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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.

[1] Riggs BL, Hartmann, N. Engl. J. Med 2003:348;618-629.

[2] Meyer U, Costantino G, Macchiarulo A, Pellicciari R, Is antagonism in FXR mediated by a noncanonical binding site? A molecular modelling study. J Med Chem 2005:48;6948-6955

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.

[1] Pellicciari, R.; Costantino, G.; Fiorucci, S. Farnesoid X Receptor: from Structure to Potential Clinical Applications. J. Med. Chem. 2005;48:5383-5403.

[2] Pellicciari, R.; Fiorucci, S.; Camaioni, E.; Clerici, C.; Costantino, G.; Maloney, P. R.; Morelli, A.; Parks, D. J.; Willson, T. M. 6alpha-ethyl-chenodeoxycholic Acid (6-ECDCA), a Potent and Selective FXR Agonist Endowed with Anticholestatic Activity. J. Med. Chem. 2002;45:3569-3572.

Other

P297

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

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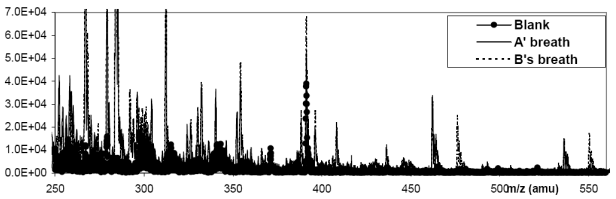
We are exploring the use of commercial atmospheric pressure source mass spectrometers (MS) coupled to atmospheric pressure chemical ionisation for the analysis of complex mixtures of vapours present in ambient air at very low concentration. After describing summarily the range of problems of interest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

Methods The atmospheric inlet pinhole to the MS (Sciex's API 365 triple quad) faces an electrospray (ES) source of acidified water releasing protonated species. A subject blows his breath through a Teflon tube, going first through a cold trap kept with ice at 0° C, and then into the ES region, where volatiles are ionised [1] and sampled into the MS.

Results Figure 1 shows the high mass region of typical mass spectra comparing a blank of clean CO_2 with the breath of two healthy subjects. One sees many peaks with signals orders of magnitude larger than the background, at masses up to 600 amu. These findings are in striking contrast with prior breath analysis literature, where background contributions are comparable to those from breath samples, and the heaviest reported mass has been 212.4 amu.



Furthermore, the relative differences previously reported in breath vs. background concentrations have been fairly small. The medical diagnostic utility of the heavier species identified here is therefore likely to be much greater than in the past, not only due to lack of background interference and clean-cut differences from subject to subject, but also because the specificity of a light species as an indicator of biological activity can be no match to that of a larger and more complex species. The most interesting species found among minor peaks (pending MS^2 confirmation) are most nucleosides (adenosine, deoxyadenosine, guanosine, deoxyguanosine, 5-methyluridine, thymidine, uridine, cytidine, deoxycytidine). No sugars or aminoacids are observed.

[1] Whitehouse, C.M., Levin, F., Meng, C.K. and Fenn, J.B., Proc.34th ASMS Conf. on Mass Spectrom. and Allied Topics, Denver, 1986, p. 507.

P298

@-Tides and Aza-@-Tides as beta-Strand Peptidomimetics: Potent Ligands for PDZ Domains

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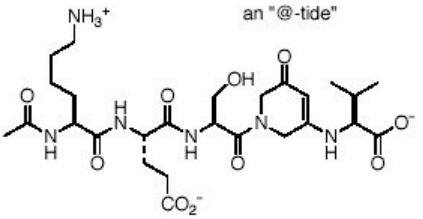
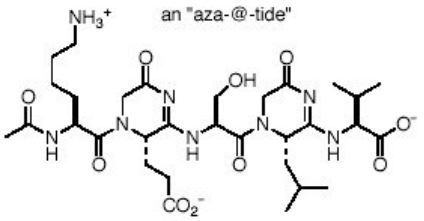
University of California, Berkeley

The search for general strategies for inhibiting protein-protein binding has been

stimulated by recognition of the key role they play in virtually every feature of living systems. Interactions that are mediated by β-strand association, as exemplified by binding to PDZ domains, present special challenges because, in contrast to β-turns and α-helices, few non-peptidic mimics of α-strands have been devised. We have described peptide analogues incorporating the "@-tide" unit, a conformationally restricted amino acid surrogate that maintains the hydrogen bonding pattern of a β-strand. These easily synthesized peptidomimetics are excellent templates for β-hairpin formation in water, and the CD Signal of the vinylogous amide reflects the extended conformation [1].



Ac-K E S @ V



Ac-K [E] S [L] V

We now describe the design and synthesis of "aza-@-tides", peptidomimetics that enable the incorporation of side chains in addition to their ability to stabilize the extended conformation. These analogues are synthesized from amino acid amides in a way that preserves both the L-stereochemistry. Their potential as mimics of a peptide β-strand was demonstrated by substituting aza-@-units at appropriate positions of the peptide acetyl-KESLV, which is a ligand of the α1-syntrophin PDZ domain [2].

Significantly enhanced binding relative to the peptide itself is observed when the peptidomimetic is substituted for a residue (e.g., the glutamate) that is in the extended conformation in the PDZ binding cleft.

[1] Phillips ST, Piersanti G, Bartlett PA. Quantifying amino acid conformational preferences and side chain-side chain interactions in β-hairpins. Proc. Natl. Acad. Sci. USA 2005; 102:13737-13742, and references therein.

[2] Schultz J, Hoffmueller U, Krause G, Ashurst J, Macias M.J, Schmieder P, Schneider-Mergener J, Oschkinat H. Specific interactions between the syntrophin PDZ domain and voltage-gated sodium channels. Nat. Struct. Biol. 1998; 5:19-24.

P299

Synthesis and Study of Biologically Active Complexes of Cd (II) with Different Ligands

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The object the presented work is synthesis and study of Cd (II) complexes with different ligands by interaction

of arsonium salts - iodide of iodmethylene trialkyl(aryl)-arsonium with the cadmium bromate(V). It was observed that the target product is obtained as small-crystalline one immediately during the mixing of above mentioned initial reagents in equal (1:1) molar ratio alcohol-water solutions at room temperature. The reaction is taking place in accordance with the following scheme:

$$[R_2As(R')CH_2I]I + Cd(BrO_3)_2 \rightarrow [R_2 As(R')CH_2 I][Cd(BrO_3)_2 \cdot I],$$

where R= n-C_3H_7, i-C_3H_7, n-C_4H_9, i-C_4H_9; R'= n-C_5H_{11}, C_6H_5.

The structure and composition of the synthesized complexes have been established using data of the elemental and IR spectral analyses. As the IR spectra showed, there are the maximums of the absorption (428 cm^{-1}, 790 cm^{-1} and 810 cm^{-1}) related to the bromate-ion, all of maximums of the absorption characteristic for the initial tetra-substituted iodides of the arsonium, are essentially retained in the spectra. This fact manifests ionic structure of the obtained complexes, which are dissociated as two-component coordination substances:

$$[R_2As(R')CH_2 I][Cd(BrO_3)_2 \cdot I] \Leftrightarrow [R_2As(R')CH_2 I]^+ + [Cd(BrO_3)_2 \cdot I]^-$$

So the electro-conductivity of the synthesized complexes varies within the interval 75-93 $om^1 \cdot cm^2 \cdot mol^{-1}$.

The thermo-stability of the synthesized complexes has been studied (DTA and TGA analyses). The feasible mechanism of thermal destruction is offered. The bioactivity of the obtained compounds is studied.

P300

Dissolution Tests of Spironolactone and Hydrochlorothiazide In Commercial Tablets: Comparison of Spectroscopic and High Performance Liquid Chromatography Methods

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Two spectroscopic methods (absorbance ratio and Vierordt) were compared with HPLC for quantitative determination in dissolution test of spironolactone (SPL) and hydrochlorthiazide (HCT) in commercial tablets. A 260 nm wavelength was chosen as the isosbestic point in the absorbance ratio method, and the absorbance ratios A_{232}/A_{260} nm for SPL and A_{269}/A_{260} nm for HCT were used for calculation of regression equations. For the Vierordt method A^1_1 values (1 %, 1 cm) obtained at 232 nm and 269 nm for both substances were used for quantitative analyses of SPL and HCT.

In the HPLC method, simultaneous determination of SPL and HCT from dissolution medium was achieved using the mobile phase containing water-methanol-phosphate buffer (71:25:4 v/v) pH: 3,1 ± 0,1 in a Luna 5 μm C_{18} (250 x 4.6 mm) reversed phase column. Mefrusid was chosen as the internal Standard and detection was carried out PDA detection at 286 nm. (Flow rate 07 ml.min-1)

Dissolution test of commercial tablets were carried out according to USP XXVI paddle method in 0,1 N HCl at 75 rpm at 37 ± 0,5 ºC. Comparison of the dissolution data from the HPLC and two spectroscopic methods indicated that spectroscopic and HPLC methods were in good correlation with each other. Therefore, it was concluded that both spectroscopic method as well as HPLC can be routine analyses SPL and HCT in dissolution tests of commercial tablets.

P301

Theoretical studies on the FXR receptor: H-12 stabilization by fexaramine.

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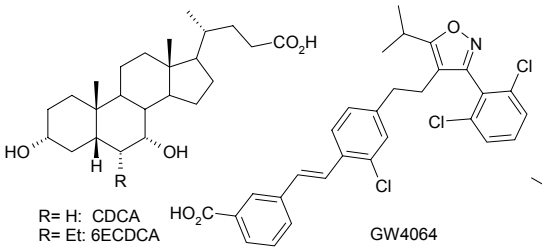
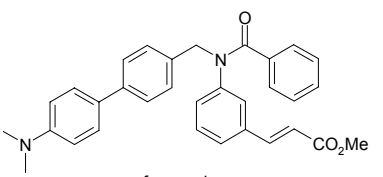
Nuclear receptors constitute a wide family of transcription factors that regulate gene expression and/or repression [1]. Their transcriptional activity involves several steps like ligand recognition, homo- or heterodimerization, co-activator recruiting and/or co-repressor releasing and, finally, recognition by the DNA.

The FXR receptor acts as a biological sensor that controls the bile acid levels and is endogenously activated by chenodeoxicholic acid (CDCA). Several agonist of this receptor are actually known, among them the 6-ethylchenodeoxicholic acid (6ECDCA, INT-747), and other non steroid compounds such as GW4064 or fexaramine.

In a previous paper, we have described the conformational changes that take place in the FXR receptor in response to the 6ECDCA binding [2]. In the present communication we describe a 12 ns molecular dynamics simulation performed on the FXR-fexaramine complex, and a mechanism for the AF2 (activation factor 2, H12) stabilization is proposed.



GW4064



fexaramine

[1] a) Wang, H.; et al. Mol. Cell. 1999, 3, 543. b) Pellicciari, R.; et al. *J. Med. Chem*. **2002**, *45*, 3569. c) Maloney, P. R.; et al. *J. Med.Chem*. **2000**, *43*, 2971. d) Downes, M.; et al. *Mol. Cell*. **2003**, *11*, 1079.

[2] Costantino, G.; Entrena, A.; Macchiarulo, A.; Gioiello, A.; Pellicciari, R. *J. Med. Chem*. **2005**, *48*, 3251.

P302

Design, Syntheses and Biological Evaluation of 1-Phenyl-1*H*-Indole-2-Carboxamides and 2-Benzoyl-1-Phenyl-1*H*-Indoles: New Selective Cyclooxygenase-2 Inhibitors

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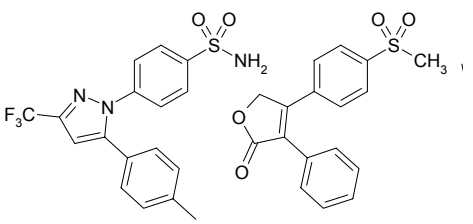
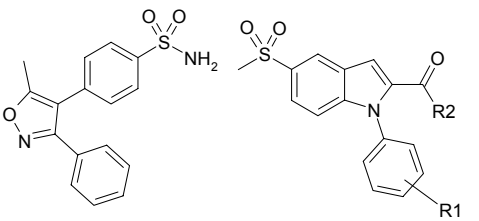
Cyclooxygenases (COXs) are key enzymes in the synthesis of prostaglandin H_2, a precursor for the biosynthesis of prostaglandins, thromboxanes, and prostacyclin [1]. Two COX isoforms exist, cyclooxygenase-1 (COX-1), constitutively expressed, and cyclooxygenase-2 (COX-2), which is inducible and expressed during inflammation, pain, and oncogenesis [2].

Many non-steroidal anti-inflammatory drugs (NSAIDs) inhibit both isoforms, among them Aspirin and indomethacin which are non-selective inhibitors. NSAIDs adverse ulcerogenic effect has been attributed to the COX-1 inhibition [3]. Current research has focussed on developing selective COX-2 inhibitors, and some of them such as celecoxib, rofecoxib, and valdecoxib have been marketed as a new generation of NSAIDs. Recent reports evidence that coxibs can lead to adverse cardiovascular effects [4], nevertheless selective COX2 inhibitors development still continues nowadays.



celecoxib **rofecoxib**



valdecoxib

1 R2 = NHR
2 R2 = Ph

In this communication we report the design, synthesis and biological assay of a series of 1-phenyl-1H-indole-2-carboxamides 1 and 2-benzoyl-1-phenyl-1H-indoles 2 derivatives as a new type of selective COX2 inhibitors.

[1] Hamberg M, Samuelsson B. Detection and isolation of an endoperoxide intermediate in prostaglandin biosynthesis. Proc Natl Acad Sci USA 1973;70:899-903.

