evaluated on human platelet function, without addressing the individual role of the a and b TP receptor isoforms. In this study, we have designed and developed a 96-well microplate test for the rapid measurement of intracellular Ca2+ mobilization elicited by stimulation of either TPα or TPβ. Our model uses Fluo-4 loaded HEK293 cell lines stably expressing either TPα or TPβ alone, and quantifies the transient fluorescent changes upon intracellular calcium mobilization triggered by U46619 (a thromboxane A2 stable agonist). Additionally, we have validated our model and then used it for the pharmacological evaluation of ten TP antagonists, of which several displayed interesting profiles. In conclusion, we have designed, developed and validated an original assay useful for the rapid pharmacological determination of compounds acting on both TPα and TPβ isoforms.

P161

Addition of carbenes as a route to 3,4- and 4,5-methanoprolines, building blocks for drug design

P. Mikhailiuk*, N. Gvozdovska*, I. Komarov*, A. Tolmachev**

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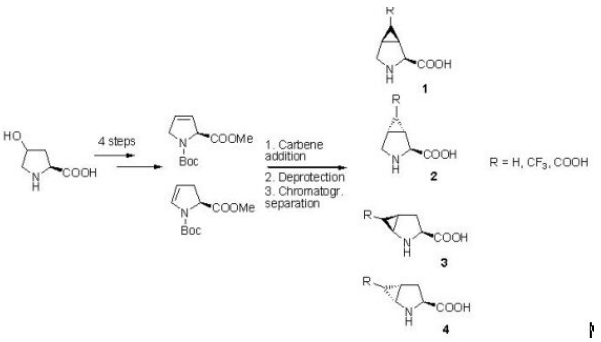
There is a continuing interest in synthesis of conformationally restricted proline analogues, both for studying them as biologically active compounds and for incorporating in peptidomimetics – potential drug candidates. Proline is unique among proteinogenic amino acids because its nitrogen atom is a part of the five-membered ring. This causes some peculiarities in the conformation of a proline-containing polypeptide chain. Proline is often found in those secondary structure elements of peptides which are responsible for biological action of peptides, therefore, conformationally restricted proline analogues are of particular interest. Here we report synthesis of proline analogues containing a cyclopropane ring, annelated at the 3,4- or 4,5-positions of the proline – cys- and trans-3,4- and 4,5-methanoprolines (1-4). The methylene bridge is the minimal structural change of the proline core, which results in conformational restriction of the molecules. Conformational restriction is an efficient tool in the design of biologically active compounds. The loss in entropy, which is achieved by the restriction, leads in favorable cases to enhancement of important intermolecular interactions responsible for biological activity.



The synthesis is based on the addition of carbenes at the double bond in 3,4- or 4,5- dehydroprolines, easily available from 4-hydroxyproline in 4 steps. The carbenes were generated from the corresponding diazomethanes using Cu(I)-catalysed decomposition:

P162

Chiral separation of 1-(2-naphthyl)ethanol and 1-(2-naphthyl)-2-(imidazol-1-yl)ethanol esters by HPLC

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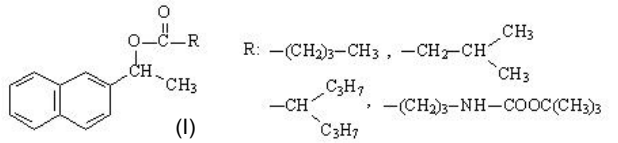
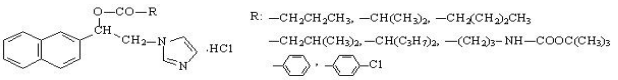
Enantiomeric separation of chiral compounds has been of great interest because the majority of synthetic drugs are chiral and enantiomers of a chiral drug may have different pharmacokinetic and pharmacodynamic effects. Chiral HPLC has proven to be one of the best methods for the direct separation and analysis of enantiomers.

In our previous study the synthesis and anticonvulsant and antimicrobial activities of 1-(2-naphthyl)ethanol (I) and 1-(2-naphthyl)-2-(imidazol-1-yl)ethanol esters (II) have been described [1]. Since these compounds have a chiral center, we aimed to separate these chiral compounds into their enantiomers by HPLC.



(I)



(II)

In this study, analytical HPLC method using derivatized cellulose stationary phase has been developed for the direct enantiomeric separation of these ester derivatives. The enantiomers of the compounds were resolved by normal-phase chromatography on silica based cellulose tris(3,5-dimethylphenylcarbamate) (Chiralcel OD) column with mobile phases consisting of mixtures of n-hexane and an alcohol (methanol, ethanol or 2-propanol) in different proportions. The effect of the concentration of alcohol in the mobil phase was studied.

Acknowledgement: This project was supported by Hacettepe University Research Fund (Project number: 05D 01 301 001).

[1] Karakurt A. Studies on synthesis, enantiomeric separation, anticonvulsant and antimicrobial activities of some new 2-acetylnaphthalene derivatives [Dissertation], Hacettepe University Institute of Health Sciences, Ankara, 2004.

P163

Solid-phase synthesis of 1,2,4-oxadiazole-based peptidomimetics

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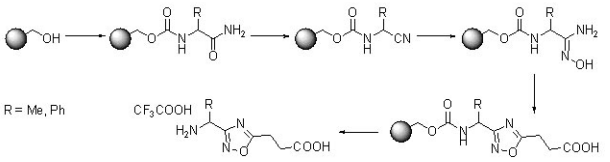
1,2,4-oxadiazoles have been shown to serve as bioisosteres of an amide bond in biologically active peptides [1]. By modifying the metabolically labile peptide bond the improved stability of resulting peptidomimetics can be obtained. Moreover, decreased conformational flexibility can result in their enhanced activity and selectivity.

