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

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GPCRs

P181

Bilastine analogues: Structure and histamine H_1 receptor affinity

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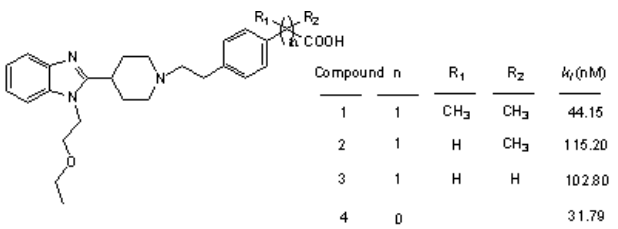
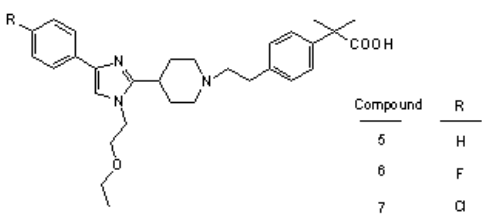
Bilastine (1) is a novel potent and selective histamine H_1 receptor antagonist [1, 2] under current clinical development for symptomatic treatment of seasonal, perennial rhinitis and chronic idiopatic urticaria. Two series of new analogues have been synthesised to determine their histamine H_1 receptor 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

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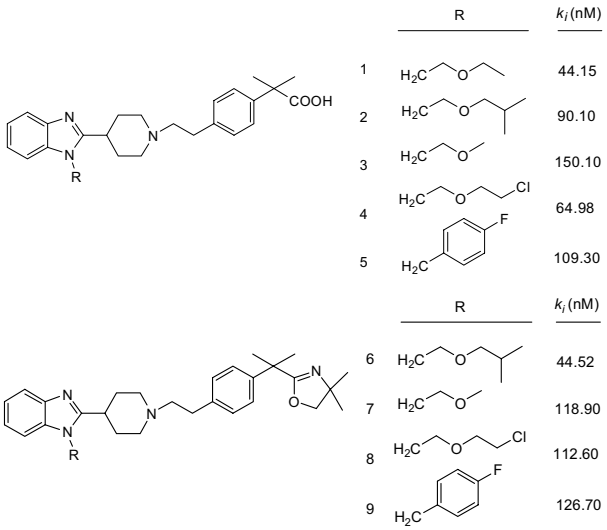
Bilastine (1) is an effective non-sedating H_1 antihistamine drug [1,2] devoid of cardiovascular effects currently in phase III clinical trials. New structural analogues have been synthesised in order to explore their pharmacological profile to compare to bilastine. Their syntheses and histamine H_1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

P183

Estimating receptor affinity for inverse agonists

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Classical methods [1,2] for the estimation of antagonist affinity constants were developed under the assumption of one unique state for the receptor. The finding of receptor constitutive activity, which implies that at least two (one active and the other inactive) receptor states coexist at equilibrium, extended the concept of antagonism by distinguishing between neutral antagonists and inverse agonists. To account for the complexity introduced in the concept of antagonism, classical Schild and Cheng-Prusoff methods are revisited within the two-state model of agonism [3,4]. The resulting [5] Schild and Cheng-Prusoff equations match the classical expressions for neutral but not for negative antagonists. In the latter case, a term depending on the concentration of the added agonist appears. The errors in affinity estimation under the Cheng-Prusoff method are greater than under the Schild analysis. Interestingly, when the agonist is a full agonist, the errors in antagonist affinity estimation under both methods are lower as greater are the concentrations of the agonist. The estimates of the antagonist-receptor dissociation constant for an inverse agonist tend to the true constant corresponding to the inactive receptor state as greater is the agonist concentration, if the Schild but not the Cheng-Prusoff equation is used. In the latter case, a term, which is dependent on the basal response, appears; the importance of this extra term increases as the level of receptor constitutive activity increases. In view of the above properties, concentrations of the agonist equal to or preferably higher than its EC50 should be used.

Because accurate measurement of antagonist potency is crucial for drug discovery, it may be concluded that routine procedures for antagonist affinity estimation on GPCRs should be revised on the light of the current knowledge of receptor theory.

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[3] Colquhoun, D. (1973) The relationship between classical and cooperative models for drug action. In A symposium on drug receptors (Rang,H.P., ed), pp. 149-182. University Park Press, Baltimore.

[4] Leff P. The two-state model of receptor activation. Trends Pharmacol Sci 1995;16:89-97.

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P184

The traffic-light controller: a three-state model for metabotropic glutamate receptors

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Metabotropic glutamate receptors (mGluRs) belong to class C G-protein-coupled receptors (GPCRs) [1]. mGluRs are composed of three main structural domains, the Venus flytrap domain (VFT) where agonists and antagonists bind, the cysteine-rich domain (CRD) that interconnects the VFT to the heptahelical domain (HD), and HD [2]. Class C GPCRs are constitutive dimers, being stabilized by a disulfide bridge in the case of mGluRs. An activation mechanism has been proposed for mGluRs, which includes three states for the VFT domain: open-open (oo), closed-open (co), and closed-closed (cc). The conformational states of VFT and HD domains are coupled. It has been suggested that in the absence of agonist, the receptor is in a resting state (Roo-HD), and switches to a partially active state upon binding of a first agonist (Aco-HD(*)), and to a fully active state upon binding of a second agonist (Acc-HD*) [3].

Mathematical models can be of prime importance in signal transduction studies for the analysis of complex mechanisms [4-7]. In this study, a mathematical model (the traffic-light controller) is constructed by including three receptor states: inactive (R), partially active (R^p), and fully active (R*). This preliminary model is redesigned by making dimeric all three states. The ability of the resulting model to represent the VFT domain is analyzed by systematic curve simulations. In particular, cooperative behaviours and biphasic curves are examined in detail.

Although the model has been developed taking the mGluRs as a reference system, it could be of general applicability to others receptors in which three states (inactive, partially active and fully active) were thought to be present.

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[4] Kenakin T. Principles: receptor theory in pharmacology. Trends Pharmacol Sci 2004;25:186-192.

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P185

A genetic algorithm for curve fitting: a possible choice for unsatisfactory gradient nonlinear regressions

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The Hill equation is likely the most used model for curve-fitting in pharmacological research [1]. However, it presents a possible drawback as it cannot account for asymmetric concentration-effect curves [2]. The Richards function solves this problem by including an additional parameter (s). Hill and Richards models are nested, being the Hill function a particular case of Richards equation if the s parameter is equal to one. A value of s different from one allows a theoretical curve to display an asymmetric shape. Regretfully, it has been found that, in a number of cases, the Richards function performs deficiently in curve fitting if gradient nonlinear regression is used [3]. The reason for that may lay in the strong correlation found between some of the parameters of the Richards model. This correlation can affect the reliability of the location, slope, and symmetry parameters yielding nonsensical values, very large errors or even failing to converge [2].

A genetic algorithm (GA) can be a useful methodology to the determination of the parameters of difficult fitting problems [4]. In particular, this technique avoids the sensitivity of local optima to the initial estimates supplied to the nonlinear regression procedure.

This study presents a GA approach for the estimation of Richards function parameters. In the cases tested, our GA provided similar or better estimates than the nonlinear regression method when the latter performed reasonably well or badly, respectively. Because the assessment of asymmetry may be important both for accurate estimation of empirical pharmacological parameters and for the mechanistic analysis of those biological systems where asymmetry is an intrinsic and relevant feature [2], our approach could be a possible choice in those situations in which gradient nonlinear regression (in particular of Richards function) is unsatisfactory.

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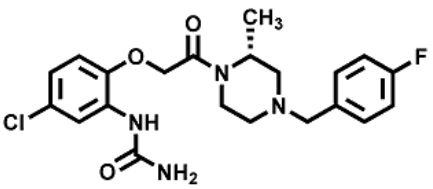
P186

Discovery of novel, orally available piperidine-containing CCr1 antagonists.

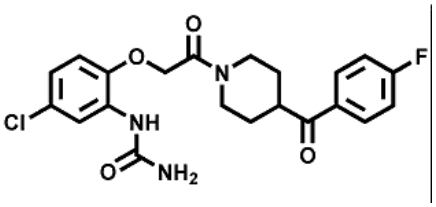
M. J. Kochanny*, S. Lu*, Y. Chou*, W. Lee*, X. Liang*, G. B. Phillips*, S. Schlyer*, R. G. Wei*, J. Xu*, H. Yu*, X, Snow Ge**, P. Harirchian**, S. Ribeiro**, B. Subramanyam**, R. Horuk***

Departments of *Chemistry, **Pharmacology and ***Immunology, Berlex Biosciences

Ligands for the CCR1 receptor (MIP-1α and RANTES) have been implicated in a number of chronic inflammatory diseases, including multiple sclerosis, rheumatoid arthritis and transplant rejection. Because these ligands share a common receptor, CCR1, we sought to discover antagonists for this receptor as an approach to treating these disorders. In the search for a follow-up to our clinical candidate BX-471, our aim was to modify the structure in order to remove the potentially labile benzyl group attached to the piperazine. These efforts led to the discovery of a new lead compound (**I**), in which the benzylpiperazine is replaced with a benzoylpiperidine. Early SAR investigation of this lead will be described and contrasted with the piperazine series. Metabolism data and pharmacokinetic profiles will be presented for selected analogs.



BX-471



P187

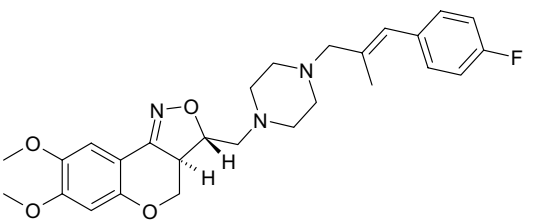
Synthesis and pharmacological activity of R226161: a potential new antidepressant that combines potent centrally and orally active serotonin reuptake inhibition and α_2-adrenoceptor antagonism

J. Andrés*, M. Bakker**, I. Biesmans**, P. Drinkenburg**, X. Langlois**, A. Megens**, T. Steckler**

Johnson & Johnson Pharmaceutical Research & Development. *Division of Janssen-Cilag, Medicinal Chemistry dept., Jarama 75, 45007 Toledo, Spain. **Division of Janssen Pharmaceutica N.V., CNS-Psychiatry dept., Turnhoutseweg 30, B2340 Beerse, Belgium

Depression is a severe mental disorder characterized by exaggerated and pervasive feelings of sadness, loss of interest and decreased energy that affect up to 10% of the population. Selective serotonin reuptake inhibitors (SSRIs) have become the standard treatment for depression. However, there are some limitations associated with the use of SSRIs including a delayed onset of action (2-4 weeks), partial treatment response (60-70%), excitation during early treatment response, nausea and sexual dysfunction. Combining serotonin (5-HT) reuptake inhibition with α_2-adrenoceptor blockade is expected to be more effective than 5-HT reuptake inhibition alone, by enhancing monoaminergic transmission in the brain.

In previous papers we have described the discovery of a new series of tricyclic isoxazolines as a new class of potential antidepressants, displaying combined 5-HT reuptake inhibition and α_2-adrenoceptor antagonistic activity [1] [2]. In this communication we will report on the identification and some preclinical data of R226161, a potent compound identified from this series that proved to be centrally and orally active for both targets, which was selected for further evaluation as a potential new antidepressant.



R226161

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[2] Andrés JI, Alcázar J, Alonso JM, De Lucas AI, Iturrino L, Biesmans I, Megens A. Synthesis of 7-amino-3a,4-dihydro-3*H*-[1]benzopyrano[4,3-*c*]isoxazole derivatives displaying combined α_2-adrenoceptor antagonistic and 5-HT reuptake inhibiting activities. *Bioorg & Med Chem* 2006; in press.

P188

Histamine H_2 receptor agonists: synthesis, pharmacological activity and brain penetration of *N*^{G}-acylated hetarylpropylguanidines

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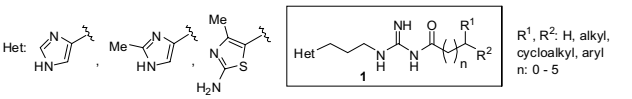
Imidazolylpropylguanidines such as arpromidine represent the most potent class of histamine H_2 receptor (H_2R) agonists known so far. Due to their strong basicity these compounds are neither orally bioavailable nor centrally active. Recently, we found that the methylene group adjacent to the guanidine can be replaced by a carbonyl group resulting in potent agonists at the guinea pig H_2R (gpH_2R). In order to further explore the structure-activity relationships, compounds of general structure **1** with branched alkanoyl and arylalkanoyl side chains were synthesized and investigated for H_2R agonism, species (human, guinea pig) and receptor selectivity (H_1, H_2, H_3, H_4), using isolated guinea pig (gp) organs and membrane preparations from Sf9 insect cells (GTPase assay) expressing the histamine receptor of interest.



N^G-(3-Phenylbutanoyl)imidazolylpropylguanidine has about 30 times the potency of histamine (gp atrium). Compared to the gpH_2R, the arpromidines as well as the corresponding acylguanidines are less potent or act as partial agonists at the human H_2R (hH_2R). Interestingly, in the acylguanidine series, H_4R agonists, H_3R antagonists and H_1R agonists were also identified. The imidazole ring can be replaced with a 2-amino-4-methylthiazole group resulting in about equipotent H_2R agonists devoid of H_3R-antagonistic activity.

Acylation lowers the basicity of the guanidines by 4-5 orders of magnitude. At physiological pH the compounds are sufficiently protonated to interact with Asp98 in TM3 of the H_2R, but a considerable portion remains uncharged. As found by HPLC-MS analysis, acylated imidazolylpropylguanidines are absorbed from the gastrointestinal tract and are capable of penetrating across the blood brain barrier in mice after systemic administration. In conclusion, the title compounds open a route to centrally active H_2R agonists which should be useful as pharmacological tools to study the role of H_2R in the brain.

P189

Design and synthesis of novel histamine H_4 receptor agonists

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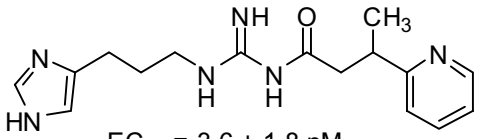
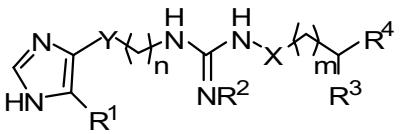
The human histamine H_4 receptor (hH_4R) is a G-protein-coupled receptor (GPCR) that is expressed in spleen, bone marrow, eosinophils and mast cells. These data suggest that the hH_4R plays a role in various inflammatory and immunological processes. Selective agonists and antagonists are required for the characterization of

the receptor and the more detailed investigation of its (patho)physiological role. In our research group N^G-acylated imidazolylpropylguanidines were synthesized as histamine H_2 receptor agonists [1-3]. Surprisingly, some of these compounds (example in Fig. 1) proved to be highly potent hH_4R agonists with nanomolar activities in the GTPase assay. This unexpected result prompted us to further explore the H_4R activities of acylguanidines and related compounds with the aim to develop selective H_4R ligands, in particular agonists, as pharmacological tools. Different analogs of these N^G-acylated imidazolylpropylguanidines were synthesized (Fig. 2) and tested in a GTPase assay for agonistic activity at the hH_4R.



EC_{50} = 3.6 ± 1.8 nM
Efficacy = 0.94 ± 0.06

Fig. 1: N^G-acylated imidazolylpropylguanidine with nanomolar activity for the hH_4R



R^1 = H, CH_3
R^2 = H, CN
R^3 = H, CH_3, Ar, Cyclohexyl
R^4 = Ar, Cyclohexyl

Y = CH_2, CH_2S
X = CH_2, C=O
n = 1, 2, 3
m = 0, 1, 2

Fig. 2: General structure of the synthesized putative hH_4R agonists

[1] Ghorai P., *PhD thesis*, University of Regensburg (2005)

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P190

Novel homo-β-proline analogues and their activities at $GABA_B$ receptors

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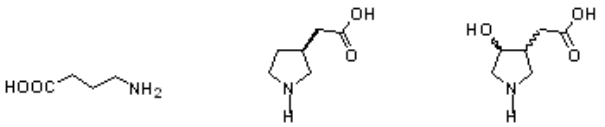
γ-Aminobutyric acid (GABA) is the most important and abundant inhibitory neurotransmitter in the brain. It maintains a balance between excitatory and inhibitory processes of cells by activating the three main subtypes of receptors: $GABA_A$, $GABA_B$ and $GABA_C$ receptors.[1,2] While GABA (**1**, Figure 1) is a structurally flexible compound that can adopt several low energy conformations upon binding at the receptors, studies of conformationally restricted GABA analogues at the receptors will provide useful information of the receptors' structure and functions. Although a number of conformationally restricted agonists and antagonists are known for the $GABA_A$ and the $GABA_C$ receptors, development of these for the $GABA_B$ receptor has been relatively slow.

(S)-(+)-*homo*-β-proline (**2**, Figure 1) a GABA analogue conformationally restrained by a pyrrolidine ring, has been shown to bind to $GABA_B$ receptors (IC_{50} = 7.8 μM), while the (R)-enantiomer is inactive at $GABA_B$ receptors (IC_{50} > 100 μM).[3] We have recently shown that the racemic (±)-*homo*-β-proline is a weak partial agonist at functional $GABA_{B1a/2}$ receptors expressed in *Xenopus* oocytes (17% of GABA (3 μM)).[4] We would now report the activities of the functionalised, enantiomerically pure β-*homo*-proline analogue, namely (3*R*)-Hydroxy-*homo*-β-proline (**3**, Figure 1).



1 2 3

Figure 1

[1] Krogsgaard-Larsen, P. *et al.*, *Eur. J. Pharmacol.*, **5**, *355*, 1997

[2] Chebib, M.; Johnston, G. A. R.; *J. Med. Chem.*, **43**, *1427*, 2000

[3] Nielsen, L. *et al.*; *J. Med. Chem.*, **33**, *71*, 1990

[4] Chebib, M. *et al.*; unpublished data

P191

Synthesis of 5-HT4 receptor ligands

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This is a continuing drug discovery project at the Department of Medicinal Chemistry, Monash University. Our aim is to discover a highly selective and safe treatment for the irritable bowel syndrome (IBS), which is a very common functional bowel condition. We are concentrating on the serotonin 5-HT4 receptor as the therapeutic target, because there is good clinical evidence that the levels of 5-HT and/or the 5-HT4 receptor are out of balance in the large intestine of IBS patients.

Currently, we have synthesized hundreds of compounds by conventional and solid phase methods. All have been designed using our two published pharmacophore models based on comparative molecular field analysis#. Some of the compounds, 50 tripeptides were synthesised using standard conditions on a PS3 automated peptide synthesiser, Protein Technology.

These peptides analogues have been screened in radioligand binding assays.

The results obtained from the radioligand binding assays and QSARs will be presented.

[1] M N Iskander , I M Coupar and D A Winkler, Investigation of 5-HT$_4$ agonist activities using molecular field analysis, *J Chem Soc Perkin Trans* 2, 153-158 (1999)

[2] Magdy N. Iskander, Lok M. Leung, Trevor Buley, Fadi Ayad, Yean Y Tan and Ian M.Coupar, Optimization of a pharmacophore model for 5-HT$_4$ agonists using CoMFA and receptor based alignment, *Euro J Medicinal Chem*, 41, 16-26, 2006.

P192

Dopamine D_2-like Receptor Ligands by Fragment-based Approach

H. Kubas[1], B. C. Sasse[1], U. R. Mach[1], J. Leppaenen[1,2], T. Calmels[3], H. Stark[1]

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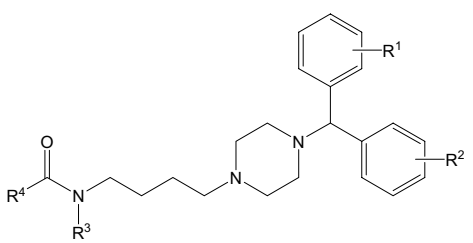
Development of potent drugs for the therapy of disorders in the central nervous system inducing minimal side effects like extrapyrimidal symptoms (EPS) is still a great challenge.

We investigated a fragment-based approach utilizing the fact that dopamine D_2-like receptor antagonists (e.g. neuroleptics) and histamine H_1 receptor antagonists show structural similarity like lipophilic/ aromatic moiety connected to a basic nitrogen atom. These relationships advise to combine them to structurally hybrid compounds expecting new interesting leads dopamine D_2-like receptors. A substructure of the well-known histamine H_1 receptor antagonist cyclizine [1] was used as rigid residue connected to arylamide moieties of several representative D_3 receptor preferring ligands like BP 897 (K_i(hD_3): 0.92 nM, K_i(hD_2)/K_i(hD_3) ratio: 66) [2]. Following to design and synthesis these new compounds were tested in a rough screening assay in order to select the most promising compounds.



The pharmacological results showed that the voluminous benzhydrylpiperazine moiety of cyclizine was well tolerated by dopamine D_2 and D_3 receptor binding sites leading to affinities in nanomolar concentration range. Selectivity could be improved by variation of the arylamide structure which led to K_i(D_2)/K_i(D_3) ratios up to 385.

By this drug-analogues fragment-based approach new structure-activity relationships could be defined enabling the finding of novel leads for dopamine D_3 receptor affine and selective ligands.

[1] Schulze, FR et al. Synthesis and Combined H_1/H_2 Antagonist Activity of Mepyramine, Pheniramine and Cyclizine Derivatives with Cyanoguanidine, Urea and Nitroethenediamine Partial Structures. Arch Pharm 1994;327:455-462.

[2] Hackling, AE et al. *N*-(*omega*-(4-(2-Methoxyphenyl)piperazin-1-yl)alkyl)carbox-amides as Dopamine D_2 and D_3 Receptor Ligands. J Med Chem 2003;46:3883-3899.

P193

Bamipine- and mianserin-based amines as dopamine D_2 and D_3 receptor ligands

O. Saur[1], B. Sasse[1], U. Mach[1], J. Leppaenen[2], T. Calmels[3], H. Stark[1]

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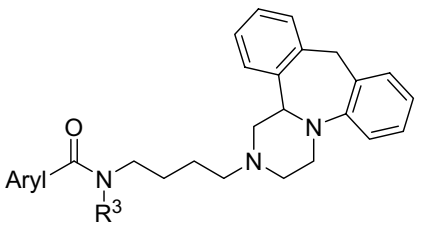
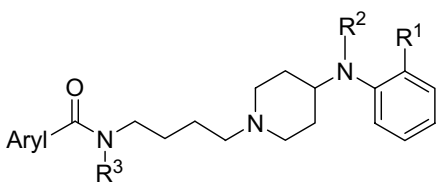
The dopamine D_3 receptor has become a promising target for antipsychotic drugs as well as for the treatment of drug abuse and Parkinson's disease [1]. Targeting high affinity and selectivity for D_3 versus D_2 receptors the partial agonist BP 897 was taken as starting lead structure [2]. Comparable structural requirements of antagonists of dopamine D_2-like receptors and histamine H_1 receptors are found in an aromatic moiety connected to an amine functionality. Since it is known that several H_1 receptor antagonists apply high affinities at D_2-like receptors we combined a dopamine D_3 receptor pharmacophore based on BP 897 with elements of H_1 receptor ligands. The 4-(2-methoxyphenyl)piperazinyl moiety of BP 897 has been replaced by substructures of the known histamine H_1 receptor antagonists mianserin (4-(2,3,4,5,10,15-hexahydro-1*H*-dibenzo[*b*:*e*]pyrazino[2,1-*g*])azepine) and bamipine (*N*-benzyl-*N*-piperidin-4-ylaniline). Further variations have been performed at the substitution pattern of the bamipine core structure and on the arylamide structure.





Several compounds have shown moderate to high affinities for dopamine hD_2 and/or hD_3 receptors with selectivity ratios >90, e.g. *N*-4-{4-[(2-methoxyphenyl)amino]piperidin-1-yl}butyl cinnamide. Although these compounds are structurally hybrids of D_3 and H_1 receptor ligands, an overlapping in the pharmacological H_1 receptor binding profile should be avoided for an improved pharmacological antipsychotic profile.

[1] AH Newman et al. Dopamine D_3 receptor partial agonists and antagonists as potential drug abuse therapeutic agents. *J Med Chem* 2005;*48*: 3663-3679. [2] AE Hackling, H Stark. Dopamine D_3 receptor ligands with antagonist properties. *ChemBioChem* 2002;*3*: 946-961.

P194

Similarity searching for selective allosteric mGluR1 antagonists using a topological atom-pair descriptor and structure-activity relationship for coumarine derivatives

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A virtual screening of a large and diverse commercial compound library for non-competitive antagonists of the metabotropic glutamate receptor 1 (mGluR1) is described. A similarity search based on a topological atom-pair descriptor [1] was applied and activity-enriched subsets were retrieved from the library. All compounds were characterized with respect to their potential to antagonize the mGluR1 receptor in both functional and binding assay. A surprisingly high overall hit rate of 26% (activity < 15μM) was obtained confirming the applicability of this method. Most of the compounds were found to be moderately active allosteric antagonists binding within the transmembrane region of the mGlu1 receptor. However, one compound has proven to be active in the nanomolar range (IC_{50}-value: 0.362 μM) based on a structurally novel scaffold (coumarine). A following optimization program of this compound facilitated the discovery of highly active and selective allosteric mGluR1 antagonists with an improved solubility profile.

[1] Schneider G, Neidhart W, Giller T, Schmid G. "Scaffold-Hopping" by Topological Pharmacophore Search: A Contribution to Virtual Screening. Angew. Chem. Int. Ed. Engl. 1999, 38, 2894-2896.

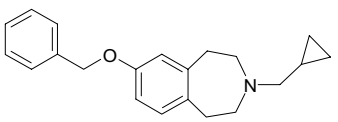
P195

A Novel Series of Histamine H_3 Receptor Antagonists

D. Dean, J. Apps, N. Bailey, M. Bamford, S. Davies, L. Harriss, A. Medhurst, T. Panchal, C. Parr, S. Sehmi, J. Steadman, A. Takle, D. Wilson, J. Witherington

*Neurology and GI Centre of Excellence for Drug Discovery,UK.

The biological effects of histamine are mediated through a family of four 7-transmembrane G-protein coupled receptors (H_1, H_2, H_3, H_4). Antagonists of the histamine H_1 and H_2 receptors, are extensively used in the treatment of allergic rhinitis and gastric acid disorders respectively. The clinical importance of the histamine H_3 receptor remains relatively unknown. Following the cloning of the receptor in 1999[1] and the discovery of non-imidazole based antagonists[2], there has been renewed interest in the target. Antagonists of the histamine H_3 receptor have been proposed as potential therapies for a number of disorders including Alzheimer's disease, schizophrenia, attention deficit hyperactivity disorder, obesity, narcolepsy and allergic rhinitis.



(I)

We identified 3-(cyclopropylmethyl)-7-[(phenylmethyl)oxy]-2,3,4,5-tetrahydro-1*H*-3-benzazepine (I) as a novel H_3 chemotype with encouraging antagonist activity at the human recombinant receptor. Optimisation of this benzazepine template to a highly potent, orally bioavailable and CNS penetrant series of antagonists suitable for further pre-clinical evaluation will be presented [3].

[1] Lovenberg, T.W. et al., *Mol. Pharmacol.,* 1999, 55, 1101.

[2] Leurs, R., Bakker, R., Timmerman, H. and Esch, I., *Nature Rev. Drug Discovery.,* 2005, 4, 107. Stark, H. *Expert Opin. Ther. Patents*., 2003, 13(6), 851. Cowart, M. et al., *Mini Rev. Med. Chem*., 2004, 4, 979.

[3] Bamford, M., Dean, D., Sehmi, S., Wilson, D. and Witherington, J. WO 2004056369.