[2] Smith WL, DeWitt DL. Prostaglandin endoperoxide H synthases -1 and -2. Adv Immunol 1996;62:167-215.

[3] Allison MC, Howatson AG, Torrance CJ. Lee FD, Russell RI. Gastrointestinal damage associated with the use of nonsteroidal antiinflamatory drugs. N Engl J Med 1992;327:749-754.

[4] Dogné JM, Supuran CT, Pratico D. Adverse cardiovascular effects of the coxibs. J Med Chem 2005;48:2251-2257

P303

Redesign of Aminoglycosides for Treatment of Human Genetic Diseases Caused by Premature Stop Mutations.

I. Nudelman[1], A. Rebibo[2], M. Hainrichson[1], L. Chen[1], V. Belakhov[1], I. Stahl, D. Shallom-Shezifi[1], T. Ben-Yosef[2] , T. Baasov[1]

1Department of Chemistry and Institute of Catalysis, Science and Technology, 2Department of Genetics, Rappaport Faculty of Medicine, Technion, Haifa 32000, Israel;

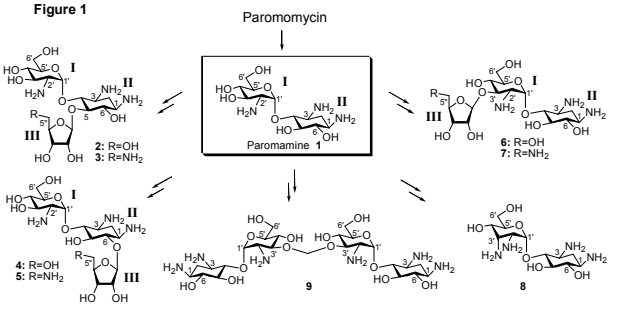
A large number of human genetic diseases result from mutations that cause premature termination of the synthesis of proteins encoded by mutant genes. Currently, hundreds of such nonsense mutations are known, and several where shown to account for certain cases of fatal diseases, including cystic fibrosis (CF), Duchenne muscular dystrophy (DMD), Tay-Sachs, and more. While several *in vitro* and *in vivo* experiments have clearly demonstrated that many of the above mutations can be functionally rescued by treatment with aminoglycoside antibiotics, especially by treatment with geneticin (G-418), gentamicin or paromomycin, still their use as therapeutic agents is highly restricted because of their high toxicity.

The main objective of this research is to develop novel aminoglycosides that will have efficient termination suppression activity, and at the same time will have reduced toxicity against mammalian cells.

For this purpose a series of new derivatives (**2-9**) (Fig. 1) were synthesized and their nonsense suppression was assayed in an *in vitro* and *ex vivo* mammalian systems. One of these structures, the pseudo-trisaccharide **3** showed significantly higher stop codon readthrough activity and lower toxicity compared to that of the parent paromomycin and gentamicin. Antibacterial tests against both Gram-negative and Gram-positive bacterial strains indicate that **3** is highly selective in its action in eukaryotic cells than in prokaryotic cells. Taken together, these results suggest that compound **3** could represent an alternative to gentamicin and paromomycin for suppression therapy. Thus, this study provides a new direc-

tion for the development of novel aminoglycoside-based small molecules that selectively target mammalian cells; this progress may offer promise for the treatment of many genetic diseases.

Figure 1



P304

Flavonoids as the promising neuroendocrine modulators

A. Levitsky*, O. Voskresensky**, O. Makarenk*

*Institute of Stomatology of AMSci of Ukraine. **National Polytechnic University

Initially, at the earliest stage of their discovery [A. Czent-Gyorgyi, 1936], the flavonoids (F) was considered, that the main, if not the only function of these compounds, is to influence the state of vascular wall, its permeability. In the subsequent development of investigations F were revealed to be of antioxidant effect, surpassing in strength the classic natural antioxidant – tocopherol. The biochemical study of F displayed their ability to regulate activity of enzymes. So, they inhibit such enzymes as proteinkinase C, tyrosin, protein kinase, muscular phosphorylases, ATP-ases, phospholipases A_2 и C, lipoxygenases, cyclooxigenases, adenylate cyclase, phosphodiesterase, DNA-topoisomerase, xanthine oxidase, hyaluronidase, ribonuclease, elastase, etc. [Middleton et al., 2000]. At the same time some F and isoflavones, in particular, induce in vivo the activity of antioxidant enzymes (SOD, glutathioneperoxidase, catalase) and some hydrolases (alkaline phosphatase) [Levitsky et al., 2002]. The study of physiological effects of F has allowed revealing the antiinflammatory characteristics, the antitoxic, hepatoprotective and cytoprotective effects. Some F display anxiolytic and other neurotropic effects [Voskresensky et al.] which are related with their binding to benzdiazepines [Griebel et al., 1999] and adenosine [Jacobson et al., 2002] receptors. The osteotropic characteristics of F such as depression of osteoclasts function, stimulation of osteoblasts, and regulation of calcium metabolism indicate on their osteoprotective properties [Makarenko et al., 2002]. Discovery of anticarcinogenic characteristics of F [Barnes, 1955, Caroll et al., 1998] induced great expectations. They are connected with capabilities of F to inhibite of protein kinase C and DNA–topoisomerase I, to stimulate of immunological control and apoptosis regulation [Hirano et al., 1995]. Some F (genistein, daidzein) have estrogenous like properties, interact with estrogenic receptors and compete with endogenous .

Other/Anticonvulsants

P305

Evaluation of Anticonvulsant Activities of Some Bis Mannich Bases and Corresponding Piperidinols Synthesized Using Ethylamine Hydrochloride and Different Arylmethylketones

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[1]Ataturk University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Erzurum Turkey. [2]Hacettepe University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Ankara, Turkey. [3]Ataturk University, Students of Faculty of Pharmacy, Erzurum Turkey

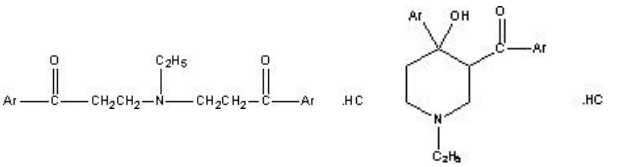
Bis-Mannich bases, bis(3-aryl-3-oxo-propyl)ethylamine hydrochlorides 1-4 **(I)**, and their corresponding structural and non-classical isomers, 4-aryl-3-arylcarbonyl-1-ethyl-4-piperidinol hydrochlorides 5-8 **(II)**, were synthesized [1].

Chemical stuructures of the compounds were confirmed by ^{1}H-NMR, ^{13}C-NMR, UV, IR and elemental analyses. Anticonvulsant activities of the compounds were evaluated by MES and scMet tests in the dose range of 30-300 mg/kg according to the procedure in literature [2,3]. Alterations in biological activity depending

Table (P305): *Anticonvulsant activities of the synthesized compounds*

	MES						ScMet						Toxicity					
Compound	1/2 hour			4 hour			1/2 hour			4 hour			1/2 hour			4 hour		
number	mg/kg			mg/kg			mg/kg			mg/kg			mg/kg			mg/kg		
	30	100	300	30	100	300	30	100	300	30	100	300	30	100	300	30	100	300
1	1/1	*	*	0/1	*	*	0/1	*	*	0/1	*	*	0/4	*	*	0/2	*	*
2	1/1	1/1	*	1/1	1/1	*	0/1	0/1	*	0/1	0/1	*	0/4	0/4	*	0/2	0/2	*
3	1/1	1/1	*	0/1	0/1	*	0/1	0/1	*	0/1	0/1	*	0/4	0/4	*	0/2	0/2	*
4	1/1	1/1	*	0/1	0/1	*	0/1	0/1	*	0/1	0/1	*	0/4	0/4	*	0/2	0/2	*
5	1/1	1/1	1/1	0/1	1/1	1/1	0/1	0/1	0/1	0/1	0/1	0/1	0/4	0/4	0/4	0/2	0/2	0/2
6	1/1	1/1	*	0/1	0/1	*	0/1	0/1	*	0/1	0/1	*	0/4	0/4	*	0/2	0/2	*
7	0/1	0/1	1/1	0/1	0/1	1/1	0/1	0/1	0/1	0/1	0/1	1/1	0/4	0/4	0/4	0/2	0/2	0/2
8	0/1	1/1	*	0/1	0/1	*	0/1	0/1	*	0/1	0/1	*	0/4	0/4	*	0/2	0/2	*

on modifications in chemical structure were also followed. Anticonvulsant activities of the compounds were shown as below.



(I) (II)

Ar : C_6H_5- (Compounds **1,5)** , p-CH_3- C_6H_4- (Compounds **2,6**), p-Cl-C_6H_4- (Compounds **3,7)**, 2-C_4H_3S- (Compounds **4,8)**.

Synthesized compounds of Bis Mannich bases and corresponding piperidinols.

Compounds **1-4**, **6**, and **8** were toxic and caused death of the animals 20 minutes after the injection. Compounds **2**, **3** and **6** were also neurotoxic at 100 mg/kg dose level. While only the compound **7** had activity in scMet test at 300 mg/kg in 4hours, all compounds showed activity in MES test at different dose levels and time periods. In conclusion, compounds **5** and **7**, which were not toxic and did not show neurotoxicity, seemed to be candidate compounds to develop new anticonvulsant drugs.

[1] Gul HI, Gul M, Erciyas E., Arzneim.-Forsch./Drug Res., 52, 628-635 (2002).

[2] Gul HI, Calis U, Vepsalainen J., Arzneim.-Forsch./Drug Res., 52, 863-869 (2002).

[3] Gul HI, Calis U, Vepsalainen J., Arzneim.-Forsch./Drug Res., 54, 359-364 (2004).

P306

Studies on the new derivatives of nafimidone and their anticonvulsant activities

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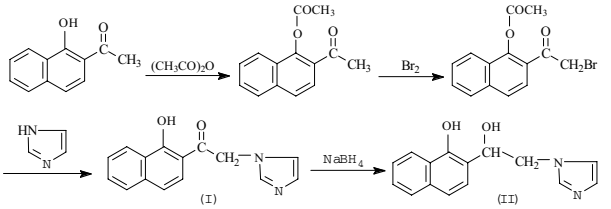
Although a large number of antiepileptic drugs have been marketed, medication toxicity and uncontrolled seizures are still the main problems of antiepileptic drug treatment. Nafimidone [1-(2-naphthyl)-2-(imidazol-1-yl)ethanone] is one of the two representatives of (arylalkyl)imidazole anticonvulsants [1]. Nafimidone alcohol is a major and active metabolite of nafimidone. In this project we prepared two new nafimidone derivatives with a hydroxyl group on the naphthalene ring to have a better protective index. The compounds **(I-II)** have been synthesized starting from 1-hydroxy-2-acetylnaphthalene as given below:



Their anticonvulsant activities were determined by maximal electroshock (MES) and subcutaneous metrazole (ScMet) tests in mice in National Institude of Neurological Disorders and Stroke (NINDS) Laboratories according to Anticonvulsant Screening Programme (ASP) of National Institute of Health (NIH).

Acknowledgement: This project was supported by Hacettepe University Research Fund (Project No: 02.02.301.002).

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P307

Synthesis and anticonvulsant activity of 5-chloro-2(3H)-benzoxazolinone-3-acetyl-2-(o/p-substituted benzal)hydrazone derivatives

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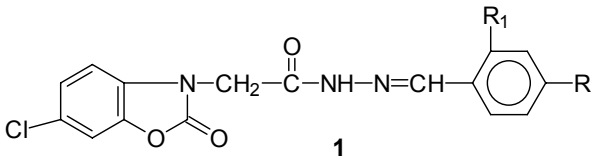
Epilepsy afflicts 1-2% of the population and often goes untreated; nearly 70% of those with a form of epilepsy fail to receive proper treatment [1]. Therefore, there is a great demand for the design of novel effective anticonvulsant to treat of epilepsy in its numerous forms. Also antiepileptic drugs may cause burdening adverse effects such as drowsiness, ataxia, gastrointestinal disturbances, hepatotoxicity, gingival hyperplasia, hirsutism and megaloblastic anemia[2].

A number of hydrazone derivatives are known to be responsible for anticonvulsant effect[3-5]. Therefore, it was thought that hydrazones of 5-chloro-2(3H)-benzoxazolinone would also exhibit significant anticonvulsant activity. It is reported in the literature that 2(3H)-benzoxazolone can exhibit diverse activities. Particularly analgesic and anti-inflammatory activities have been scrutinized intensively[6-8]. Also anticonvulsant activity of 2(3H)-benzoxazolone derivatives have been reported[9-11].

These data encouraged us to synthesize new 5-chloro-2(3H)-benzoxazolinone-3-acetyl-2-(o/p-substituted benzal)hydrazones **4** and test their anticonvulsant activity. In this study, fourteen new hydrazones of 5-

chloro-2(3H)-benzoxazolinone-3-acetyl hydrazide were synthesized. Chemical structures of synthesized compounds have been established by IR, ^{1}H-NMR spectral analyses and elementary analyses. Their anticonvulsant activities were tested by penthylenetetrazole induced seizure test.



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P308

Application of Molecular Topology in Descriptor – based Virtual Screening for the Discovery of New Anticonvulsant Agents

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From a training set made of clinical anticonvulsant drugs whose mechanism of action involves sodium channels blockade [1,2], several topological models for recognition of anticonvulsant activity were generated. The models were validated through randomization test, leave-one-out cross – validation and external validation. The model that best performed in the validation steps was applied in the virtual screening of a library of 500,000 compounds. These structures were analyzed to assure normal distribution regarding the parameters included in Lipinski´s "rule of five" [3]: molecular weight, number of H bond donors, number of H bond acceptors and log P; in this way we verified both the structural diversity and the drug-like properties of the screened compounds. Some of the drugs selected in the virtual screening were bioassayed in the Maximal Electroshock test with good results, which arises as strong evidence of the efficacy of the employed methodology.