Several methods for the solid-phase synthesis of 1,2,4-oxadiazoles have been described, mostly following approaches known from solution oxadiazoles chemistry [2]. In our work we focused on developing a method which would enable us to use simple amino acid derivatives as the starting point and thus enable us the parallel synthesis of short peptides mimetics.

In a model reaction sequence, L-Ala and L-Phe amides were coupled to Wang resin. Dehydration yielded corresponding nitriles which were transformed to amidoximes by addition of hydroxylamine. Acylation with anhydrides and cyclisation were performed in one step, giving desired oxadiazoles, which were cleaved under standard conditions. The optimal reaction conditions included carbonyldiimidazole as a coupling reagent, trifluoracetic anhydride as a dehydrating agent and microwave heating for the cyclisation.



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[2] Hebert N, Hannah A, Sutton S. Synthesis of oxadiazoles on solid support. Tetrahedron Lett 1999; 40: 8547-8550.

P164

Synthesis and 1H NMR Spectrum of Some 1H-Benzimidazoles Derivatives

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In our days many benzimidazole derivatives are used for various biological activities such as selective Dopamin D3 antagonist activity, inhibitors of cholesterol biosynthesis, 5-HT3 receptor antagonist activity, and aromatase inhibitory activity [1-4].

This variation is provided through different substitutions particularly at positions 2-, 4-, 5-, 6- and 7-. A set of compounds having at position 2-, p-ethoxyphenyl group carrying different amine groups at the end of the chain has been prepared. Thus 2-(4-(1H-benzo[d]imidazol-2-yl)phenoxy)-N,N-diethylethanamine, dimethylamine, di-isopropylamine, N-piperidyl, N-morfolinyl derivatives of the respective molecule have been obtained. Their structures have been elucidated through instrumental methods. However on the examination of 1H-NMR spectra of the compounds no regular pattern of splitting of the protons that should be sitting at positions 4-, 5-, 6- and 7- has been observed although the integration always indicated one hydrogen at each site. In order to secure the interpretation with a better method we applied HMQC and HMBC techniques. Upon careful analysis of the spectra it was concluded that tautomerization between positions 1- and 3- of the benzimidazole ring causes such an ambiguity [5].

Key Words: 1H-Benzimidazole derivatives, synthesis, 1H NMR spectra

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P165

Simultaneous Determination of Spironolactone and Hydrochlorothiazide in Tablets by Spectrophotometric and HPLC Methods

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A sample selective, sensitive and reliable spectroscopic methods (absorbance ratio and Vierordt), compared with HPLC for quantitative determination of spironolactone (SPL)-hydrochlorothiazide (HCT) in commercial tablets. A wavelength 260 nm was chosen as the isobestic point in the absorbance ratio method and the absorbance ratios A269/A260 nm for HCT and A232/A260 nm for SPL were used for calculation of regression equations. For the Vierordt method A11 values (1 %, 1 cm) obtained and 232 and 269 nm for both substances were used for quantitative analyses of HCT and SPL. Linearity range for SPL and HCT was 2-12 μg.ml-1 and 2-15 μg.ml-1 respectively for both methods.

The relative deviations for absorbance ratio and Vierordt method were found to be 1,58 %-1,32 % for HCT and 1,26 % - 1,43 % for SPL respectively. In the HPLC method HCT and SPL were determined by isocratic system using water-methanol-phosphate buffer (71:25:4) mobile phase, luna 5? C18(250x4,1 mm) reversed phase column. Mefrusid was chosen as an internal standard and detection was carried out for PDA detection at 286 nm (flow rate 0,7 ml.min-1). Linear concentration was obtained as 5-25 μg.ml-1, 2-15 μg.ml-1 for HCT and SPL respectively. Therefore, it was concluded that both spectroscopic method, as well as HPLC can be used in routine analyses of HCT-SPL in commercial tablets.

Enzymes

P166

Chemistry of DPPIV inhibitors derived from 2-cyanoazetidine

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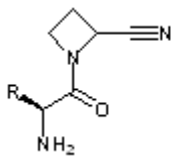
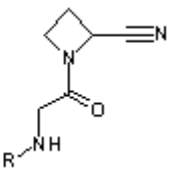
The pyrrolidine (or 1,3-thiazolidine) moiety is a fundamental part of the pharmacophore of most potent DPPIV inhibitors. The change of the ring size can drastically diminish their inhibition [1]. Additional boost in potency is provided by the introduction of cyano group (i.e., 2-cyanopyrrolidine) which acts as a “serine hook” [1] at the DPPIV catalytic site.

We have established that 2-cyanoazetidine-based inhibitors (CAI) also exhibit substantial levels of activity against DPPIV. In contrast with related 2-cyanopyrrolidine derivatives, stereochemistry of the 2-position has almost no effect on the potency of CAI. This finding was rationalized by molecular modeling, which indicated that azetidine ring fits in the S1 pocket in such a way that the distances between cyano group and Ser-OH are nearly equal for both S- and R-isomers of CAI.





Removal of BOC protecting group during conventional preparation of C-substituted CAI using HCl induces their rapid cyclization into corresponding iminoketopiperazines and, in addition, causes racemization of CAI, while TFA deprotection smoothly leads to desirable salts. A similar effect was observed for 2-cyanopyrrolidine derivatives as well.

[1] Wiedeman PE, Trevillyan JM. Dipeptidyl peptidase IV inhibitors for the treatment of impared glucose tolerance and type 2 diabetes. Current Opin Investig Drugs 2003; 4: 412-420.