P196

In silico screening on the homology model of the human histamine H4 receptor

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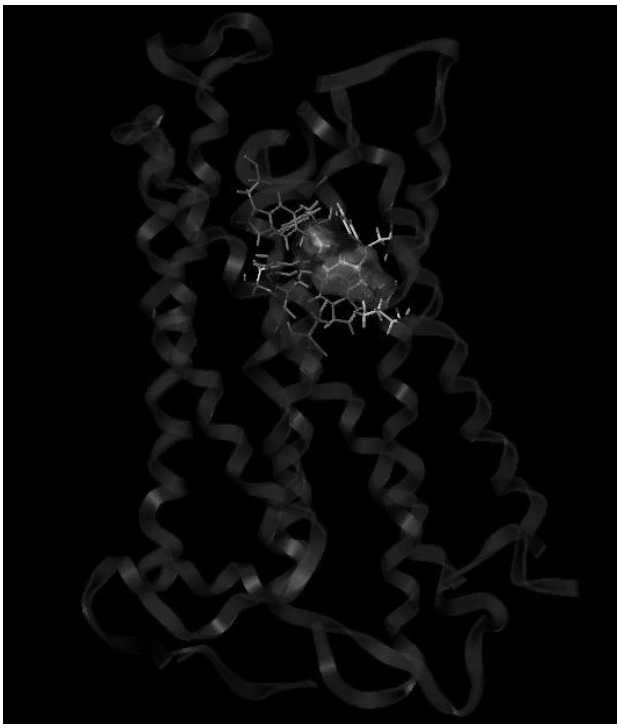
The histamine H4 receptor (H4R) is the novel member of the histamine receptor family. Experimental results indicate a major role for H4R in inflammation and allergy, therefore it seems to be an interesting target for drug design.

Three dimensional model of the human histamine H4 receptor (HH4R) was developed by means of homology modeling. Six models were build by Modeller 6.2. The quality of the models were tested by validation softwares (Procheck, Whatcheck), and available mutational data from the literature.



The binding site of histamine was mapped, and several interaction points of histamine were explored. Histamine and other known H4 agonists and antagonists were docked successfully into the homology model with FlexX.

To evaluate whether our model is suitable for selecting HH4R active compounds from a dataset containing known actives and inactives, enrichment studies were carried out with diverse and druglike sets of molecules. We used FlexX in combination with the C-Score package, and were able to get enrichment factors of about 50 with the most appropriate scoring function combination. After these encouraging results, we decided to set up the world's largest structure based in silico screening. We collected almost 8 millions of compounds from different resources. Until now, about 6 millions of compounds have been docked into our model by FlexX. The best scored compounds from the virtual high throughput screening, will be also tested in vitro.

P197

Analogues of Clobenpropit as potent histamine H_4 receptor ligands

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Information from the human genome has been used to identify the H_4 receptor, which is the latest addition to the histamine receptor family. The selective expression of the H_4 receptor on hematopoietic cells and the H_4 receptor effects on cellular function indicate that these receptors have a role in mediating either immune or inflammatory responses [1]. We have recently described that the isothiourea-based H_3 receptor ligand Clobenpropit also binds to the H_4 receptor [2]. Interestingly, Clobenpropit acts as inverse agonist at the human H_3 receptor while being a potent partial agonist at the human H_4 receptor. We have prepared a series of clobenpropit analogues to explore the structural requirements for binding and intrinsic activity at both the histamine H_3 and H_4 receptors.

[1] de Esch IJP, Thurmond RL, Jongejan A, Leurs R. The histamine H_4 receptor as a new therapeutic target for inflammation. Trends Pharmacol Sci 2005; 26: 462-469.

[2] Lim HD, van Rijn RM, Ling P, Bakker RA, Thurmond RL, Leurs R. Evaluation of histamine H_1-, H_2-, and H_3-receptor ligands at the human histamine H_4 receptor: identification of 4-methylhistamine as the first potent and selective H_4 receptor agonist. Pharmacol Exp Ther. 2005; 314: 1310-1321.

P198

Identification and Structure–Activity Relationships of a new series of CXCR3 receptor antagonists

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*UCB Celltech, Granta Park, Great Abington, United Kingdom

The chemokine receptor CXCR3 has been of interest for many years as a potential target for treatment of inflammatory diseases such as Multiple Sclerosis [1] and Rheumatoid Arthritis [2]. Blocking strategies have demonstrated efficacy in several murine disease models including arthritis, transplant rejection, asthma and inflammatory bowel disease.

For this reason, we initiated a high throughput screen and identified several structurally distinct hit compounds. One such hit compound was chosen for hit to lead development, through which potent CXCR3 antagonists with activity against both murine and human receptors were developed.

The SAR for this series and the biological evaluation of selected analogues will be discussed.

[1] Goldberg, S. H., van der Meer, P., Hesselgesser, J., Jaffer, S., Kolson, D. L., Albright, A. V., González-Scarano, F. & Lavi, E. (2001) CXCR3 expression in human central nervous system diseases. *Neuropathology & Applied Neurobiology* 27 (2), 127-138.

[2] Patel D.D.; Zachariah J.P.; Whichard L.P. (2001) CXCR3 and CCR5 Ligands in Rheumatoid Arthritis Synovium *Clin. Immunol.* 98: 39-45.

P199

Structure Activity Relationship at 5-HT_{1A} receptors within a novel series of spiro-1,3-Dioxolane-based ligands.

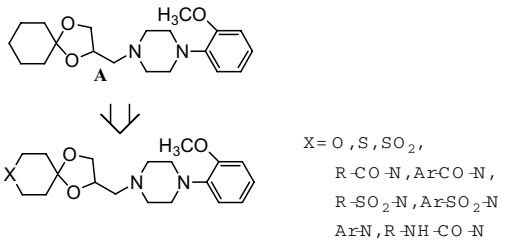
S. Franchini[1], A. Prandi[1], A. Baraldi[1], P. Angeli[2], G. Marucci[2], M. Buccioni[2], A. Leonardi[3], E. Poggesi[3], G. Motta[3], L. Brasili[1].

*Dipartimento di Scienze Farmaceutiche, Universita' di Modena e Reggio Emilia, Italy. **Dipartimento di Scienze Chimiche, Universita' degli Studi di Camerino, Italy. ***Divisione Ricerca e Sviluppo, RecordatiS.p.A., Italy.

The family of receptors that are activated by the neurotransmitter serotonin (5-HT) consists of, at least, seven types and, among them, the $5HT_{1A}$ receptors are, to date, one of the best characterized subtypes. They are involved in the pathophysiology of several neuropsychiatric disorders such as depression, anxiety, bulimia, schizophrenia, aggressive behavior in Alzheimer disease and, more recently, in Parkinson's disease.

Meanwhile in the past years many $5HT_{1A}$ agonists and partial agonist have been disclosed, antagonist at presynaptic and postsynaptic receptors are still scarce.

Recently we discovered a new class of $5\text{-}HT_{1A}$ ligands bearing a 1,3-dioxolane structure and, among them, compound **A** displayed high $5\text{-}HT_{1A}$ receptor affinity (pKi=8.29) and antagonist activity. This compound shows also some affinity to alpha1 adrenoceptor subtypes.



In order to gain new insight into the structural features required for antagonist activity and in the attempt to improve selectivity we adopted the strategy of in-parallel synthesis to rapidly explore derivatives of the lead compound A. These molecules were obtained by introducing hydrogen bond acceptors or electron acceptor/donor moieties, as depicted above. The library was designed to explore a relatively diverse groups of such derivatives taking advantage of the well-understood chemistry of amides and sulfonamides.

The pharmacological results of the newly synthesized compounds will be extensively discussed during the congress.

In Silico ADME

P200

In Silico ADME Modelling : Computational Models to Predict Human Intestinal Absorption Using Sphere Exclusion and kNN QSAR methods

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*Life Sciences R&D Division, Advanced Technology Centre,India.

Investigation of the causes of late stage failures in drug development revealed that inappropriate ADMET [Absorption, Distribution, Metabolism, Excretion and Toxicity] properties were responsible for these failures. The later these failures are identified, the higher is the cost of development. Often, the liability affecting the compounds are not identified until a compound reaches the clinic, the most expensive phase of Pharma R&D. Over the last few years, a number of in vitro and high-throughput methods have been developed to provide experimental evaluation of these properties as early and rapidly as possible . However, it is important to note that for some assays, the time, throughput or compound requirements make it impossible to make the desired measurements on all desired compounds. Consequently, the interest on in silico estimates has increased dramatically, as compared to the in vitro or in vivo counterparts. In silico models are useful to rationalize a large number of experimental observations, offer potential for virtual screening applications and consequently can help in reducing time and cost of the drug discovery and development process. The potentials of in silico ADME models have created enormous interest among researchers from pharmaceutical industry and academia and thus it stands as an area of intense research.

Human intestinal oral absorption is one of the most important ADMET properties that determines the success or failure of a drug candidate during development. Modelling of human intestinal absorption (HIA) data of 175 diverse drugs and 336 calculated descriptors is performed to develop global predictive models that are applicable to the whole medicinal chemistry space. For this aim, we employed two automated procedures a). sphere exclusion algorithm to select members of the training and tests sets based on structural dissimilarity and b). k-nearest neighbors (kNN-QSAR) method to select significant descriptors having high significance to HIA. This methodology helped us to derive optimal quantitative structure-property relationship (QSPR) models based on three and four descriptors. The best three descriptor model is based on Delta Chi Index of order 3 (Cluster), Hydrogen type E-State index ShsOH, AlogP99 [Q^2_{LOO}=0.7401, Q^2_{Ext} = 0.7989 and R^2 = 0.6102]; the best four variable model is based on Auto-correlation descriptor (Moran) weighted by Atomic weights – Order 7, AI-State_Indices_AISssssC, Number of hydrogen bond acceptors, AlogP99 [Q^2_{LOO}=0.8196, Q^2_{Ext} =0.6999 and R^2 =0.6437]. Extensive validation tests of the models and comparison of their performance with other reported models in literature suggest that they have excellent predictive power. Finally, as the models reported herein, are based on computed properties, they appear a valuable tool in virtual screening, where selection and prioritisation of candidates is required.

The presentation will discuss the predictive approaches employed by us and the scope and limitations of our models for virtual screening studies.

P201

Balancing Structural Modification and Physicochemical Properties in Lead Optimization

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*Advanced Chemistry Development UK Ltd.,England. **Advanced Chemistry Development, Inc. (ACD/Labs). Canada

In the lead optimization process the main goal is to optimize potency and selectivity of a drug candidate while simultaneously minimizing pharmacokinetic, metabolic, and other liabilities. This cannot be done in isolation and, in many cases, is at the expense of favorable ADME properties that determine successful delivery of the drug to the target site. The challenge thus becomes multi-dimensional, requiring alignment of structural modifications with molecular properties. *In-vivo* activity of a drug relies on physicochemical properties (such as solubility, log*P*, and pK_a) that determine its ease of transport through many physiological barriers such as absorption through the GI tract, penetration of the blood brain barrier, and oral bioavailability. *In-silico* tools commonly used in early hit-to-lead discovery have been further developed into a software suite to aid the lead optimization process—the software combines physicochemical property predictors with a database of substituents compiled from compounds successfully entering Phase II clinical trials. The medicinal chemist can now easily align lead optimization with these important parameters. We will discuss the application of this powerful tool on pharmaceutically relevant compounds.

P202

Prediction of Activation Energies for Hydroxylation of Drugs by Cytochromes P450

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The cytochromes P450 form a ubiquitous protein family with functions including synthesis and degradation of many physiologically important compounds, as well as degradation of xenobiotic compounds, e.g. drugs. Considering the importance of the cytochromes P450 in the metabolism of drugs, it would be highly desirable to have a method that could predict if and in what way a drug candidate will be metabolised by these enzymes. For this, a method is needed that predicts the activation energy accurately for the reaction of a drug candidate with compound I (the reactive species in the P450s reaction cycle). Such energies can be estimated by DFT calculations, but they are quite time-consuming, especially for molecules of the size of a typical drug (weeks of CPU time).

We have calculated hydroxylation activation energies for a diverse set of 24 different substrates with state-of-the-art DFT methods. We then investigate if these energies can be predicted by computationally less demanding methods. We show that the activation energies can be reproduced by calculations with small organic mimics of compound I with both DFT and the semiempirical AM1 method (within 3–4 kJ/mol). We have also tested 20 different molecular descriptors of the substrate or the corresponding radical. The best predictions are obtained with the bond dissociation energy, calculated without relaxation of the radical (DFT) or estimated from three-point fit to a Morse potential (AM1), with a mean absolute error of 4 and 5 kJ/mol respectively. However, for simple molecules, the activation energy can be predicted directly from the chemical environment of the groups with an error of only 2.5 kJ/mol.

P203

The Use of Physicochemical Properties to Predict Non-Specific Hepatic Microsomal Binding

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*Scientific Development, Simcyp Ltd, Blades Enterprise Centre, John Street, Sheffield, S2 4SU, United Kingdom. *Academic Unit of Clinical Pharmacology, University of Sheffield, Royal Hallamshire Hospital, Glossop Road, Sheffield, S10 2JF, United Kingdom

An important aspect of the extrapolation of *in vivo* drug clearance from data obtained with hepatic microsomes or those from recombinant enzyme expression systems is a knowledge of unbound drug concentrations in the *in vitro* system. The prediction of clearance can be improved considerably by accounting for the non-specific microsomal binding of substrate, expressed as the unbound drug fraction in the incubation mixture (fu_{mic}).

Here we consider the prediction of hepatic fu_{mic} from readily available physicochemical properties *viz*. acid-base-neutral class, ionisation state and octanol-buffer logP. A previous model[1] pooled data for acids, bases and neutral compounds to produce a single linear relationship between logK_{mic} (the logarithm of the microsome-buffer partition coefficient) and a mixed lipophilicity term, logP *or* logD (logP|D), chosen according to acid-base status. For ionised compounds the logP|D term clearly mixes data with different physicochemical meaning. Consequently, we have developed models relating logK_{mic} and logP separately for each compound class; logP alone was chosen having considered both the properties of octanol with respect to ionised compounds and that there exists a large public resource of reliable logP values. A large, diverse, unpublished dataset (n = 102) was collected from Simcyp Consortium members (www.simcyp.com) and supplemented with data from the literature and academia (n = 54). For each compound class a significant correlation of logK_{mic} with $logP_{o:w}$ was obtained (r^2 range: 0.68 to 0.83). The models were validated using repeated cross-validation and by making predictions for compounds unavailable at the time of model construction. The results obtained represent a significant improvement on a widely

used fu_{mic}-prediction model[1]. We also comment critically upon a model in which a squared logP|D term has been incorporated[2].

[1] Austin et al (2002) *Drug Metab Dispos* **30**: 1497;

[2] Hallifax and Houston (2006) *Drug Metab Dispos* **34**: 724.

P204

Prediction of intestinal absorption of drugs using computed physicochemical descriptors and PLS statistics

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*Centre for Drug Research, Universiti Sains Malaysia. **School of Science & Technology, Universiti Malaysia Sabah

Oral administration is a convenient approach to administer drugs. Prediction of membrane permeability of these drugs can be computed acceptably using their molecular properties [1,2].

In this study we report a fast and inexpensive way for modelling permeability using physicochemical descriptors and PLS multivariate statistics. Compounds for the training set were chosen from the previously reported data [1]. Descriptor values of LogMW, LogS, LogP, LogD2.5, LogD7.4, LogPSA, Log HBD and LogHBA were computed using an online PreADME server and used as independent parameters for PLS modelling. By employing Unscrambler statistical software (Ver 9.0), relationship between the intestinal absorption of drugs in humans and the theoretically computed molecular descriptors was defined. These models were cross-validated using a different set of drug compounds. Good statistical models were derived with acceptable RMSEP values when compared with the validation set, permit an excellent predictive ability for intestinal absorption following oral administration of drugs to humans.

[1] Palm K, Sternberg P, Luthman K, Artursson P. Polar molecular surface properties predict the intestinal absorption of drugs in humans. Pharm Res 1997; 14: 568-571.

[2] Norinder U, Osterberg T, Artursson P. Theoretical calculation and prediction of Caco-2 cell permeability using MolSurf parametrization and PLS statistics. Pharm Res 1997; 14: 1785-1790.

In Vitro ADME

P205

A rapid and sensitive HPLC method for the determination of 2-hydroxyflutamide in human plasma

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The non- steroidal pure antiandrogen, flutamide, is clinically used for the treatment of prostatic cancer [1]. Flutamide is rapidly metabolized to 2-hydroxyflutamide by first-pass metabolism in the liver [2]. The biological activity of flutamide is associated with its metabolite. Low and highly variable plasma concentrations of flutamide, resulted in monitoring 2-hydroxyflutamide levels in human plasma to study the relative bioavailability of flutamide in various drug formulations.

In this study 2-hydroxyflutamide extracted from human plasma with dichloromethane and determined by HPLC using UV detection. The chromatographic system consisted of Nova-Pak CN cartridge, an isocratic mobile phase of acetonitrile-water (37.5: 62.5, v/v) and UV detection at 300 nm.

2-Hydroxyflutamide and internal standard were eluted at about 4.2 and 5.8 min, respectively. The method was linear over the range of 10-1000 ng/ml of 2-hydroxyflutamide in plasma (r^2 > 0.9998).The within-day and between-day precision values were in the range of 1.96-6.06 %. The limit of quantification of the method was 10 ng/ml. The method was successfully applied for the study of the pharmacokinetics of flutamide in healthy volunteers.

[1] Mcleod DG, Benson RCJr, Eisenberger MA, Crawford ED, Bulmenstein BA, spicer D, Spaulding JT. The use of flutamide in hormone-refractory metastatic prostate cancer. Cancer 1993; 72: 3870-3873.

[2] Schulz M, Schmoldt A, Donn F, Becker H. The pharmacokinetics of flutamide and its major metabolites after a single oral dose and during chronic treatment. Eur J Clin Pharmacol 1988; 34 (6): 633-636.

P206

Quantitative Determination of Imatinib in Human Plasma by High Performance Liquid Chromatography-UV Detection

G. Şahin[1], Z.İ. Diler[1],R. Ceyhan[1], D. D. Erol[1]

*Faculty of Pharmacy, Yeditepe University, İstanbul, Türkiye

The aim of this study was to develop a rapid and sensitive HPLC method with UV detection for the estimation of imatinib (4-[(4-Methyl-1-piperazinyl)methyl]-N-[4-methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]benzamide methanesulfonate) from the plasma of patients with chronic myeloid leukemia (CML) or for pharmacokinetic studies.

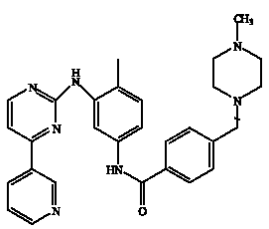

Figure: Chemical structure of imatinib

The method was developed using Agilent analytical column (Zorbax Eclipse XDB-C8) with a Waters guard column (Symmetry C18). An isocratic elution in a 0.02M potassium dihydrogen phosphate-acetonitrile mixture was adjusted to a pH of 4.50 with orto phosphoric asid as the mobile phase. The UV detection was set at 260 nm and Clozapine is used as internal standart. [1,2]The compounds were separated with a simple liquid-liquid extraction. The method was shown to be linear at a concentration range of 30-3000ng/ml for imatinib. Under these chromatographic conditions of the method, all plasma interferences were resolved from drugs.

The method is accurate, sensitive and simple for routine therapeutic drug monitoring (TDM), and for routine analysis of bioavailability and bioequivalence studies in humans.

[1] Velpandian, T., Mathur, R., Agarwal, N.T., Arora, B., Kumar, L., Gupta, S.K. Development and validation of a simple liquid chromatographic method with ultraviolet detection for the determination of imatinib in biological samples, J. Chromatogr. B, 804, 431-434, 2004.

[2] Widmer, N., Beguin, A., Rochat, B., Buclin, T., Kovacsovics, T., Duchosal, M.A., Leyvraz, S., Rosselet, A., Biollaz, J.,Decosterd, L.A., Determination of imatinib (Gleevec) in human plasma by solid-phase extraction-liquid chromatography-ultraviolet absorbance detection, J. Chromatography B, 803, 285-292, 2004.

P207

Quantitative analysis of trimetazidine in human plasma for clinical pharmacokinetic study using gas chromatography coupled to mass spectrometry

Z. M. Bah[2], N. Zakaria[2], S. Ramanathan[1], S. M. Mansor[1], Y. Hassan[3], M. N. Mordi[1]

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Trimetazidine or 1-[2,3,4-trimethoxybenzyl] piperazine dihydrochloride (TMZ) is a clinically effective antiischemic drug for the treatment of stable angina pectoris [1]. Previous method of analysis for TMZ in biological fluid is lack of sensitivity and requires complex extraction technique.

We have developed a rapid, sensitive and specific gas chromatography/mass spectrometry (GC/MS) based on a single ion monitoring and retention time locking technique to quantify TMZ using external standard method. Employing a one step extraction and derivatization approach, the analyte was extracted from plasma by liquid-liquid extraction using a mixture of hexane and dichloromethane solvent (3:1) and derivatized at the same time with isobutyl chloroformate. Chromatographic separation of the analyte was achieved using a HP-5MS capillary column with a chromatographic run time of the analyte of 8.0 min. A linear calibration curve over the range 1-100 ng ml^{-1} (r^2>0.9996) and the limit of quantification of 1 ng ml^{-1} were observed. Due to the sensitivity and the repeatability of the assay, this method is currently used for the bioequivalence study of various TMZ preparations.

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P208

Combined use of LC-SPE-NMR and LC-MS for the metabolites characterization of ATP-sensitive potassium channel openers belonging to 3-alkylamino-4*H*-1,2,4-arylthiadiazine 1,1-dioxides.

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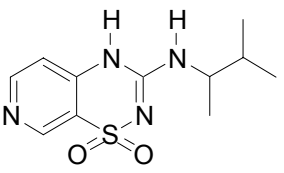
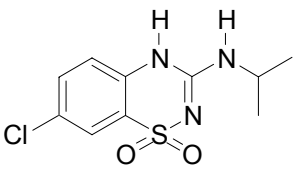
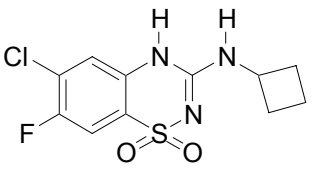
Pancreatic ATP-sensitive potassium channel openers (pancreatic PCOs) have been demonstrated to be of clinical value in the treatment of several metabolic disorders, including type I and type II diabetes, obesity and hyperinsulinemia. Taking into account these promising therapeutic interests, we developed series of 3-alkylamino-4*H*-1,2,4-arylthiadiazine 1,1-dioxides. Among those series, compounds such as BPDZ 44, 73 and 256 were demonstrated to be very potent and selective pancreatic PCOs and to be good potential drug candidates. However, knowledge of their *in vitro* metabolic fate is a fundamental requirement in the view of the future *in vivo* development and the lead optimisation process of these series.



BPDZ 44



BPDZ 73



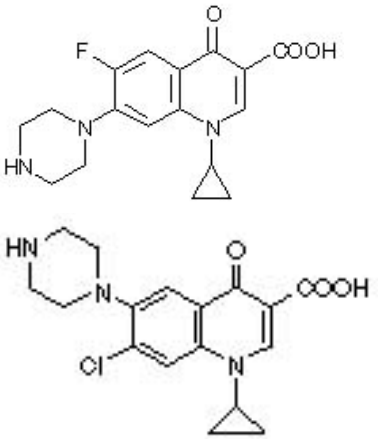
BPDZ 256

Several compounds of interest were incubated in the presence of Phenobarbital-induced rat liver microsomes. These microsomes are considered as the most active and supposed to produce the highest amounts of phase I metabolites.

The major metabolites issued from this incubation were then analysed not only by liquid chromatography (LC) coupled to a Time of flight mass spectrometer (Q-TOF MS/MS) but also by LC-SPE-NMR [a Solid Phase Extraction (SPE) system used as an interface between LC and NMR spectrometer equipped with a special flow probe].

The methodology consisted of selectivity the most appropriate MS/MS and NMR conditions and developing a LC method adapted to an efficient separation of the parent compound and its potential metabolites in order to identify the biotransformation pathways and to determine the chemical structure of the major metabolites. The SPE trapping conditions were optimized.

Even if NMR is poorly sensitive, it provides a powerful toolkit to probe the molecular structure of organic compounds. Combined use of an exact mass LC-MS/MS platform and a LC-SPE-NMR system allow the complete identification of metabolites. The metabolites of several PCOs belonging to 3-alkylamino-4*H*-1,2,4-arylthiadiazine 1,1-dioxides were fully identified and their structure completely determined using these combined hyphenated techniques.



[1] D. M. Campoli-Richards, J.P. Monk, A. Price, P. Benfield, P. A. Todd, A. Ward, Drugs 35 (1988) 373

[2] F. Hundrea, C. Grosset, J. Alary, M. Bojita, Pharmazie 52 (1997) 516

[3] M. J. Montgomery, P. M. Beringer, A. Aminimanizani, S. G. Louie, B. J. Shapiro, R. Jelliffe, M. A. Gill, Antimicrob. Agents Chemother. 44 (2000) 1674

P209

Ruggedness testing of an HPLC method for the determination of ciprofloxacin

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The possibility of optimization of an HPLC method for the determinations of ciprofloxacin and ciprofloxacin impurity C was investigated according to the British Pharmacopoeia, using ruggedness testing. Four factors were selected to be tested in this ruggedness test (temperature of the column, volume of acetonitrile in the mobile phase, volume of the aqueous phase in the mobile phase and pH of the aqueous phase in the mobile phase). Seven responses were determined in each design experiment: retention times, peak heights, peak widths, number of theoretical plates, peak areas, peak areas RSD (%) and selectivity. A three level design was used. The optimal conditions for the chromatographic procedure determined as the column temperature 42 °C and the acetonitrile to aqueous ratio in the mobile phase 14:86 (v/v). The HPLC method using the optimal conditions was tested for selectivity, linearity, precision, accuracy, limit of quantitation and limit of detection. The applicability of the suggested method was, as well, tested on the stability of ciprofloxacin in pharmaceutical preparations (tablets and infusion solutions, products of Zdravlje-Actavis, Serbia) under stress stability data.

P210

Development of a high-performance liquid chromatographic method for the determination of vardenafil in human plasma

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Vardenafil or [2-[2-ethoxy-5-(ethyl-piperazine-1-sulfonyl)-phenyl]-5-methyl-7-propyl-3H-imidazo[5,1-f][1,2,4]-triazin-4-one (Fig.1), is a new potent and highly selective inhibitor of phosphodiesterase Type V that increases blood flow to the penis during sexual stimulation and helps to restore the ability to achieve and sustain an erection in men with erectile dysfunction. Vardenafil was developed specifically to be an effective and safe oral medication for the treatment of erectile dysfunction, with potential advances over existing therapies [1-3].

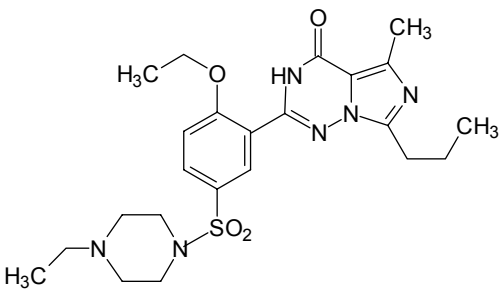

Fig.1

A sensitive high-performance liquid chromatographic (HPLC) method with ultraviolet detection (230 nm) was developed for the determination of vardenafil in human plasma. A liquid-liquid extraction procedure was performed to recover vardenafil from 1.0 mL of human plasma. A Phenomenex C_{18} column (150 x 4.6 mm I.D., 5μm) was used as stationary phase. The mobile phase used consisted of 30% acetonitrile and 70% potassium dihydrogen phosphate (70 mM; pH 4.0) at a flow-rate of 1.0 mL/min. The HPLC method has been successfully used in studies involving treatment with vardenafil.