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Other/Antihistaminics

P309

Synthesis and biological activities of some thiazolidin-4-ones

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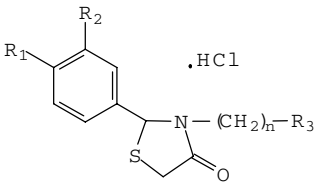
H_1-Histamine antagonists, such as ethylenediamines, aminoethyl ethers, propyl- and propenylamines, phenothiazines, piperidines and piperazines, have been discovered to have remarkable chemical and geometric similarities [1, 2]. Tiyazolidin 4-on derivatives have been evaluated for their antihistaminic activities due to the structural similarity with this group and it has been found that they have shown considerable antihistaminic activity [3].

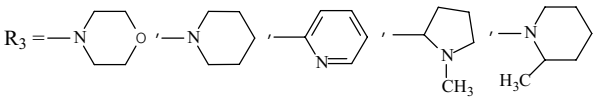
In this study, fifteen 2,3-disubstituted-4-thiazolidinone derivatives were synthesized by the reaction of Schiff bases and α-mercaptoacetic acid.

The structures of the compounds were elucidated by IR, ^{1}H-NMR, ^{13}C-NMR, mass spectral data and elementary analysis. The antihistaminic and anticholinergic activities of the compounds determined by the tests performed on isolated guinea pig trachea in comparison with aminophilline. When activity results have been examined, it has been seen that most of the compounds have anti-

histaminic activity. Additionally all of the compounds have been evaluated for their anticholinergic activity and it has been determined that they haven't had any anticholinergic activity.



n = 2, 3
R_1 = OCH_3, CH_3
R_2 = H, OCH_3



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Other/Antioxidants

P310

Synthesis And Antioxidant Properties Of Novel N-Substitued Indole-3- Carboxamide, Acetamide And Propionamide Derivatives

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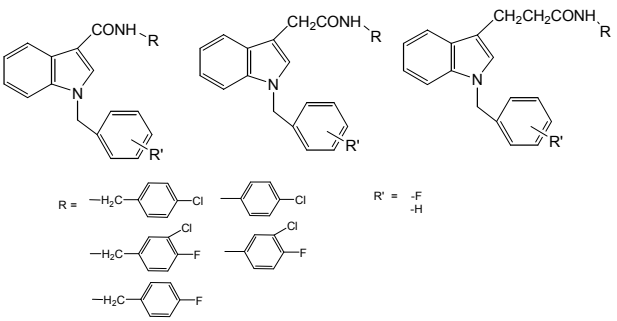
The serious consequences of free radical action on biological systems, and the multiple complex aspects of their intervention in a series of inflammatory and several cancers, explain the increasing interest being given to antioxidant medication and to obtaining compounds which can reduce the effects of these radicals. An increasing understanding of specific protein kinases that are over expressed in some tumors and their inhibition has afforded a new paradigm for cancers treatment. It was found that protein kinases can be activated by oxidative stress and inhibited by antioxidant. It was also reported that cyclooxygenase increase the reactive oxygen species (ROS) production, thus the enzyme can participate in oxidative stress. The broad spectrum of the observed antioxidant activity of indole-2- and 3-carboxamides suggested us that they can scavenge oxygen free radicals, and some of them also scavenge O_2 produced by the cyclooxygenases. Several indole derivatives synthesized in our laboratories as tyrosine kinase and COX-2 inhibitors were also found as important anti-oxidant compounds by determining their effects on superoxide anion inhibition and lipid peroxidation level.

The above-mentioned results stimulated our interest in design novel N-substituted-indole-3-carboxamide, acetamide, propionamide analogues and evaluate the antioxidant capacities. N-substituted indole-3-carboxylic, acetic and propionic acids were synthesized by substitution of 1H of indole nitrogen with benzyl bromides or p-F-benzyl bromides and following the hydrolysis of esters. (Murakami et al., synthesis, 738, 1984). The final compounds were synthesized by the reaction of acylated acids with substituted aryl or benzyl amines.



The antioxidant properties of synthesized compound were determined by compared with á- tocopherol. R groups were chosen halogen substituted aromatic ring in order to identify the possible structure-antioxidant activity relationships of the compounds and guide perceptively the design of new analogues. The effects of carboxylic, acetic and propionic acid chains were also evaluated by comparing with those synthesized N-substituted indole amides previously.

P311

CANCELED

Pain & Inflammation

P312

Synthesis of certain substituted pyrrolidines as analgesics

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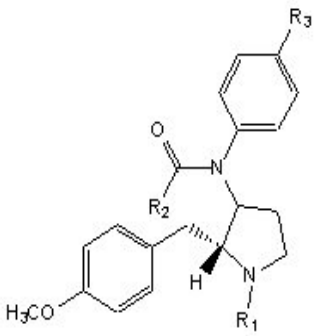
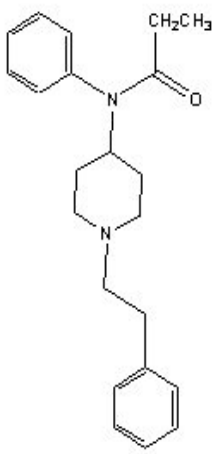
Synthesis of N-[1-alkyl or phenyl alkyl-2-(4-methoxy-benzyl)-pyrrolidin-3-yl]-N-(substituted phenyl)-acetamide or propionamide **(6a-l)** where R_1 = alkyl; R_2 = methyl or ethyl and R_3 = H or alkyl group was achieved. The aim of their preparation is to evaluate their analgesic activity as they are structurally related to N-(1-Phenethyl-piperidin-4-yl)-N-phenyl-propionamide **(Fentanyl®, 7)** [(1)].



6a-l



7

The pathway of preparation for these compounds was achieved through Dieckmann cyclization to afford 1-acetyl-4-ethoxycarbonyl-2-(4-methoxy-benzyl) pyrrolidin –3-ones[(2)] followed by deethoxycarboxylation, then condensation with the appropriate arylamine to the schiff's bases. There after a two step reduction was carried out followed by reaction with the appropriate acid anhydride which afforded **6a-l**. The analgesic profile of the synthesized compounds will be studied.

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Key Words: Pyrrolidines, Fentanyl, Analgesics.

P313

Development of CXCR2 receptor antagonists with in vivo efficacy for inflammatory disorders

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Pharmacopeia Drug Discovery Inc., 3000 Eastpark Blvd., Cranbury, NJ 08512 Schering-Plough Research Institute, 2015 Galloping Hill Rd, Kenilworth, NJ 07033-0539.

Neutrophil infiltration is implicated in a variety of inflammatory diseases. CXCR1 and CXCR2 are chemokine receptors that upon activation mediate neutrophil trafficking to sites of inflammation. This presentation details the discovery of a novel series of CXCR2 receptor antagonists, chemical optimization, and selection of a clinical candidate for chronic obstructive pulmonary disease (COPD).

P314

Opioid and serotonergic activity of 8-aryl-3,4(8H)dioxo-6,7-dihydroimidazo[2,1-c][1,2,4]triazines in view of their tautomeric equilibrium.

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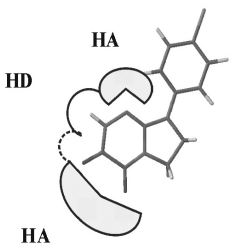
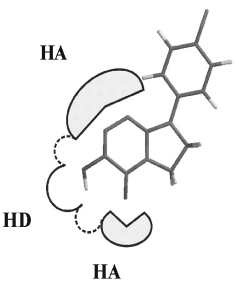
Dioxo derivatives of fused imidazolidine ring systems express significant pharmacological activity especially in

the central nervous system (antinociceptive and serotonergic) [1,2]. Their activity is structure dependant. Selectivity of affinity for opioid MOP and serotonergic 5-HT_2 receptor for most of them depends on the location of the hydrogen bond acceptor centers (HA).

It was proved that tauthomeric equilibrium (keto-enol and amido-imido) for fused heterocyclic system of 3,4-dioxo-imidazo[2,1-c][1,2,4]triazine affects its cns activity. Quantum chemical calculations and X-ray analysis for 3-Cl and 4-Cl derivatives confirmed that their tauthomeric equilibrium strongly depends on position of substituent present in the N-8 aromatic ring. For these compounds strong influence of the prevailing tauthomeric form on the pharmacological activity (opioid or serotonergic) was confirmed [3].



Opioid activity



Serotonergic activity

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P315

Docking studies of 3-carboxamides benzothiazines 1,1-dioxide derivatives with COX II

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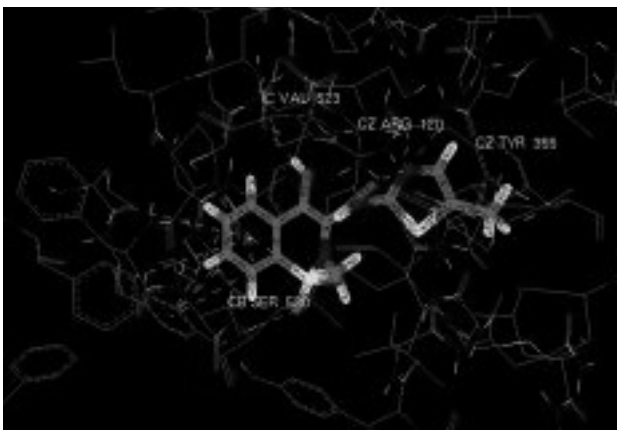
In 1996 meloxicam outstood and was commercialized due to its 8 times greater selectivity to COX-2. Last year some compounds of the Coxibs family were retired from the pharmaceutical market due to adverse events detected. Since this event the oxicams family selective to COX II should be considered again as a potential candidate to develop new leaders in this therapeutic area. [1]

Due that no oxicams - COX complex cristallographies could be achieved until now, this work tries to explain as a first approach the chemical-molecular basis of action and selectivity of oxicams to COX II, performing a computational docking of Piroxicam (non selective to COX II) and meloxicam (selective to COX II) with the COX II using the FlexX-Pharm program. The crystallographic data of the complexes of SC-558-COX-2 (6 COX) were obtained from the Protein data bank [2]. The definition of the starting model of the active site and active site surface were performed by the FlexX-Pharm program with a 7.5 Å radius.



As a result of the docking of the meloxicam -COX II we observed a localized and strong interaction in terms of energy with the corresponding COX-2 active site and active site “pocket” residues. In the case of the piroxicam-COX II complex, it shows multiple interactions but weaker than the other ones. This fact would explain the lack of selectivity of piroxicam to COX-2. From this observations meloxicam analogues were planned tending to a major selectivity towards COX II. They are being syntethised and will be pharmacologically evaluated to confirm the stated predictions.

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[2] Protein Data Bank, Chemistry Department building, 555 Brookhaven National Laboratory, P O Box 5000 USA.

P316

Developing of new methodologies for preparing non-hydrolyzable sulfocarbohydrate analogs

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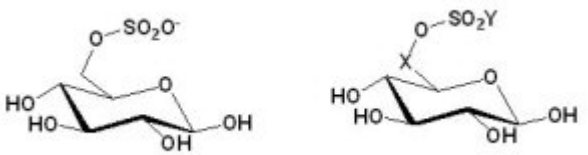
The selectins represent novel targets for the development of anti-inflammatory agents [1]. A variety of sulfated oligosaccharides have been reported to be recognized by selectins [2]. Nonhydrolyzable analogues of glucose-6-sulfate in which the oxygen in the labile O-S bond was replaced by CH_2, CHF, and CF_2 **1-6**, were prepared. The fluorinated compounds were prepared by electrophilic fluorination of α-sulfonyl or α-sulfonamide carbanions of protected **1** and **4**. The monofluorination reaction was highly diastereoselective and the diastereoselectivity could be controlled by the cation of the base used to generate the α-carbanions.



1: X=CH2, Y=O- 4: X=CH2, Y=NH2
2: X=CHF, Y=O- 5: X=CHF, Y=NH2
3: X=CF2, Y=O- 6: X=CF2, Y=NH2

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P317

Design and synthesis of new water-soluble tetrazolide derivatives of celecoxib and rofecoxib as selective cyclooxygenase-2 (COX-2) inhibitors

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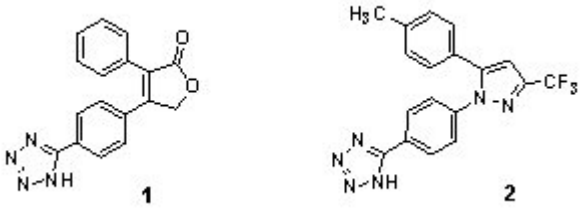
In the management of severe or moderately severe pain, parenteral treatment are preferred because of the rapid onset of action. Recent surveys have shown that postoperative pain is considered poorly managed, due to side-effect limitations of available injectable medications, such as opioids and nonsteroidal anti-inflammatory drugs (NSAIDs) [1,2]. The discovery of a second isoform of cyclooxygenase (COX-2) that is expressed in inflammatory cells, but not in gastric mucosa, offers the possibility of developing anti-inflammatory and analgesics that lack the gastrointestinal side-effects of currently available NSAIDs [3].