P167

L-ascorbic acid derivatives as inhibitors of bacterial and mammalian hyaluronidases

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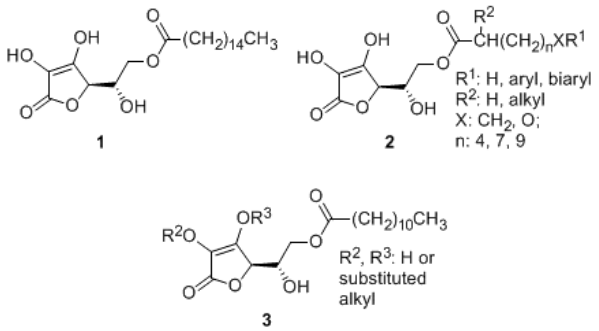
University of Regensburg, Germany

Hyaluronidases are enzymes degrading hyaluronan, an important component of the extracellular matrix. As the role of bacterial and mammalian hyaluronidases is far

from being understood, potent and selective inhibitors, which are not known to date, are required as pharmacological tools. Moreover, such inhibitors might be useful as drugs for the treatment of various diseases.

Very recently we demonstrated by investigation of ascorbic acid hexadecanoate (**1**) that the weak inhibitory activity of vitamin C on the bacterial hyaluronate lyase from *S. pneumoniae* is considerably increased by additional hydrophobic interactions [1]. Whereas bovine testicular hyaluronidase (BTH) and another bacterial hyaluronidase from *S. agalactiae* (hylB4755) were inhibited, interestingly, **1** did not inhibit the human hyaluronidase Hyal1, which was recently recombinantly expressed in insect cells and purified in our workgroup. These results prompted us to further explore the structure-activity relationships (SAR) of 6-*O*-acylated ascorbic acid derivatives. The alkanoyl chain was varied by introduction of aromatic groups and branching (**2**). In addition, the enediole system was alkylated (**3**).



The synthesized compounds were investigated for inhibition of human Hyal1, BTH and hylB4755 in a turbidimetric assay [2]. The activity of the most potent inhibitors was in the low micromolar range at the BTH and the bacterial enzyme depending on the chain length of the alkanoyl residue at O-6. Moreover, first moderately active inhibitors of the human enzyme were identified. The synthesis and SAR are presented on the poster.

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[2] Salmen S et al., Sulphated oligosaccharides as inhibitors of hyaluronidases from bovine testis, bee venom and Streptococcus agalactiae. Planta Med 2005; 71:727-732

P168

Recombinant expression, purification and characterisation of human hyaluronidase PH-20

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Hyaluronic acid, an important glycosaminoglycan of the extracellular matrix, is catabolised by â-1,4-endoglycosaminidases termed (E.C. 3.2.1.35) hyaluronidases. PH-20, one of six subtypes of human hyaluronidases, is primarily located on the head of sperms facilitating penetration of the ovum through the zona pellucida, which is rich in hyaluronan [1]. Bovine PH-20 has been used for many years as an antidote to treat extravasates of vinca alkaloids due to its function as a “spreading factor”. However, investigations on hyaluronidases suggest a role in signal transduction events directly correlated with tumour growth and cell migration [1]. Yet correlations with disease progression are controversial due to missing information on enzyme properties and the lack of specific inhibitors as pharmacological tools.

This work is part of a project to develop inhibitors of human hyaluronidases. Here we present a new method for the production of a soluble form of human PH-20 in Drosophila Schneider-2 cells. PH-20 was fused C-terminally to a V5 epitope and a His-tag, which enabled purification of the enzyme by ion-metal-affinity-chromatography (IMAC). Western blotting confirmed the identity of PH-20 as a single band of 57 kDa. The purified PH-20 was characterised with respect to its enzymatic properties and compared with a preparation of bovine PH-20. For both enzymes the pH dependent degradation of hyaluronic acid and chondroitin sulphate is similar, but the latter is catabolised at a much slower rate. Assay dependent differences in the pH profile have been described for bovine PH-20 [2] and were also observed for human PH-20.

The recombinant expression provides the basis for the large-scale production of human PH-20 for biochemical and biophysical characterisation studies as well as for the design and synthesis of specific inhibitors.

[1] Stern R. Glycobiology 2003, 13: 105R-115R

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P169

Synthesis of the Novel Series of Bispyridinium Compounds bearing (E)-but-2-ene Linker and Evaluation of their Reactivation Activity against Tabun-Inhibited Acetylcholinesterase

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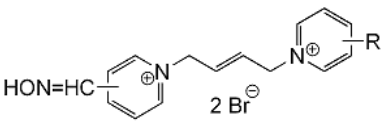
The reactivators of acetylcholinesterase (AChE, EC 3.1.1.7) are very important components in the treatment of intoxications caused by organophosphate inhibitors

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]. Therefore, development of more potent compounds able to reactivate broader spectrum of inhibitors is a major challenge actual from the point of view of accidents, terroristic attacks or war operations.

Potential AChE reactivators were synthesized using modification of currently known synthetic pathways [3]. Their potency to reactivate AChE inhibited by nerve agent tabun was tested *in vitro*. According to the results, there are promising reactivators of tabun-inhibited AChE. The reactivation potency of these compounds depends on structural factors such as constitution of the linking chain between both pyridinium rings, position of the oxime moiety at the pyridinium ring and presence of quaternary nitrogens [4].



R = H, C_6H_5, $CH_2C_6H_5$, CH=NOH, CN, COOH, COOMe, COOEt, $CONH_2$, Me, etc.