[1] Keating G.M., Scott L.J., Vardenafil: a review of its use in erectile dysfunction. Drugs 2003; 63: 2673-2703.

[2] Kendirci M., Bivalacqua T.J., Hellstrom W. J. Vardenafil: a novel type 5 phosphodiesterase inhibitor for the treatment erectile dysfunction. Expert Opin Pharmacother 2004; 5: 923-932.

[3] Kuthe A., Phosphodiesterase 5 inhibitors in male sexual dysfunction. Curr Opin Urol 2003; 13: 405-410.

P211

Therapeutic drug monitoring: analysis of the novel antipsychotic aripiprazole in human plasma

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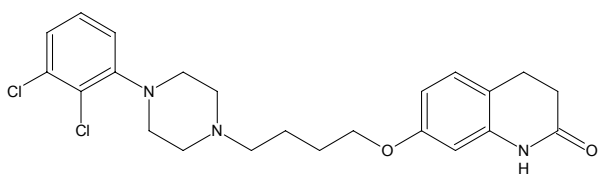
Aripiprazole (Abilify®, 7-[4-[4-(2,3-dichlorophenyl)-1-piperazinyl]butoxy]-3,4-dihydro-2(1H)-quinolinone) is one of the most recent atypical antipsichotics available on the market. Aripiprazole usual doses range from 10 to 30 mg daily with drug plasma levels between 80 and 450 ng/mL. Aim of this study is the development of analytical methods for the analysis of aripiprazole in plasma of schizophrenic patients for therapeutic drug monitoring purposes.



The first method is based on HPLC with UV detection. The stationary phase is a C8 column, while the mobile phase is a mixture of phosphate buffer and acetonitrile (65/35). Using haloperidol as the Internal Standard a complete chromatographic run lasts about 9 minutes. The pretreatment of plasma samples is carried out by solid-phase extraction (SPE) on cyano cartridges obtaining good extraction yield values and satisfactory sample purification. The method is now being validated and preliminary results are encouraging.

The second analytical method, based on capillary electrophoresis with diode array detection, is currently under development. Good analytical performances have been obtained using an uncoated silica capillary (50 μm I.D., 33.0 cm total length, 24.5 cm effective length) and a background electrolyte composed of phosphate buffer at pH 2.5. Under these conditions aripiprazole is detected within 4 minutes at 217 nm. Loxapine is used as the internal standard. Assays are in progress in order to implement a suitable sample pretreatment procedure for this method.

P212

Maintenance treatment with buprenorphine: HPLC determination of buprenorphine and its main metabolite in plasma of opiate addicted patients

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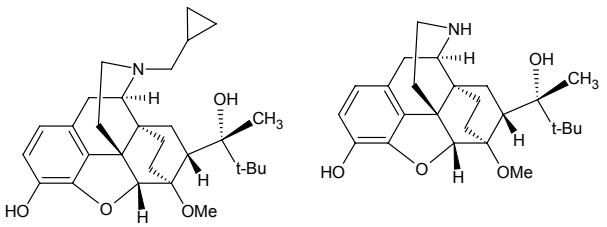
Buprenorphine [(αS,5α,7α)-17-(cyclopropylmethyl)-α-(1,1-dimethylethyl)-4,5-epoxy-18,19-dihydro-3-hydroxy-6-methoxy-α-methyl-6,14-ethenomorphinan-7-methanol] is a recent semi-synthetic opioid with mixed agonist and antagonist effects. It is used in the treatment of pain and for abstinence from illicit opioids. Due to its high potency, long half-life and long duration of action it can be administered every other day at doses up to 40 mg; the main active metabolite is norbuprenorphine.



Therefore, the simultaneous determination of both compounds is useful for therapeutic monitoring purposes. Aim of this study is to develop an original HPLC-DAD method to carry out a reliable determination of the analytes in human plasma. The method uses a C8 (250x4.6 mm, 5 μm) reversed-phase column as the stationary phase and a phosphate buffer / methanol / acetonitrile (40/10/50) mixture as the mobile phase (flow rate of 1 mL/min and injection by a 50-μL loop).

For analytical purposes, the detection is carried out at 214 nm; DAD spectra are used to confirm peak identity and purity. Sample pretreatment is carried out by means of solid-phase extraction (SPE): 500 μL of plasma are loaded onto a C8 cartridge and the analytes are eluted with methanol. Method validation is currently in progress; the method seems to be promising for the determination

of therapeutic levels of buprenorphine and norbuprenorphine in human plasma.

P213

Structure-activity relationship of novel 4,5-diarylimidazoline derivatives regarding their antagonistic $P2X_7$ receptor activity

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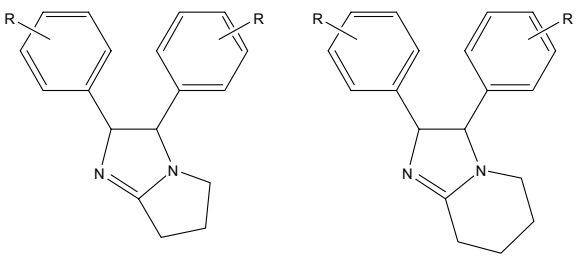
The purinergic P2 receptors comprise an ionotropic ($P2X_{1-7}$) and a metabotropic ($P2Y_{1-11}$) family [1]. One of the most interesting members of the ionotropic P2X family is the $P2X_7$ receptor. Found on a wide range of inflammatory and immunomodulatory cells, activation of the $P2X_7$ receptor by extracellular ATP leads to the processing and release of the proinflammatory cytokine interleukin-1β (IL-1β) among other cellular responses [2]. Therefore, it is implicated in a number of autoimmune and inflammatory diseases. Thus it is anticipated that antagonists of the $P2X_7$ receptor will show anti-inflammatory activity and may be of interest to therapeutic use for diseases with this component [3].

Among the very few $P2X_7$ receptor antagonists described in literature some 4,5-diarylimidazolines with various phenylalkyl residues in position 2 have been found showing inhibitory activity (Merriman et al.) [3].

Therefore, we focused our attention on a SAR study in the class of 4,5-diarylimidazolines by varying substituents in position 2,3 and 4 of the aryl residues as well as exchanging the phenylalkyl residues (Merriman et al. [3]) for cyclic substituted analogues.

The following figure shows a selection of synthesized compounds. The evaluation of the inhibitory activity is in progress



R= -H, -Cl, -F, $-OCH_3$, $-CF_3$

[1] P.G. Baraldi et al., *Synthesis of conformationally constrained analogues of KN62, a potent antagonist of the $P2X_7$-receptor*. Bioorg. Med. Chem. Lett. 10 (2000) 681-684

[2] A. Baxter et al., *Hit-to-lead studies: The discovery of potent adamantane amide $P2X_7$ receptor antagonists*. Bioorg. Med. Chem. Lett. 13 (2003) 4047-4050

[3] G.H. Merriman et al., *Synthesis and SAR of novel 4,5-diarylimidazolines as potent $P2X_7$ receptor antagonists*. Bioorg. Med. Chem. Lett. 15 (2005) 435-438

P214

Chemometric Analysis Of Capillary Electrophoretic Data In Metabonomic Studies Of Nucleosides And Modified Nucleosides In Urine

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With a growing interest in metabonomic studies, there is a need for development of robust methods for the analysis of intracellular metabolites profiles in real samples like biological fluids *e.g.* serum, urine. The concentration of different metabolites in tissues or body fluids is closely related to variations in phenotype that are relevant to human growth, development and health. Recently, urinary profiles of modified and normal nucleosides have been actively studied as cancer markers and for monitoring of the progress of neoplastic disease. In presented studies, different chemometric methods were compared for preprocessing of data from capillary electrophoretic (CE) analysis of nucleosides and modified nucleosides in urine of healthy volunteers and cancer patients. Analyzed spontaneous urine samples were collected from healthy adults and cancer patients with diagnosed kidney, prostate, bladder and testicular cancer. First, CE method for the analysis of nucleoside metabolites was developed and characterized in terms of reproducibility, linearity and sensitivity. Than, for determination of selected nucleosides in urine the established CE method was applied and appropriate metabolic profiles were collected. Different baseline correction, denoising and peak matching algorithms were used to analyze the obtained electrophoretic data. Two datasets with different level of variability were employed in order to verify the capability and efficiency of the applied pretreatment steps. Finally, principal component analysis on transformed profiles was performed to reveal the structure of data and evaluate the differences between two groups of profiles: healthy controls and cancer patients.

P215

Validation Of An HPLC And UV-VIS Method Of Quantitation Of Ketoprofene

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The aim of this work had consisted of a validated HPLC and UV/Vis. method of quantitation of Ketoprofene in a 100 mg Profenid® suppository.

The HPLC separation and proportioning was performed on a µBondarpack CI8 column (10µm); 125 A°; 3.9x300 mm using methanol (99.8%) for analysis as a mobile phase. A flow-rate of 1ml/min was used and the analyte was monitored at 254 nm. The injection volume was 20µ/l under 6 bars pressure at 25°C.

Those parameters were optimized to produce the most sensitive and reproducible results.

The standard solution was prepared by dissolving 100 mg of Ketoprofen standard in a 100ml mixture of methanol/water (80/20v/v). After shaking, 1 ml of the solution was diluted in 100 ml.

The study of the repeatability led to the following results: CV= 0,2 and 0,1 for the standard and the suppository solutions respectively.

In the other side, the study of the reproducibility had allowed to reach the following results: CV= 0,3 and 0,2 for the standard and the suppository solutions respectively.

The linear calibration curves were obtained in the range of 2.5 x 10^{-4} and 2 x 10^{-1}g/ml

The method was applied successfully to the analysis of Pharmaceutical suppository with Ketoprofen. The recoveries were 100% for peak area and 97.45% for peak height .

The coefficients of correlation were 0.9990 and 0.9966 respectively

The validation of an UV method of their proportioning was performed under 255nm.The method was simply, rapid and linear.

The coefficients of correlation was 0.9997 and 0.9988 for the standard and the suppository solutions respectively.

The average coefficients of variation of within-and between-day were: 0.22 and 0.20 for the standard and the suppository solutions respectively.

Detection limit for this active ingredient range was found to be 2.5 x 10^{-4} g/ml.

The results obtained by HPLC show a very good correlation with those obtained by U.V visible.

The proposed method is sensitive, reproducible and very suitable for the determination of Ketoprofen in pharmacokinetic studies.

[1] Aboul-Enein H.Y, Dal A.G., Tuncel M. A validated method development for ketoprofen by a flow-injection analysis with UV-detection and its application to pharmaceutical formulations. Farmaco 2003; 58:419-22

[2] Bannier A ; Brazier J.L, Ribon B. Determination of ketoprofen in plasma using high-performance liquid chromatography. Comparison with gas—liquid chromatography (author's transl)]. J. Chrom. 1978; 55:371-8.

P216

Analysis of lamotrigine and its two main metabolites in human plasma

F. Bugamelli[1], R. Mandrioli[1], A. Musenga[1], M. Amore[2], S. Fanali[3], M. A. Raggi[1]

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Lamotrigine (6-(2,3-dichlorophenyl)-1,2,4-triazine-3,5-diamine) is a recent antiepileptic drug whose mechanism of action probably involves the inhibition of brain sodium channels. It is also often used in Psychiatric Clinics during antipsychotic therapy as a mood stabiliser, at doses betwen 50 and 250 mg/day. Lamotrigine is extensively metabolised and the main metabolite is lamotrigine N2-glucuronide (which constitutes more than 60% of the excreted dose); another important metabolite, which seems to have cardiactive properties, is N2-methyl-lamotrigine. Aim of this study is the development of a reliable HPLC method with diode array detection (DAD) for determining lamotrigine and its two metabolites in plasma of schizophrenic patients. The stationary phase is a C8 (150x4.6 mm I.D., 5 µm) reversed phase column and the mobile phase is a phosphate buffer / methanol mixture (68/32) containing triethylamine. Melatonin is used as the internal standard. The DAD detector is set at 220 nm for lamotrigine, the methylated metabolite and the internal standard and at 270 nm for the glucuronide. The plasma sample pretreatment is carried out by means of a two-step purification procedure. Firstly, the sample is subjected to protein precipitation with methanol; then, the supernatant is subjected to solid-phase extraction on C2 cartridges after eliminating methanol. Preliminary results indicate that the method allows to obtain high extraction yield values and good precision.

Ionotropic Neurotransmitter Receptors

P217

2,3-Benzodiazepine-based noncompetitive AMPAR antagonists: design, synthesis, binding affinity and mechanism of inhibition of the GluR2Q$_{flip}$ homomeric receptor channel

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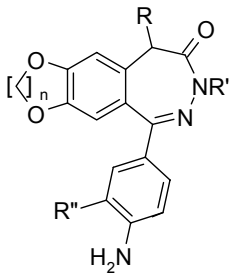
AMPA receptors (AMPARs) are a group of glutamatergic receptors that are implicated as causative in the pathogenesis of numerous acute and chronic neurological disorders. There are a number of pharmacological agents that affect AMPAR function through interactions outside of the agonist-binding domain. Among them are 2,3-benzodiazepine noncompetitive AMPAR antagonists which demonstrated significant anticonvulsant and neuroprotective action. These antagonists bind at the interface between the S1 and S2 glutamate binding core and channel transmembrane domains, specifically interacting with S1-M1 and S2-M4 linkers, thereby disrupting the transduction of agonist binding into channel opening [1].

In our ongoing studies aimed at identifying AMPAR antagonists, we synthesized a number of 4*H*-2,3-benzodiazepin-4-ones, which display high affinity at AMPARs. Recently, we evaluated the effect of the introduction of a substituent at C-5 of the diazepine nucleus and demonstrated an enantioselective interaction of these compounds with the noncompetitive binding site of the AMPARs [2].

Taking into account these interesting results, we synthesized a number of new derivatives in order to make an overview on this class of 2,3-benzodiazepines as AMPAR antagonists.



The involvement of AMPARs has been assessed by means of binding assay. Furthermore, we report the kinetic investigation of the mechanism of inhibition of the $GluR2Q_{flip}$ AMPA receptor channel by the prototype of our class of 2,3-benzodiazepine derivatives.

[1] Balannik V, Menniti FS, Paternain AV, Lerma J, Stern-Bach Y. Molecular mechanism of AMPA receptor noncompetitive antagonists. Neuron 2005;48:279-288

[2] Zappalà M, Postorino G, Micale N, Caccamese S, Parrinello N, Roda G, Menniti FS, Ferreri G, De Sarro G, Grasso S. Chiral resolution and enantiopharmacology of a potent 2,3-benzodiazepine as noncompetitive AMPA receptor antagonist. J Med Chem 2006;49:575-581.

P218

Solid Phase Synthesis of Polyamine Spider Toxins JSTX-3 and ArgTX-636 and Their Analogues

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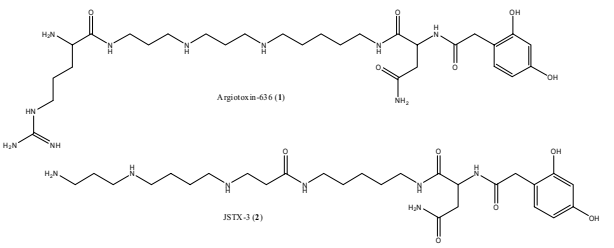
Polyamine spider toxins represent a diverse class of neuroactive compounds, including Argiotoxin-636 (**1**), and Joro spider toxin JSTX-3 (**2**). These compounds non-competitively antagonize Ca^{2+}-permeable ionotropic Glutamate receptors (iGluRs)[1]. Their high specificity and potency lend them to be excellent lead compounds for the design of neuroprotective agents. To this end, we developed the first synthesis of polyamine spider toxins ArgTX-636 and JSTX-3 and their analogues on solid phase. The synthesis incorporated a variety of solid- and solution-phase techniques including reverse peptide couplings (NàC direction) and selective amine alkylation strategies. We were thus able to examine the role of the individual functionality within the polyamine and peptide portions of these compounds.



This allowed us to build a small library of functionalized polyamines, enabling the elucidation of the structure-activity relationship for this ever-important class of substances. Reactions were optimized to develop a robust, routine synthesis of these compounds and to exploit them for their full potential in the synthesis of a diverse class of potential iGluR antagonists.

[1] Strømgaard, K. and I. Mellor, *AMPA Receptor Ligands: Synthetic and Pharmacological Studies of Polyamines and PolyamineToxins.* Medicinal Research Reviews, 2004. **24**(5): p. 589-620.

P219

Synthesis and Pharmacological Profile at Glutamic Acid Receptors of the Enantiopure Forms of *Erythro*- and *Threo*-Tricholomic acid

A. Pinto,[1] P. Conti,[1] C. De Micheli,[1] G. Roda,[1] L. Tamborini,[1]U. Madsen,[2] H. B.Osborne,[2] M. De Amici[1]

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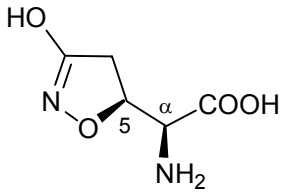
The conformational rigidification of the endogenous ligand (*S*)-glutamic acid (Glu) is an efficient strategy to achieve selectivity towards the different Glu receptor subtypes. Numerous structurally rigidified amino acids acting as Glu receptors ligands are natural compounds, among them kainic acid, domoic acid, ibotenic acid, and tricholomic acid.

Erythro-L-tricholomic acid is a flycidal substance produced by different species of mushrooms, e.g. *Tricholoma muscarium*, *Amanita strobiliformis* and *Ustilago maydis.* Its biological activity, evaluated on rat cortical neurones and on giant neurones of an African giant snail turned out to be similar to that displayed by Glu. However, since these studies were performed about 30 years ago, the pharmacological profile of tricholomic acid at the different ionotropic and metabotropic Glu receptors was never evaluated. To this end, we tackled the synthesis of tricholomic acid [(5*S,* αS)-**1a**], and its stereoisomers (5*R,*αR)-**1a**, (5*R,*αS)-**1b**, and (5S,αR)-**1b**.

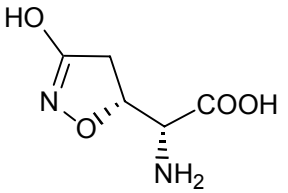
The two isomers with (*R*)- configuration at the α-amino acidic center, i.e. (–)-**1a** and (+)-**1b**, were fully selective for the NMDA receptor; in particular, (–)-**1a** displayed a relevant affinity in the binding assay (K_i = 0.60

μM). The two isomers having (*S*)- configuration at the α-amino acidic center, i.e. (+)-**1a** and (–)-**1b**, interacted with different iGlu as well as mGlu receptor subtypes. Nevertheless, the natural tricholomic acid [(5*S*,α*S*)-**1a**] displayed a 40-50 times higher affinity for AMPA-KA vs NMDA receptors, and it showed only a marginal agonist activity at mGluR1 (EC_{50} = 610 μM).

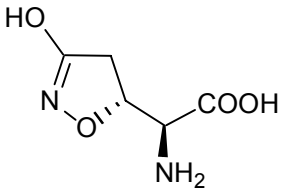


Tricholomic acid

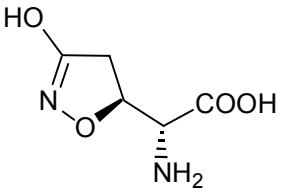
(+)-(5*S*,α*S*)-**1a**



(–)-(5*R*,α*R*)-**1a**



(–)-(5*R*,α*S*)-**1b**



(+)-(5*S*,α*R*)-**1b**

P220

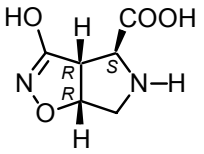
Enantiomeric Forms of (±)-HIP-A and (±)-HIP-B: Synthesis and Evaluation of their Activity at the Excitatory Amino Acid Transporters

L. Tamborini,[1] S. Colleoni,[1] A. Pinto,[1] P. Conti,[1] M. De Amici,[1] M. Gobbi,[2] T. Mennini,[2] C. De Micheli[1]

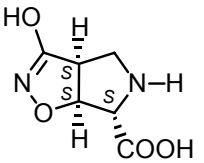
*Istituto di Chimica Farmaceutica e Tossicologica, University of Milano, Viale Abruzzi, Milano, Italy. **Istituto di Ricerche Farmacologiche "Mario Negri",Via Eritrea, Milano, Italy

In a previous study we investigated the affinity/activity profiles at glutamate receptor subtypes of two conformationally constrained aspartate and glutamate analogs, (±)-HIP-A and (±)-HIP-B. The two bicyclic amino acids displayed low affinity for AMPA and Kainic acid receptors and were inactive at NMDA and metabotropic glutamate receptors, either as agonists or antagonists [1]. More recently, we characterized the interaction of the same compounds with the excitatory amino acid transporters (EAATs) in rat brain cortical synaptosomes [2]. The two racemic amino acids behaved as potent and noncompetitive inhibitors of [^{3}H]L-glutamate uptake with IC_{50} values (17-18 μM) close to that of the potent EAAT inhibitor DL-*threo*-β-benzyloxyaspartic acid (TBOA: IC_{50} = 14.5 μM). Based on these results, we prepared and tested the two enantiomeric pairs of (±)-HIP-A and (±)-HIP-B.

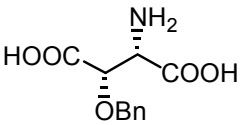
Inhibition of the [^{3}H]L-glutamate uptake in rat brain cortical synaptosomes is mainly due to (–)-HIP-A (IC_{50} = 4.0 μM) and (+)-HIP-B (IC_{50} = 8.7 μM). Noticeably, both eutomers are characterized by the (S) configuration at the amino acidic stereogenic center. By comparing the above-reported results with those of the corresponding distomers (+)-HIP-A and (–)-HIP-B (IC_{50} > 1 mM and 68 μM, respectively), it emerges a remarkably higher degree of enantioselectivity of HIP-A over HIP-B, i.e. a ratio >250 versus 8.



(±)-**HIP-A** (*R**,*R**,*S**)
(–)-**HIP-A** (*R*,*R*,*S*)
(+)-**HIP-A** (*S*,*S*,*R*)



(±)-**HIP-B** (*S**,*S**,*S**)
(+)-**HIP-B** (*S*,*S*,*S*)
(–)-**HIP-B** (*R*,*R*,*R*)



(±)-**TBOA**

[1] Conti, P. et al. *J. Med. Chem.* **1999**, *42*, 4099-4107.

[2] Funicello, M. et al. *Mol. Pharmacol.* **2004**, *66*, 522-529.

P221

Efficacy of 8-chloro-substituted 2,3-benzodiazepine AMPA receptor antagonists

J. Barkóczy, I. Ling, Z. Greff, G. Gigler, A. Simó, K. Móricz, M. Ágoston, M. Végh, G. Kapus, S. Kertész, G. Lévay, L.G. Hársing, Jr., G. Szénási, G. Simig

EGIS Pharmaceuticals Ltd., 1475 Budapest, POB. 100, Hungary

Excessive glutamate receptor activation due to ischaemia or trauma leads to necrotic and apoptotic neuronal death (excitotoxicity). 2,3-Benzodiazepines with a 4-amino-phenyl moiety are selective, non-competitive antagonists at glutamatergic AMPA (2-amino-3[3-hydroxy-5-methyl-4-isoxazolyl] propionic acid) receptors possessing good anticonvulsant and neuroprotective activity. We have shown recently that hindering N-acetylation by introducing a methyl group ortho to the amino group on the 4-amino-phenyl moiety improved metabolic stability of 2,3-benzodiazepines. Our current goal was to evaluate the efficacy of 8-chloro-substituted 2,3-benzodiazepines in comparison with that of their 7,8-methylenedioxy parent compounds. In general, 8-chloro-substition resulted in a twofold decrease in AMPA antagonist activity (range 1,3-2,5) as assessed by measuring kainate-evoked whole cell AMPA current in cultured rat telencephalon neurons and AMPA-induced spreading depression in the chicken retina, *in vitro*. As a result, 8-chloro-substited compounds were less effective than 7,8-

methylenedioxy parent compounds in maximal electroshock and audiogenic seizure models in mice. On the contrary, 8-chloro agents protected against neuronal loss at similar or lower doses. EGIS-11229, the best 8-chloro-substituted compound decreased pyramidal cell necrosis in the CA1 area of the hippocampus by about 50 % at 10 mg/kg i.p. in Mongolian gerbils and at 5 mg/kg i.p. in rats at 4 days after global cerebral ischemia, while EGIS-10608, its 7,8-methylenedioxy counterpart produced similar effects only at 20 mg/kg i.p. EGIS-11229 reduced the volume of cerebral infarction with a minimum effective dose (MED) of 0.3 mg/kg i.p. after permanent middle cerebral artery occlusion in rats, whereas MED of EGIS-10608 was above 10 mg/kg i.p. In conclusion, 8-chloro-substition in 2,3-benzodiazepines seems to alter subtype selectivity at AMPA receptors resulting in a small reduction in AMPA receptor and anticonvulsant activities but resulting in an improved neuroprotective action.

P222

Tetrahydroisoquinoliniums as potential SK channel blockers: synthesis and radioligand binding studies

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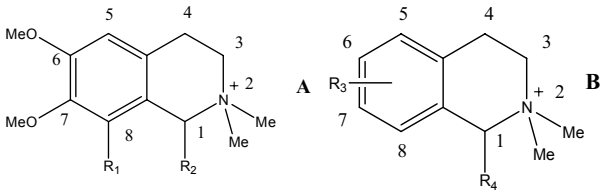
[1]Laboratory of Medicinal Chemistry, Drugs Research Center, University of Liège (ULg), B-4000 Liège 1, Belgium. [2]Laboratory of Pharmacology, Research Center for Cellular and Molecular Neurobiology, University of Liège (ULg), B-4000 Liège 1, Belgium

Neuronal action potentials are followed by an afterhyperpolarization (AHP) which is mediated by small conductance Ca^{2+}-activated K^+ channels (SK channels or KCa2 channels). AHP plays an important role in regulating neuronal excitability and agents modulating AHP amplitude have a potential therapeutic interest. N-methyllaudanosine (**NML**) shows reversible blocking properties for SK channels [1]. Therefore, the synthesis and binding evaluation of compounds related to **NML** scaffold were performed in order to find more potent isoquinolinium derivatives.



R1= H, MeO
R2= n-Pr, iBu, 3,4-diMeObenzyl, 3,4-diMeOphenethyl
NML: R1= H and R2=3,4-diMeOBenzyl

R3= 5-Br, 5-Cl, 8-Br, 8-Cl, 8-Me,8-Et,8-iPr, 8-MeO
R4= 3,4-diMeObenzyl

Compounds **A** were obtained by the Bischler-Napieralski synthesis and compounds **B** were prepared by using Reissert compound pathway. The 3,4-dimethoxy-benzyl group is the most effective fragment in C-1 position (**A**). Derivatives with an alkyl group in C-1 position (**A**) have no affinity [2]. Halogenated derivatives (**B**) possess a low affinity. The presence of a bulky alkyl group in C-8 position (**B**) is very favourable for the affinity of the isoquinolinium derivatives [3]. 8-Methoxy-NML (**A**) is two times more potent than **NML** (Ki = 1.5 µM) but the 8-methoxy derivative (**B**) with a 3,4-dimethoxybenzyl group in C-1 is totally inactive. So C-1 and C-8 positions appear to be of interest for further investigations.