In an attempt to prepare a new water soluble, parenteral COX-2 inhibitor, rofecoxib (**1**) and celecoxib (**2**) analogues, in which the respective SO_2Me and SO_2NH_2 hydrogen-bonding pharmacophores were replaced by a tetrazole ring, were designed and synthesized for evaluation as selective COX-2 inhibitors with in vivo anti-inflammatory activity. The rofecoxib (**1**) and celecoxib (**2**) analogues exhibited a good in vitro selectivity relative to the reference drug celecoxib and also showed high aqueous solubilities at pH higher than 7 and good anti-inflammatory activities in a carrageenan-induced rat paw edema assay.



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P318

Site-directed mutagenesis study of human and rat P2X7 receptors and the effect on ligand activity

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The P2X7 receptor is a ligand-gated ion channel of interest for the treatment of inflammatory conditions, including rheumatoid arthritis[1,2] and inflammatory and neuropathic pain[3]. Although a number of classes of P2X7 receptor antagonist have been reported, identification of compounds with good activity against both human and rat

receptors, which share ca 80% protein identity, has proved problematic. To understand the species differences in the activity of ligands at the molecular level, mutant rat/human genes were prepared. These genes were recombined into BacMam viruses to facilitate efficient functional delivery to U-2 OS osteosarcoma cells. The activity of P2X7 ligands was investigated with these chimeric receptors as compared to native human and rat P2X7 BacMam virus. Several interesting observations were made leading to the identification of key amino acid residues for single point mutation work. The results of this study and their contribution to an increased understanding of the binding sites and species selectivity of P2X7 ligands will be presented.

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P319

Chromone derivatives as novel calpain inhibitory compounds

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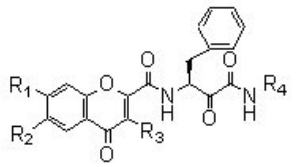
Calpains are intracellular cysteinyl proteolytic enzymes and require calcium ions for activation. However, excessive calpain activation can lead to serious cellular damage or even cell death and has been found in a number of pathological conditions, such as cerebral ischemia, myocardial infarction, Alzheimer's disease and inflammation [1]. For this reason, considerable efforts have been focused on the design and synthesis of calpain inhibitors as a novel therapeutic principle. Most of reported calpain inhibitors are derived from peptides like Z-Val-Phe-H (MDL 28,170) [2]. The present study, therefore, selected a chromone ring as a replacement of a-amino acid moiety, Z-Val within MDL 28,170 to reduce peptide character and coupled with amino-alcohols derived from L-phenylalanine followed by oxidation of hydroxyl group to lead new calpain inhibitors.

Among the synthesized, compound **1** ($R_1 = R_2 = R_4 = H$, $R_3 = CH_3$) showed the most potent inhibitory activity ($IC_{50} = 0.24 \pm 0.11$ μM) comparable to the inhibitory activity of MDL 28,170 ($IC_{50} = 0.20 \pm 0.03$ μM). On the other hand, compound **1** exhibited 14.4% and 22.4% inhibition on the activities of cathepsin B and cathepsin L, respectively, at 0.2 μM concentration, while MDL 28,170 inhibited these cysteinyl proteases by nearly 100% at the same concentration, demonstrating high selectivity of chromone derivatives for μ-calpain. The synthesis of chromone carboxamide and their biological evaluation and binding model with calpain will be discussed in the presentation.



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[2] Wang KKW, Yuen PW. Calpain inhibition: an overview of its therapeutic potential. Trends Pharmacol Sci 1994;15:412-419.

P320

Valid screening procedures to evaluate new anti-inflammatory drugs

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The biochemical mechanisms being responsible for inflammatory processes are complex. Beside mediators such as leukotrienes and prostaglandins generated in the arachidonic acid cascade by 5-LOX and COX-1/COX-2 we focus on pro-inflammatory cytokines (IL-1, IL-6, TNF-α) which play a decisive role. Not only their effect on prostaglandin production but also the activation of MMPs (matrix metalloproteinases) and adhesion molecules involved into the extravasation of leukocytes into the endothelium make them a promising target for the development of new anti-inflammatory drugs [1]. Therefore valid screening procedures are an indispensable tool to evaluate active compounds. A human whole blood assay allows the simultaneous determination of COX-2 derived PGE_2 by EIA, whereas cytokines can be measured via EIA, flow cytometry or a Proteo Plex™ array kit.

For the primary screening to quantify leucocyte adhesion phenomena to endothelial cells under static conditions BAECs provide a basis for a cell-based, computerized in-vitro assay [2]. If a reduction of leukocyte adhesion is found, then subsequently the expression of CAMs such as E-selectins, ICAM-1 or VCAM-1 are investigated by FACS in order to determine the exact points of attack of the compounds [3].

[1] Diaz-Gonzalez F, Sanchez-Madrid F. Inhibition of leukocyte adhesion: an alternative mechanism of action for anti-inflammatory drugs. Immunol Today 1998;4:169-172

[2] Ulbrich H, Prech P, Luxenburger A, Dannhardt G. Development of a computerized in vitro assay for the investigation of anti-adhesive compounds. Biol. Pharm. Bull. 2005;28:718-824

[3] Ulbrich H, Soehnlein O, Xie X, Eriksson EE, Lindbom L, Albrecht W, Laufer S, Dannhardt G. Licofelone, a novel 5-LOX/COX-inhibitor, attenuates leukocyte rolling and adhesion on endothelium under flow. Biochem. Pharmcol. 2005;70:30-36

P321

Quinazolines as orally active small molecule IL-12 production inhibitors

Z. Demko, E. Kostik, G. Liang, L.S.N. Tatsuta, Y. Wada, Q. Wang, Y. Wu, and D. Zhou

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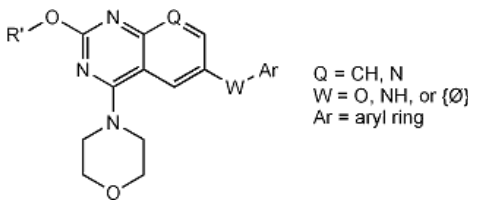
Interleukin-12 (IL-12), a p35/p40 heterodimeric cytokine, has been shown to play a critical role in a number of inflammatory disorders, including Crohn's disease, rheumatoid arthritis, and psoriasis. The possibility of using selective inhibition of IL-12 overproduction as a therapy for these diseases has been validated in a recent publication in which it was disclosed that administration of a human monoclonal antibody against IL-12 to patients suffering active Crohn's disease resulted in significant rates of remission.



Our lead compound, STA-5326, is currently in clinical trials for treatment of Crohn's disease, rheumatoid arthritis, and CVID. Herein we describe the synthesis and SAR studies of a next generation series of novel small molecule IL-12 production inhibitors. Optimized quinazoline based inhibitors demonstrate potent *in vitro* activity against IL-12 production in human PBMC with an IC_{50} less than 100 nM.

[1] Gately *et al*. (1998) *Ann. Rev. Immunol*. 16: 495.

[2] Abbas *et al*. (1996) *Nature* 383: 787.

[3] Adorini *et al*. (1997) *Chem. Immunol*. 68: 175.

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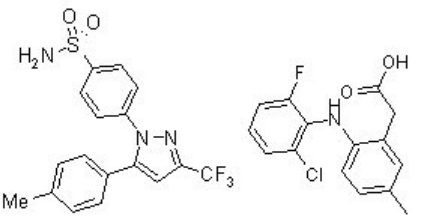
P322

3-substituted-5-aryl-imidazo[4,5-b]pyridine and 9-substituted-2-aryl-purine derivatives as selective cyclo-oxygenase-2 (COX-2) inhibitors

R. Gleave, P. Beswick, L. Chambers, D. Livermore, A. Naylor, L. Page, J. Reeves, J. Skidmore, A. Stevens, M. Swarbrick, S. Vile

Neurology and Gastrointestinal Centre of Excellence for Drug Discovery, GlaxoSmithKline, New Frontiers Science Park, Harlow, Essex, United Kingdom

COX-2 inhibitors are clinically efficacious in treating inflammatory pain. The majority of compounds described to date are either 1,2 diaryl heterocycles eg celebrex (1) or NSAID derivatives eg Lumiracoxib (2). There is considerable current interest in selective inhibitors with different structural templates. We will describe two series of monoaryl heterocylic inhibitors.



(1) (2)

Compounds were prepared derived from imidazopyridine and purine cores. A detailed investigation of structure activity relationships was undertaken and will be presented, together with in vitro data. Several analogues had a COX-2 microsomal IC_{50} <150nM and selectivity > 900 fold.

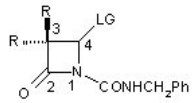
P323

Correlation Between Chemical Reactivity and Serine Protease Inactivation in C-4 Substituted β-Lactams

J. Mulchande, L. Martins, R. Moreira

CECF, Faculdade de Farmácia, Universidade de Lisboa, Av. Forças Armadas, 1600-083 Lisboa, Portugal

Human leukocyte elastase (HLE) is a serine protease that has been implicated in the chronic tissue matrix proteins destruction associated with inflammation, arthritis and emphysema. [1] Monocyclic β-lactams as compound **1** behave as inhibitors of HLE, acylating the catalytic serine residue with expulsion of the leaving-group, LG, at C-4, in a suicide-type inhibition pathway [2].



1

R = H, Et; LG = OC_6H_5, SC_6H_5, $SO_2C_6H_5$, $SCH_2C_6H_5$, $SO_2CH_2C_6H_5$, H

It has been suggested that the alkaline hydrolysis can be used as a guide to determine the inhibitory potential of an enzyme acylating agent [3]. A series of b-lactams **1** with substituents raising specific enzyme recognition and susceptibility towards nucleophilic attack were evaluated

against at HLE and PPE. A good correlation was obtained between the second-order rate constants for alkaline hydrolysis, k_{OH}, and the second-order rate of enzyme inactivation. These results suggest that k_{OH} can be used as a guide to select a potential inhibitor as an enzyme acylating agent. We now report that strong electron-withdrawing groups, such as sulfones, at C-4, can be used to improve inhibitory potency.

[1] Konaklieva M.I., *Current Medicinal Chemistry – Anti-Infective Agents*, 2002, *1*, 3, 215-238

[2] Firestone, R.; Barker, P.; Pisano, J.; Ashe, B.; Dahlgren, M.; Tetrahedron, 1990, 46, 2255.

[3] Sykes, N.; Macdonald, S.; Page, M., J. Med. Chem., 2002, 45, 2850- 2856.

JM and LM thank to Fundação para a Ciência e Tecnologia (FCT, Portugal) to Ph.D grants SFRH/BD/17534/2004 and SFRH/BD/6499/2001, respectively.

P324

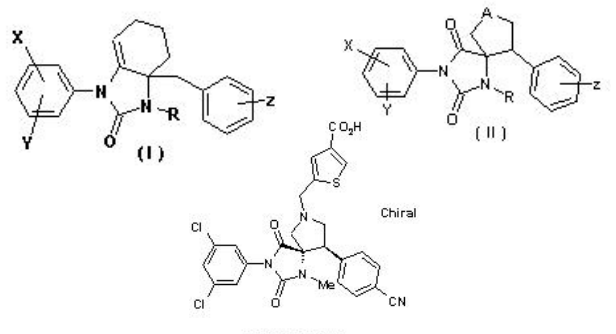
New conformationally constrained LFA-1 antagonists

D. Potin*, M. Launay*, E. Nicolai*, A. Fouquet*, F. Chevallier*, F. Monatlik*, P. Malabre*, F. Caussade*, D. Besse*, D. Stetsko**, S. Skala**, M. Mckinnon**, J. Barrish**, E. Iwanowicz**, S. Suchard**

*CEREP, 19 avenue du Québec, 91951 Courtaboeuf, FRANCE. **Bristol Myers Squibb Pharmaceutical Research Institute, Princeton, NJ 08543-4000, USA

LFA-1 (Leukocyte Function Associated Antigen-1), is a member of the Beta$_2$-integrin family and is expressed on all leukocytes. The LFA-1/ICAM interaction promotes tight adhesion between activated leukocytes and the endothelium, as well as between T cells and antigen-presenting cells. Because of the strong evidence from both animal models and clinical trials for LFA-1/ICAM as a target in several different inflammatory diseases, there has been an intense effort to identify orally available, small molecule inhibitors of this interaction.

This poster will describe the design, synthesis and SAR of conformationally restrained LFA-1 antagonists based on the tetrahydro-benzimidazolone (I) and the hydantoin (II) scaffolds that led to the identification of BMS-587101.



P325

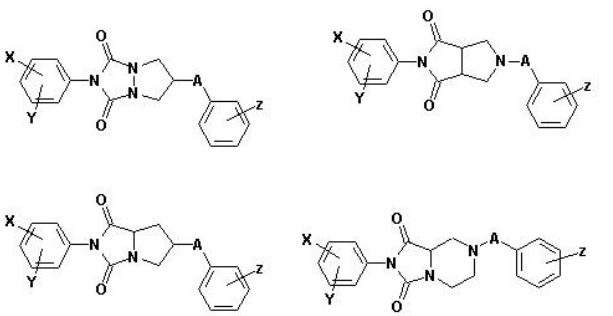
SAR around small molecules as LFA-1 antagonists

D. Potin*, M. Launay*, E. Nicolai*, M. Maillet*, A. Fouquet*, P. Malabre*, F. Monatlik*, F. Caussade*, D. Besse*, S. Skala**, D. Stetsko**, D. Hollenbough**, M. Mckinnon**, J. Barrish**, E. Iwanowicz**

*CEREP, 19 avenue du Quebec, 91951 Courtaboeuf, FRANCE. **Bristol Myers Squibb Pharmaceutical Research Institute, Princeton, NJ 08543-4000, USA

LFA-1 (Leukocyte Function Associated Antigen-1), is a member of the b$_2$-integrin family and is expressed on all leukocytes. The LFA-1/ICAM interaction promotes tight adhesion between activated leukocytes and the endothelium, as well as between T cells and antigen-presenting cells. Because of the strong evidence from both animal models and clinical trials for LFA-1/ICAM as a target in several different inflammatory diseases, there has been an intense effort to identify orally available, small molecule inhibitors of this interaction.