[1] Bajgar J. Organophosphates/nerve agent poisoning: mechanism of action, diagnosis, prophylaxis, and treatment. Adv Clin Chem 2004;38:151-216.

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The work was supported by the grant of Grant Agency of Charles University No. 302/2005/B-CH/FaF, by the grant of Ministry of Defence of Czech Republic No. FVZ0000501 and by Zentiva a.s. (Czech Republic).

P170

The Effects of Two L-Proline Mimetics on Potency, Lipophilicity and Binding Kinetics of Prolyl Oligopeptidase Inhibitors

E. M. Jarho*, J. I. Venäläinen**, J. Garcia-Horsman**, J. A. M. Christiaans*, **** M. M. Forsberg**, Tomi Järvinen*, P.T. Männistö**,*** and Erik A. A. Wallén*

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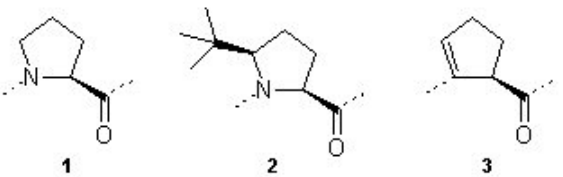
Prolyl oligopeptidase (POP) is a serine peptidase that cleaves oligopeptides at the carboxyl side of a prolyl residue. POP has been implicated in cognitive disorders, affective and eating disorders, and Chagas' disease. POP inhibitors have been shown to enhance cognition in monkeys [1] and to improve performance in verbal memory tests in humans [2].

In the present study, the L-prolyl group **1** in the middle of the POP inhibitor structure (at the P2 position) was replaced by a 5(*R*)-*tert*-butyl-L-prolyl group **2** and a (*R*)-cyclopent-2-enecarbonyl group **3** [3,4]. The effect of the mimetics on potency, lipophilicity and binding kinetics was studied. The IC_{50} and K_i values and the binding kinetics were determined for porcine POP, which serves as a good model for human POP since all differences in the amino acid sequence are far from the active site. Both replacements with L-proline mimetics resulted in POP inhibitors that were equipotent with the parent compounds. Both L-proline mimetics increased lipophilicity but the effect of the 5(*R*)-*tert*-butyl-L-prolyl group was more pronounced. While the 5(*R*)-*tert*-butyl-L-prolyl group increased the half-life of the enzyme-inhibitor complex, the (*R*)-cyclopent-2-enecarbonyl group decreased it. It was shown that these mimetics can be used to modify the lipophilicity and the binding kinetics of POP inhibitors.



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P171

Design, synthesis and biological evaluation of 8-Biarylquinolines: A novel class of PDE4 inhibitors

M. Gallant, L. Boulet, N. Chauret, D. Claveau, S. Day, D. Deschênes, D. Dubé, Z. Huang, P. Lacombe, F. Laliberté, S. Liu, D. Macdonald, J. Mancini, P. Masson, D. Nicholson, D. A. Nicoll-Griffith, H. Perrier, M. Salem, A. Styhler, R. N. Young, Y. Girard

Merck Frosst Centre for Therapeutic Research, 16711 Trans Canada Hwy., Kirkland, Québec, Canada, H9H 3L1

The structure-activity relationship of a novel series of 8-Biarylquinolines acting as type 4 phosphodiesterases (PDE4) inhibitors is described herein. Prototypical compounds from this series are potent and non-selective inhibitors of the 4 distinct PDE4 (IC50 < 10 nM) isozymes (A to D). In a human whole blood *in vitro* assay, they inhibit (IC50 < 0.5 μM) the LPS-induced release of the cytokine TNF-α. Optimized inhibitors were evaluated *in vivo* for efficacy in an ovalbumin-induced bronchoconstriction model in conscious guinea pig. Their propensity to produce an emetic response was evaluated by performing pharmacokinetic studies in squirrel monkeys. This work has led to the identification of several compounds with excellent *in vitro* and *in vivo* profiles, including a good therapeutic window of efficacy over emesis.

P172

Synthesis and testing of Nδ-methylcitrulline as a novel inhibitor of nitric oxide synthases

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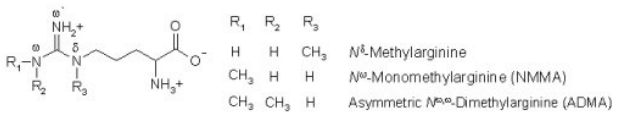
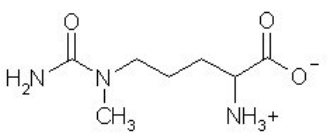
Nitric oxide is generated from the amino acid L-arginine by the action of the nitric oxide synthase (NOS), which can be blocked by endogenous inhibitors such as asymmetric dimethylarginine (ADMA) and monomethylarginine (NMMA). These natural occurring amino acids represent potential mediators of atherosclerotic complications in patients with coronary heart disease and cause an increase in vascular resistance and blood pressure [1]. Both ADMA and NMMA are methylated at the N-omega-position whereas not much is known about N-delta-methylated arginine [2].



Within the scope of investigating the physiological role of N-delta-substituted arginines we found N-delta-methylcitrulline to be a competitive inhibitor of all nitric oxide synthase isoenzymes.



Nδ-Methylcitrulline

In a standard in vitro assay format IC50-values were determined with recombinant human NOSs as follows: endothelial NOS: 2,0 mM, inducible NOS: 2,4 mM and neuronal NOS: 3,0 mM. Distinct synthetic approaches have been pursued in order to obtain N-delta-methylated derivatives of ornithine, citrulline or arginine, respectively [2, 3]. Therefore, extensive protection group chemistry was required and its usability explored. In particular, simultaneous protection of the alpha-amino acid moiety by formation of boroxazolidinones proved to be a convenient option to perform side chain modifications.