[1] Scuvée-Moreau J, Liégeois JF, Massotte L, Seutin V. J Pharmacol Exp Ther 2002; 302: 1176-1183

[2] Graulich A, Scuvée -Moreau J, Seutin V, Liégeois JF. Bioorg Med Chem 2005; 13: 1201-1209

[3] Graulich A, Scuvée -Moreau J, Seutin V, Liégeois JF. J Med Chem 2005; 48: 4972-7982

Acknowledgments: AG is Research Fellow of the “Fonds pour la formation à la Recherche Industrielle et Agricole (FRIA)” and JFL is Senior Research Associate of the “Fonds Nationnal de la Recherche Scientifique de Belgique (FNRS) ”. This work was financially supported in part by FNRS and ULg.

P223

Synthesis and pharmacological activity of novel α-conotoxin analogues containing substituted proline derivatives

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α-Conotoxins are isolated from the venom of carnivorous marine snails and are highly potent and selective inhibitors for different subtypes of nicotinic acetylcholine receptors (nAChR). They are characterised by the presence of two disulfide bonds that gives rise to two loops of intervening amino acids and a single conserved proline residue in loop 1, which contributes to a very rigid and well defined three dimensional structure.

Acetylcholine binding protein (AChBP) shares sequence homology with the ligand binding domain of nAChRs and provides a valuable tool for the rational design of novel conotoxin analogues with improved pharmacological properties. Recent crystal structures of α-conotoxins ImI and PnIA complexed with AChBP (1,2) shows that upon ligand binding, the conserved proline in loop 1 is brought into close proximity to a hydrophobic binding pocket consisting of three aromatic residues. Therefore substituting the 4-position of the conserved proline with various substituents may lead to the development of potent new nAChR ligands with novel pharmacological properties, without compromising the native conformation.

We have synthesised a series of analogues of α-conotoxins ImI, AuIB and GID, with hydroxyl, phenyl,

guanidino, and *quaternary*-amino substituents on the 4-*cis* and *trans* positions of the conserved proline. The peptides were assembled in parallel using Fmoc-solid phase peptide synthesis and the correct disulfide bond isomers formed by air mediated oxidation in aqueous buffer following cleavage from the resin with TFA. The compounds were investigated at various subtypes of nAChRs and the shown to possess unique pharmacology.

[1] Celie, P. H. N., Kasheverov, I. E., Mordintsev, D. Y., Hogg, R. C., van Nierop, P., van Elk, R., van Rossum-Fikkert, S. E., Zhmak, M. N., Bertrand, D., Tsetlin, V., Sixma, T. K., and Smit, A. B. (2005) Nature Struc. Mol. Biol. 12, 582-588

[2] Hansen, S. B., Sulzenbacher, G., Huxford, T., Marchot, P., Taylor, P., and Bourne, Y. (2005) EMBO J. 24, 3635-3646

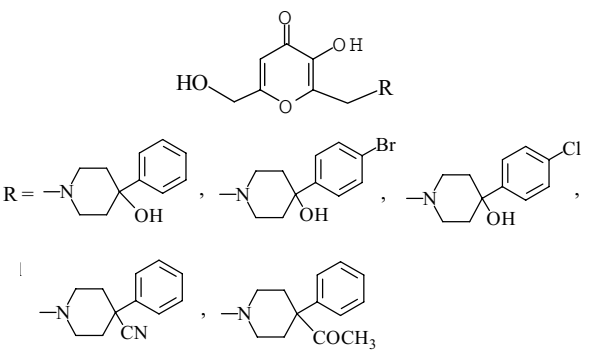
P224

Synthesis and anticonvulsant activity of new kojic acid derivatives

M. Dilsiz Aytemir, E. Septioğlu, Ü. Çalýş

Hacettepe University, Faculty of Pharmacy, Pharmaceutical Chemistry Dept., 06100, Sıhhiye, Ankara, Turkey

Epilepsy one of the most frequent neurological disorders requires special medical attention. Since uncontrolled seizures and expense of significant toxic side effects are some of the main problems still associated with antiepileptic drug therapies, the development of new anticonvulsant compounds with greater specificity and fewer toxic side effects is still a highly desirous goal [1-2]. It has been reported that 3-hydroxy-6-methyl-2-substituted-4*H*-pyran-4-one derivatives have antimicrobial and anticonvulsant activities [3]. In this study, five new 3-hydroxy-6-hydroxymethyl-2-substituted-4*H*-pyran-4-one derivatives synthesized and examined their anticonvulsant activity. Mannich bases were prepared by the reaction of appropriate substituted piperazine derivatives with kojic acid and formaldehyde. The chemical structures of the compounds were proved by IR, ¹H-NMR and elemental analysis data.



Their anticonvulsant activities were examined by maximal electroshock (MES) and subcutaneous metrazol (ScMet) tests according to the ADD (Antiepileptic Drug Development) program Phase I. Neurotoxicity of the compounds was determined by rotarod toxicity test [4]. All the compounds tested against maximal electroshock and pentilentetrazole in male mice. This study is supported by Hacettepe Univ. Research Center Office (Project No: 03 02 301 001).

[1] Schmidt D, in: Eadie M J, Vajda FJE (Eds)., The search for new anticonvulsants. Handbook of experimental pharmacology: Antiepileptic drugs, Pharmacology & Therapeutics, Springer Verlag, Berlin, 1999.

[2] Cosford NDP, McDonald IA, Scweiger EJ. Recent progress in antiepileptic drug research. Ann Rep Med Chem 1998;33: 61-70.

[3] Aytemir MD, Çalış Ü, Özalp M. Synthesis and evaluation of anticonvulsant and antimicrobial activities of 3-hydroxy-6-methyl-2-substituted 4*H*-pyran-4-one derivatives. Archiv Pharm Pharm Med Chem 2004;337: 281-288.

[4] Krall RL, Penry JK, White BG, Kupferberg HJ, Swinyard EA. Antiepileptic drug development: II.Anticonvulsant drug screening. Epilepsia 1978;19:409-28.

P225

Design, Synthesis and Pharmacological Evaluation of Novel 7-Substituted 3,4-Dihydro-2*H*-1,2,4-benzothiadiazine 1,1-Dioxides acting as AMPA potentiators

P. Francotte[1], P. De Tullio[1], P. Fraikin[1], S. Counerotte[1], E. Goffin[1], L. Danober[2], P. Lestage[2], P. Renard[2], D.H. Caignard [2], B. Pirotte[1]

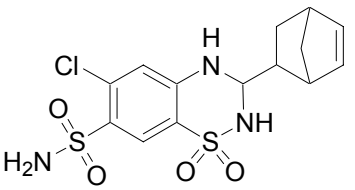
[1] Natural and Synthetic Drugs Research Center, Dept of Medicinal Chemistry, University of Liège, Av. de l'Hôpital, 1, B36, 4000 Liège, Belgique. [2] Institut de Recherches Servier, 125, Chemin de Ronde, F-78290 Croissy sur Seine, France

Amongst the ionotropic receptors responding to glutamate appears the AMPA subtype, which mediates fast excitatory transmission in the brain. It is now well established that AMPA receptors are involved in the expression and the maintenance of long-term potentiation, a phenomenon that has been closely linked to learning and memory processes. Hence the AMPA receptors are considered as an attractive target to develop cognitive enhancers.

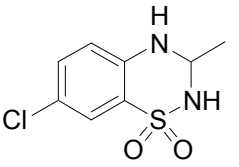
AMPA agonists were first proposed for this purpose, but were found to cause adverse effects. Positive allosteric modulators acting on AMPA receptors ("AMPA potentiators" or "AMPA PAMs") represent an interesting alternative to agonists, since they are able to only potentiate AMPA signals in the presence of the endogenous neurotransmitter. Experimental and clinical data from the last decade have proven the therapeutic interest of AMPA PAMs as cognitive enhancers. Additional works have recently highlighted their potential value as a novel approach for the management of schizophrenia or depression.

Benzothiadiazine 1,1-dioxides constitute a chemical class currently investigated for the discovery of new AMPA PAMs. Starting from the structure of cyclothiazide

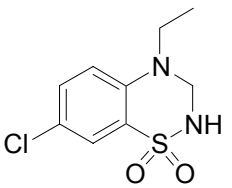
(1) and IDRA-21 (2), our team previously synthesized *in vitro* active 2,3-dihydro-4*H*- benzothiadiazine 1,1-dioxides from which emerged BPAMPA 50 (3), bearing a short alkyl chain in the 4-position.



Cyclothiazide (1)



IDRA-21 (2)



BPAMPA 50 (3)

This work presents the preparation of novel AMPA PAMs structurally related to BPAMPA50 (3) and focalises on structure-activity relationships deduced from their *in vitro* activity. Particular attention is paid to the influence of the substituent in the 7-position, although substituents at the 2- and 4-positions of the heterocycle are orientated based on the structure-activity relationships previously established by our team. Biological evaluations are realized *in vitro* on *Xenopus* oocytes expressing AMPA receptors.

P226

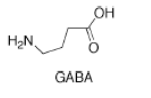
5-Substituted imidazole-4-acetic acid GABAC agonists: synthesis, modeling and site-directed mutagenesis.

B. Frølund[1], C. Madsen[1], A.A. Jensen[1], U. Kristiansen[1], M. Larsen[2], B. Bang-Andersen[2] and T. Liljefors[1].

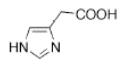
Department of Medicinal Chemistry [1], The Danish University of Pharmaceutical Sciences, Denmark and Departments of Medicinal Chemistry, H. Lundbeck A/S, Denmark [2] .

The ionotropic GABA receptors, the $GABA_A$ and the $GABA_C$ receptors, are involved in a wide variety of CNS functions and visual processing, respectively. The overall architecture of the ligand-binding site of the $GABA_A$ and the $GABA_C$ receptors seems to be very similar since most $GABA_A$ agonists seem to have some agonist/antagonist action at $GABA_C$ receptors. In order to study the structural determinants for $GABA_A$ and $GABA_C$ activation and level of efficacy, we have synthesized a series of ring-substituted analogues of imidazole-4-acetic acid (IAA), a partial $GABA_A$ and $GABA_C$ agonist.

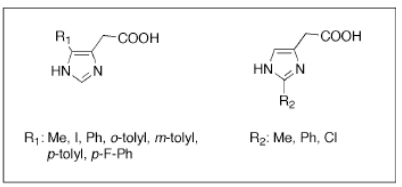
The pharmacological characterization of the new compounds was carried out using receptor-binding assays, the FLIPR Membrane Potential assay on the >1-HEK293 cell line and electrophysiological experiments using two-electrode voltage clamp on <1®3©S expressing oocytes. All of the compounds were without $GABA_A$ activity, whereas compounds containing small substituents or *p*-substituted phenyl substituents in the 5-position (R1) of the imidazole ring were shown to be equipotent or less potent (22–420 μM) than IAA (13 μM) as $GABA_C$ agonists. Interestingly, the pharmacological profile of the compounds under study has changed from partial agonism for IAA to full agonists. To unravel the observed pharmacology a mutation study was performed on the basis of docking the compounds to homology modeled structures of the $GABA_A$ and $GABA_C$ receptor ligand-binding domain.



GABA



Imidazole-4-acetic acid (IAA)



P227

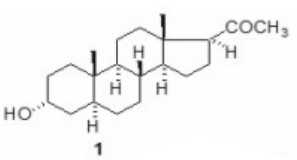
Activity of B-nor analogs of neurosteroids on the GABAA receptor in primary neuronal cultures

C. Suñol *, D. A. García*, J. Bujons**, Z. Krištofíková***, L. Matyáš***, Z. Babot*, A. Kasal***

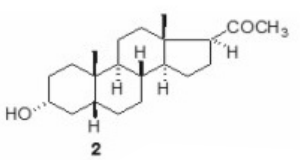
*Institute of Biomedical Research of Barcelona, Consejo Superior de Investigaciones Científicas, Barcelona, Spain. **Department of Biological Organic Chemistry, Institute of Chemical and Environmental Research of Barcelona, Consejo Superior de Investigaciones Científicas, Barcelona, Spain. ***Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Prague, Czech Republic.

Neurons using neurotransmitter receptor systems are mainly excited by glutamate and inhibited by γ-aminobutyric acid (GABA). The inhibition of the transport of signals through the neural system reduces the sensation of pain, anxiety and epileptic seizure susceptibility, and thus, compounds that potentiate the activity of GABA may be useful as analgesic, anesthetic, anxiolytic, and anticonvulsant agents. Neurosteroids such as allopregnanolone (**1**) or pregnanolone (**2**), are positive modulators of $GABA_A$ receptors with proved anticonvulsant, anesthetic, antidepressant and neuroprotectant properties.

New types of neuroactive steroids have been widely sought and structure modification of allopregnanolone has been examined in light of the vast family of GABA receptor subtypes within the brain, and in order to overcome the problems derived from its very fast metabolism.



1



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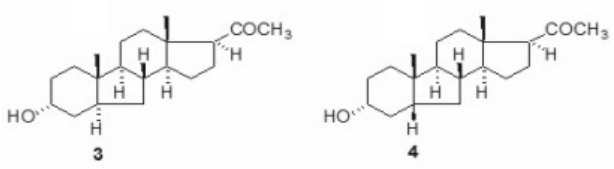


The activity of different B-nor neurosteroid analogs (*i.e.* **3** and **4**) has been evaluated by determining their effects on chloride influx and on flunitrazepam binding in intact cultured cortical neurons from mice. Computational analysis of the structures of the active and inactive neurosteroid analogs has allowed the proposal of pharmacophoric models that contain the features required for neurosteroid interaction with the $GABA_A$ receptor.

Kinases

P228

The Development p38 MAP Kinase Inhibitors: Current Understanding and Future Directions

K. Leftheris

Department of Discovery Chemistry, Bristol-Myers Squibb, USA

Inflammatory diseases such as rheumatoid arthritis (RA) result from overproduction of cytokines including TNF-α and IL-1β. These cytokines are known to be regulated by the stress-activated p38 MAP kinase pathway. Because of this, inhibition of p38 MAP kinase has been one of the most compelling targets for the treatment of inflammatory disease. Over the last 10 years, numerous groups have reported on the development of p38 MAP kinase inhibitors. X-ray co-crystallization with the enzyme suggests a receptor propensity to accommodate structurally diverse molecules. Regions of the binding site are known to be unique to p38 vs other kinases, enabling the development of p38 selective molecules. Reports of successful preclinical development with p38 inhibitors are numerous. However, few molecules to date have been successful in clinical trials. It has been suggested that several early compounds to enter the clinic may have had off target activities responsible for some of the side-effects observed. This lecture will describe the current understanding of p38 inhibitors, what is structurally required for binding the p38 enzyme as well as what is known about the current status of compounds in clinical trials.

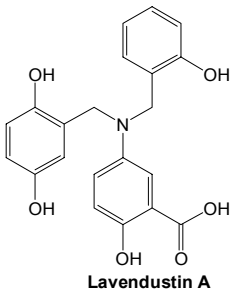
P229

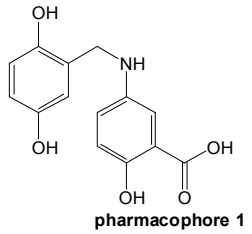
Syntheses of Substituted Lavendustin A Derivatives as New EGFR-Tyrosine Kinase Inhibitors

S. Wöge, R. Albuschat and W. Löwe

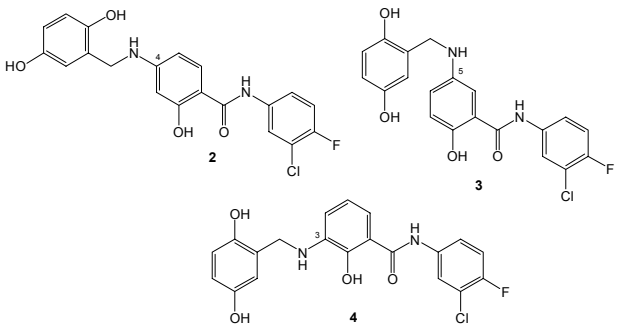
Institut für Pharmazie, Freie Universität Berlin

Investigations in the 1980s led to the use of Lavendustin A and its pharmocophore **1** as leading structures for the design of EGFR-TK-inhibitors.





We present the synthesis of the Lavendustin A derivatives **2 – 4,** which are derived from different aminosalicylic acids.



These three compounds **2 – 4** show significant EGFR-TK-inhibition activities and a high antiproliferative activity, too, which are independent of the structure of the aminosalicylic moiety.

A detailed discussion of the syntheses will be presented together with the spectral and pharmacological results.

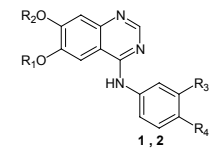
P230

Syntheses of Substituted 4-(Indol-3-yl)quinazolines, a New Class of EGFR-Tyrosine Kinase Inhibitors

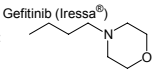
A. Lüth and W. Löwe

Freie Universität Berlin, Institut für Pharmazie. Freie Universität Berlin, Institut für Kristallographie

Earlier investigations with inhibitors of the epidermal growth factor receptor (EGFR) family of tyrosine kinases led to the highly active 4-anilinoquinazolines Gefitinib **1** (Iressa®; Astra Zeneca) and Erlotinib **2** (Tarceva®; Genentech/OSI Pharmaceuticals/Roche).



1 Gefitinib (Iressa®)

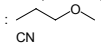
R_1 : 

R_2 : CH_3

R_3 : Cl

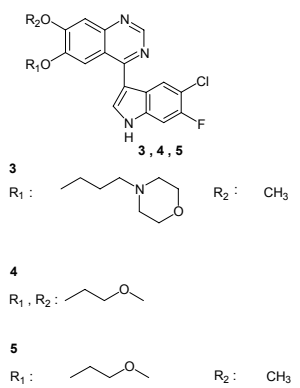
R_4 : F

2 Erlotinib (Tarceva®)

R_1 , R_2 : 

R_3 : CN

R_4 : H



We present the syntheses of the compounds **3**, **4** and **5** in which the 4-anilino-moiety is exchanged by a substituted 3-indolyl heterocycle. Compounds **3**, **4** and **5** represents a more fundamental change in the pharmacophore and claim an excellent EGFR-tyrosine kinase- and a HER-2-tyrosine kinase inhibition activity.

A detailed discussion of the synthetic results will be presented together with NMR-spectral and x-ray results.

P231

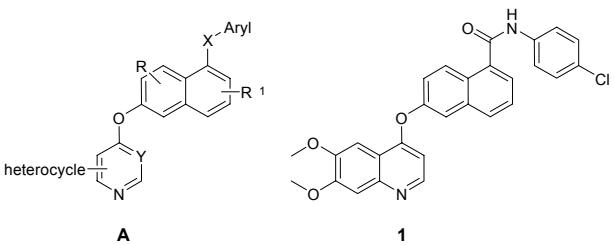
1,6-Naphthyls as a new class of potent KDR inhibitors

M. Potashman, M. Bajpai, D. Bauer, S. Bellon, G. Borg, J. Bready, D. Choquette, A. Coxon, T. DeMelfi, L. DiPietro, N. Doerr, J. Estrada, S. Flynn, J. Germain, J.C. Harmange, D. La, M. Morrison, V. Patel, S. Van Der Plas, A. Polverino, M. Potashman, D. Powers, C. Starnes, M. Weiss, D. Whittington, R. Zanon

AMGEN INC, Cambridge, MA; AMGEN INC, Thousand Oaks, CA.

Inhibition of VEGF-mediated angiogenesis has proven to be an effective therapeutic approach to treating human tumors in clinical trials. Endothelial cell proliferation and migration, two crucial steps in angiogenesis, are mediated through a specific VEGF receptor, the Kinase insert Domain containing Receptor (KDR). Inhibition of KDR kinase activity by small molecules has been shown to be a very promising way to interdict this VEGF pathway in human cancers.

Using a structure-based drug design approach, we identified a new series of KDR inhibitors. We will present the structure activity relationship for a series of 1,6-naphthyl-based KDR inhibitors (**A**). The pharmacokinetic properties and pharmacology of lead compound **1** will also be discussed.



P232

Inhibition of alpha, betal, delta, heta and zeta Protein Kinase C Isoforms by Xanthones: Improvements Towards Selectivity

M. Pinto[1], M.E. Sousa[1], R. Castanheiro[1], L. Saraiva[2,3], G. Pereira[2] and J. Gonçalves[2]

CEQOFFUP, Faculdade de Farmácia, Universidade do Porto, Rua Aníbal Cunha Portugal. [1]Serviço de Química Orgânica. [2]Serviço de Farmacologia. [3]Serviço de Microbiologia

The PKC isoenzymes are related to very important biological actions mainly in tumorigenesis. Clarification of the role of individual PKC isoforms in the cell has been difficult by the lack of selective PKC activators and inhibitors [1]. Xanthone derivatives have been reported to mediate several important biological activities, namely PKC modulation [2-4]. Molecular modifications in the xanthone scaffold were developed in order to improve potency and/or selectivity towards PKC modulation on the activators **1-2** and inhibitors **3-4** (Fig. 1).

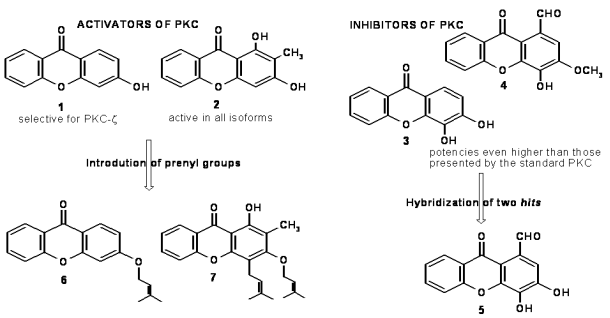


Fig. 1. Strategy for improving potency /selectivity in PKC modulators.

The modulatory activity of 1-formyl-3,4-dihydroxyxanthone (**5**) and two prenylated xanthone derivatives (**6-7**), on isoforms α, βI, δ, η and ζ of protein kinase C (PKC) was evaluated using an *in vivo* yeast assay. Xanthones **5** and **7** caused an effect compatible with PKC inhibition, similar to that elicited by known PKC inhibitors (chelerythrine and NPC 15437), and presented differences on their potency and selectivity towards the distinct isoforms tested. The present results revealed 1-hydroxy-2-methyl-4-(3-methylbut-2-enyl)-3-(3-methylbut-2-enyloxy) (**7**) xanthone as a new η-isoform-specific PKC inhibitor.

[1] Webb BLJ, Hirst SJ, Giembyez MA. Br J Pharmacol 2000;130:1433-1452.

[2] Saraiva L, Fresco P, Pinto E, Sousa E, Pinto M, Gonçalves J. Bioorg Med Chem 2003;11:1215-1225.

[3] Saraiva L, Fresco P, Pinto E, Sousa E, Pinto M, Gonçalves J. Bioorg Med Chem 2002;10:3219-3227.

[4] Saraiva L, Fresco P, Pinto E, Sousa E, Pinto M, Gonçalves J. J Enzyme Inhib Med Chem 2003;18:357-370.

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

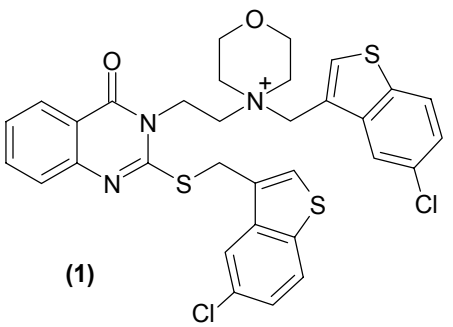
P233

Synthesis and biological profiling of novel antibiotic agents targeting UMP kinase

M. Braun, M. Bandera, R. Löwe, L. Schmitt, P. Schneider, H. Laue, S. Hawser, K. Islam

Arpida AG, Switzerland

Uridine Monophosphate Kinase (UMPK) is an essential and well-conserved enzyme in the bacterial kingdom but absent in eukaryotes. It has been shown to be essential for example in *E.coli* [1] and *S.pneumonia* [2] and could constitute an ideal target for novel antibacterial agents. We have developed a high throughput assay and screened a library of compounds which exhibited microbiological activity. This screen identified a small molecular weight compound **1** which demonstrated a potent low micromolar inhibition of UMPK from *Staphylococcus aureus*. Compound **1** was also screened for microbiological activity against a panel of Gram-positive and Gram-negative bacteria and for the potential for resistance development. The compound was microbiologically active and neither spontaneous nor induction of resistance methods resulted in mutants suggesting a low or no potential for resistance development. Based on this information we initiated the design and synthesis of focused series of analogues in order to study the SAR and SSR (structure-activity and structure-selectivity relationship).



(1)

In addition, to the inhibitory activity of these new compounds on *S.aureus* UMPK and *E.coli* UMPK enzymes we also determined the MICs (minimum inhibitory concentrations) against a panel of Gram-positive and Gram-negative bacteria. The compounds were also profiled against three different eukaryotic kinases such as the human UMP/CMP, and showed good selectivity for the bacterial enzyme. Subsequently, the compounds were further profiled using a larger panel of kinases to determine specificity [3]. The data on these compounds will be described with respect to improving the lead-likeliness as well as the activity and selectivity profile following bioisosteric functional group replacement and chemotype switching.

[1] Fassy et al. Biochem J., 2004, 384, 619-627.

[2] Yamanaka et al. J. Bact., 1992, 174, 7517-7526.

[3] P.Cohen. Nat. Rev. Drug Discovery, 2002, 1, 309-315.

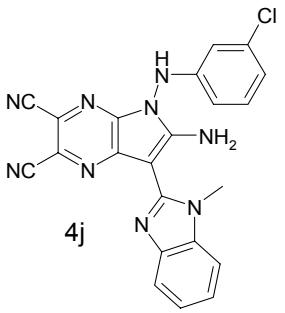
P234

Novel 5,7-Disubstituted 6-Amino-5H-Pyrrolo[3,2-b]Pyrazine-2,3-Dicarbonitriles, the Promising Protein Kinase Inhibitors with Antiproliferative Activity

G.G. Dubinina*, M.O. Platonov**, S.M. Golovach*, P.Î. Borysko**, A.Î*. Tolmachov, Y.M. Volovenko

*Kiev National university Ukraine. **Enamine ltd. Ukraine

New derivatives of pyrrolo[2,3-b]pyrazine were synthesized and tested on a panel of cultured human cancer cell lines. It was found that 6-amino-5-(3-chlorophenylamino)-7-(1-methyl-1H-benzo[d]imidazol-2-yl)-5H-pyrrolo[3,2-b]pyrazine-2,3-dicarbonitrile (**4j**) exhibited a significant antiproliferative activity: GI_{50} for cell lines RXF 393 (renal cancer) and BT-549 (breast cancer) were 14 nM and 82 nM, respectively.



4j

To identify possible molecular targets, docking of the most active compounds into the active sites of cyclin-dependent kinases was performed. Molecular modeling of the inhibitor-enzyme complexes showed the differences in the binding poses of the new pyrrolo[2,3-b]pyrazine inhibitors called aloisines (Fig.2). the patterns of drug kinase interaction correlated well with antiproliferative activity of novel derivatives. Key interactions and binding mode of docked compounds are discussed.