This poster will describe the design, synthesis and SAR of some conformationally restrained LFA-1 antagonists based on the hydantoin, urazole and pyrrolidine-2,5-dione scaffolds.



P326

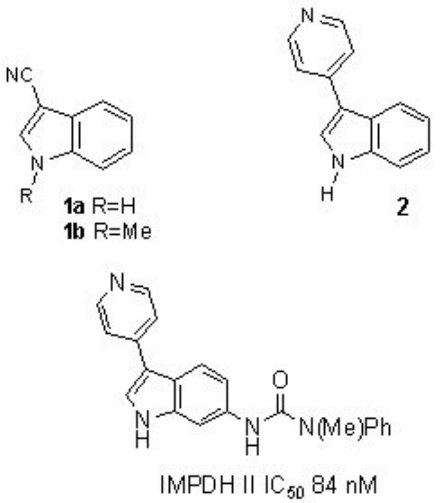
The Discovery of Non-Oxazole containing Indole Inhibitors of Inosine Monophosphate Dehydrogenase (IMPDH) via Fragment Optimisation

R. Beevers, G. Buckley, E. Frost, N. Kinsella, F. Galvin, D. Hannah, A. Haughan, K. Jenkins, S. Mack, W. Pitt, A. Ratcliffe, M. Richard, V. Sabin, J. Fraser, A. Sharpe, S. C. Williams

UCB, Granta Park, Great Abington, Cambridge, CB16GS, UK

Proliferation of T and B lymphocytes is dependent on access to a large cellular pool of guanine nucleotides. Within the de novo purine biosynthesis pathway a key rate limiting step is oxidation of inosine-5'-monophosphate to xanthosine-5'-monophosphate by the NAD dependent enzyme inosine monophosphate dehydrogenase (IMPDH). Two isoforms of the enzyme have been identified and designated type I and type II. Of these isoforms it is IMPDH II that is upregulated in actively proliferating cell types. As a consequence inhibition of IMPDH II has become an attractive immunology target for the

treatment of transplant rejection, psoriasis, systemic lupus erythematosus and rheumatoid arthritis.



IMPDH II IC_{50} 84 nM

This poster describes our preliminary efforts to discover non-oxazole containing IMPDH II inhibitors using a fragment optimisation approach. A series of indole fragments were synthesised or obtained from commercial sources. Two hit templates, namely the cyanoindoles (**1a**) and (**1b**) and 3-(4-pyridyl)indole (**2**) were highlighted for further investigation. The elaboration of these templates is described, discussing synthesis, in vitro inhibitory values for IMPDH II, PBMC proliferation and physicochemical properties.

[1] D.R.Hannah *et al.* Low Molecular Weight Indole fragments as IMPDH Inhibitors. *Bioorg. Med. Chem. Lett*. In press.

[2] D.R.Hannah *et al*. Novel Indole Inhibitors of IMPDH from Fragments: Synthesis and Initial Structure-Activity Relationships. *Bioorg. Med. Chem. Lett*. In pre

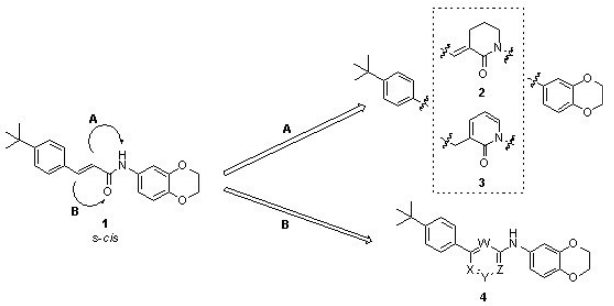
P327

Conformationally Restricted Analogues of trans-Cinnamides: Discovery of Substituted Pyrimidines as Potent TRPV1 Antagonists

M.H. Norman,[1] N. Chen,[1] E.M. Doherty,[1] C.H. Fotsch,[1] N.R. Gavva,[3] N. Nishimura,[1] T. Nixey,[1] V.I. Ognyanov,[1] R. Rzasa,[1] M. Stec,[1] R. Tamir,[3] V. Viswanadhan,[2] J. Zhu,[1]and J.J.S. Treanor[3]

[1]Department of Chemistry Research & Discovery; [2]Department of Molecular Structure; [3]Department of Neuroscience, Amgen Inc, Thousand Oaks, CA

The vanilloid receptor-1 (TRPV1 or VR1) is a member of the transient receptor potential (TRP) family of ion channels and plays a role as an integrator of multiple pain-producing stimuli. From a high-throughput screening assay, measuring calcium uptake in TRPV1-expressing cells, we identified an N-aryl trans-cinnamide (**1**) that acts as a potent TRPV1 antagonist. We have demonstrated the antihyperalgesic properties of **1** in vivo and have also reported the discovery of novel, orally bioavailable cinnamides derived from this lead. Herein, we describe the synthesis and biological evaluation of a series of conformationally constrained analogues of the s-cis conformer of **1** as isosteric replacements of the trans-cinnamide core (i.e., lactam **2**, pyridone **3**, and various amino-heterocycles **4**, wherein W, X, Y, and Z = CH or N). These investigations resulted in the identification of a novel series of 4,6-disubstituted pyrimidines as potent TRPV1 antagonists.



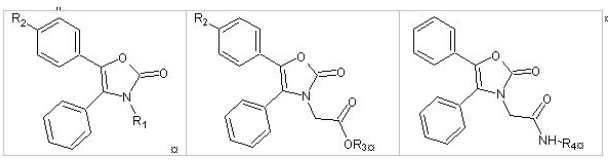
P328

Synthesis of new 4,5-diphenyl-2-oxo-3H-1,3-oxazole derivatives as inhibitors of cyclooxygenase enzymes

Y. Dündar, S. Ünlü, E. Banoglu, N. Noyanalpan

Gazi University Faculty of Pharmacy Department of Pharmaceutical Chemistry

Prostaglandins (PGs) are important biological mediators of inflammation, originating from biotransformation of arachidonic acid catalyzed by cyclooxygenases (COX). Two isoenzymes of COX have been identified, namely COX-1 and COX-2. While the COX-1 isoform provides normal production of PGs which have protecting effect in the gastrointestinal tract, the inducible form (COX-2) was found almost exclusively in inflamated tissues as a cause of inflammatory stimuli. PGs may also mediate a variety of neuropathologic phenomena including inflammation-associated disorders in brain cells such as Alzheimer's disease (AD) [1]. Many reports suggest that 1,2-diphenyl substituted heterocyclic moiety as the essential core structure for selective inhibition of COX-2 isoenzyme [2].



R_1: H, Methyl,
R_2: H, Aminosulfonyl,
R_3: H, Ethyl,
R_4: Phenylethyl, octyl, piperidinyl, 2-thiazoyl, 4-chlorophenyl, 4-methoxyphenyl, morpholinyl.

The objective of this study was to develop new compounds with the inhibitory potency and the enzyme selectivity against COX-2. Therefore, we prepared the 3-

unsubstituted/substituted-4,5-diphenyl-4-oxazoline-2-one derivatives as potential COX-2 inhibitors. *In vitro* inhibitory potency of synthesized compounds on COX enzymes will be reported in this study.

[1] L. A. Teather, R. J. Wurtman, Neuroscience Letters, (2003), 340, 177–180.

[2] J. Van Ryn, G. Trummlitz, M. Pairet, Current Medicinal Chemistry, (2000), 7, 1145-1161.

This study was supported by Gazi University BAP. (Project Number 02/2005-22).

P329

Synthesis, Characterization and Preliminary Screening of Regioisomeric 1-(3-Pyridazinyl)-3-arylpyrazole and 1-(3-Pyridazinyl)-5-arylpyrazole Derivatives towards Cyclooxygenase Inhibition

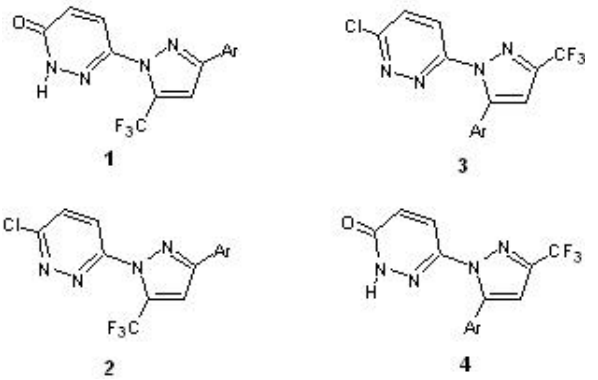
S. Ünlü*, E. Banoğlu*, S. Ito**, T. Niiya***, G. Eren*, B. Ökçelik*, M. Şahin*

*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Gazi University 06330 Etiler, Ankara, Turkey. **Institute of Biomaterials and Bioengineering, Tokyo Medical and University, 2-3-10, Surugadai, Kanda, Chiyoda-Ku, Tokyo 101-0062, Japan. ***Faculty of Pharmaceutical Sciences, Fukuoka University, Fukuoka 814-0180, Japan

The discovery of two distinct cyclooxygenase (COX) isoforms, namely COX-1 and COX-2, made it possible to separate the pharmacological effects from the general side effects of traditional non-steroidal anti-inflammatory drugs (NSAIDs). As well documented, the COX-1 is expressed constitutively in most cells and tissues and responsible for the synthesis of PGs that are important for gastric protection and vascular homeostasis while the COX-2 is mostly expressed as a result of mitogenic and inflammatory stimuli. Hence, this discovery suggested that the inhibition of COX-2 but not COX-1 was of importance for designing compounds that lack the gastrointestinal and renal side effects of currently used NSAIDs. This major advantage in inflammation has led to the development of selective COX-2 inhibitors which expected to be effective and safe NSAIDs.

As a well known knowledge, a number of 1,5-diaryl substituted pyrazoles are known to possess COX-2 inhibitory activity, and one of them known as celecoxib was used in the therapy of inflammation. During the last decade, the most studied part of these compounds was the replacement of the central ring with other carbocyclic or heterocyclic ring systems. Therefore, the objective of this study was the replacement of one of the aryl groups in celecoxib template with pyridazine to evaluate the significance of the aryl substituents of the central pyrazole ring. With this purpose to evaluate the biological consequences of incorporation of a pyridazine/pyridazinone ring as one of the aryl substituents, and also the effect of a 1,3- or 1,5-diarylsubstitution pattern around the pyrazole ring on the *in vitro* COX inhibitory potency, we required the synthesis of 1-(pyridazin-3(2H)-on-6-yl)-3-arylpyrazoles (**1**), 1-(6-chloro-3-pyridazinyl)-3-arylpyrazoles (**2**), and the other regioisomers, 1-(3-pyridazinyl)-5-arylpyrazoles (**3**), 3(2H)-pyridazinones (**4**).

The results of inhibitory potency against COX-1 and COX-2 in a human whole blood assay of synthesized compounds will be discussed during the congress.



P330

Discovery of new ligands of the C3a receptor. Part 1. Arginine derivatives

F. Denonne, S. Binet, M. Burton, A. Dipesa, M. Eckert, D. Ene, T. Ganguly, M. Gillard, A. Giannaras, P. Higgins, T. Hullinger, S. Kumar, J. Leonard, B. Levine, T. Lewis, F. Maounis, J.M. Nicolas, T.Mansley, P.Pasau, D.Preda, S.Rao, K. Stebbins, A. Volosov, D. Zou.

UCB Chemin du Foriest - 1420 Braine-l'Alleud - Belgium

The synthesis and structure-activity relationships on the C3a receptor of these arginine derivatives (Figure 1) will be presented.

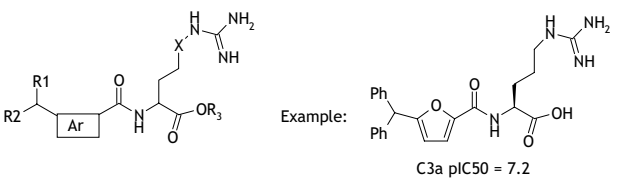


Figure 1.

P331

Synthesis of 3-[1-(3-pyridazinyl)-5-phenyl-pyrazole-3-yl]propanoic acid as potential anti-inflammatory compounds

B. Çalışkan Ergün*, B. Cakir**, E. Banoglu*

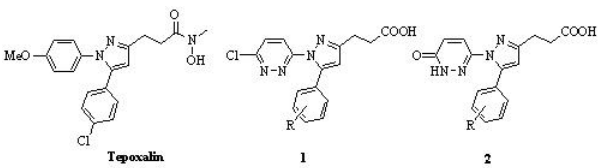
*Gazi University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, 06330 Etiler, Ankara, Turkey. **Gazi University, Faculty of Pharmacy, Department of Pharmacognosy, 06330 Etiler, Ankara, Turkey

Nonsteroidal anti-inflammatory drugs (NSAIDs) widely used in treating pain and the symptoms of arthritis have

limitations due to drug associated side effects, including life threatening ulceration and renal toxicity. Common NSAIDs work by blocking the activity of cyclooxygenase (COX), an enzyme that converts arachidonic acid into prostanoids. Two forms of COX are now known, a constitutive isoform (COX-1) and an inducible isoform (COX-2) of which expression is upregulated at sites of inflammation. While COX-1 appears to be responsible for gastrointestinal and renal protection, COX-2 is believed to be a predominant isoform present in inflammation conditions. This major advantage in inflammation has led to the development of selective COX-2 inhibitors which expected to be effective and safe NSAIDs. However, although these drugs are better tolerated than classical NSAIDs with improved gastric safety profile, their prolonged use caused cardiovascular side-effects which limited their therapeutic benefit. Based on these results, dual inhibition of COX and 5-lipoxygenase (5-LOX) emerged as a new strategy to provide effective and safer NSAIDs lacking the drug associated GI and cardiovascular side-effects.