P173

Synthesis and Evaluation of 1H-Benzimidazoles as Topoisomerase I Inhibitors

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Benzimidazole is one of the most important heterocyclic rings showing various biological activities, such as, antibacterial, antifungal, antimicrobial, antiprotozoal and antihelmintic activities [1-5]. Several benzimidazole derivatives are also active as type I DNA topoisomerase inhibitors [6]. DNA topoisomerase I has a significant role in DNA metabolism and chromosome structure with an important role in almost all stages of the cell cycle. DNA topoisomerase I is also shown to be an important enzyme as anticancer drug target. In this study, six 1*H*-benzimidazole derivatives with different electronic characteristics at positions 5- and/or 6- have been synthesized [4-(1*H*-benzo[d]imidazole-2-yl)phenol **(1)** and 5-chloro **(2)**, 5-methyl **(3)**, 5-carboxylic acid **(4)**, 5-nitro **(5)**, 5,6-dimethyl **(6)** 1*H*-benzimidazole derivatives] and evaluated for their mammalian type I DNA topoisomerase inhibitory activity, via *in vitro* supercoil relaxation assays. For the structure elucidation of compounds, melting points, UV, IR, ^{1}H NMR, ^{13}C NMR and mass spectral data were interpreted. Among the compounds we covered, 5-nitro derivative **(5)** manifested the most potent topoisomerase I inhibition.

Key Words: *1H-Benzimidazole derivatives, type I DNA topoisomerase, plasmid supercoil relaxation assays,*

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Epigenetics

P174

Derivatives of 8-Bromo-1-phenyl-1,2-dihydro-benzo-[*f*]chromen-3-on as new potent inhibitors of human SIRT2

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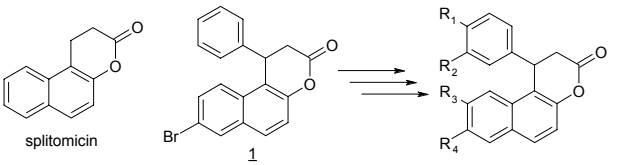
Histone Deacetylases (HDACs) are divided in three classes. Inhibitors of class I and II HDACs are already established in cancer chemotherapy. However, only a few inhibitors, one of them being splitomicin [1], are known of class III HDACs, the so called sirtuins. They play an important role in the regulation of the activity of tumour suppressor p53 [2] and the HIV-tat protein [3]. Hence, sirtuins represent an emerging target for anticancer and antiviral drugs.

We have synthezised 8-Bromo-1-phenyl-1,2-dihydro-benzo[f]chromen-3-on, 1, which turned out to be the first SIRT2-selective inhibitor at low micromolar regions. This compound also inhibits HCT-116 cancer cells at 75 µM while not affecting fibroblasts.



From syntheses which are based on molecular modelling studies we have discovered more potent and more selective inhibitors of SIRT2 by introducing substituents on the â-phenyl moiety and varying the substitution pattern of the naphthalin system of our lead structure. Detailed structure-activity relationships will be presented.

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[2] North BJ, Verdin E. Sirtuins: Sir2-related NAD-dependent protein deacetylases. Genome Biol. 2004; 5: 224

[3] Pagans S, Pedal A, North BJ, Kaehlke K, Marshall BL, Dorr A, Henklein P, Frye R, McBurney MW, Hruby H, Jung M, Verdin E, Ott M. SIRT1 regulates HIV Transcription via Tat deacetylation. PLoS Biol. 2005; 5: e41

P175

Synthesis and pharmacological characterization of new nicotinic ligands

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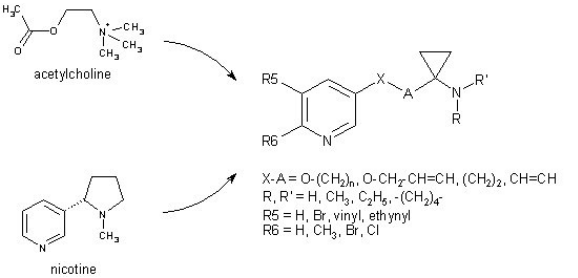
Nicotine has been shown in a variety of studies to improve aspects of cognitive performances in humans and animals. Recently, nicotinic acetylcholine receptor (nAChRs) subtypes and their role in disease and therapy have received a particular attention. However, the clinical use of nicotinic ligands as therapeutic agents is severely limited by their cardiovascular and neuromuscular side effects mainly resulting from a non selective activation of different nAChRs subtypes. To obtain novel selective nAChR ligands with marked effects on cognition but devoid of nicotine-like side effects, our own efforts have been concentrated on the synthesis of new compounds including elements from both acetylcholine and nicotine and characterized by a restricted conformational mobility provided by a cyclopropane ring.



Binding studies using rat membranes indicated that most of these molecules are specific ligands for $\alpha4\beta2$ nAChR *vs* muscarinic receptors and $\alpha7$ nAChR. Influence of the nature of the linker between the cyclopropane ring and the pyridine moiety, as well as the variation of the substitutions of the amino group and of the pyridine ring have been investigated. Preparation from an unique key cylopropane alcohol intermediate and pharmacological evaluation will be presented and discussed.