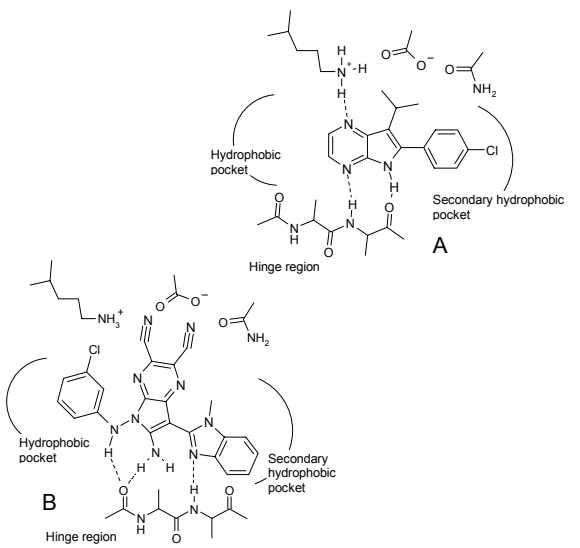


Fig. 2. Schematic representation of the binding mode of the **Aloisine B** (A) and compound **4j** (B) by the Standard Kinase Interaction Pattern

Medicinal Chemistry Teaching & Training

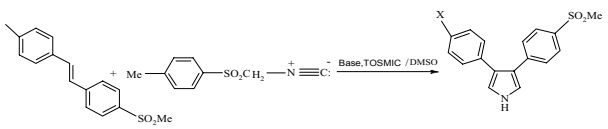
P235

New derivatives of 3, 4- diaryl-(1H)- pyrroles as potential COX-2 inhibitors and anti-inflammatory compounds

A.R. Bekhradnia[1], A. Shafiee[2]

[1]Department of Chemistry, Faculty of Pharmacy, Mazandaran University of Medical Sciences, Sari, Iran. [2]Department of Chemistry and Pharmaceutical Science Research Center, Tehran University of Medical Science, Tehran, Iran

New derivatives of 3, 4- diaryl-(1H)- pyrroles as potential COX-2 inhibitors and anti-inflammatory drugs, from tosylmethyl isocyanide and commercially available or readily synthesized arylalkenes are reported (1-2) (Scheme 1). Optimal conditions were found to be NaO *t*-Bu in dry DMSO. The methodology was particularly efficient electron poor aryl groups were attached to the alkenes (Scheme 2).



X=F, Cl, CH_3, OCH_3, H

[1] Unverferth K., Engel J., Hofgen N., Rostock A., Gunther R., Lankau H.J., Menzer M., Rolfs A., Liebscher J., Muller B., Hofmann H.J., Synthesis, Anticonvulsant Activity, and Structure-Activity Relationships of Sodium Channel Blocking 3-Aminopyrroles. J Med Chem 1998; 41: 63-73.

[2] Nicholas A., Meanwell J.L., Romine, M. J., Rosenfeld S.W., Martin A.K., Trehan J.J., Kim Wright M.F., Malley J.Z., Gougoutas C.L., Nonprostanoid prostacyclin mimetics. 5. Structure-activity relationships associated with [3-[4-(4,5-diphenyl-2-oxazolyl)-5-oxazolyl]phenoxy]acetic acid. J Med Chem 1993; 36: 3884-3903.

P236

Molecular analysis of 6-ethyl-4-(3-methylphenyl)-5-(methoxycarbonyl)-3,4-dihydropyrimidine-2(1*H*)-thione

Y. Köysal[1], Ş. Işık[1], İ.S. Zorkun[2], S. Saraç[2]

[1]Department of Physics, Faculty of Arts and Sciences, Ondokuz Mayıs University, 55139 Kurupelit, Samsun, Turkey. [2]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Hacettepe University,06100 Sıhhiye, Ankara, Turkey

In recent years dihydroprimidinones and their sulfur analogs (DHPMs) have attracted significant attention due to their diverse range of biological properties [1]. In our previous study, synthesis and calcium channel blocker activities of 4-aryl-3,4-dihydropyrimidine-2(1*H*)-thiones have been reported [2]. In this study the crystal structure of 6-ethyl-4-(3-methylphenyl)-5-(methoxycarbonyl)-3,4-dihydropyrimidine-2(1*H*)-thione ($C_{15}H_{18}N_2O_2S$) has been characterized by X-ray diffraction. The crystal is monoclinic, space group $P2_1/n$, with a= 12.965(2)Å, b= 7.3141(7)Å, c= 17.398(3)Å, â= 108.461(13)° and Z= 4. In the title molecule, C8/C9/C10/C11/N1/N2 ring system makes a dihedral angle of 88.90(18)° with the C2/C3/C4/C5/C6/C7 ring, showing that these rings are almost perpendicular to each other. The high values of the displacement parameters of the O2 and C13 atoms indicate possible disorder of these atoms. Following a sequence of refinements and difference Fourier synthesis, disordered atoms O2 and C13 were recognized in a 57(2):43(2) ratio. Their atomic displacement parameters are only slightly larger than those of the other atoms. The crystal structure is stabilized by N-H…S, N-H…O and C-H…O type hydrogen bonds.

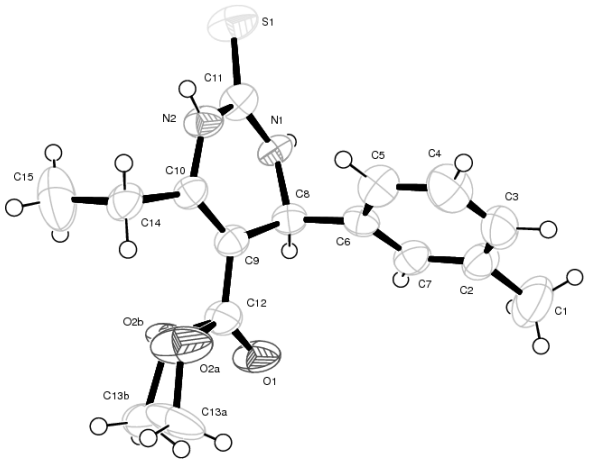


Fig. The molecular structure of title compound showing the atom-numbering scheme and displacement ellipsoids drawn at the 50% probability level. Both disordered components are shown.

[1] Kappe CO. Recent advances in the *Biginelli* dihydropyrimidine synthesis. New tricks from an old dog. Acc Chem Res 2000;33:879-888.

[2] Zorkun İS. Studies on 4-aryl-3,4-dihydropyrimidine-2(1*H*)-thione derivatives. Hacettepe University Health Sciences Institue MS Thesis in Pharmaceutical Chemistry, Ankara, 2005.

P237

Medicinal Chemistry for Undergraduate Chemistry Students

R. Sanyal

Department of Chemistry, Faculty of Arts and Sciences, Bogazici University, 34342 Bebek, Istanbul, Turkey

A unique background with a BS in Chemical Engineering and PhD in Chemistry followed by working as a medicinal chemist in a pharmaceutical company

gave me the opportunity to combine my knowledge and enthusiasm about all three fields to be able to assemble a course which is attractive to students in different departments, namely Chemistry, Biology and Chemical Engineering. Although a variety of "Medicinal Chemistry" courses are offered by Pharmacy Departments, most often chemistry or chemical engineering students are not eligible to register for them. On the other hand with the increasing number of graduates finding jobs in pharmaceutical industry, students seek to be more prepared for such a situation. A medicinal chemistry course for this purpose should involve pharmacology, organic synthesis, drug delivery and biology to understand a variety of diseases.

In this presentation two courses, one undergraduate and one graduate level Medicinal Chemistry course taught in Bogazici University Department of Chemistry will be exemplified. Material taught in the course, problems of designing such a course, multi media tools utilized during the course, students' reasons for taking such a course, their responses after taking the course and a snapshot of students' final presentations will be demonstrated.

Membran Proteins and Drug Design

P238

Integrin Modulators with a Triazine Template

K. Nadrah[1] and M.S. Dolenc[1]

[1]Faculty of Pharmacy, Ljubljana, Slovenia

Integrins are heterodimeric transmembrane glycoprotein receptors that mediate cell-cell, cell-extracellular matrix, and cell-pathogen interactions. Integrins $\alpha_{IIb}\beta_3$, $\alpha_V\beta_3$, $\alpha_V\beta_5$ and $\alpha_5\beta_1$ often collaborate in the pathogenesis of several disorders, such as cancer, cardiovascular diseases, neovascularization retinopathy and osteoporosis and are therefore interesting candidates for dual targeting [1]. Their modulators show promise in the treatment of these disorders and may be useful when incorporating biomaterials into human body, such as bone implants. Targeting only one integrin is often inadequate for significant biological effect. Modulators with dual/triple action are especially promising in such cases [3]. Design and development of new integrin modulators is largely based on the Arg-Gly-Asp (RGD) sequence recognized by these four integrins and is common to their natural ligands [2].

We chose a cycloRGDfV lead from the crystal structure of a $\alpha_V\beta_3$-cycloRGDfV complex [4][3]. The Arg, Asp and Phe group form a triangle with a basic, acidic and aromatic angles. The 1,3,5-triazine provides a similar, but a more rigid structure with the three substitution sites forming the angles of the triangle. We substituted the 1,3,5-triazine at sites 2, 4 and 6 with various groups in order to achieve similar spatial orientation of pharmacophores to the lead cycloRGDfV. The substituents were chosen to mimic the acidic and basic properties of Asp and Arg, respectively, and the aromatic nature of Phe. We designed new modulators of integrins with a triazine core and evaluated their *in vitro* biological activity on $\alpha_{IIb}\beta_3$, $\alpha_V\beta_3$, $\alpha_V\beta_5$ and $\alpha_5\beta_1$, especially their potential to act on several integrins simultaneously.

[1] Springer T.A., Wang J.-H. In Cell Surface Receptors, ed. KC Garcia. San Diego, CA: Elsevier, 2004, 68: 29.

[2] Shimaoka, M., Springer, T. A. Nat. Rev. Drug. Disc., 2003, 2 :703.

[3] Nadrah, K., Sollner Dolenc, M. Curr. Med. Chem., 2005, 12: 1449.

[4] Xiong, J., T. Stehle, R. Zhang, A. Joachimiak, M. Frech, S.L. Goodman, M.A. Arnaout, Science, 2002, 296: 151.

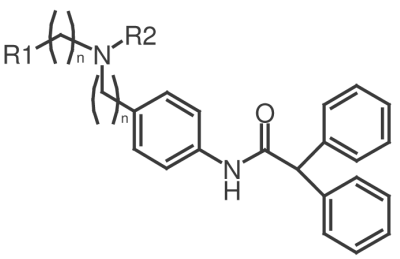
P239

Synthesis and biological evaluation of arylamide derivatives as melanin-concentrating hormone receptor 1 antagonists

Javier Ceras[1], Nuria Cirauqui[1], Silvia Galiano[1], Silvia Pérez[1], Laura Juanenea[1], Gildardo Rivera[1], Ignacio Aldana[1], Antonio Monge[1]

[1]Unidad en Investigación y Desarrollo de Medicamentos, Centro de Investigación en Farmacobiología Aplicada (CIFA), Universidad de Navarra, C/Irunlarrea, s/n, 31080 Pamplona, Spain. e-mail: silvia.galiano@gmail.com

Melanin-concentrating hormone (MCH), a cyclic nonadecapeptide, has been implicated in the control of appetite and energy balance regulation through several lines of evidence. Genetic ablation of either the MCH prohormone or MCH receptor 1 (MCH-R1) results in leanness. En adition, MCH, injected ICV, stimulates feeding acutely and chronic infusions lead to the development of obesity [1]. The anorexigenic effects of MCH-R1 antagonists molecules reported in recent years [2] support the hypothesis that MCH-R1 antagonists provides a novel target for the treatment of obesity.



1

As a part of a general proyect with the aim of searching MCH-R1 antagonists and based on the identification of a hit compound of our library, we have synthesized and evaluated for in vitro binding to 1R receptor a new serie of arylamide derivatives (**1**) in order to develop SAR. Prior hit-to lead efforts resulted in the identification of compounds with good activity.

[1] Vasudevan A, Wodka D, Verzal MK, Souers AJ, Gao J, Brodjian S, Fry D, Dayton B, Marsh KC, Hernandez LE, Ogiela CA, Collins CA, Kym PR. Synthesis and evaluation of 2-amino-8-alkoxy quinolines as MCHr1 antagonists. Part 2. Bioorg Med Chem Lett 2004;14:4879-4882.

[2] Takekawa S, Asami A, Ishihara Y, Terauchi J, Kato K, Shimomura Y, Mori M, Murakoshi H, Kato K, Suzuki N, Nishimura O, Fujino M. T-226296: a novel, orally active and selective melanin-concentrating hormone receptor antagonist. Eur J Pharm 2002;438:129-135.

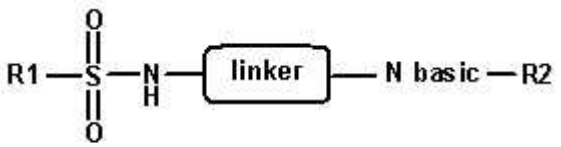
P240

Arylsulfonamide derivatives as melanin-concentrating hormone receptor 1 antagonists

S. Galiano[1], N. Cirauqui[1], J. Ceras[1], S. Pérez[1], L. Juanenea[1], G. Rivera[1], I. Aldana[1], A. Monge[1]

[1]Universidad de Navarra, Spain.

The key role of melanin-concentrating hormone (MCH) in the regulation of mammalian energy balance and feeding behavior has been confirmed by findings of lean phenotype of mice with targeted deletion of the MCH and MCH receptor 1 (MCH-R1) whereas the overexpression of the MCH-gene gives an obese and insulin resistance phenotype [1].



Arylsulfonamide derivatives

In recent years, the publication of novel selective antagonist with anorectic and antiobesity effects [2] have suggested that pharmacological blockade of MCH-R1 is a useful therapeutic approach for the treatment of obesity.

As a continuation of our research with the aim of identifying potential antiobesity drugs and based on the bibliographic antecedents, a new serie of arylsulfonamide derivatives has been synthesized and evaluated for in vitro binding to 1R receptor in order to develop SAR. Prior hit-to lead efforts resulted in the identification of compounds with good activity. Subsequent investigations were focused on improving the functional potency of these compounds.

[1] Ludwig DS, Tritos NA, Mastaitis JW, Kulkarni R, Kokkotou E, Elmquist J, Lowell B, Flier JS, Maratos-Flier EJ. Melanin-concentrating hormone overexpression in transgenic mice leads to obesity and insulin resistance. J Clin Investig 2001;107:379-386.

[2] Receveur JM, Bjurling E, Ulven T, Little PB, Nørregaard PK, Högberg T. 4-Acylamino- and 4-ureidobenzamidees as melanin-concentrating hormone (MCH) receptor 1 antagonists. Bioorg Med Chem Lett 2004;14:5075-508

P241

Design and synthesis of multiple ligands: new arylamines and their binding affinity toward 5-HT transporter (SERT) and 5-HT$_7$ receptors

L. Berrade[1], S.Perez [1], P. Egea[1], L. Giurato[2], S. Guccione[2], I. Aldana[1], G. Molinaro[3], F. Nicoletti[3] and A. Monge[1]

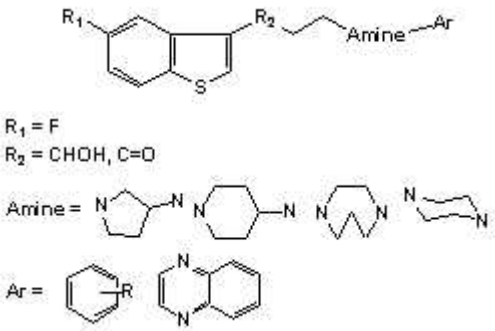
[1] Universidad de Navarra. Spain. [2] University of Catania. Italy. [3] Instituto Neurologico Mediterraneo Neuromed. Camerelle, Italy.

The role of serotonin (5-HT) in depression disorders has been well investigated generating very interesting therapies. Disturbances in central serotonin system have been associated with the pathogenesis of depression and the antidepressant effect of the selective 5-HT reuptake inhibitors (SSRIs) is believed to be due to an enhancement of postsynaptic 5-HT levels [1,2].

Years ago, our group synthesised dual compunds: 5-HT reuptake inhibitors and postsynaptic 5-HT$_{1A}$ antagonists, obtaining wonderful results [3].

Hypotheses driving current research indicate that the 5-HT$_7$ receptor might be involved in mood regulation, suggesting that this receptor is a putative target in the treatment of depression [2].

Nowadays our work consits of the synthesis of new multiple compounds, 5-HT reuptake inhibitors and with affinity towards 5-HT$_7$ receptors. We have synthesised arylamine series according the structure:



Results of receptor binding studies performed on rat brain tissue with these new compounds will be presented along with molecular modelling studies combining *state of art* softwares and homology modelling with the additional aim to investigate the 5-HT$_7$ *vs*. 5-HT$_{1A}$ binging.

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P242

Piperazinylalkylpyrazoles as T-type calcium channel blockers (I)

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Korea Institute of Science and Technology (KIST), Korea. Biochemicals Research Center, [1]Biomedicals research center, [2]Center for Calcium and Learning,

Since Mibefradil, the first marketed T-type calcium channel blocker, was withdrawn from market due to toxicity from drug-drug interaction, numerous series of compounds have been studied in search for new T-type calcium channel blocking drug candidates. Recently, 1,3-dihydroquinazolines and piperazinylalkylisoxazoles have been reported to be good candidates for T-type calcium channel blockers.

In this study, a new series of piperazinylalkylpyrazole derivatives was designed, synthesized and evaluated for calcium channel inhibitory activity. A focused piperazinylalkylpyrazole library consisting of over 160 compounds was constructed through parallel synthesis protocol. Of them, 123 compounds were evaluated for their inhibitory effects on α_{1H} T-type calcium channels expressed in Xenopus oocytes and HEK 293 cells. Several compounds showing good activity against T-type calcium channels were also screened against α_{1B} N-type calcium channels.

Consequently, a number of compounds exhibiting excellent T-type calcium channel blocking activity were evaluated for visceral pain responses.

P243

Photodegradation and identification of photoproducts of hexahydroquinoline derivatives – a new group of calcium channel blockers

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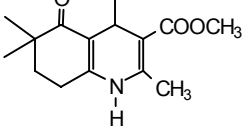

R: Cl, NO_2, CF_3, CH_3, OCH_3

The calcium channel antagonists is one of the groups of drugs applied in the treatment of the circulatory system disorders [1-4]. The calcium channel antagonists have also been found effective in the treatment of other diseases in nephrology, gynaecology, gastroenterology or central nervous system [5].

Applying a modified synthesis of Hantsch, many compounds have been synthesised a series of compounds belonging to the derivatives of methyl 2,6,6-trimethyl-4-aryl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate derivatives, containing different substituents in the phenyl ring (-Br, -F, -Cl and -NO_2) [6-10]. The synthesised HHQ derivatives have been subjected to pharmacological and pharmacokinetic studies to test their activity as calcium antagonists.

Unfortunately, similarly as DHP derivatives also this group of compounds is characterised by high photosensitivity. As the photochemical properties of this group of compounds are poorly recognised, the aim of the study was investigation of the photodegradation of the hexahydroquinoline derivative with the -Br, -F, Cl and -NO_2 substituents in different position of the phenyl ring.

The photodegradation was carried out in the conditions recommended in the first version of the Document issued by the International Conference on Harmonization (ICH). The ultraviolet spectrophotometry and HPTLC permitted determination of the kinetic parameters of HHQ photodegradation described as a dependence ln c = f (t). Quantitative assessment of photodegradation of HHQ derivatives was also made on the basis of determination of quantum yields of the photochemical reactions which was performed using Reinecke salt as chemical actinometer. To get the real quantum yield the apparent quantum yields were extrapolated to zero exposition time. For the compounds studied the quantum yields were in the range 10^{-3}–10^{-5}, which indicates occurrence of secondary photochemical reactions.

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P244

7-substituted hexahydroquinolne derivatives and their effects on calcium channels

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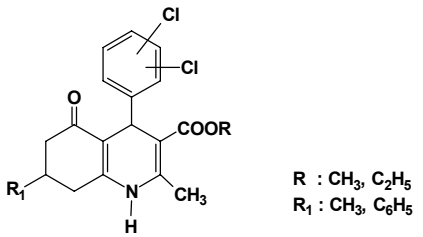

Dihydropyridine derivatives have calcium modulatory activity and been used as cardiovascular agents. Nifedipine is the prototype of this group. Active analogs have been obtained by introducing the 1,4-dihydropyridine structure to condensed systems. These analogs have also exerted calcium antagonistic activity. The aim of this study was to synthesise new 1,4-dihydropyridine derivatives. These derivatives were synthesised by Hantzsch method. In order to synthesize the compounds, 5-methyl (phenyl)-1,3-cyclohexanedione, appropriate dichlorobenzaldehyde were refluxed by methyl (ethyl) aminocrotonoate in methanol for 6 h. After this period forming crystals were separated. The structure of the compounds were elucidated by spectral methods such as IR, ^{1}H-NMR, ^{13}C-NMR, mass spectra, COSY, DEPT, HMQC, X-ray and elementel analysis. The calcium modulatory activity of these compounds were investigated on isolated rat ileum and rat thorasic aorta.

The crystal structure of methyl 2,7-dimethyl-4-(2,4-dichlorophenyl)-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate and methyl 2-dimethyl-4-(2,4-dichlorophenyl)-5-oxo-7-phenyl-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate were determined by X-Ray diffraction. X-ray analysis results showed that the 2,3-dichlorophenyl ring is oriented such that the chloro substituents are in a syn-periplanar orientation with respect to the 1,4-DHP ring plane and the oxocyclohexene ring has a slightly distorted envelope conformation. Both structures exhibit the same intermolecular N—-H...O hydrogen bonding motif in which the molecules are linked into chains by interactions involving the carbonyl oxygen atom of the oxocyclohexene ring. Finally, the elemental analysis results are also consistent with the postulated structures.

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P245

Pyrazolone methylamino piperidine derivatives as novel CCR3 antagonists

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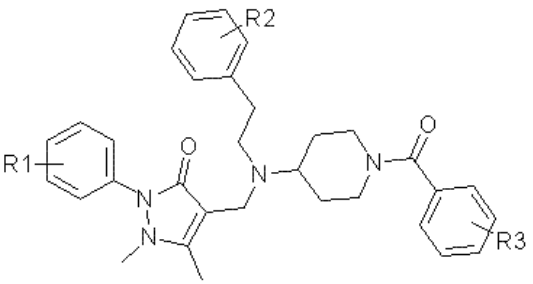
The prevalence of asthma is increasing in industrialised countries and symptoms are not completely controlled for many patients. There is a need for new therapies against this chronic inflammatory disease of the airways. Pulmonary eosinophil recruitment is thought to be closely related to the symptoms of allergic asthma. Eosinophils, via their CC chemokine receptor 3 (CCR3), are attracted in response to chemoattractants such as eotaxin, Rantes, MCP-3, MCP-4 released in the airways of asthmatics [1]. Inhibition of pulmonary eosinophilia by blocking the CCR3 receptor with small molecule antagonists should lead to a reduction in the inflammation and the airway responsiveness seen in asthma.

We present here the discovery and optimisation of a novel class of potent CCR3 antagonists: the pyrazolone methylaminopiperidine derivatives Full details of synthesis and SAR are given together with some ADME properties of selected compounds. We show that an optimal balance between activities, physicochemical properties and *in vitro* metabolic stability can be reached by the proper choice of substituants.



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P246

Quinuclidine Derivatives as Potent and Selective Muscarinic Antagonists for the Treatment of COPD

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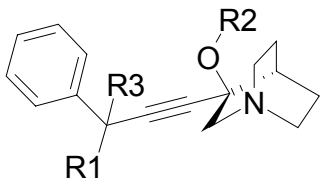
[1] General Pharmacology [2] Global Chemistry [3] In Vitro Pharmacology. R&D, UCB S.A., Chemin du Foriest, Belgium

COPD is a chronic, progressive and poorly reversible condition characterized by impaired expiratory outflow and abnormal inflammatory response of the lungs to noxious particles and gases. COPD is one of the most common chronic diseases worldwide, it affects 4-6 % of people older than 45 and is predicted to be the third leading cause of death by 2020.[1] There are currently no drug therapies able to slow down the progression of the disease. Bronchodilator drugs are the current mainstay of treatment for symptoms relief. Anticholinergic bron-

chodilators, particularly selective muscarinic M_3 antagonists (ipratropium, tiotropium), are currently the preferred choice for the symptomatic management of COPD.

We have reported, a few months ago, the synthesis and biological evaluation of a novel family of alkyne-quinuclidine derivatives [2] displaying potent anticholinergic properties as potential new drugs for the treatment of overactive bladder.

In this poster, we present the discovery and optimization of new analogues displaying high muscarinic affinities and a particularly interesting kinetic selectivity for the M_3-receptor rendering them particularly suitable for the treatment of COPD. We will describe the structure-activity relationships that underlie a rational improvement in the kinetics and binding profile selectivity for the M_3-R. We examined the correlation between M_3-R K_{off} and the persistence of anti-bronchoconstrictor effects *in vitro* (isolated guinea pig trachea).



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P247

Novel Potent H3 Antagonists: Therapeutics for the Treatment of Dementia including Alzheimer's Disease

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Neurology and GI Centre of Excellence for Drug Discovery, GlaxoSmithKline, UK.

Histamine H3 receptor antagonists have potential as therapeutic agents for the management of CNS mediated disorders[1] including cognition impairment (Alzheimers, MCI), narcolepsy, ADHD, schizophrenia, epilepsy, pain and obesity.

This poster will describe the design and discovery of a novel series of H3 antagonists. Optimisation of analogues within the constraints of a self-imposed molecular budget designed to control molecular weight, logP and polar surface area produced a series that exhibited excellent developability characteristics including high Br:Bl ratio, low clearance and good oral bioavailability.

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Metabolic Diseases

P248

The benefit effect of vitamin E on zinc deficiency in experimental diabetes

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To investigate the benefit effect of vitamin E on zinc deficiency in experimental diabetes (IDDM). Forty male alloxan-diabetic rats albino (Wistar) rats of 8 weeks of age were fed a complete purified diet containing either 54 µg zinc/gram diet (control) or 1 µg zinc/ gram diet (deficient). Half of the animals in each diet group received supplemental vitamin E(500 mg/kg diet). Body weight gain was recorded regularly. On day 21, after an over night fasting, animals were killed and blood glucose, serum zinc, serum cholesterol and serum triglycerides concentrations and serum glutamic oxalic transaminase (GOT) and serum glutamic pyruvic transaminase (GPT) activities were determined. Body weight gain and serum zinc of low-zinc diabetic animals at the end of three weeks of dietary manipulation were significantly lower than those of the control diabetic animals. Dietary zinc intake significantly increased blood glucose, serum cholesterol, and serum triglycerides of low zinc diabetic rats. Also the consumption of low-Zn diet led to increasing of GOT and GPT and decreasing alkaline phosphatase activities. However, vitamin E was ameliorated all the previous parameters. To conclude, the present study demonstrates that vitamin E presumably acting as an antioxidant, which significantly reduced the severity of low zinc effect in experimental diabetes in other word that vitamin E supplementation might be led to an improvement of insulin activity.

Key words: Diabetic rats, Alloxan, Low zinc, Vitamin E, GOT, GPT, Alkaline phosphatase.

P249

A Novel Stimulator of Osteoblast differentiation: Synthesis and Biological Evaluation of Triazolopyridazine Derivatives

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While searching for novel stimulators of osteoblast differentiation, we found a new series of triazolopyridazine derivatives that induced potent cellular alkaline phosphatase (ALP) activity in mouse osteoblastic cell line MC3T3-E1. Among them, 6-(4-fluoro-1-piperidinyl)-3-(6-methoxy-2-pyridinyl)[1,2,4]triazolo[4,3-b]pyridazine (YM-271338) showed more potent ALP induction activity than TAK-778. In an osteoporosis model in rats, the combined use of YM-271338 and incadronate synergistically increased the bone mineral density, thickness and strength of cortical bone when compared to incadronate alone by oral administration. The synthesis and the structure-activity relationships of these triazolopyridazine derivatives are presented.