These finding have stimulated us to develop dual COX and 5-LOX inhibitors which maintain the high anti-inflammatory activities without common side-effects. In our continuing efforts towards the synthesis of tepoxalin (dual COX/5-LOX inhibitor) related 1,5-diarylpyrazole anti-inflamamatory agents, we required the synthesis of 3-[1-(6-chloro-pyridazin-3-yl)-5-phenyl-pyrazole-3-yl]propanoic acids (**1**) and 3-[1-(3(2H)-pyridazinon-6-yl)-5-phenyl-pyrazole-3-yl]propanoic acids (**2**) .

The results of the in vitro COX inhibitory activity as well as in vivo anti-inflammatory activities in carrageenan-induced air porch model of the synthesized compounds will be discussed during the congress. 5-LOX inhibitory activity of the compounds are currently under investigation in our laboratory.



Tepoxalin 1 2

P332

New 1-(3-pyridazinyl)-5-aryl-pyrazole derivatives as potential analgesic compounds

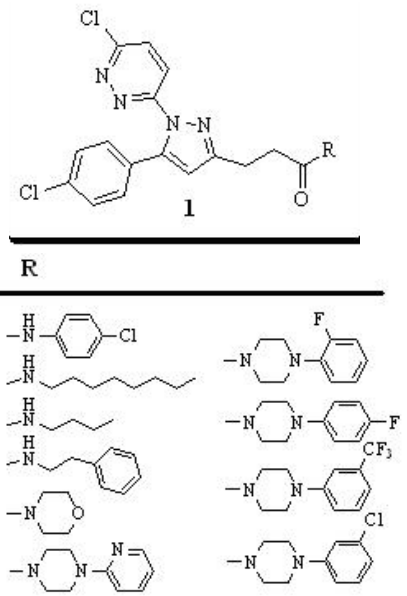
M. Şüküroglu, B. Çalışkan Ergün, E. Aypar, M. Ark, E. Banoglu

Drug Discovery Group, Departments of Pharmaceutical Chemistry and Pharmacology, Faculty of Pharmacy, Gazi University, 06330 Etiler, Ankara, Turkey

Interest in the identification of potent anti-inflammatory drugs has been intense since the demonstration that early nonsteroidal anti-inflammatory drugs (NSAIDs) caused typical side effects such as gastrointesitinal toxicity and kidney damage which limited their therapeutic use. Emerging evidence suggests that these adverse reactions are attributed to COX-1 inhibition with prolonged use of NSAIDs. Meantime, development of COX-2 inhibitors resulted in clinical efficacy equivalent to NSAIDs with a much improved gastric safety profile thus fulfilling the promise for this class. However, COX-2 selective inhibitors intrinsically lack anti-thrombotic activity and some cardiovascular liabilities have been associated in clinical use of this drug class. Therefore, a strategy for developing new drugs which are able to block both COX and 5-LOX pathway has become of interest for many laboratories.

These finding have stimulated us to develop dual COX and 5-LOX inhibitors which maintain the high anti-inflammatory activities but less side-effects. Our past research activities in this area showed us that 6-(1-pyrazolyl)-pyridazinone derivatives having acetamide side-chain resulted in potent in vivo analgesic and anti-inflammatory activities. Using this information, we recently have designed 1-(3-pyridazinyl)-5-phenyl-pyrazoles having propanamide side-chain (1) towards dual inhibition of both COX and 5-LOX enzymes.

The results of the in vitro COX inhibitory activity as well as in vivo anti-inflammatory activities of the synthesized compounds will be discussed during the congress. 5-LOX inhibitory activity of the compounds are currently under investigation in our laboratory.



P333

Discovery of new ligands of the C3a receptor. Part 2. Aminopiperidine derivatives

F. Denonne, S. Binet, M. Burton, S. Defays, A. Dipesa, M. Eckert, D. Ene, M. Gillard, A. Giannaras, P. Higgins, T. Hullinger, S. Kumar, J. Leonard, B. Levine, J.M. Nicolas, P. Pasau, C. Pégurier, D. Preda, S. Rao, K. Stebbins, N. Van Houtvin, A. Volosov, D. Zou

* UCB Chemin du Foriest - 1420 Braine-l'Alleud - Belgium

The synthesis and structure-activity relationships on the C3a receptor of these aminopiperidine derivatives (Figure 1) will be presented.

Figure 1.

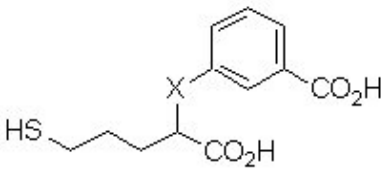
P334

Structural Optimization of Thiol-based Inhibitors of Glutamate Carboxypeptidase II by Modification of the P1' Side Chain

P. Majer, B. Hin, D. Stoermer, J. Adams, W. Xu, B. Duvall, G. Delahanty, Q. Liu, M. Stathis, K. Wozniak, B. Slusher, T. Tsukamoto

MGI Pharma, Inc. 6611 Tributary Street, Baltimore, MD 21224

A series of thiol-based inhibitors containing a benzyl moiety at the P1' position have been synthesized and tested for their abilities to inhibit glutamate carboxypeptidase II (GCP II). 3-(2-Carboxy-5-mercaptopentyl)benzoic acid **6c** was found to be the most potent inhibitor with an IC_{50} value of 15 nM, 6-fold more potent than 2-(3-mercaptopropyl)pentanedioic acid (2-MPPA), a previously discovered, orally active GCP II inhibitor. Subsequent SAR studies have revealed that the phenoxy and phenylsulfanyl analogs of **6c**, 3-(1-carboxy-4-mercaptobutoxy)-benzoic acid **26a** and 3-[(1-carboxy-4-mercaptobutyl)-thio]benzoic acid **26b**, also possess potent inhibitory activities towards GCP II. In the rat chronic constriction injury (CCI) model of neuropathic pain, compounds **6c** and **26a** significantly reduced hyperalgesia following oral administration (1.0 mg/kg/day).



6c (X = CH_2) IC_{50} = 15 nM
26a (X = O) IC_{50} = 14 nM
26b (X = S) IC_{50} = 32 nM

P335

The Synthesis of Some New Isoxazolo[4,5-d]pyridazin-4(5H)-one Derivatives

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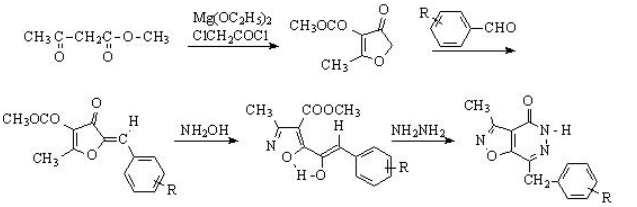
It is well known that the therapeutic use of classical non-steroidal anti inflammatory drugs is often limited due to their gastrointestinal side effects such as hemorrhage and ulceration. In view of this fact, the research have been directed at designing compounds with different structural features that are able to act on other biological targets involved in the anti inflammatory response.

Dal Piaz and co-workers synthesized and evaluated the analgesic activities of a series of isoxazolo[3,4-d]- and isoxazolo[4,5-d]pyridazinone derivatives. They reported that 5-{[4-(3-chlorophenyl)piperazin-1-yl]propyl}-3-methyl-7-phenylisoxazolo[4,5-d]pyridazin-4(5*H*)-one showed higher analgesic activity than morfine [1,2]. These results led us to synthesize a new series of isoxazolo[4,5-d]pyridazin-4(5*H*)-ones. Synthetic procedure was given in below :



R: -H, -Cl, -Br, -CH_3, -OCH_3

Sheme: Synthetic procedure of the compounds

In the first step of the synthetic procedure, 4-carbmethoxy-5-methyl-3(2H)-furanone was prepared by the reaction of methyl acetoacetate and chloroacetyl chloride. Then 4-carbmethoxy-5-methyl-3(2H)-furanone was reacted with substituted benzaldehydes to obtain 2-substitutedbenzylidene-4-carbmethoxy-5-methyl-3(2H)-furanones. These furanone derivatives were treated with hydroxylamine to yield methyl 5-(2-substitutedphenyl-1-hydroxyethenyl)-3-methylisoxazolo-4-carboxylates. In the last step, the isoxazole derivatives obtained, were converted into 3-methyl-7-substitutedbenzylisoxazolo[4,5-d]pyridazin-4(5H)-ones by reacting with hydrazine hydrate.

[1] Giovannoni M., Vergelli C., Ghelardini C., Galeotti N., Bartolini A., Dal Piaz V., [(3-Chlorophenyl)piperazinyl-propyl]pyridazinones and Analogues as Potent Antinociceptive Agents, J. Med. Chem. 2003; 46; 1055-1059.

[2] Dal Piaz V., Vergelli C., Giovannoni M., Scheideler M., Petrone G., Zaratin P., 4-Amino-3(2*H*)-pyridazinones bearing arylpiperazinylalkyl groups and related compounds: synthesis and antinociceptive activity, Il Farmaco 2003; 58(11); 1063-1071

P336

Preparation and Antiinflammatory-Analgesic Activity of Some 5-Aryl-3-alkylthio-1,2,4-trizoles and Corresponding Sulfones

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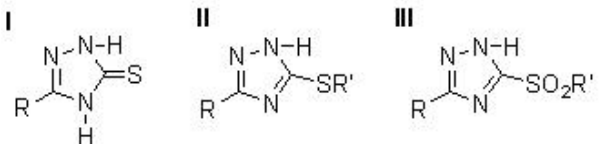
Nonsteroidal antiinflammatory drugs (NSAIDs) are among the most widely used therapeutics, primarily for the treatment of pain and inflammation in arthritis. Unfortunately, therapeutic effects and side effects of them are closely related to their biochemical mechanism of action. As a consequence, administration of NSAIDs in long term may lead to development of threatening GI ulcers, bleeding and renal disorders. Therefore, although there are a number of antiinflammatory-analgesic drugs available in the market, development of new compounds having antiinflammatory and analgesic activity without side effects is still a necessity.

In continuation to our lasting interest towards chemistry and pharmacological properties of 1,2,4-triazoles we have designated and synthesized a series of 5-aryl-3-alkylthio-1,2,4-triazole derivatives (**II**) and their corresponding sulfones (**III**).

The starting compound, 5-aryl-1,2,4-triazole-3-thiones (**I**) were obtained by the reaction of 4-acylthiosemicarbazide with KOH 10% under reflux, followed by the acidification with concentrated hydrochloric acid. Alkylation of starting compounds with iodomethane/or ethane provided the methyl/ethyl thioether intermediates (**II**). Treatment of 3-alkylthio compounds with $KMnO_4$ in the acetic acid at 20 ºC temperature resulted in the oxidation of sulphur to sulfone (**III**). Chemical structures of the synthesized compounds were confirmed by spectral and elemental analysis.



The analgesic and antiinflammatory activities and ulcerogenic risk of the compounds synthesized is currently under investigation. Detail of the results will be discussed in poster.

P337

Biarylcarboxybenzamide derivatives as Potent Vanilloid Receptor (VR1) Antagonistic Ligands

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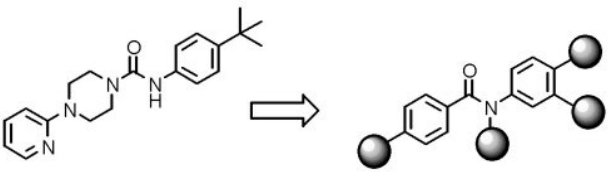
Vanilloid receptor (VR1) is a nonselective cation channel placed in the plasma membrane of peripheral sensory neurons, which has been regarded as a new target for the treatment of pain. The agonists desensitize the peripheral sensory neurons by influx of cations, especially Ca2+, into neuronal cell, which leads analgesic effect. However, their initial excitatory side effects, such as initial irritation, hypothermia, bronchoconstriction, and hypertension, derived from its inherent mechanism, make it hard to develop as systemic analgesics. In order to avoid the side effects from agonist, competitive antagonists have been pursued as novel analgesic drugs. So far, several synthetic and semi-synthetic antagonists were introduced and their pharmacological potential for pain treatment were evaluated. Recently Purdue Pharma research group disclosed 4-(2-pyridyl)piperazine-1-carboxybenzamides as potent VR1 antagonists. In this poster, we report the synthesis and functional assay on VR1 receptor of biarylcarboxybenzamide derivatives, based on molecular modeling studies.



P338

Synthesis of new 5-methyl-3-(4'-substituted benzoylmethyl) and 3-[2-(4'-substituted phenyl)-2-hydroxyethyl]-2-benzoxazolinones as analgesic/antiinflammatory agents

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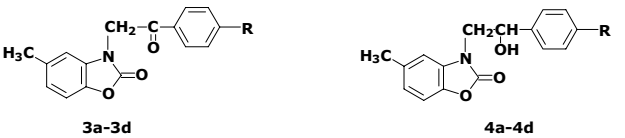
Non-steroidal antiinflammatory drugs (NSAIDs) are widely used. Despite their large number, none is very effective therapeutically, and almost all have several undesired, often serious, side effects and so long term administration is not advisable. Thus the need for new anti-inflammatory drugs is obvious. Therefore, there has been renewed interest in antiinflammatory agents endowed with either more selective mechanisms or novel modes of action. One of these novel action modes is inhibition of inducible nitric oxide synthase, which contributes to acute and chronic inflammation[1-2]. In this context, it has been shown that some benzoxazolinone derivatives, especially 5-methyl analogs inhibit NOS and they constitute a novel class of non-aminoacid NOS inhibitors[3]. In view of these facts and as a continuation of the previous efforts on 2-benzoxazolinone moiety carried out in our laboratory, it was thought worthwhile synthesizing a new series of 5-methyl-3-(4′-substituted benzoylmethyl)-2-benzoxazolinones and their reduction products and investigating the effect of such molecular variation on the analgesic-antiinflammatory activities.