P176

N,N'-bis-Benzylidene-benzene-1,4-diamines and *N,N'-bis*-Benzylidene-naphthalene-1,4-diamines as SIRT2 Inhibitors

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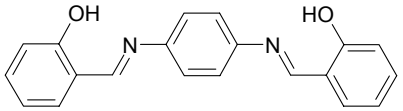
Silent Information Regulator 2 (SIR2) protein is a nicotinamide adenine dinucleotide dependent protein and it belongs to the histone deacetylase class III protein family. SIR2 is widely distributed in organisms [1]. There are seven human sirtuin type (SIRT) homologs. SIRT2 colocalizes with cytoplasmic microtubules and deacetylates lysine-40 in α-tubulin both *in vitro* and *in vivo* [2]. SIRT2 seems also to participate in the control of the mitotic exit in the cell cycle, probably by regulating the spindle microtubules.

The crystal structure of SIRT2 has been used as a starting point for molecular modeling and virtual screen-

ing. A search in the Maybridge database resulted in two hit compounds which showed high inhibitory activity for SIRT2 [3]. These compounds have structural backbones that are new for SIRT2 inhibitors. The new structural backbones were combined and a series of *N,N'-bis*-benzylidene-benzene-1,4-diamines and *N,N'-bis*-benzylidene-naphthalene-1,4-diamines were synthesized and tested *in vitro* against SIRT2 [4]. *N,N'-bis*-(2-Hydroxybenzylidene)-benzene-1,4-diamine **1** with an IC_{50} value of 58 mM was equipotent with the most potent hit compound and one of the most potent known SIRT2 inhibitors, sirtinol.



1

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[2] North BJ, Marshall BL, Borra MT, Denu JM, Verdin E. The human Sir2 ortholog, SIRT2, is an NAD^+-dependent tubulin deacetylase. Mol Cell 2003; 11: 437-444.

[3] Tervo AJ, Kyrylenko S, Niskanen P, Salminen A, Leppänen J, Nyrönen TH, Järvinen T, Poso A. An in silico approach to discovering novel inhibitors of human sirtuin type 2. J Med Chem 2004; 47: 6292-6298.

[4] Kiviranta PH, Leppänen J, Kyrylenko S, Tervo AJ, Suuronen T, Kuusisto E, Järvinen T, Salminen A, Poso A, Wallén EAA. *N,N'-bis*-Benzylidene-benzene-1,4-diamines and *N,N'-bis*-benzylidene-naphthalene-1,4-diamines as SIRT2 inhibitors. (*manuscript*)

P177

Novel Curcumin Derivatives as p300 Histone Acetyltransferases inhibitors

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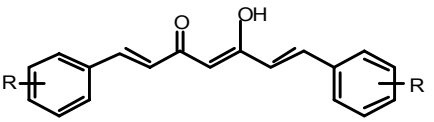
Histone acetyltransferase (HATs) are a group of enzymes that play a significant role in regulation of gene expression. The level of chromatin organization is mainly determinated by the covalent modification of histones. Histone acetyltransferase covalently modify the N-terminal lysine residues of histones by the reversibile addition of acetyl groups from acetyl-CoA[1]. Histone acetylation facilitates the access of the trascription machinery to DNA by loosening the interactions between both adjacent nucleosomes and DNA, as well as by serving as a recognition site for the recruiment of accessory regulatory factors. In addition to histones, HAT have been described to acetylate other proteins, of both cellular and viral origin, such as transcription factors and non-histone-chromatin-associated proteins. More recently, have been demostrated that the carboxy-terminus of Integrase specifically binds p300, one of the HAT family members.[2] Thus, any aberration that occurs in the HAT activity may have severe repercussions like cancer, neurodegenerative diseases and may also activate latent HIV infections. Here we describe the design, synthesis and action against p300HAT of several curcumin analogs and their related restrained cinnamoyl derivatives. Some compounds were proven potent inhibitors of p300HAT at micromolar concentrations. These derivatives are the first synthetic inhibitors of p300HAT, characterized by simple chemical structures. These results should open up new possibilities to design better chemotherapeutics in future.



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[2] Cereseto, A.; Manganaro, L.; Gutierrez, M.I.; Terreni, M.; et al.; *The EMBO Journal ,* **2005**, *24*, 3070-3081.

P178

Synthesis and Biological Evaluation of New Apicidin Derivatives as Potent Histone Deacetylase [HDAC] Inhibitors

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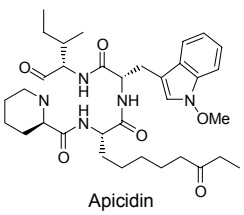
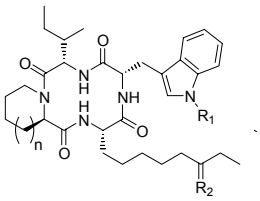
A natural product, apicidin isolated from cultures of *Fusarium Pallidoroseum*, belongs to a rare group of cyclic tetrapeptiol fungal metabolites.[1] Apicidin inhibits protozoal HADC and is orally active against *Plasmodium berghei* malaria in mice. The biological activity of apicidin appears to be apicocomplexan HDAC at low nanomolar concentrations. At present, we modified the ketone moiety of apicidin to various imine derivatives in consideration of interaction with HDAC. As part of our program toward the development to new antitumor agents, we synthesized its derivatives systemically, and then studied their structure-activity relationship.[2] We discovered that apicidin and its derivatives have a mild antitumor activity and change the morphology of tumor cells to the one of normal cells