P250

Novel characteristic anti-bone resorption activity agent : 5-Bromo-2-(4-chlorobenzoyl)-3-(*Z*)-2-(cyano-3-hydroxybut-2-enonyl)aminobenzo[*b*]furan (MU-314)

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We have synthesized several types of original compounds and evaluated their anti-bone resorptive activity by pit assay (By coculture of fresh bone marrow pre-osteoclasts expressing the receptor activator of NF-κB (RANK) with calvarial osteoblasts that express the ligand for RANK (RANKL), bone resorbing osteoclasts developed and formed resorption pits on a dentin slice. PGE_2 stimulated pit formation, and estrogens (e. g. estrogen 2 (E_2)) inhibited PGE_2-stimulated pit formation by suppressing the RANKL effect.). Among the compounds tested, 5-bromo-2-(4-chlorobenzoyl)-3-(*Z*)-2-(cyano-3-hydroxybut-2-enonyl)aminobenzo[*b*]furan (MU-314) showed potent inhibition comparable to E_2.

Subsequently, we studied some evaluations concerning MU-314 using OVX rats *in vivo* assays (peritoneal and oral administration). MU-314 showed inhibition of decrease of bone mineral density. (Fig. 1) On the other hand, MU-314 did not effect to uterine, and inhibited increase of body and adipocyte tissue weight on OVX rats. In conclusion, MU-314 showed estrogenic action to bone and fat metabolism and anti-estrogenic action to uterine. These data suggested MU-314 would be new potent candidate for novel osteoporosis agent.

P251

Conformational analysis of the new obesity related peptide Obestatin

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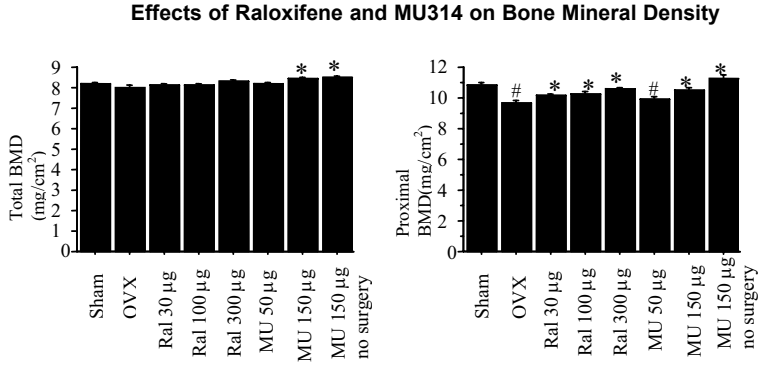
Obestatin is a new endogenous ghrelin associate peptide, involved in the regulation of food intake and weight gain. Although obestatin and ghrelin originate from a common precursor of 117 residues, named propreghrelin, they are reported to exert opposing physiological roles by binding distinct receptors belonged to subgroup of type A GPCRs.

Obestatin is anorexigenic, decreases food intake, gastric emptying and jejunal motility. It was found to be the natural ligand of the orphan GPR39 receptor. GPR39 is meanly expressed in jejunum, duodenum, stomach, pituitary and hypothalamus and has a peculiar constitutive activity, probably induced by an aromatic cluster on the inner face of the extracellular ends of TMs VI and VII. Elucidation on the mechanism of action of obestatin could clarify its potential in metabolic diseases leading to develop new specific drugs against obesity. On this way, we synthesized mouse obestatin (FNAPFDVGIKLSGAQYQQHGRALNH$_2$) and its related C-terminal subfragment (LSGAQYQQHGRALNH$_2$), and investigated their conformational behaviour in aqueous solution and in membrane mimicking environments, by means of Nuclear Magnetic Resonance (NMR) and Circular Dichroism (CD). The data indicate that the C-terminal portion of obestatin has a peculiar ordered structure, suggesting its involvement in the biological activity of the peptide.



P250 Fig. 1 MU : MU-314 Ral : raloxifene

Multiple Drug Resistance

P252

Synthesis of elacridar analog as chemosensitizer in multidrug resistance in cancer

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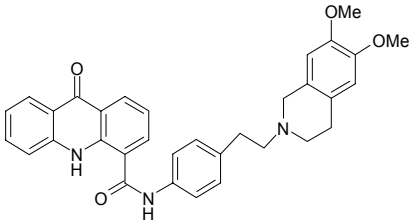
K.M.Kundanani College of Pharmacy,Mumbai,India

Elacridar exhibits Potent, selective chemosensitizing activity with low toxic potential. [1] The present work involves the synthesis of novel elacridar analog.



Synthesis of Elacridar analog with structure 4-[2-(6',7'-dimethoxytetrahydroisoquinoline-2-yl)ethyl]acridone-4-carboxanilide involved alkylation of 6,7-dimethoxy tetrahydroisoquinoline with 2-(4-nitrophenyl) ethyl bromide to get an intermediate with structure 6', 7'–dihydroxy–2[2-(4-nitrophenyl) ethyl] tetrahydroisoquinoline which was then methylated to 6', 7'–dimethoxy–2[2-(4-nitrophenyl) ethyl] tetrahydroisoquinoline. The nitro group of dimethoxy compound was then reduced to corresponding amine by using ammonium sulfide (Yield=78.35%) or tin and hydrochloric acid (Yield=57.8%) to get 4–[2-(6',7'-dimethoxytetrahydroisoquinoli-2-yl) ethyl] benzenamine. The amine and acridone–4–-carboxylic acid were then condensed by using DCC to get the final Elacridar analog (yield=53.8%, M.P.=173ºC)

The intermediates involved were synthesized using alternate methods than that reported in literature [2]. Progress of all reactions was monitored by thin layer chromatography. The compounds were characterized by infrared and ^{1}H NMR spectroscopy. Reaction steps were optimized with respect to yield and reaction time.

The work involved optimization of yield and reduction of reaction steps, use of alternate catalyst/reducing agents and effective cost reduction

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P253

Computational 3D Elucidation of the human ABC-Transporter P-Glycoprotein in the post-hydrolytic state

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Human P-glycoprotein is a 170kDa membrane-spanning, ATP dependent efflux pump, which plays a critical role in confering multi-drug resistance and in mediating toxicologically important drug-drug interactions. The power stroke for this transport function is considered to be provided by binding and hydrolysis of ATP. To investigate the conformational changes, which occur upon nucleotide binding and hydrolysis, we used the 4.2 Å resolution x-ray structure of the bacterial Lipid A transporter MsbA of Salmonella typhimurium (SalTy) as a template for a comparative homology model of P-gp in the post-hydrolytic state.

After the structural evaluation and refinement of our template, we established a homology model of P-gp using the modelling tool implemented in the MOE software package. Finally, the quality of the protein model was improved by an energy minimization in the AMBER94 force field and the proper side chain configuration was adjusted by using SCWRL 3.0. The resulting model shows more than 98% of the amino acids in allowed regions of the Ramachandran plot and provides a Cα-RMSD of 0.862 towards MsbA-SalTy.

Unfortunately, our model does not fit some of the experimental post-hydrolytic cross-linking data in putative transmembrane-segments, which are considered to be relevant for substrate translocation. Therefore, the model is further refined via molecular dynamics simulations in a lipid environment with distance constraints that obey the experimentally determined data.

P254

A structural model for ABCG2

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The breast cancer resistance protein (BCRP, MXR, ABCG2) currently represents one of the most important targets for anti-cancer drug development. It is a 72-kDa, membrane bound multidrug efflux transporter, which transports structurally and functionally diverse cytostatic agents out of tumour cells. ABCG2 consists of two halves, each containing a transmembrane domain (TMD) and a nucleotide binding domain (NBD). However, in contrast to other members of the ABC-transporter family, ABCG2 shows a reverse domain organisation starting with the NBD followed by the TMD. Thus, the currently available structures of bacterial ABC-transporter show only limited applicability as templates for protein homology modelling of ABCG2.

We created a protein model of ABCG2 by creating the 6 transmembrane helices de novo and superimposing them with the P-gp helices derived from our homology model. However, according to sequence similarity and the reverse domain organisation, we mapped helix 1 of ABCG2 onto helix 2 of P-gp and helix 6 of ABCG2 onto helix 1 of P-gp. The loops were added by using the loop database from the Swiss-PDB-Viewer. Finally the NDB was established on basis of the Malk-1 NBD (1Q1E). The resulting protein structure was evaluated using Procheck in order to validate the stereo chemical properties. The structure correlates well with biochemical data derived from site specific mutagenesis and cysteine bridge scanning and thus may serve as a versatile tool for target-based drug design studies.

P255

Molecular Dynamics Simulation Studies on Nucleotide-free P-glycoprotein in a POPC Bilayer

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One of the major causes of multidrug resistances is the over expression of membrane-embedded ABC-Transporters like P-glycoprotein (P-gp, ABCB1). Although many biochemical, structural, and functional data of ABCB1 are available, detailed information on the atomic level is still missing. Thus, design of inhibitors of P-gp, which represent a promising concept to overcome multiple drug resistance in tumour therapy, has to rely exclusively on ligand-based information. In order to complement our structure-activity studies on propafenone-type inhibitors of P-gp with structural informations we used Molecular Dynamics simulations of our previously published homology model. P-glycoprotein was embedded in a fully hydrated bilayer lipid membrane and two MD-runs for 5 and 10 ns were performed using distance restraints taken from Loo and Clarke. A second protocol was applies for the system setup in which we gently released the position restrains for an more carefull system preparation. All simulations gave stable protein conformations after 4 ns and show improved values in protein validation tools.

The best structures with the lowest rmsd values were optained with the improved equilibration protocol without additional restraints. This model will further be used for docking studies of propafenone type inhibitors.

P256

Chemosensitization of a Multidrug-Resistant *Leishmania tropica* Line by Sesquiterpenes from *Zinowiewia costaricensis*

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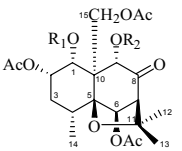
Protozoan parasites are responsible for some of the most important and prevalent diseases of human and domestic animals, threatening the lives of nearly one-quarter of the human population, being leishmaniasis the second worldwide cause of death among parasitic diseases, mainly because of the appearance of drug-resistance mechanisms. The MDR phenotype due to the increased expression of P-glycoprotein (Pgp)-like transporters has been characterized in tumor cells and protozoan parasites, including *Plasmodium* and *Leishmania* spp. The inhibition of the activity of these proteins represents an interesting way to control drug resistance both in cancer and in infectious diseases. Most conventional mammalian Pgp-MDR modulators are ineffective in the modulation of Pgp activity ín the protozoan parasite *Leishmania*. Consequently, there is a necessity to find effective modulators of Pgp-MDR for protozoan parasites.



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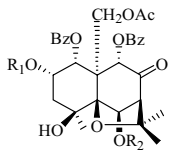
2	Ac	Bz
3	H	Bz
4	Bz	Ac
5	Ac	Ac



	R_1	R_2
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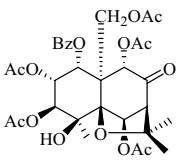
7	Ac	Ac
8	Bz	Ac



9

We report the activity of nine dihydro-β-agarofuran compounds (**1-9**) which have been tested on a MDR *Leishmania tropica* line overexpressing a Pgp-like transporter to determine their ability to revert the resistance phenotype, and to modulate intracellular drug accumulation. These compounds were isolated from the leaves of *Zinowiewia costaricensis.* Their structures were elucidated by means of ^{1}H and ^{13}C NMR spectroscopic studies and the absolute configuration of them were determined by CD studies, chemical correlations or biogenetic grounds.

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Dihidro-â-Agarofuran Sesquiterpenes: A New Class of Reversal Agents of the Multidrug Resistance Phenotype Mediated by P-Glycoprotein in the Protozoan Parasite *Leishmania.* Current Pharmaceutical Design, 2005,11:3125-29

Natural Products

P257

A study on morphine levels in poppy seeds

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Poppy seeds are the ripe seeds harvested from the capsules of Papaver Somniferum. Owing to their content of fatty oil (40–60 %) and protein (15–24 %), the seeds have been a popular source of food (for example in poppy seed cake, desserts etc.). In poppy seeds, in contrast to other parts of the plant, the alkaloid rich latex (milky juice) is not found. Therefore, poppy seeds contain the alkaloids only in traces according to the scientific literature. Partially the same plants from which the seeds derive are used to obtain opium alkaloids. In addition to the primary alkaloid, morphine, there are secondary alkaloids such as codeine, thebaine, noscapine and papaverine found in opium.

In this study, 20 poppy seed samples were tested. Their morphine levels were detected by HPLC. Thus, Morphine levels detected were <20 µg/g in 4 samples, were as high as >20 µg/g up to a maximum of 50 µg/g in 13 samples, were as >50 µg/g in 3 samples. After the seeds washed with water, They were dried and again analysed. Their morphine levels were <10 µg/g in all samples.

Tests have shown that, poppy seeds may contain very different amounts of these natural constituents. The possible causes of high opiate levels sometimes found in poppy seeds include the contamination of poppy seeds with capsule fragments and the botanical varieties. Thus, morphine levels could be drastically reduced by washing of the seeds. Maximum limits for admissible levels of the above opium alkaloids in poppy seeds are 10 µg morphine/g poppy seed for morphine. The manufacturers ought to make every effort to reduce the concentrations of all pharmacologically active opium alkaloids in poppy seeds to the lowest level technologically achievable.

Key Words: Poppy, Seed, Morphine, HPLC.

P258

Selective Transformations of Licorice Triterpenoids as a Basis for the Production of New Medicinal Agents with Target Biological Activity

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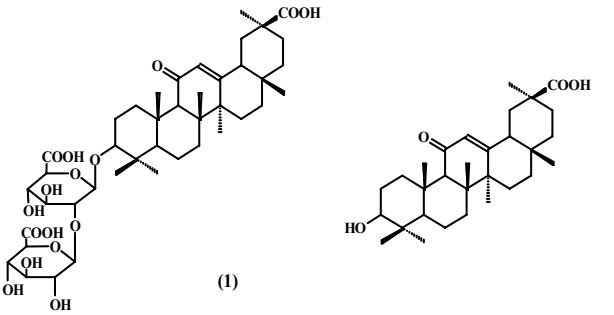
Naturally occurring triterpenoids and their glycosides are often found as the major components in medicinal and food plants. They have a range of pharmacological and biological activities (hemolytic, antifungal, antiviral, hypolipidemic, anticarcinogenic, anti-inflammatory, antiulcerogenic etc.). The molecular mechanisms of most types of biological activities of these substances is not yet entirely clear. The well-known plant's triterpene saponin, Glycyrrhizic Acid (GA) (1) and its aglycon, Glycyrrhetinic Acid (GLA) (2) to be the main components of licorice root (Glycyrrhiza glabra L., Gl. uralensis Fisher) are of special interest as a basic compounds to produce novel antiviral preparations due to their low toxicity and availability from plant's raw materials.



To study the molecular mechanism of antiviral activity of GA and structure-activity relationships we carried out selective chemical transformations of GA, GLA and related compounds. Modified GA analogues containing olean-9(11),12(13)-diene system in aglycone were synthesized by the reduction of glycoside with $NaBH_4$. Sulfates and acylates of 18á-GA (nicotinate, salycilate, cynnamate etc.) were synthesized for the SARs studies. New amides of GLA were synthesized with hexamethylendiamine spacer. A-nor-derivatives of minor triterpenoids of licorice root (11-desoxo-GLA, 18,19-dehydro-GLA) were prepared via dehydration with PCl_5 and selective oxidation with ozone or dimethyldioxirane were carried out to produce A-nor-epoxy and -oxo-derivatives. A-aza-derivatives were synthesized by the Beckmann rearraignment of oximes.

This work was supported by grants of Rosnauka (02.434.11.7060).

P259

Carnosic acid and carnosol, phenolic diterpenes of *Salvia offic.* and *Rosmarinus offic.* are multiple target compounds, being activators of PPARγ as well as inhibitors of lipoxygenase and show additional anti-inflammatory properties

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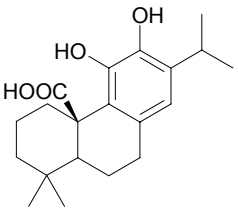
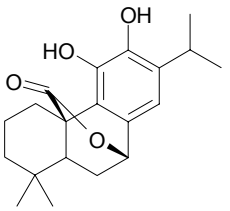
Carnosic acid and carnosol were recently reported, to show anti-inflammatory, apoptotic and prodifferential effects. Moreover, carnosol was reported to be an inhibitor of 5-lipoxygenase. Here we describe different targets for carnosic acid and carnosol, whereat both compounds show a different target specifity pattern.



carnosic acid



carnosol

Peroxisome proliferator activated receptor gamma (PPARγ) is a ligand activated transcription factor. Activation of the PPARγ leads to lowered blood levels of fatty acids and glucose. Hence, synthetic activators of PPARγ are clinically used for the therapy of type 2 diabetes, furthermore they show beneficial anti-inflammatory effects.

We determined carnosic acid and carnosol to activate PPARγ with EC_{50} values of 19.6 µM and 41.2 µM, respectively.

Additionally, we were able to verify the reported inhibition of 5-lipoxygenase for carnosol, and in addition, we also found inhibition of 12-lipoxygenase. Carnosic acid on the other hand, proved to less potently inhibit 5-lipoxygenase, but did not inhibit 12-lipoxygenase.

Since both rosemary and sage were reported to contain carnosic acid and carnosol in the lower percent range, our results provide evidence for the traditional use of rosemary and sage as antidiabetic agents. Moreover, our observations could explain anti-inflammatory effects reported for both compounds previously. Carnosic acid seems to be a promising lead structure for medicinal chemistry optimisation of anti-inflammatory drugs.

P260

Prenylflavone derivatives from *Psoralea corylifolia* inhibit nitric oxide synthase expression through the inhibition of Ik-B degradation in activated microglial cells

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The overproduction of nitric oxide (NO) by inducible nitric oxide synthase (iNOS) plays as neurotoxic effector in central nervous system, resulting in the neurodegenerative diseases. From the alcoholic extracts of *Psoralea corylifolia*, we purified two inhibitors of NO production in lipopolysaccharide (LPS)-activated microglia by activity-guided purification along with two inactive compounds. The active compounds were identified as 7,8-dihydro-8-(4-hydroxyphenyl)-2,2-dimethyl-2H,6H-benzo-(1,2-b:5,4-b')dipyran-6-one **(1)** and 4-hydroxylonchocarpin **(2),** and the inactive two compounds were identified as bavachinin **(3)** and bavachalcone **(4)** by spectral analysis. The compound **(2)** was isolated first time from this plant. Compounds **(1)** and **(2)** inhibited the production of NO in LPS-activated microglia in a dose dependent manner (IC_{50} was 11.4, 10.2 µM, respectively), and also suppressed the expression of protein and mRNA of iNOS in LPS-activated microglial cells at 20 µM as observed in Western blot analysis and RT-PCR experiment. Furthermore they inhibited the degradation of IκBα in activated microglia. These results imply that compounds **(1)** and **(2)** may have neuroprotective activity through the inhibition of NO production in activated microglial cells.

P261

UVA-activated furocoumarins induce accumulation of γ-globin mRNA in human erythroid cells

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The search for potential therapeutic agents in haematological diseases, including β-thalassemia and sickle cell anemia (SCA), focuses on the pharmacologically mediated regulation of the expression of human γ-globin genes, leading to the production of fetal haemoglobin (HbF). In this context, many studies have been started to find natural or synthetic compounds capable of augmenting HbF levels in humans. In particular, emphasis has been given to DNA-binding agents, which appear of special interest. Among molecules able to interact with DNA, psoralen and related compounds could be relevant. In this study we have analysed several linear and angular psoralens after UVA irradiation, employing two experimental cell systems, such as the human erythroleukemia K562 cell line and the two phase-liquid culture of human erythroid progenitors isolated from normal donors for further analysis on the most active compounds.

The obtained results show that psoralen, angelicin and several structurally related compounds (trimethylangelicin, 5-methoxypsoralen, 4-methylpsoralen and 5'-methylpsoralen) are powerful inducers of erythroid differentiation evaluated by benzidine staining and γ-globin mRNA accumulation both in K562 cells and in human erythroid precursors. Interestingly the activity of some compounds such as angelicin was found higher than that displayed by hydroxyurea, a drug commonly used as HbF inducer in β-thalassemia and SCA patients. These results could have a practical relevance, because pharmacologically mediated regulation of the expression of human γ-globin genes leading to increased HbF production is con-

sidered a potential therapeutic approach in haematological disorders, including β-thalassemia and SCA.

P262

Volatile components of South *algerian nigella sativa linn.* oil extracted by hydrodistillation and microwave process

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In Algeria, among medicinal and condiment seed which are widely cultivated appears the *Nigella sativa*, commonly called black seed or sanoudj. That last, is intended for medicinal purpose and cultivated in many others Asiatic and African areas. Volatile fraction represents 0.38 to 0.50 % of seed mass and 1.4% to 1.9% of fixed oil. That seed have a wide application in traditional medicine recently exposed in several pharmacological and biological researches. The high content of active components and antioxidants as thymoquinone, thymol, carcavol, carvone, and p-cymene has encouraged the researchers to investigate the black seed effects in many medical research domains like gastric activity immunopharmacology and cancerology.

Extraction of essential oil from *Nigella sativa* seed has been conducted by two different procedures: hydrodistillation (Oh) and microwaves extraction process (Om). The last method gives the best yields with reduction of extraction time and high amount of volatile fraction. The composition of the volatile oil has been investigated by capillary gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS).

p-Cymene (26.60%, 34.91%, Oh and Om respectively), α-thujene (15.31-16.60%), sabinene (12.95-7,53%), thymoquinone (5.91-14.7%), carvacrol (4.59-1.93%), trans-sabinene hydrate (2.78-2.19%), δ-terpinene (2.76-1.94%) longifolene (1.15-3.09%) and α-longipinene (1.6-1.10%) were the major components representing more than 72.05% (Oh) and 83.46% (Om) of the oil.

MAP process constitutes the adequate technique for the extraction operations from the yields and the high content in major component, and allows minimizing considerably the energy consumption and heating time, what causes the formation of artefact products. So, it's profitable to treat some plants and seeds using this process for preserve their thermolabile components.

In term of selectivity, the microwaves allowed to extract monoterpene and sesquiterpene hydrocarbons, and carbonyls compounds considered generally such as active and antioxidant components of essential oil. The proposed method provides more valuable essential oils, moreover the thymoquinone percentage extracted is increased, and this offers oils biologically active. The microwave extraction is a green technique since reduces environmental burden (less CO_2 in the atmosphere).

P263

Functional Design of Plant's Derived Glycosides

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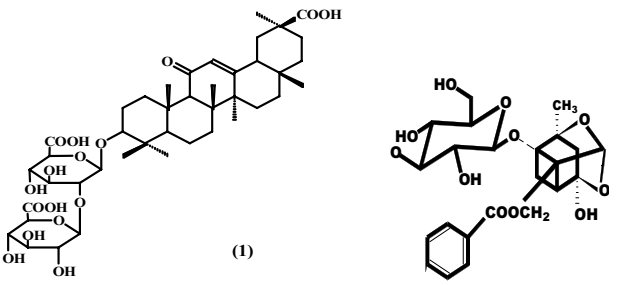
*Bashkir State Medicinal University, Institute of Organic . hemistry Ufa Research Centre of RAS. **Institute of Organic Chemistry Ufa research Centre of RAS. ***Institute of Organic Chemistry Ufa Research Centre of RAS. ****Bashkir State Medicinal University

Functional design of natural compounds is one of the perspective root to produce new bioactive compounds for medicine. Available plant's derived poly functional glycosides are of interest as basic compounds for the chemical modifications. Among them the main attention is given to triterpene glycoside, Glycyrrhizic acid (GA) (1), to be the main bioactive component of Glycyrrhiza glabra and Gl. uralensis Fisher roots [1]. GA has a range of pharmacological and biological activities (antiviral, hypolipidemic, anticarcinogenic, anti-inflammatory, antiulcerogenic etc.). The second glycoside is of interest for transformations is peoniflorin (PF) (2) isolated from Paeonia anomala which is known due to the high antitumor activity.



To study the structure-activity relationships among GA and PF derivatives we carried out chemical modification of carbohydrate part of GA and PF. Diglucuronide part of GA were changed by D-glucosamine or glycosylamines and neutral sugars. To change the lipophylity of glycosides different acylated derivatives (nicotinates, cinnamates, acetylsalycilates etc.) and sulphates of β-D-Glcp-(1-2)-β-D-Glcp analogue of GA and PF were produced by using acid chlorides method. To design of novel triterpene glycosides to be GA mimetics we carried out 3-O-glycosylation of Licorice triterpenoids by using acetylated sugars in the presence of $SnCl_4$. Structures of new glycosides derivatives were confirmed by the high resolution NMR spectra.

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P264

Nicotinic receptors as therapeutic targets

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Acetylcholine, released from cholinergic nerves centrally and peripherally, plays a major role in many physiological functions. The discovery of nicotine and muscarine provided the agents needed to define the nicotinic and muscarinic receptors that subserve acetylcholine-mediated functions. The nicotinic receptors consist of hetero- and homo-pentameric cation channels, while the five muscarinic receptors are G-protein-coupled to second messenger systems, involving either phospholipase C or adenylyl cyclase. Nearly twenty nicotinic receptor subunits have been cloned. Combinations of alpha and beta subunits form a variety of heteropentomeric nicotinic receptors in the central nervous system. There is also one major homopentomeric nicotinic receptor in the central nervous system, which is comprised of alpha7 subunits.The natural products nicotine, cytisine, anatoxin, anabaseine, epibatidine, erysodine, methyllycaconitine, and galanthamine have provided lead compounds for synthetic efforts to develop selective nicotinic agents with biomedical potential.. A wide range of compounds have been synthesized and investigated with different subtypes of nicotinic receptors. Potential therapeutic targets include chronic pain, Alzheimer's disease, Parkinsonism, epilepsty, anxiety, depression, drug addiction, and schizophrenia. The peptidic conotoxins provide yet another class of nicotinic ligands

P265

The effects of olive oil and usnic acid on glutathione and lipid peroxidation levels in gastric damaged tissues induced by indomethacine in rats

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ABSTRACT: In our previous research, we investigated the gastroprotective effects of 25, 50, 100 and 200 mg/kg doses of usnic acid isolated from a lichen species (*Usnea longissima*), and olive oil on indomethacin-induced gastric damage. Our previous results showed that all doses of usnic acid dissolved in olive oil and alone olive oil significantly reduced the gastric damages in stomach of rats induced by indomethacin. Today, it is well known that reactive oxygen species (ROS) have an important role in the pathogenesis of the gastric damage. Therefore, in the present study, in all stomach tissues, the levels of glutathione (GSH) and lipid peroxidation (LPO) were studied. The present results showed that indomethacin (IND) caused oxidative damage in stomach tissues of rats by decreasing the levels of GSH and increasing the level of lipid peroxidation as compared with healthy rat group. Contrarily, the administrations of all doses of UA and alone olive oil reversed the trend, inducing a significant decrease in LPO level and subsequent increase in GSH level. These results suggested that usnic acid and olive oil have decreasing effects on the levels of reactive oxygen species in stomach tissues. Thus, the gastroprotective effects of usnic acid and olive oil can be attributed to their reducing effect on the levels of ROS.