5-Methyl-2-benzoxazolinone converted into 3-(4′-substituted benzoylmethyl)-5-methyl-2-benzoxazolinones

(3a-3d) by refluxing a-bromo-4-substituted acetophenone with 5-methyl-2-benzoxazolinone. The obtained compounds were then submitted to reduction reaction by treatment with $NaBH_4$, which yielded 3-[2-(4′-substituted phenyl)-2-hydroxyethyl]-5-methyl-2-benzoxazolinones.



3a-3d 4a-4d

R: H, Cl, Br, CH_3

Among the compounds examined in this study, compounds **4a** and **4c** possessed the most clear and consistent antiinflammatory activity. Of the compounds which were given at 100 mg/kg body weight dose level as orally, only the compound 4a showed more than a 20% inhibitory effect.

Acknowledgement: This study was supported by Hacettepe University Research Fund (Project number: 05 D 301002).

P339

Synthesis and docking of novel series of 7-substituted xanthine derivatives as phosphodiesterase isoenzyme inhibitors

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There are five main families of tissue dependent cyclic nucleotide phosphodiesterase isoenzymes (PDE) which catalyze the hydrolysis of cyclic AMP and cyclic GMP to 5' nucleotides [1]. PDE4 represents the major class expressed in human inflammatory cells and particularly in macrophages, eosinophils and neutrophils, the main cell types present in the lungs of asthmatic and chronic obstructive pulmonary disease (COPD) patients [2]. Selective inhibitions of particular types have been shown to produce bronchodilatation in asthma, COPD [3] and inhibit mediator release from inflammatory cells [4]. Theophylline, rolipram, cilomilast and roflumilast are clinically used therapeutics for asthma and COPD based on inhibition of PDE isoenzymes with various side effects.

In this study, based on the knowledge that theophylline and rolipram are inhibiting the PDE4 non-selectively and selectively, we synthesized a new group of 7-substituted xanthine structures to enhance the selectivity of theophylline to this subtype. Docking studies of these compounds to catalytic domain of human phosphodiesterase 4B were made by using autodock and dock programs to find the possible modes and sites of binding. To enhance the properties of structures program GRID was used. Present molecular modeling studies provide guidelines for the design and pharmacological activity of novel selective isoenzyme inhibitors.

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[2] Wang P., Wu P., Ohleth K.M., Egan R.W., Billah M.M.;Mol. Pharmacol., 1999, 56: 170-174.

[3] Torphy T.J.;New Anti-asthma Drug ed. O' Donnell, S.R. & Persson, C.G.A. 1988, pp. 37-53. Basel: Birkhauser.

[4] Lagente V., Martin-Chouly, C., Boichot, E., Martins, M.A.,Silva, P.M.R.; Mem Inst Oswaldo Cruz, 2005;100: 131-136.

P340

Identification and SAR of Drug-like mixed P2X3/P2X2/3 Antagonists for the Treatment of Pain

[2]D.S. Carter, [3]D.A. Cockayne, [2]M.P. Dillon, [1,3]A.P.D.W. Ford, [3]M.F. Jett, [1]J.R. Gever, and [4]B.D. Koch

[1]Biochemical Pharmacology, Roche Palo Alto. [2]Medicinal Chemistry, Roche Palo Alto. [3]Neuroscience, Roche Palo Alto. [4]Discovery Sciences and Technologies, Roche Palo Alto. 3431 Hillview Ave, Palo Alto, CA 94304, U.S.A.

P2X purinergic receptors are ligand-gated ion channels activated by ATP. There are currently seven receptor subunits ($P2X_{1-7}$) which can form homo- and heteromultimeric channels that are believed to exist as trimers[1]. Both the $P2X_3$ and $P2X_{2/3}$ receptor subtypes have been shown to play an important role in the regulation of sensory function[2]. The lack of suitable dual antagonists with drug-like properties led to a high throughput screen of the Roche compound collection. Rat $P2X_3$ receptors were expressed in CHO-K1 cells and the cytosolic calcium flux evoked by the agonist α,β-Me-ATP was measured using FLIPR. Four chemotypes were selected for hit to lead activities, eventually yielding two series that were advanced. Subsequent optimization resulted in the discovery of RO-3, a potent, selective and drug-like dual $P2X_3/P2X_{2/3}$ antagonist[3]. Profiling of this compound and analogs in a number of preclinical pain models confirms the potential therapeutic benefit of this compound class. This poster will outline the HTS campaign and present some of the preclinical pain data generated using these molecules.

[1] Khakh, BS et al., (2001) Pharmacol. Rev. 53:107-118

[2] Liu, XJ and Salter, MW, (2005) Curr. Opinion Invest. Drugs 6:65-75

[3] Ford, APDW et al., (2006) Br. J. Pharmacol. 147:S132-143

P341

Synthesis of new 4-alkyl/aryl substituted-1-[(5-methyl-2-benzoxazolinone-3-yl)acetyl]3-thiosemicarbazide derivatives

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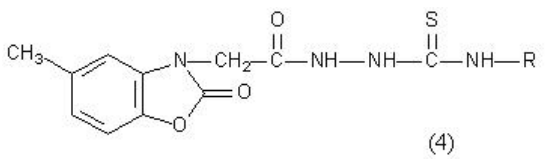
Recently researchers have revealed that different pharmacodynamic moieties of benzoxazolinones possess potent biological activities such as dopamine receptor agonist, cardiotonic, antihypertensive, antiulcer activities. Many reports indicate that compounds containing the benzoxazolinone ring possess analgesic-antiinflammatory activities and it has been suggested that N-phenylacetamide and propionamide derivatives of 2-benzoxazolinone ring can be the starting compounds to obtain potent inhibitors of human leucocyte myeloperoxidase activity.

It is well known that 1-acyl-4-substituted thiosemicarbazide and their corresponding cyclized 1,3,4-oxadiazole, 1,3,4-thiadiazole and 1,2,4-triazole derivatives have diverse pharmacological activities such as fungicidal, insecticidal, bactericidal, herbicidal, antitubercular, antitumor, analgesic-antiinflammatory and there are numerous reports that highlight their chemistry and use.

Structural variations bring about new physical and biological properties. The molecular manipulation of a promising lead compound is stil a major line of approach for the discovery of new drugs. Molecular manipulation involves the efforts to combine separate groups having similar activity in one compound. In the present study analgesic-antiinflammatory activities associated with both 2-benzoxazolinone and 1,3,4-oxadiazole, 1,3,4-thiadiazole and 1,2,4-triazole moieties prompted us to synthesize new benzoxazolinone derivatives carrying the biodynamic heterocyclic systems (1,3,4-oxadiazole, 1,3,4-thiadiazole and 1,2,4-triazole) at position-3 with an objective to obtain biheterocycles of enhanced biological activities.



(4)

R: CH_3, C_2H_5, C_3H_5, C_6H_5

The synthesis of 5-methyl-2-benzoxazolinone (1) was carried out by the reaction of 4-methyl-2-aminophenol with urea. Compound 1 was reacted with ethyl chloroacetate in acetone under basic conditions to obtain ethyl (5-methyl-2-benzoxazolinone-3-yl)acetate (2). (5-methyl-2-benzoxazolinone-3-yl)acetohydrazide (3) was obtained by the reaction of 2 with hydrazine hydrate in ethanol. The hydrazide thus obtained was reacted with 4-substituted-3-thiosemicarbazide derivatives to obtain the title compounds (4). Physical and chemical properties of synthesized compound have been confirmed by utilizing their melting points, IR, ^{1}H-NMR, mass and elemental analysis.

P342

Synthesis and analgesic and anti-inflammatory activity of (6-acyl-2-oxo-3H-benzothiazol-3-yl)propanamide derivatives

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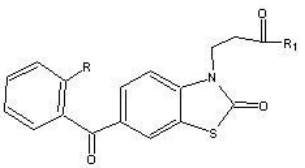
The search for new analgesic compounds devoid of the side effects such as respiratory depression, constipation, and physical dependence in morphine-like opioid agonists as well as of the gastrointestinal irritation and kidney damage associated with nonsteroidal anti-inflammatory drugs have attracted considerable attention in recent years. Among these compounds, oxobenzothiazolines have generated particular interest.

Some 6-acyl-2-oxobenzothiazoline derivatives have been reported as potent analgesic agents. In this respect, Ferreira reported the antinociceptive activity 6-benzoyl-2-oxobenzothiazoline in 1995, and concluded that it might release an endogenous opiod-like substance from the adrenal glands which exerts the antinociceptive activity [1]

Yous and co-workers concluded that 6-benzoyl-2-oxobenzothiazoline represents a new type of antinociceptive agent acting in periphery by inhibiting the cyclooxygenase pathway and promoting the release of an opioid peptide [2].

Based on above information, the synthesis of twelve new (6-benzoyl-2-oxobenzothiazoline-3-yl) propionamide derivatives (1) are reported in this study. The compounds were tested for analgesic and antiinflamatory activity using p-Benzoquinone-induced writhing and Carrageenan-induced hind paw edema methods. Among these compounds, 1-[3-(6-(2-fluorobenzoyl)-2-oxo-3H-benzothiazolon-3-yl)propanoyl]-4-(4-fluorophenyl)piperazine has been found to be significantly more active than the other compounds synthesized.



R: H, F

R_1: Morpholine, 4-phenylpiperazine, 4-(4-fluorophenyl)piperazine, 4-(4-chlorophenyl)piperazine, 4-piperonylpioerazine, 4-benzylpiperazine

References

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P343

"Bridged" Resveratrol-Analogues Show Potent and Selective COX-1 Inhibition

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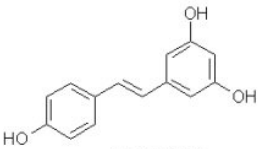
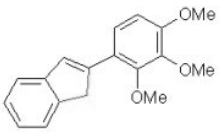
Resveratrol ((*E*)-3,4',5-trihydroxystilbene), a phytoalexin found in grapes and other plants, shows a broad spectrum of biological activities. Among various effects it exerts relaxation on isolated smooth muscle preparations (terminal ilea, aortic-, arteria pulmonalis rings; i. e. decrease of force of contraction (f_c) with a concentration of 100 µM resveratrol on arteria pulmonalis rings to -53.56 ± 7.10 %) and a non-selective COX-inhibition (IC_{50}(COX-1) = 0.535 µM; IC_{50}(COX-2) = 0.996 µM) [1]. By modifying the stilbene scaffold we wanted to obtain more potent and selective compounds, which are presented here.



Resveratrol



1

In a recent paper [1] we published polymethoxy- and polyhydroxystilbenes which showed potent and selective COX-2 inhibition. In continuation of our studies we synthesized novel "brigded" stilbenes (i. e. compound **1**) and tested them for their biological effects. In contrast we obtained compounds (i. e. 2-(2,3,4-trimethoxyphenyl)-1*H*-indene (**1**)) showing potent and selective COX-1 inhibition (IC_{50}(COX-1) = 0.03 µM; IC_{50}(COX-2) = 1.33 µM for compound **1**) but no significant activity on smooth muscle preparations (i.e. decrease of f_c with a concentration of 100 µM **1** on arteria pulmonalis rings to -11.92 ± 11.17 %).

The effects were also confirmed by similar molecules bearing a "bridged" resveratrol scaffold.

[1] Murias M, Handler N, Erker T, Pleban K, Ecker G, Saiko P, Szekeres T, Jaeger W. Resveratrol analogues as selective cyclooxygenase-2 inhibitors: synthesis and structure-activity relationship. Bioorg Med Chem 2004;12:5571-5578.

P344

Synthesis of 3-(3,5-di-tert-butyl-4-hydroxybenzyl)-2,1,3-benzothiadiazines as potential inhibitors of PDE4 enzyme

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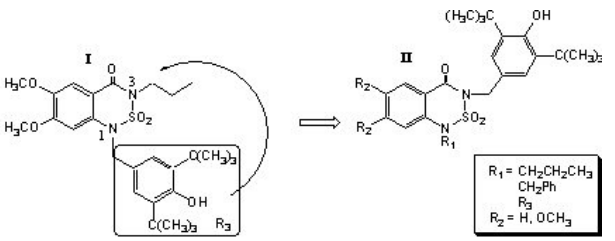
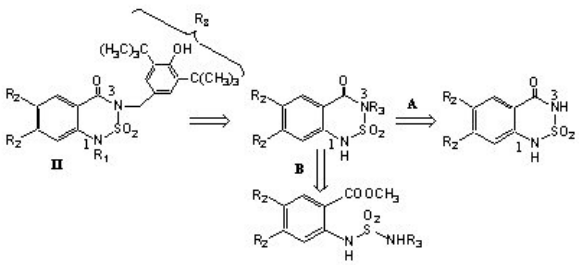
Phosphodiesterases (PDEs) are represented by 7 types of intracellular enzymes involved in the signal transduction 2nd messenger mediated pathway. In particular PDE4 and 7 play a fundamental role in the regulation of pathological processes such as inflammation (acute and chronic) and cancer proliferation1. Consequently their selective inhibition would be very useful for the improvement of the therapy in these pharmacological areas.