Apicidin

n = 0, 1

R_1 = H, OMe
R_2 = O, $NNHCONH_2$, $NNHCONHNH_2$, $NNHCSNH_2$ $NNHCSNHNH_2$, $NNHCONHCH_3$, $NNHCSNHCH_3$, $NHCOCH_3$, $NHCOOCH_3$, $NHCH_2CH_2OH$ $NNHCOCH_2CH_2OH$, $NNHCSCH_2CH_2OH$, CHCN, CHCOOEt, $CHCH_2OH$



n = 0, 1

R_1 = H, OMe
R_2 = O, $NNHCONH_2$, $NNHCONHNH_2$, $NNHCSNH_2$ $NNHCSNHNH_2$, $NNHCONHCH_3$, $NNHCSNHCH_3$, $NHCOCH_3$, $NHCOOCH_3$, $NHCH_2CH_2OH$ $NNHCOCH_2CH_2OH$, $NNHCSCH_2CH_2OH$, CHCN, $CHCOOEt$, $CHCH_2OH$

In this presentation, we discuss the synthesis and biological evaluation of various apicidin derivatives. Compared to SAHA, one of the typical HDAC inhibitor at a nanomolar level, several apicidin derivatives we have developed show potent and selective activity in vitro against HeLa cell at low nanomolar concentrations.

[1] Darkin-Rattray SJ, Gurnett AM, Myers RW, Dulski PM, Crumley TM, Allocco JJ, Cannova C, Meinke PT, Colletti SL, Bednarek MA, Singh SB, Goetz MA, Dombrowski AW, Polishook JD, Schmatz D M. Apicidin : a novel antiprotozoal agent that inhibits parasite histone deacetylase.PNAS 1996,93, 13143-13147.

[2] Lee, HW, Jung, YH, Han, JW, Lee, SY, Lee, YW, Lee, HY, Zee, OP. Preparation and formulation of apicidin derivatives for use as antitumor agents. PCT Int. Appl. 2002, 24pp.

P179

Chromatin modifiers as new epigenetic regulators: protein arginine methyltransferase (PRMT) inhibitors

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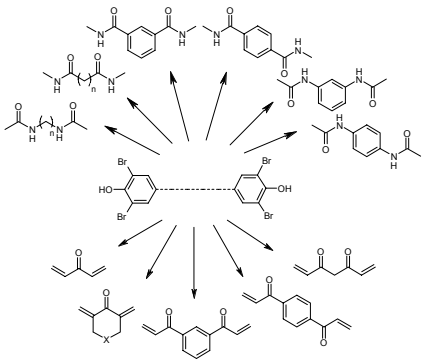
Protein Arginine *N*-Methyl Transferases (PRMTs) are implicated in a variety of processes, including nuclear receptor-regulated transcription, signal transduction, chromatin regulation, gene silencing, protein repair, and protein trafficking [1]. Among HKMTs, SET7/9 has been reported to act not only on histones, but it also methylates the tumor suppressor p53 leading to gene silencing. PRMT dimerization is essential for enzymatic activity: on the basis of these knowledges we have designed and synthesized a series of compounds bearing two 1-hydroxy-2,6-dibromophenyl moieties connected by a spacer, to obtain simmetric molecules. We have hypothized that the 1-hydroxy-2,6-dibromophenyl group could act as a pharmacophore in these molecules; in fact, the 2,6-dibromophenol moiety likely could be able to make hydrogen bond with one or both the Glu side chains (E144 and E153) contained in the active site of PRMTs giving the inactivation of enzymes [2]. These compounds have been evaluated against PRMT1, yeast RmtA, HKMTs and their inhibitory potency is in the low micromolar range.



[1] Cheng, X.; Collins, R. E.; Zhang, X. Structural and sequence motifs of protein (histone) methylation enzymes. Annu Rev Biophys Biomol Struct 2005, 34, 267-294.

[2] Zhang, X.; Cheng, X. Structure of the Predominant Protein Arginine Methyltransferase PRMT1 and Analysis of Its Binding to Substrate Peptides. Structure 2003, 11, 509-520.

P180

Non-hydroxamate HDAC inhibitors as new potent cytodifferentiating agents

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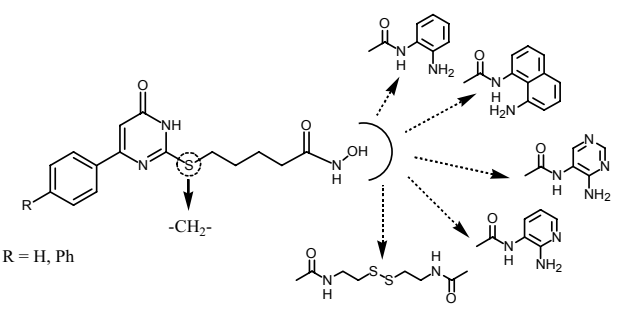
Starting from the pharmacophore model for histone deacetylase inhibitors (HDACi) design, we have recently reported a novel class of hydroxamates as (sub)nanomolar HDACi named Uracil-Based Hydroxyamides (UBHAs) [1]. Although hydroxamates are well known to provide the most effective HDAC inhibition, the limited *in vivo* efficacy due to their metabolically labile nature prompted us to look for alternative zinc binding groups suitable for use in our uracil-based template. So, we prepared a series of analogs in which the hydroxamic acid was replaced by (hetero)arylanilide and disulfide moieties. Since thioether linkage between uracil and alkyl chain *in vivo* could represent a potential metabolic instability site, we also replaced the C_2 sulphur atom with a methylene unit.



Despite these compounds exhibited a reduced efficacy in enzymatic assays, they displayed very promising cytodifferentiating and anticancer properties in U937 human leukemia cells.

[1] Mai A, Massa S, Rotili D, Pezzi R, Bottoni P, Scatena R, Meraner J, Brosch G. Exploring the connection unit in the HDAC inhibitor pharmacophore model: novel uracil-based hydroxamates. Bioorg Med Chem Lett 2005;15:4656-4661.