P266

Effects of the Methanol Extract of *Lobaria pulmonaria* on Glutathione *S*-Transferase Activity and Gastric Damages in Rats

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ABSTRACT: In the present study, the gastroprotective effects of methanol extract of a lichen species, *Lobaria pulmonaria* (L.) Hoffm. was investigated in indomethacine (IND)-induced gastric ulcer models in rats. Gastroprotective effect of 50, 100, 200, 500 and 1000 mg/kg body wt. doses of the extract were determined by comparing with IND (25 mg/kg body weight) group. While the gastric lesions were significantly reduced by all doses of the extract and ranitidine (RAN), the highest gastroprotective effect was observed for 1000 mg/kg body wt. dose with 77.2 % inhibition.

The *in vivo* glutathione *s*-transferase levels in the stomach tissues of all animal groups were also evaluated. The present results showed that the administration of IND significantly decrease the GST activity ($p<0.05$). In contrast to IND, the administration of 100, 200, 500 and 1000 mg/kg doses of the extract and RAN significantly increased the GST activity in tissues. In addition, the *in vitro* antioxidant properties of the extract were determined and the extract showed significant antioxidant activity.

The present results indicate that the methanolic extract of *L. pulmonaria* has a gastroprotective effect on gastric ulcer induced by IND, which can be attributed to its antioxidant potential.

P267

Sesquiterpene lactones and other constituents from *Matricaria chamomilla*

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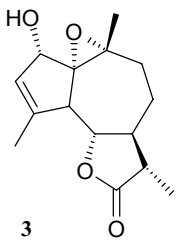
Key words: Sesquiterpene lactones, Monoterpene glucoside, Natural products, Matricaria camomilla, Asteraceae.

Abstract. The genus *Matricaria* (Asteraceae, tribe anthemideae), is used as a valuable ingredient of many galenic preparations [1]. In the present work, we investigated *Matricaria chamomilla* L. which was collected from Oued Tonga near El-Kala in the eastern Algeria in June 2000. After separation and purification by successive chromatographic methods we isolated a new sesquiterpene lactone, matricolone **4**, and a new acyclic monoterpene glucoside, chamol **6**, together with four known compounds from the chloroform soluble part of the aqueous methanol extract of the aerial parts of this plant.

The structures of the known compounds were identified as stigmasterol **1** [2]**,** ethyl caffeate **2** [3]., 2α-hydroxy-arborescin **3** [4] and dihydroridentin **5** [5]. The structure of **4** and **6** were established by spectroscopic methods notably HRFABMS, ^{1}H NMR, ^{13}C NMR, 2D NMR experiments (COSY, HSQC, HMBC, ROESY).



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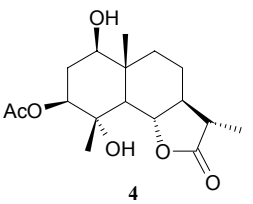

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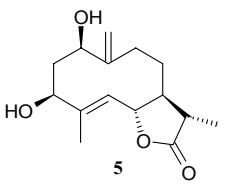

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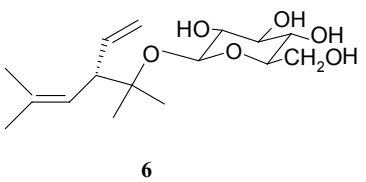

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P268

Polyphenols of Food as Ligands of Neurotransmitters and Hormones Receptors: a Resource of Drug Discovery

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A role of vitamins as exogenous obligatory cofactors (coenzymes) and bioantioxidants is well-known. Experiments in animals discovered affinities of different plant polyphenols (P) to steroid receptors, particularly – estrogens receptors [1]. An affinity of some P to benzodiazepines and adenosine receptors is found too [2,3]. It was supposed that nutritional plant P are obligate only in conditions of influence of unfavourable factors.

The goal of study: exploration of resistance of white rats to stress-factors at different level of receiving of plant polyphenols. Models P deficiency – semisynthetic experimental diet with complete vitamin mixture, which is deprived of plant P; mental stress of anxiety by Desiderato and oral mucosa damage. Methods: biochemical, morphometrical and hystological and tests of behaviour of rats.

Results. Experimental plant polyphenol deficiency not induces any functional or morphological alterations. But the rats with plant P deficiency have increased sensibility to mental stress, to toxical effects of genotoxicants (chloroquine or ftoruracyl) and to oral damage. Different protective adaptogenic effects of different plant preparations: quercetin, genistein, phytosed and flavon-glycosides of wheat and rice were observed. The observed adaptogenic effects of P were explorated by SAR methods [4] and probably targets of P were predicted.

Conclusion. Polyphenols – stilbene derivatives, anthocyanes, catechins and flavonoids of nutritional plants are physiological agonists/antagonists of neurotransmitters and hormoness receptors, and these properties provide their normalizing effects on neurohumoral regulation at adaptation syndrome. Selective affinities of different P to the diverse receptors are the base for development of different stress-protective medicines (adaptogens) and food additions.

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P269

Biotransformation of carvone by environmentally isolated *Chlorella vulgaris*

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Biotransformation of carvone was carried out by locally isolated unicellular microalgae, *Chlorella vulgaris*. The microalgal strain was isolated during a screening program from soil samples collected from paddy-fields of Marvdasht, south of Iran. The *Chlorella vulgaris* was cultured in 500 ml conical flasks, each containing 100 ml of BG-11 liquid medium, incubated at a temperature of 28±2°C and illuminated continuously with fluorescent lamps with shaking at 80 rpm. The samples were taken and extracted for analysis at 2, 4, 6, 12, 24 and 48 hours. The metabolites were identified by thin layer chromatography and GC/MS.

The results showed that *Chlorella vulgaris* has the ability to convert carvone into *cis* / *trans* -dihydrocarvone via reducing C=C bond of cyclohexene ring.

P270

New Flavonoids and other constituents from *Centaurea africana* (Asteraceae)

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Key words: Flavonoids, *Centaurea africana*, Asteraceae, Compositae.

Abstract: As a part of our studies on Algerian Centaurea [1-6], we investigated *Centaurea africana* (Asteraceae) an endemic species to North africa [7],collected from El-Kala in the eastern Algeria in June 2000. The ethylacetate soluble part of the hydro ethanolic extract of this species afforded two new flavonoid derivatives : centaureidin-7-glucoside **15** and comiculatucin 4',3-dimethyl ether(6" synapilglucoside) **14** and 4'-methyl gossypetin **10** which was described for the first time as native natural compound , together with nine known compounds : Methyl-3 (4- hydroxyl phenyl propanoate) **4**, Ethyl -3,4 dihydroxybenzoate **5** , 3'-hydoxyflindulatin **6**, Chrysoeriol **7**, Hispidulin **8**, Jaceosidin **9**, 8-methylgossipetin **11**, nepetin **12** and ferrulic acid **13**. Three other known compounds were isolated from the chloroforme soluble part : β-sitosterol **1**, centaureidin **2** and 3-methyl (4-hydroxyphenyl propanoate) **3**. The structures of the new compounds were established through 1D 1H and 13C experiment and 2D NMR including COSY-45, ROESY, NOESY, HMQC and HMBC spectra as well as with EIHRMS and UV spectroscopy using chelating agents . The known compounds were identified with comparison with literature data and were not reported before from this species.

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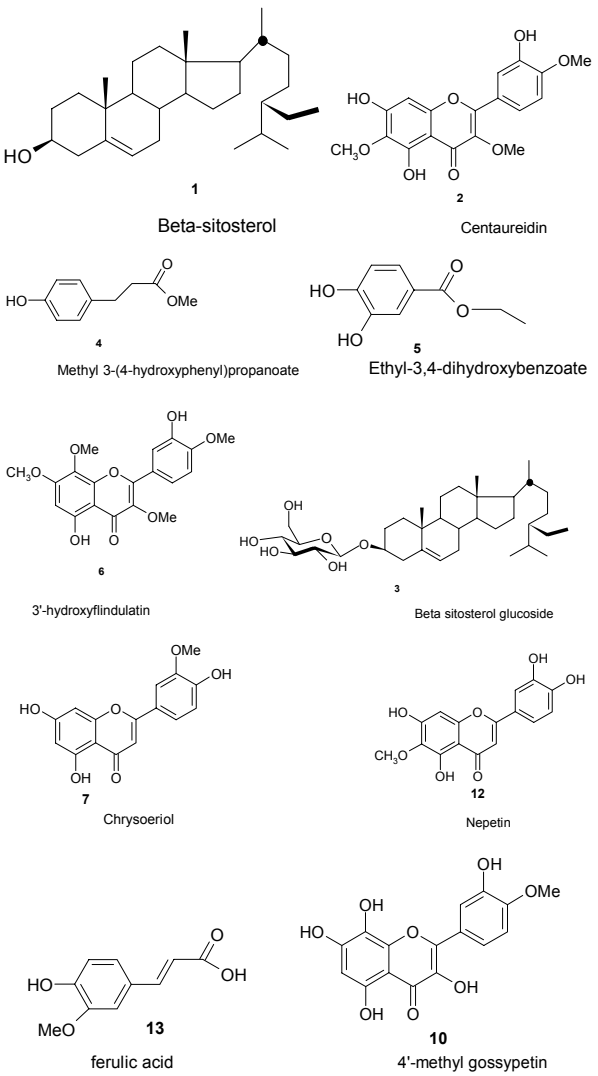
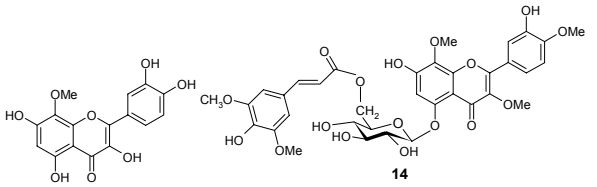
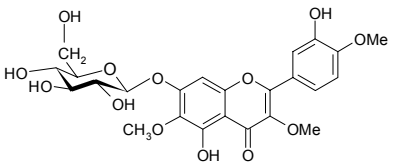




11
8-methyl gossypetin

14
Corniculatucin 4',3-dimethyl ether 5-(6"-sinapylglucoside)



15
Centaureidin 7-glucoside

P271

Biotechnology of *Echium* genus for the production of medicinally important fatty acids

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Highly unsaturated fatty acids (HUFAs) of the ω-3 & ω-6 series play important roles in several pathologies, specially to prevent cardiovascular diseases. Furthermore, stearidonic acid (SDA) and gammalinolenic acid (GLA), are precursors of HUFAs (AA, araquidonic acid; EPA, eicosapentaenoic acid and DHA, docosahexaenoic acid), compounds which aid to suppress the growth of some tumors and proliferation of breast cancer and melanoma. The genus *Echium* (*Boraginaceae*) is represented by 23 endemic species in the Canary Islands. These constitute the largest plant source of SDA and GLA. By biotechnological tools, induction and establisment of hairy root cultures of *E. acanthocarpum* have been achieved. These cultures produced, among other fatty acids, SDA and GLA at different amounts following growth. Furthermore, from the liquid nutrient medium a novel compound, a sequiterpene quinone named acanthoquinone, has been isolated and structurally elucidated. In order to increase the accumulation of SDA and GLA, the overexpression of Δ6 desaturase, which converts linoleic acid into GLA and linolenic acid into SDA, will be attempted in transgenic *E. acanthocarpum* hairy roots. Furthermore, a complete phytochemical study of this culture system is underway, as well as the induction and establishment of other *Echium* species hairy roots.

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P272

Search of new bioactive compounds from *Celastraceae* species: phytochemical studies and biotechnological approach.

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As part of our research on bioactive compounds from South American medicinal plants, we studied *Maytenus ilicifolia* (*Celastraceae*), broadly distributed in Southern Brazil, used as antiulcer, antiseptic, anticonceptive, antiasthmatic and as antitumour agent [1,2]. The n-hexane:Et_2O root bark extract was repeatedly chromatographed to yield three new compounds (a triterpene phenol with tingenol skeleton, an octacyclic sesquiterpene-triterpene hetero Diels-Alder adduct and a dimer composed of one quinoid-type triterpene and one aromatic triterpene derived from pristimerine), and several known bioactives molecules. The n-hexane extract of *Plenckia integerrima* (*Celastraceae*) roots, yielded several new agarofuran sesquiterpenes and other known bioactive compounds. Their structures were determined by means of ^1H and ^{13}C NMR spectroscopy studies, including ^1H-^{13}C heteronuclear correlation, (COSY, ROESY, HSQC and HMBC). *In vitro* culture of *M. canariensis*, a *Celastraceae* species endemic to the Canary Islands which present bioactive agarofuran sesquiterpenes with antitumoral, anti-MDR and antifeedant activities [3,4], and the establishment of hairy roots have been attempted. This culture system would provide a continuous source of these valued metabolites. Results on the phytochemical studies of *M. ilicifolia* and *P. integerrima* as well as results on the biotechnological approach with *M. canariensis* are presented.

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P273

Bioassay-guided isolation of leishmanicidal compounds from *Piper elongatum*

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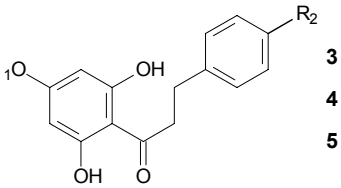
The genus *Piper* (Piperaceae), widely distributed in the tropical and subtropical regions of the world, is often used as food flavouring agents and traditional medicines [1]. Phytochemical investigations of *Piper* species have led to the isolation of several classes of physiologically active compounds such as alkaloids, amides, pyrones, dihydrochalcones, flavonoids, phenilpropanoids, lignans and neolignans [2].

As part of our research aiming to uncover leishmanicidal compounds from Bolivian *Piper* species, we have studied the CH_2Cl_2 soluble fraction of the ethanol extract of *Piper elongatum* Vahl, which showed *in vitro* leishmanicidal activity and was subjected to bioassay-guided fractionation, resulted in the isolation of five compounds, a phenilpropanoid (**1**), a prenylated benzoic acid (**2**) and three dihydrochalcones (**3**-**5**). The structures of these compounds were elucidated on the basis of spectroscopic data and chemical evidence.



3 $R_1 = CH_3$, $R_2 = H$

4 $R_1 = CH_3$, $R_2 = OH$

5 $R_1 = H$, $R_2 = H$

All compounds were assayed for their *in vitro* leishmanicidal activity against promastigote forms of three *Leishmania* strains. The most active compound was the dihydrochalcone **4** (IC_{50} 5 μg/ml), also more active than the positive control pentamidine (IC_{50} 10 μg/ml).

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Neurodegeneration

P274

Development of 2-Amino-Pyrimidines as Selective Adenosine hA_{2a} Receptor Antagonists

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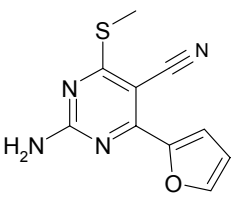
Antagonists of the human adenosine A_{2a} receptor show potential as therapeutic agents in the treatment of a number of disorders of the central nervous system, such as Parkinson's Disease [1]. A random screen of the Roche compound library led to the identification of several 2-amino-5-cyano-pyrimidines – as exemplified by RO-19-2712 (**1**) – possessing moderate binding affinity at the hA_{2a} receptor but lacking selectivity *vs* the other adenosine receptor sub-types. Systematic investigation of the structure-activity relationships (SAR) guided the optimization of this class to provide compounds (**2**) having significantly enhanced binding affinity at the A_{2a} receptor ($Ki \leq 1$ nM) and improved selectivity (100-1000 fold) *vs* the hA_1 receptor [2]. Many of these compounds displayed A_{2a} antagonistic activity in animal behavioural models.



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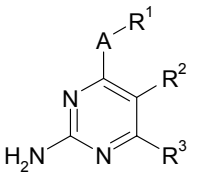

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P275

Discovery of novel, potent and fully selective MAO-B inhibitors

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Mononamine oxidases (MAO) are flavin adenine dinucleotide (FAD) containing enzymes located in the outer membrane of mitochondria. They catalyze oxidative deaminations involving the reduction of FAD to $FADH_2$ and the conversion of substrate amine to an aldehyde by formation of hydrogen peroxide from oxygen.

MAO-A and B catalyze the oxidative deamination of brain neurotransmitters such as dopamine and serotonin as well as a variety of biogenic and xenobiotic amines.

The first generation of MAO inhibitors were irreversible and unspecific for the two MAO isoforms. A fully selective and reversible MAO-B inhibitor would have a

better safety profile and therapeutic scope in a range of neurodegenerative diseases [1] such as Parkinson´s disease (PD) and Alzheimer´s disease (AD) and in anti-addiction therapies such as smoke cessation.

A high throughput screening (HTS) campaign was initiated with the goal to discover a novel molecular class with potential to rapidly be transformed into a development candidate.

We wish to disclose, for the first time in detail, our successful efforts in the discovery of an attractive Lead Series. We will describe a process that facilitated the rapid identification of low molecular weight, highly selective, potent and *in vivo* active compounds demonstrating a broad SAR within a range of novel chemotypes emerging from exploration of the parent hit series.

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P276

Synthesis of novel condensed 3-oxo[1,2,4]triazoles as potential jnk-3 inhibitors.

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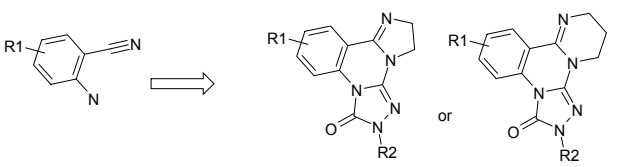
The c-Jun N-terminal protein kinases (JNKs) are members of the mitogen-activated protein kinase (MAPK) group of serine/threonine protein kinases and play critical role in a wide range of disease. JNK3, which is predominantly found in CNS neurons, has been implicated in several neurodegenerative diseases, including Parkinson's disease Alzheimer's disease, and stroke. Because of the involvement of JNK3 in neuronal diseases, the inhibition of this enzyme is an attractive therapeutic target.

A number of inhibitors of JNK3 have been reported about in the last three years [3],[4].

Screening of our internal compound collection for inhibitors of JNK3 led to the identification of two novel classes of condensed 3-oxo[1,2,4]triazoles as potent JNK3 inhibitors. Mentioned templates were synthesized based on commercially available 2-cyano-anilines in 6 steps Further SAR is currently under investigation.



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P277

β-(γ-)Carbonyldihydropyridines as neuroprotectors and privileged structures

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β-(γ-)Carbonyldihydropyridines can be regarded as biomimetics:

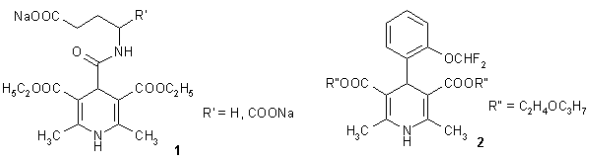
1) as analogues of NAD(P)H, possessing electron- and hydrogen-donating properties resulting in antioxidant activity;

2) as functional peptidomimetics: possessing neuroprotective properties;

3) as calcium channel antagonists, anti-inflammatory compounds, immunoprotectors.

1,4-Dihydropyridine (DHP) structure is proposed as a "privileged pharmacophore" capable of being appropriately "decorated" to provide a number of ligands and drugs [1].

DHP derivatives **1** comprise moieties of unsaturated cyclic amino acids, they can be regarded as analogues of dipeptides. Like peptidomimetics they possess long-lasting neuroprotective, memory enhancing, anti-alcohol properties at low concentrations. Comp. **1** (R'=COONa) and its DHP moiety possess anti-mutagenic properties [2] and antioxidant activity. Also, 4-aryl-1,4-DHP derivatives, e.g., **2** (*cerebrocrast*) reveal bioregulatory properties. *Cerebrocrast* possesses a selective cerebrovasodilating activity.



Mitochondria can be considered as a target for **2** neuroprotective action. So, in MPP$^+$ neurotoxicity model, *cerebrocrast* completely protected cells from alterations in mitochondrial bioenergetics (leading to cell death by affecting mitochondrial metabolism and free radical production) by preventing neurons from oxidative damages.

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P278

Polycyclic amine and guanidine structures as novel entities for NOS inhibition

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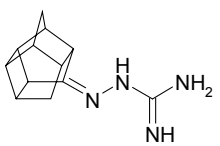
Nitric oxide (NO) has various roles in normal physiology. Elevated levels of NO however, contribute to neurodegeneration and have been implicated in diverse pathological conditions. Inhibition of nitric oxide synthase (NOS) can thus be of value in the treatment of neurodegenerative diseases. As intracellular concentrations of l-arginine are typically in excess of its Km value for NOS, potent inhibitors are necessary for significant inhibition.

Amino guanidine (AG) displays high isoform selectivity but has very low potency[1]. This along with the absence of a transport mechanism for guanidino compounds into the brain led to the incorporation of AG and related structures on polycyclic hydrocarbons with known blood-brain barrier permeability[2].



8-imine-N-guanidino-pentacyclo[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}]undecane

Conjugation of pentacycloundecane-8-one and pentacycloundecane-8,11-dione with guanidine and amine structures yielded the desired test compounds. The oxyhaemoglobin assay was employed to measure the activity of the compounds *in vitro*.

IC_{50} values of the novel guanidines compared favourably to that of AG and the presence of the carbamidine moiety was found to be essential for activity. The combination of AG and the polycyclic cage compounds can thus be applied to achieve increased activity and thus better therapeutic inhibition of NOS.

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

P279

A new approach to the synthesis of derivatives of 4–amino 5,6,7,8–tetrahydrothieno [2,3-b] quinolines , analogues of tacrine a drug against Alzheimer disease

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Alzheimer's disease (AD) is characterized by a progressive loss of cognitive functions and has been associated with the impairment of the cholinergic system. Currently, one therapeutic intervention in AD involves the enhancement of the levels of the neurotransmitter acetylcholine (Ach) through the administration of acetylcholinesterase inhibitors [1]. TACRINE (5-amino 1,2,3,4-tetrahydroacridine) [2] was the first drug (COGNEX®) authorized since 1993 for AD therapy.

In a first part of our work [3], we did show that the synthesis of new 9-amino 5,6,7,8 – tetrahydrothieno [3,2-b] quinoline 8-ols and new 4-amino 5,6,7,8- tetrahydrothieno [2,3-b] quinoline 5-ols as analogues of VELNACRINE, the 1-hydroxy derivative of TACRINE is possible by condensing aminocyanothiophenes with alicyclic 1,3- diketones.

In the new approach, we condensed 4-substituted 2-amino 3-cyanothiophenes obtained with very good yields by a one or two step procedure including GEWALD-reaction with alicyclic ketones through a FRIEDLÄNDER-reaction [4] obtaining thus in very good yields (> 80%) 4-amino 5,6,7,8 – tetrahydrothieno [2,3-b] quinolines as analogues of TACRINE in one single step.

There, condensation of aromatic cycles [5] or aromatic heterocycles (this work) with amino and cyano functions in an ortho position, opens a new and very fast way to realize a library of analogues of TACRINE and VELNACRINE. The examination of acetylcholinesterase inhibition properties of the new compounds by the ELLMANN protocol is underway.

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P280

Monoamine Oxidase B substrate properties and neurotoxicity of 1-methyl-3-phenyl-3-pyrrolines

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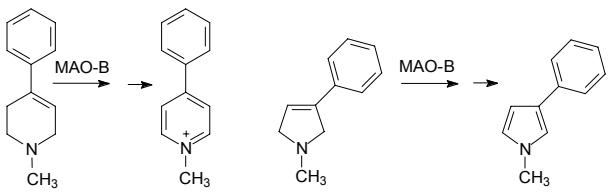
1-Methyl-3-phenyl-3-pyrroline is an analogue of the pro-neurotoxin, MPTP, and like MPTP, has been reported to be a substrate for monoamine oxidase B (MAO-B) [1]. In this report we describe the synthesis of additional 1-methyl-3-phenyl-3-pyrrolines and their evaluation as MAO-B substrates. Selected 1-methyl-3-phenyl-3-pyrrolines were also evaluated for neurotoxicity in mice. Unlike MPTP, which is bioactivated by MAO-B to the charged toxic species MPP^+, the 1-methyl-3-phenyl-3-pyrrolines are not metabolized to permanently charged end-products. As expected, these compounds did not mimic MPTP's neurotoxic action.



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P281

Antioxidative quercetin 3-methyl ether derivatives as potential anti-ischemic compounds

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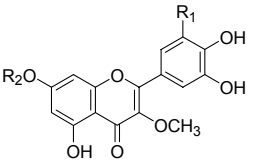
Free radical generation is known to be involved in the pathophysiologic mechanisms of ischemic stroke, particularly in ischemia-reperfusion injury [1]. Quercetin 3-methyl ether (QME, **1**), an antioxidative flavonoid, was shown to exhibit potent neuroprotective activities against the oxidative injuries induced in cortical neuronal cell cultures [2]. The present study, therefore, examined *in vivo* neuroprotective effects of antioxidative derivatives of QME in a rat model of 2-hr transient focal cerebral ischemia. Compounds were administered intravenously at a dose of 10 mg/kg 30 min after onset of ischemia. Infarct size was measured 24 hr after onset of ischemia. QME significantly reduced both corrected total infarct volume and edema by 49 and 51%, respectively, compared with a vehicle-treated control group. 3',4',5,5',7-Pentahydroxy-3-methoxyflavone (**2**) and 3-methoxy-7-(2-(piperidin-1-yl)ethoxy)-3',4',5-trihydroxyflavone (**3**) hydrochloride, which had antioxidative activities similar to QME, also produced neuroprotective effects similar to QME. However, 7-(2-(diethylamino)ethoxy)-3',4',5,5'-tetrahydroxyflavone (**4**) hydrochloride, which had much lower superoxide anion radical scavenging activity than QME, produced the weakest neuroprotective effect compared to the other compounds.



1, R_1 = H, R_2 = H
2, R_1 = OH, R_2 = H
3, R_1 = H, R_2 = 2-(piperidin-1-yl)ethyl
4, R_1 = OH, R_2 = 2-(diethylamino)ethyl

The synthesis and correlation of antioxidative activities with neuroprotective effects of QME derivatives in a transient focal cerebral ischemic rat model will be presented.

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P282

A New Aβ Modulator, NP0361, As A Potential Disease-Modifying Agent For The Treatment Of Alzheimer's Disease

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Neuropharma S.A., Avda. Industria 52, 28760 – Tres Cantos (Madrid), Spain

Alzheimer's disease (AD) is a neurodegenerative disorder that currently affects nearly 2% of the population in industrialized countries. Treating AD is the biggest unmet medical need in neurology. Current drugs are safe and improves symptoms, but do not have profound disease-modifying effects. Therapeutic strategies aimed at lowering levels of Aβ peptides as well as reducing Aβ deposition in the brain constitute a very promising approach to search for disease-modifying effects. In that regard, we have recently shown evidence that our Aβ modulator, namely NP0361 is able to reverse memory deficits in the Morris water maze paradigm as well as to reduce the amyloid plaque load in the brains of hAPPsw transgenic mice after 3 months oral treatment. NP0361 is a rationally designed synthetic compound based on AD molecular targets. To gain insight into the precise mechanism by which NP0361 might act, *in vitro* Aβ aggregation experiments have been performed showing that this compound

indeed inhibits AchE-induced Aβ aggregation with the highest potency reported so far. NP0361 also displays high inhibitory potency against human AchE and high selectivity regarding BuChE. Here we will present also evidence showing that NP0361 is able to significantly reduce Aβ production in several APP-transfected cell lines. In summary, we believe that all these data indicate that NP0361 is a promising disease-modifying agent of great potential therapeutic value for the treatment of AD.

P283

Annelated Xanthine Derivatives as A_1 and A_{2A} Adenosine Receptor Ligands

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[1]Department of Technology and Biotechnology of Drugs, Medical College, Jagiellonian University, Kraków, Poland. [2]Pharmaceutical Institute Poppelsdorf , University of Bonn, Bonn, Germany. [3] Institute of General and Ecological Chemistry, Technical University of Łódź, Łódź, Poland

Compounds envisaged as constrained bioisosteric analogs of 8-styrylxanthines were designed: the group of oxygen- or nitrogen-containing annelated theophylline derivatives. Obtained, pharmacologically tested (A_1, A_{2A}AR activity) and spatially examined, were tricyclic derivatives with oxygen and nitrogen heteroatoms in the third cycle.