GPCRs

P181

Bilastine analogues: Structure and histamine H_1 receptor affinity

M. Bordell, V. Rubio, G. Canal, A. Innerárity, A. Berisa, L. Labeaga, R. Mosquera, A. Orjales

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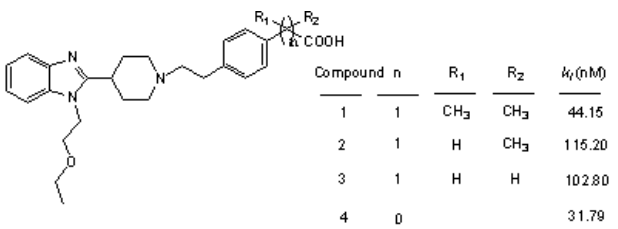
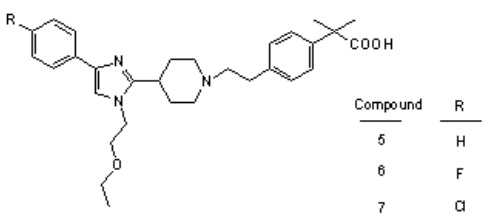
Bilastine (1) is a novel potent and selective histamine H_1 receptor antagonist [1, 2] under current clinical development for symptomatic treatment of seasonal, perennial rhinitis and chronic idiopatic urticaria. Two series of new analogues have been synthesised to determine their histamine H_1 receptor affinities in relation to bilastine and obtain more information on the structure-activity relationship in this family of benzimidazole compounds.

In one series modifications on the carboxylic fragment were carried out by diminishing the number of methyl groups and shortening the chain. In the other series the benzimidazole nucleus of bilastine was changed by an imidazole ring linked to an unsubstituted or substituted phenyl group. While compounds in the first series maintained a good affinity for the histamine H_1 receptor, compounds of the second series showed no affinity at all.

This work has been funded in part by Ministerio de Industria, Turismo y Comercio of Spain and Consejería de Industria, Comercio y Turismo of the Basque Government.



Compound	n	R_1	R_2	k_i(nM)
1	1	CH_3	CH_3	44.15
2	1	H	CH_3	115.20
3	1	H	H	102.80
4	0			31.79



Compound	R
5	H
6	F
7	Cl

[1] Orjales A, Rubio V, Bordell M. Patent US 5877187.

[2] Corcóstegui R, Labeaga L, Innerárity A, Berisa A, Orjales A. Drugs R D 2005; 6 (6): 371-384.

P182

Synthesis and histamine H_1 receptor affinity of new analogues to bilastine

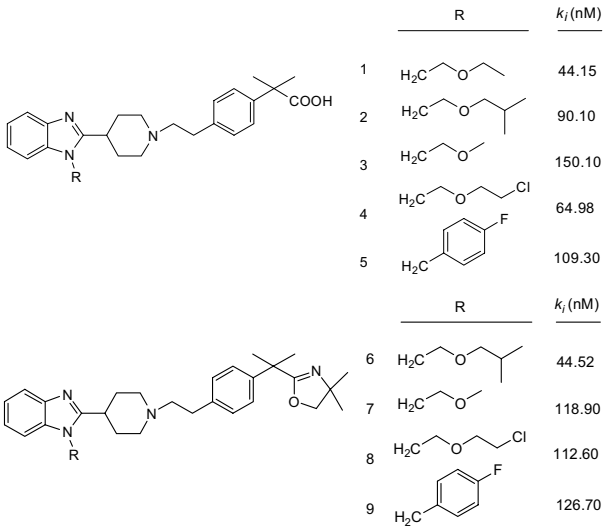
A. Orjales, V. Rubio, M. Bordell, G. Canal, A. Innerárity, A. Berisa, L. Labeaga, R. Mosquera

Department of Research, FAES FARMA, S. A. Apartado Spain

Bilastine (1) is an effective non-sedating H_1 antihistamine drug [1,2] devoid of cardiovascular effects currently in phase III clinical trials. New structural analogues have been synthesised in order to explore their pharmacological profile to compare to bilastine. Their syntheses and histamine H_1 receptor affinity values of some of these compounds (2-9) are described.

Compounds 2-5 were obtained respectively from 6-9 by acidic hydrolysis in fair yields. Synthesis of compounds 6-9 was carried out in several steps starting from 4-bromophenylacetic acid. All of them were tested to establish their affinities for the histamine H_1 receptor. Calculated K_i values indicate that they have good affinity for this receptor and are potentially useful molecules as antihistamines.

This work has been funded in part by Ministerio de Industria, Turismo y Comercio of Spain and Consejería de Industria, Comercio y Turismo of the Basque Government.



	R	k_i(nM)
1	H_2C–O–	44.15
2	H_2C–O–CH(CH$_3$)$_2$	90.10
3	H_2C–O–CH$_3$	150.10
4	H_2C–O–CH$_2$–CH$_2$–Cl	64.98
5	H_2C–(4-F-C$_6$H$_4$)	109.30

	R	k_i(nM)
6	H_2C–O–CH(CH$_3$)$_2$	44.52
7	H_2C–O–CH$_3$	118.90
8	H_2C–O–CH$_2$–CH$_2$–Cl	112.60
9	H_2C–(4-F-C$_6$H$_4$)	126.70

[1] Orjales A, Rubio V, Bordell M. US Patent 5877187.

[2] Corcóstegui R, Labeaga L, Innerárity A, Berisa A, Orjales A. Drugs R D 2005; 6 (6): 371-384.

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Estimating receptor affinity for inverse agonists