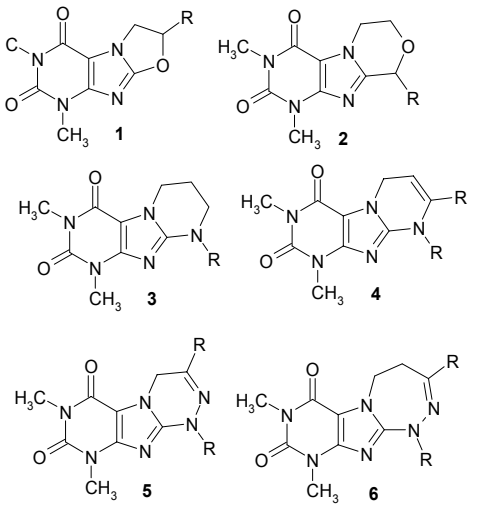
Oxygen containing tricyclic xanthine derivatives can be subdivided into two categories: five-membered and six-membered. Oxazolo[3,2-*a*]purinediones (**1**) have shown moderate micromolar affinity toward A_{2A}AR. Within this group the longer alkyl-substituent in oxazole ring, the more active derivative was [1]. Compounds containing phenyl ring substituent were low affinity ligands. The ring-enlarged analogues [1,4]oxazino[4,3-*a*]purinediones (**2**) have shown improvement in affinity.

Nitrogen-containing ligands fall into two groups: pyrimidine and triazine-derivatives. Pyrimido[1,2-*a*]purinediones (**3)** appeared to be more active then oxygen containing ligands. These compounds with the N-alkylphenyl substituent have shown submicromolar affinity and selectivity toward A_{2A}AR. Introduction of oxygen or nitrogen into the spacer connecting phenyl ring with tricyclic structure, has led to decrease in affinity toward both A_1 and A_{2A}ARs [2]. In the group with N-benzyl substituents, 1,3-dipropyl analogues possessed nanomolar affinity toward A_1AR but at the same time they have lost their selectivity. 6,9-Dihydropyrimido[1,2-*a*]purinediones (**4**) possessing N-alkyl substituent showed better affinity than benzyl analogues, halogen substituents in the phenyl ring are not favourable for the affinity.

Triazino-derivatives in turn can be subdivided into two categories: triazino- and triazepinopurinediones. [1,2,4]Triazino[4,3-*a*]purinediones (**5**) have shown selective affinity toward A_1AR. Compounds with C3-phenyl substituent were the most active, introduction of N-acyl group has led to the improvement of selectivity. In turn, N-phenyl derivatives were inactive toward both AR subtypes. The N-unsubstituted analogues in the group of 1,2,4-triazepino[4,3-*f*]purinediones (**6**) are A_{2A}AR ligands, while the N-substituted compounds are A_1AR ligands. The character of N-substituent is important for the selectivity while the length is important for affinity. Generally, compounds possessing ethyl moiety at C3-atom of triazepine ring were more active.

To summarise, the nitrogen containing xanthine derivatives have shown better activity than their oxygen analogues. Six-membered third ring was more favourable for binding then five-membered [2]. Long chain alkyl substituents in the fused cycle were good for affinity, but not for selectivity.

The research was partly supported by Polish Committee of Scientific Research (Grant No 2 PO5F 014 28, No 2 PO5F 022 26)



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P284

The Development of Novel Glutamate Carboxypeptidase II Inhibitors

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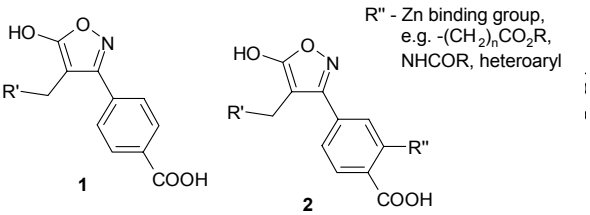
Glutamate carboxypeptidase II is Zn containing metallopeptidase that, in central nervous system (CNS), cleaves dipeptide *N*-acetylaspartylglutamate (NAAG), producing *N*-acetylaspartate (NAA) and *L*-glutamate (Glu) [1]. GCP II is believed to be responsible for the regulation of levels of the major CNS neurotransmitter- Glu

and that of endogenous agonist of mGluR3 receptor - NAAG. Consequently, GCP II inhibitors have a potential for the treatment of neurodegenerative diseases associated with pathological levels of glutamate, including amyotropic lateral sclerosis (ALS), schizophrenia, Alzheimer`s disease, Parkinson`s disease and neuropathic pain [1]. Potent GCP II inhibitors have been developed exhibiting inhibitory activity at nanomolar range [2, 3]. However, known inhibitors contain at least two carboxylic groups and are highly polar, preventing the blood-brain-barrier penetration.



We focused our research on the development of less polar and metabolically stable GCP II inhibitors. Screening of nonionizable compound library and preliminary hit optimization resulted in a series of compounds with general formula **1** with inhibitory activity within the range of IC_{50}= 1 – 10 μM.

We believe that further modification of the scaffold in order to improve activity could be done by introducing Zn binding groups (like $–(CH_2)_nCO_2R$, -NHCOR, heteroaryl) to the molecule. Herein we report our endeavour to develop synthetic route toward 3-(4-carboxyphenyl)-5-hydroxyisoxazole **2** substituted in the 3rd position of the benzene ring with potential Zn binding groups.

This work has been partly supported by the European Social Fund within the National Programme “Support for the carrying out doctoral study programm`s and post-doctoral researches project “Support for the development of doctoral studies at Riga Technical University”.

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[3] Alan P. Kozikowski, Jiazhong Zhang, *J. Med. Chem.,* 2004, *47*, 1729-1738.

P285

A novel neuroprotective agent with antioxidant and nitric oxide synthase inhibitory action

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Nitric Oxide (NO) and reactive oxygen species (ROS) act independently as well as cooperatively to induced neuronal death in neurological disorders. Inhibition of neuronal nitric oxide synthase (*n*NOS) and inhibition of lipid peroxidation induced by ROS have been proposed as neuroprotective strategies [1-2]. N^{α}-vanillyl-N^{ω}-nitroarginine (**N-1**) that combines the active functions of natural antioxidant and nitric oxide synthase inhibitor was developed and investigated for its neuroprotective properties. Compound **N-1** exhibited protective effects against hydrogen peroxide-induced cell damage and the inhibitory effect on nitric oxide production in NG 108-15 neuroblastoma cells. The result showed that **N-1** at 0.1 μM significantly protected neurons from H_2O_2-induced cell death in NG 108-15 cells and decreased nitric oxide production induced by calcium ionophore significantly at 100 μM. **N-1** inhibited the constitutive NOS isolated from rat cerebellar in a greater extent than constitutive NOS from human endothelial cells. Low binding energy (-10.2 kcal/mol) obtained from docking **N-1** to nNOS supported the additional mode of action of **N-1** as an nNOS inhibitor. The *in vivo* neuroprotective effect on kainic acid-induced nitric oxide production in rat brain was investigated via microdialysis. Rats were injected intra-peritonially with **N-1** at 75 μmole/kg before a kainic acid injection (10 mg/kg). The significant suppression effect on kainic acid-induced NO was observed in the hippocampus at 40 minutes after the induction. The quantitative analysis of the histological damage showed that **N-1** increased the number of surviving cells in the CA1 and CA3 cell layers significantly when compared with KA-treated group. The results indicate that **N-1** should be a promising candidate for treatment of neurodegenerative disorders that involve overproduction of nitric oxide and reactive oxygen species.

[1] Dawson VL. Potent neuroprotectants linked to bifunctional inhibition. Proc Natl Acad Sci USA 1999; 96:10557 – 8.

[2] Spinnewyn B, Cornet S, Auguet M, Chabrier PE. Synergistic protective effects of antioxidant and nitric oxide synthase inhibitor in transient focal ischemia. J Cereb Blood Flow Metab 1999; 19:139 – 43.

P286

Design, Molecular Modeling Studies, Synthesis and Preliminary Biological Evaluation of Novel PARP-1 Inhibitors on the Road of Antiischemic Agents.

E. Camaioni[1], P. Sabbatini[1], G. Costantino[1], D. Bellocchi[1], A. Chiarugi[2], F. Moroni[2], A. Wood[3], R. Pellicciari[1]

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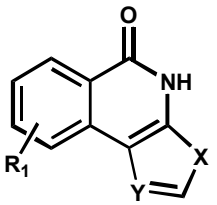
Poly(ADP-ribosyl)ation is a transient post-translational modification which takes place in eukariotes in response to exposure to DNA-damaging agents involved

in fundamental processes related to the preservation of genomic integrity such as DNA repair, chromatin decondensation, and, under certain circumstances, cell necrosis, and apoptotis. Potent PARP inhibitors have been shown to be endowed with neuroprotective properties in experimental models of brain ischemia, thus representing a promising new class of anti-ischemic agents. By taking advantage of the X-ray structures of the catalytic domains of PARP-1 and PARP-2, in silico docking and virtual screening studies were carried out [1]. The integration of these results with the structure activity relationships based on our potent thieno[2,3-c]isoquinolin-1-one derivative (TIQ-A) [2], allowed the design and synthesis of a series of novel inhibitors which, tested for their ability to inhibit PARPs, showed high potency and selectivity.



X, Y= CH, S, O, N;

R_1= H, F, Cl, OH, OCH_3, NH_2.

Some of the new potent derivatives were selected for further characterization and found to be endowed with neuroprotective properties in models of cerebral ischemia.

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P287

Design and synthesis of acetylcholinesterase inhibitors

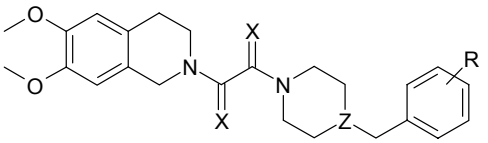
R. Gitto[1], A. Chimirri[1], B. Pagano[1], E. Francica[1], S. De Grazia[1], G. De Luca[2]

[1]Department of Medicinal Chemistry, University of Messina, Italy. [2]Department of Biochemical, Physiological and Nutritional Sciences, University of Messina, Italy

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by a pronounced degradation of the cholinergic system and by alteration in other neurotransmitter system such as glutamatergic one.

The AD treatment has been dominated by the use of acetylcholinesterase inhibitors (AChEIs) [1] which increase the acetylcholine concentration. Recently, it has pointed out that AChE is also involved in the proaggregating activity toward β-amyloid peptide (Aβ) thus suggesting that it plays a key role in the development of the senile plaques. In particular the peripheral anionic binding site (PAS) of AChE is responsible for amyloid fibril formation. For these reasons AChEIs interacting with catalytic and peripheral binding site represent a newer therapeutic approaches [2] to contrast hypofunction of cholinergic system and to avoid Aβ aggregation.

In the search for new agents useful in AD therapy and on the basis of main structural requirements characterizing the AChEIs we designed a new class of potential ligands (**1**) acting as dual binding site enzyme inhibitors.



X = O, H_2 Z=CH, N **1**

A solution-phase parallel approach for the synthesis of a small library of type **1** derivatives has been set up. The results of the evaluation of their inhibitory potency will be reported and discussed.

[1] Lleo A, Greenberg SM, Growdon JH. Current pharmacotherapy for Alzheimer's disease. Annu Rev Med 2006; 57: 513-533.

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P288

Design and Synthesis of Novel Histamine H3 Antagonists: Potential Therapeutics for the Treatment of Alzheimers Disease

V. Johnstone, L. Abberley, J. Bailey, M. Briggs, G. Bruton, A. Huxley, A. Medhurst, B. Orlek, G. Stemp, B. Trail, D. Wilson.

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Alzheimer's disease can affect up to 30% of patients over 80 years of age. It is a debilitating disease that places a huge burden of care on the family and community. As such there is a clear unmet medical need for a more effective treatment.

Histamine H3 receptor antagonists are attractive therapeutic targets for the management of CNS mediated disorders[1]; including cognition impairment (Alzheimer's Disease), narcolepsy, ADHD, schizophrenia, epilepsy, pain and obesity. Preclinical data[2] indicate a positive correlation between Histamine H3 receptor blockade and an increase in cognitive function. These data suggest that novel H3 antagonists could be useful in the treatment of Alzheimer's disease.

This poster will describe the discovery of novel and highly effective H3 antagonist motifs. Optimisation of analogues within the constraints of a self-imposed molecular budget designed to control Mw, logP and PSA, produced

a series that exhibited excellent developability characteristics including high Br:BI ratio, low clearance and good oral bioavailability.

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P289

Investigation of in vitro antioxidant behaviour of some 2-phenylindole derivatives: discussion on possible antioxidant mechanisms and comparision with melatonin

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Ankara University, Faculty of Pharmacy, [a]Department of Pharmaceutical Chemistry, [b]Department of Pharmaceutical Toxicology, 06100, Tandogan, Ankara, Turkey.

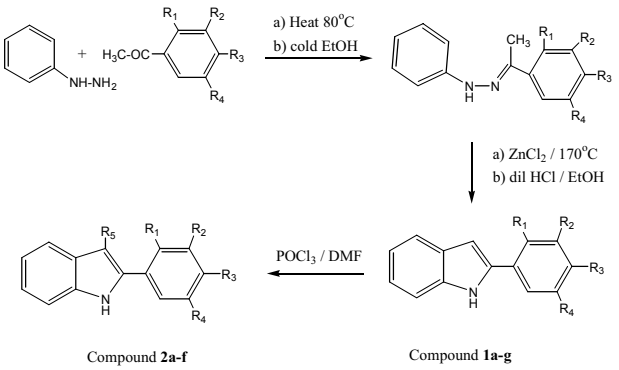
Oxidative stress has been implicated in the development of many neurodegenerative diseases and also responsible from aging and some cancer types. . Indolic compounds are a broad family of substances present in microorganisms, plants and animals. They are mainly related with tryptophan metabolism, and present particularities that depend on their respective chemical structures. Due to free radical scavenger and antioxidant properties of indolic derivatives such as indolinic nitroxides and melatonine, a series of 2-phenyl indole derivatives were prepared and their *in vitro* effects on rat liver lipid peroxidation levels, superoxide formation and DPPH stable radical scavenging activities were determined against melatonin, BHT and α-tocopherol.

The inhibitory effects of the compounds on LP levels were determined by measuring the formation of 2-thiobarbituric acid reactive substances. All the compounds were evaluated against melatonin which was chosen as reference compound. The reason using melatonin was its high anti-oxidant capacity. Compounds **1a-f, 2e, 2f** significantly inhibited **(72-98 %**) LP at 10^{-3} M and 10^{-4} M concentrations.

The superoxide anion radical scavenging activities of 2PI and 2PIA derivatives at different concentration were investigated. The results showed that compounds **1f** and **1g** have strong scavenger effect on superoxide anion at 10^{-3} M concentration. Additionally, these compounds had comparable scavenger effects on superoxide radical as that of SOD (89 % inhibitor at 45 IU).

The scavenging effect of 2PI and 2PIA derivatives on the DPPH radical was examined as well. Compounds **1e** and **1f** have highest DPPH scavenger activity at 10^{-3} M and 10^{-4} M concentrations (88-96 %). These compounds were found to be as effective as the BHT, a well known antioxidant utilized as positive control.

The *in vitro* experimental findings of the indole derivatives show that there are many derivatives that has good in some cases better antioxidant properties than melatonin. It would be considerable to synthesised more derivatives to find out their free radical scavenger activities and make biological evoluation of the synthesized compounds.



Compound **2a-f** Compound **1a-g**

Comp.	R_1	R_2	R_3	R_4	R_5
1a	H	H	H	H	H
1b	H	H	F	H	H
1c	H	H	Cl	H	H
1d	H	Cl	Cl	H	H
1e	H	H	NH_2	H	H
1f	H	NO_2	Cl	H	H
1g	OH	H	H	CH_3	H
2a	H	H	H	H	CHO
2b	H	H	F	H	CHO
2c	H	H	Cl	H	CHO
2d	H	Cl	Cl	H	CHO
2e	H	H	NH_2	H	CHO
2f	H	NO_2	Cl	H	CHO

P290

Neuroprotective effects of substituted 9-aminoacridines

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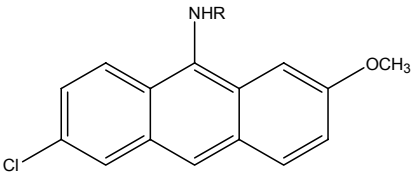
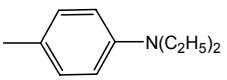
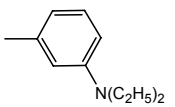
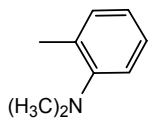
Quinacrine has been reported to abolish increases in cytoplasmic phospholipase A_2 (PLA_2) mRNA levels in the rat hippocampus after kainate (KA)-induced neuronal injury [1]. This neuroprotective action may stem from the ability of quinacrine to block the upregulation of PLA_2 after KA treatment. In order to establish an understanding of the structural features of quinacrine associated with this activity, various 9-substituted aminoacridines were synthesized and evaluated for their ability to protect neuronal cells from KA-induced neurotoxicity [2].

The results showed that the nature of the substituent on the 9-amino function of the 2-methoxy-6-chloroacridine ring influenced activity to a great extent. When tested at 25 μM, aliphatic substituents on the

amino function (compounds 1-4) were less effective compared to aromatic substitution (compounds 5-7). From this series, greatest protection against kainate-induced toxicity was found for compound 6. A regioisomeric effect was evident in that locating the diethylamino substituent at the meta position was preferable to either a para or ortho location.



Compound	R
1	$-(CH_2)_3N(C_2H_5)_2$
2	$-(CH_2)_3N(CH_3)_2$
3	$-(CH_2)_2N(C_2H_5)_2$
4	$-(CH_2)_4N(C_2H_5)_2$
5	
6	
7	

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Nuclear Receptors

P291

Novel synthetic derivatives of pirinixic acid as potent dual PPAR α/γ agonists

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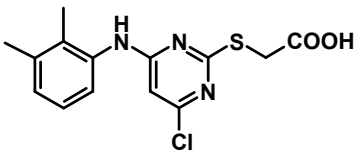
The **P**eroxisome **P**roliferator-**A**ctivated **R**eceptors (PPARs) α, γ and β/δ are nuclear hormone receptors, that regulating fatty acid and glucose homeostasis. PPARá ligands include the fibrate drugs which are used in patients with hyperlipidemia and which reduce triglycerides, increase HDL cholesterol and reduce inflammation. Ligands of PPARγ include the thiazolidinedione (TZDs) class of agents used in the treatment of type 2 diabetes.

Muraglitazar and tesaglitazar, collectively known as the glitazars, are a new class of antidiabetic drugs that activate both PPARα (as do fibrates) and PPARγ (as do TZDs); PPARα/γ dual agonists.

Pirinixic acid (Wy 14643) has been recognized before as a moderate activator of both PPARα and PPARγ. This compound activates hPPARα with an EC_{50}-value of 39.9 µM, and 53.7 µM at hPPARγ.



Wy 14643

Here we show that introduction of aliphatic residues in α-position of pirinixic acid leads to a series of potent dual activators of human PPARα and PPARγ, exemplified by the most potent dual PPARα/γ activators YS_{85} (α=*n*-butyl), YS_{103} (α=dimethyl) and YS_{121} (α=*n*-hexyl). α-substitution with an aliphatic chain leads to a 30-fold higher activity on PPARα and about 20-fold induction of PPARγ activity in comparison to pirinixic acid. Replacement of the 2,3-dimethylaniline by a 6-aminoquinoline and 3,5-Bis-(2,2,2-trifluoro-ethoxy)-phenylamine structure leads to a series of potent activators of PPARα and PPARγ, exemplified by LP_{120}, LP_{121} and LP_{105}. Introduction of 6-aminoquinoline without simultaneously alkyl-substitution in α-position failed to activate PPARs.

In summary, we have identified novel pirinixic acid derivatives as potent dual PPARα/γ agonists.

P292

Synthesis and Biological Evaluation of Thiocarbamate-based 2-Ethoxypropinoic Acids as Novel PPAR / Dual Agonists

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As members of the nuclear hormone receptor super family of ligand-activated transcription factors, the PPARs(peroxisome proliferator-activated receptors) exert a role in regulating various cellular process including the storage and catabolism of fats and carbohydrates. In particular, PPAR γ has attracted public attention because it has been considered as the primary receptor modulating the antidiabetic activity of TZDs through amelioration of insulin resistance. In addition, PPAR α has been known to regulate lipid homeostasis via fatty acid catabolism. Considering the significantly increased risk of metabolic disorder related with lipid catabolism among patients with

type 2 diabetes, it has been postulated that a PPAR α/γ dual agonist might present an outstanding agent for the treatment of type 2 diabetes and dyslipidemia. Our paper reports design, synthesis and biological evaluation of a series of novel thiocarbamate-based 2-ethoxypropanoic acids as potent PPAR α/γ dual agonists. In addition, their structure-activity will be also discussed.

P293

SAR of novel selective, partial PPARδ agonists with good pharmacokinetic properties in vivo. PPARδ subtype selectivity obtained by only modification of the acidic part of the molecule.

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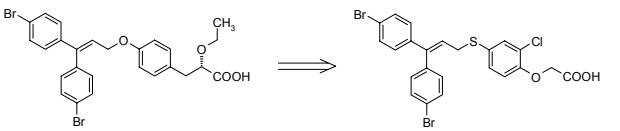
The aim was to identify a novel selective, partial (in transactivation assay) PPARδ agonist with full efficacy on fatty acid (FFA) oxidation in vitro and good oral pharmacokinetic properties in vivo. Using the triple PPARα,γ,δ agonist **1** as the structural lead we wanted to investigate the possibility of obtaining selective PPARδ agonists by modifying only the acidic part of **1**, while holding the lipophilic half of the molecule constant. The Structure-Activity Relationship was guided by in vitro transactivation data in human PPAR receptors, FFA oxidation efficacy in rat muscle L6 cell line and in vivo rat pharmacokinetic properties. Compound **7** ([4-[3,3-bis-(4-bromo-phenyl)-allylthio]-2-chloro-phenoxy]-acetic acid) was identified as a selective, partial agonist with good oral pharmacokinetic properties in rat. These data suggest that **7** could be a useful tool for investigations of the pharmacology of selective, partial PPARδ agonists.



PPARpan agonist (**1**) Selective, partial PPARδ agonist (**7**)

P294

Structure-Activity Relationship of Telmisartan and Derivatives Regarding their PPARγ Activity

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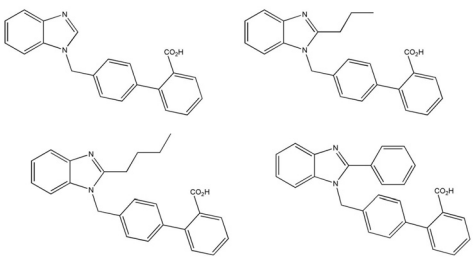
The peroxisome proliferator-activated receptors (PPARs) are members of the nuclear receptor superfamily of ligand-activated transcription factors [1]. A series of coactivators/ corepressors are involved in ligand-induced transcription of genes allowing a tissue and ligand specific activation of these target genes by PPARs [2]. The most abundant isoform in adipose tissue, PPARγ, plays an important role in the regulation of insulin sensitivity. Glitazones or thiazolidinediones are high affinity ligands for this receptor and are currently used in the treatment of type 2 diabetes mellitus. Given the side effects of glitazones like weight gain, edema and fluid retention, the characterization of new PPARγ ligands that retain metabolic efficacy without exerting adverse actions plays a central role in the development of new therapeutic strategies for insulin resistance and type 2 diabetes mellitus [3].

A promising new group of such ligands are SPPARγMs (Selective PPARγ Modulators), compounds that activate only a subset of the functions induced by cognate ligands or act in a cell-type selective manner [4]. It has been shown by Schupp et al., that a subgroup of angiotensin II receptor type 1 blockers (ARB) induce PPARγ activity [5].

The aim of this SAR-Study is the identification and characterization of new selective PPARγ activating compounds, based on the structure of Telmisartan, which attends to be the most potent PPARγ activating ARB. We focused our attention on the variation of the position 2 of the 1H-benzimidazol-1'-ylmethyl-[1,1'-biphenyl]-2-carboxylic acid and its role on PPARγ activation. Following figure shows a selection of synthesized compounds, which show PPARγ activity in a Dual-Luciferase Reporter Assay System.



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P295

Nuclear Receptors: Identifying possible noncanonical binding sites

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Nuclear receptors (NR) are ligand dependent transcription factors that play crucial roles in the regulation of expression of genes controlling physiological events in development, homeostasis and cellular life, and are clinically exploited by the use of selective nuclear receptor modulators. [1] There is increasing demand for understanding the molecular basis of the interaction of nuclear receptor modulators with their macromolecular counterpart and the way in which selective modulation of NR is achieved is the object of ongoing researches. In this contribution we investigated the existence of unprecedented binding sites in NR by a first systematic visual inspection of the crystal structures, which - as a proof of concept - secondly was followed by docking studies of small model steroid compounds (guggulsterone (1) and progesterone (2).

This structure based molecular modeling study covered the full available range of available nuclear receptor crystal structures. As a first result, for some NR group members an unprecedented binding site, build up by elements of the loop helix 1 and helix 2, the helix H3, the helix 5 and helix 8, able to coexist with the canonical binding site of nuclear receptors was established. This structural feature shows close structural similarities to a binding site for guggulsterone-like compounds established recently for FXR by docking studies [2]. The possibile physiological role in modifying NR and importance for designing a future generation of selective nuclear receptor modulators will be hypothesized.

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P296

In Search for FXR Gene Selective Modulators: Synthesis, Biological Activity and Docking Studies of 23-N-Carbocinnamyloxy-Norchenodeoxycholanylamine (UPF838)

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The discovery of modulators for the Farnesoid X Receptor (FXR) has recently attracted a considerable interest in view of the therapeutic opportunities offered by this bile acid sensor in the area of bile acid and cholesterol related human diseases [1]. While a number of potent and selective FXR agonists have already been reported, with the semi-synthetic bile acid 6-ECDCA (INT747) [2] already in Phase I clinical studies, less is known on FXR partial agonists and antagonists. Herein, we report that carbamate derivatives of 3α,7α-dihydroxy-24-nor-5β-cholan-23-amine represent a novel class of FXR partial agonists endowed with favourable gene selective modulating properties. One of them, in particular, UPF838 has been shown to induce BSEP, whose increased expression is potentially useful in cholestasic diseases, and to not induce SHP, another FXR target gene known to be responsible for the undesired triglycerides level increase. Docking results have revealed that the side chain of the UPF838 almost perfectly fits a so far unexploited receptor cavity localized near the 'back door' of FXR. Thus, for the first time we demonstrate that a fine tuned modulation of FXR can be achieved by modifying a part of the molecule (i.e. the extended side chain) which is predicted to not directly affect the H12 orientation. The significance of this observation may be of great relevance towards the design of selective FXR modulators (SBARMs) and can in principle be extended to other members of the nuclear receptor superfamily.

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Other

P297

Use of APCIMS for analysis of ambient volatiles. Application to the detection of heay vapors in human breath

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We are exploring the use of commercial atmospheric pressure source mass spectrometers (MS) coupled to atmospheric pressure chemical ionisation for the analysis of complex mixtures of vapours present in ambient air at very low concentration. After describing summarily the range of problems of interest to us, we illustrate the potential of the technique through the analysis of human breath.