Recently we synthesized a series of N-1,3 disubstituted 2,1,3-benzothiadiazines bearing a *2,6-di-tert-butylphenol* moiety at N-1 position. This residue appears to be important for the inhibitory activity toward PDE4 enzyme2,3 and conferes antioxidant properties to these molecules. Among them the 6,7-dimethoxy substituted derivative (**I**) has revealed the best antioxidant activity. Moreover a molecular docking study2 was carried out and three main interactions into the catalytic domain of PDE4D subtype were observed.



On the light of these results and in order to evaluate if the N-1 position of the *antioxidant group* is essential for the PDE4 activity, new compounds have been designed in which this group has been moved to N-3. Two ways of synthesis (**A**, **B**) of this novel class of 2,1,3-benzothiadiazines will be presented and discussed in terms of yields, purity and time saving results together with biological evaluation.



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[3] Tait, A.; Luppi, A.; Avallone, R.; Baraldi, M.; *Il Farmaco*, **2005**, 60, 653-663.

P345

Synthesis and Analgesic and Antiinflammatory Activity of 6-Substituted-3(2H)-pyridazinone- 2-yl Acetic Acide Derivatives

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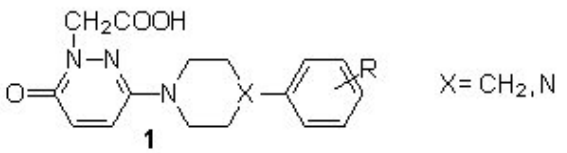
Despite an ever growing body of knowledge of endogenous nociceptive and antinociceptive systems, many pain syndromes like rheumatoid arthritis and certain advances cancers are still unsatisfactorily treated. Moreover, there are certain kinds of pain, such as neuropatic pain, associated to amputations, which at present have no adequate treatment. The main objective in current pain research is to develop improved non-opioid analgesic which are as effective as the opioid but without their side effects. In this regards a considerable number of pyridazinone derivatives endowed with analgesic properties have been reported recently

Our experience in the pyridazinone field [1-4] , together with the observation that some pyridazine derivatives such as emorfazone[5], on the market in Japan, and compounds bearing an alkylpiperazinyl alkyl moiety on the pyridazinone ring[6-9], show interesting antinociceptive activity not related to effects on prostaglandins or opioid system, led to design and synthesize a series of pyridazinone derivatives as potential analgesic drugs.

In this study a series of 6-substituted-3(2H)-pyridazinone-2-yl acetic acide **1** derivatives were synthesized and evaluated for analgesic and anti-inflammatory activities. The structures of these new pyridazinone derivatives were confirmed by their IR, ^{1}H-NMR spectra and elementary analysis. Analgesic and anti-inflammatory activities of title compounds have been evaluated.



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[2] M. Gökçe, M. F. Şahin, E. Küpeli, E. Yeşilada, Arzneimittel Forschung Drug Research, 2004, 54, 396-401.

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P346

Synthesis and Analgesic and Antiinflammatory Activity of 6-(Substituted-phenyl piperazine/piperidine)-4-methyl-3(2H)-pyridazinone Derivatives

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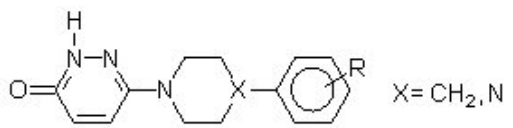
The design and study of new molecules potentially useful in the control of pain and particularly in the management of oncological pain is a very important target today. It is well known that the mechanism of pain transmission is very complex and involves numerous neuromodulators of pain response [1]. The therapeutical approach consists in the use of non steroidal anti-inflammatory drugs (NSADIs) and opiates, both characterized by many side effects.

3(2H)-pyridazinone derivatives are known analgesic and anti-inflammatory activities [2-4]. Previously we have reported that 6-substituted-3(2H)-pyridazinone derivatives can exhibit good analgesic and anti-inflammatory activities and low gastric ulcerogenic activity [5-6]. As the extension of our interest for the search of new heterocyclic moieties as potent anti-inflammatory agents and at the same time they are devoid of side effects like ulcerogenic activity, we have synthesized series of 6-(Substituted-phenyl piperazine/piperidine)-4-methyl-3(2*H*)-pyridazinone derivatives



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The structures of these new pyridazinone derivatives were confirmed by their IR, ^{1}H-NMR spectra and elementary analysis. Analgesic and anti-inflammatory activities of title compounds have been evaluated. The pharmacological study of these compounds confirms that modification of the chemical group at position 6 of 3(2H) pyridazinone system influences analgesic and anti-inflammatory activities. None of the compounds showed gastric ulcerogenic effect compared with reference nonsteroidal anti-inflammatory drugs (NSAIDs).

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[3] B. Ökçelik, S. Ünlü, E. Banoglu, Arch. Pharm.et. al. Pharm. Med. Chem., 336, 406-412 (2003).

[4] F. Rohet, C.. Rubat, P. Coudert, et al.Bioorganic & Medicinal Chemistry, 5(4), 655-659 (1997).

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P347

The Quantitative Determination of Etodolac in Human Plasma by HPLC/UV System

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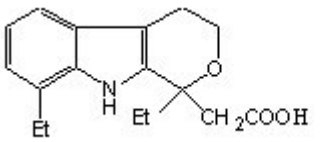
Etodolac is a non steroidal anti-inflammatory drug. The mechanism of action of etodolac is due to blockade of prostaglandin synthesis via inhibition of cyclooxygenase enzyme. Chemically, it is (+)-1,8-Diethyl-1,3,4,9-tetrahydropyrano-[3,4-b] indole-1-acetic acid. With an empirical formula is $C_{17}H_{21}NO_3$.



Etodolac

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

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

Results of analysis showed that the retention times for etodolac and diclofenac (internal standard) were 10.4 and 13.5 minutes respectively. Recovery (absolute) was 99.72%. The calibration range was 0.2-60 mg/ml; with a LOQ 0.2 mg/ml. After five days validation process, coefficient correlation was 0.99718 – 0.99981. In quality control samples, within -batch and batch- to batch accuracy were 88.33 – 109.33% and 95.90 – 107.67%, precision values were 0.66-4.63 and 3.15 – 5.10 respectively. In calibration standard samples, batch to batch accuracy were 92.75 – 112.76%, precision were 1.87 – 7.53%. Our whole study was conducted according to FDA regulations about bioanalytical method validation of FDA,2001[2].

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

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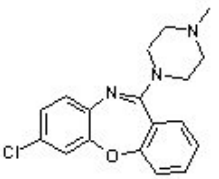
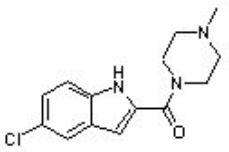
P348

Design and synthesis of non-imidazole antagonists for the histamine H4 receptor

Since the discovery of the human histamine H_4 receptor (H_4R) in 2000, considerable progress has been made in unravelling its exact physiological role and its potential as a drug target. Recently it was shown that blocking the H_4R with selective antagonists was beneficial in various models of chronic inflammatory disease such as inflammatory bowel disease and asthma. We have constructed a pharmacophore model for histamine H_4 receptor ligands that is based on VUF 6884 and JNJ 7777120. This model has been used to design, synthesize and screen a novel series of compounds leading to a new class of potent H_4R antagonists.



VUF 6884 $pK_i = 7,6$



JNJ7777120 $pK_i = 7,7$

P349

Synthesis and Biological Evaluation of 2-[5,6-Diphenyl-3(2H)-pyridazinone-2-yl]acetamide and 3-[5,6-Diphenyl-3(2H)-pyridazinone-2-yl]propanamide Derivatives as Potential Analgesic and Anti-inflammatory Agents

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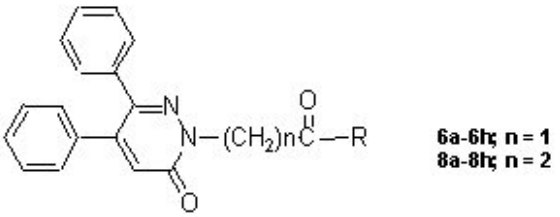
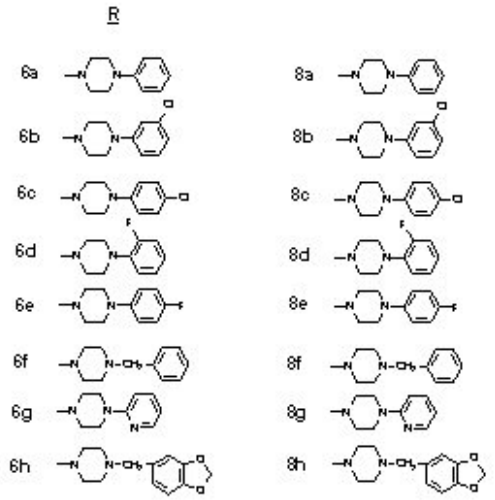
In this study, new 2-[5,6-diphenyl-3(2H)-pyridazinone-2-yl]acetamide and 3-[5,6-diphenyl-3(2H)-pyridazinone-2-yl]propanamide derivatives were synthesized and tested their *in vivo* analgesic and anti-inflammatory activities by using *p*-benzoquinone-induced writhing test and carrageenan-induced hind paw edema model, respectively. Propanamide derivatives were found more potent than acetamide derivatives in terms of anti-inflammatory activity. Inhibitory activity of the active compounds on cyclooxygenase isoforms was also investigated by using *in vitro* human whole blood assay and found that these derivatives did not exert their analgesic and anti-inflammatory activities through COX inhibition and other mechanisms might be involved.





Phosphatases

P350

Discovery of potent and selective inhibitors of fructose-1,6-bisphosphatase with potential as a new class of agents to treat type 2 diabetes

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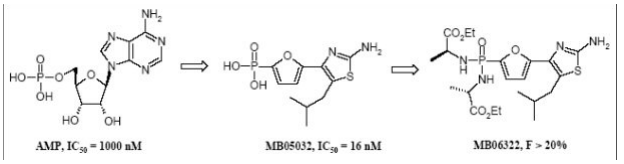
Fructose-1,6-bisphosphatase (FBPase) is a key rate-limiting enzyme of gluconeogenesis (GNG), which has been shown to contribute significantly to the up-regulated hepatic glucose output in type 2 diabetes (T2DM). Consequently, inhibition of FBPase has been explored as a potential approach to control blood glucose levels in T2DM. However, past drug discovery programs targeting the AMP binding site of FBPase have not identified potent and selective FBPase inhibitors as clinical candidates, which can be attributed to the highly hydrophilic nature of the AMP site and the fact that AMP is often used to regulate enzymes and receptors naturally.

Using a structure-guided drug design strategy, we discovered a series of potent and selective FBPase inhibitors that mimic AMP but with little structural resemblance. Moreover, a novel bisamidate prodrug approach was introduced to deliver these phosphonates orally. MB06322 (CS-917) was identified and is in Phase IIB clinical trials as a potential treatment for T2DM. TQhe medicinal chemistry aspects on the design and SAR of our FBPase inhibitor program will be presented.



Process R & D

P351

Asymmetric Synthesis of Clemastine

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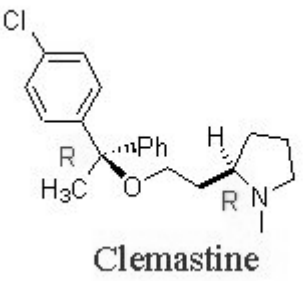
College of Pharmacy, Sookmyung University, Seoul, Korea

In connection with the asymmetric synthesis of chiral 1,2-diol, we report here the novel synthesis of H_1-receptor antagonist (+)-clemastine using diastereoselective alkylation featuring tridentate chelation-controlled asymmetric alkylation of a-hydroxyketone, in which the chiral auxiliary is attached to the hydroxyl group as ether linkage. The starting D-glyceraldehyde acetonide was converted (S)-[(4R)-2,2-dimethyl -1,3-dioxolan-4-yl](4-methoxyphenyl)methanol. Then, the alcohol was successively transformed to (+)-clemastine in 4 steps via *O*-alkylation, deprotection, and deoxygention.



P353

Enantioselective Synthesis of (R)-alfa-Alkylcysteine and (S)-alfa-Alkylcysteine via Phase-Transfer Catalytic Alkylation

T. Kim, Y. Lee, B. Jeong, J. Lee, M. Kim, H. Park, S. Jew

College of Pharmacy, Seoul National University

As one of α,α-dialkyl amino acids, α-alkylcysteines are valuable building blocks for the biologically active peptidomimetics since they not only resist the enzymatic degradation but also form the stabilized preferred conformations of the peptide backbone. Furthermore, they could form further constrained cyclic peptide structure by disulfide bond formation. Besides, there are several natural products involving α-alkylcysteine moiety such as tantazoles, mirabzoles, and thiangazole, which exhibit

antitumor and anti-HIV-1 activities. A number of enantioselective synthetic methods for α-alkylcysteines have been reported so far. Their main synthetic strategy could be classified as follows: 1) thiomethylation of bislactim ether prepared from valine as a chiral auxiliary, 2) neuclephilic ring opening of chiral aziridine or chiral β-lactone with thiolates, 3) self-reproduction of chirality using oxazolidinone or thiazolidinone derivatives, 4) enzymatic desymmeterization of monomethyl dimethylmalonate. But since the most of the reported methods employed chiral starting materials or chiral auxiliary, their applications to the industry process for mass production of chiral α-alkylcysteines might be not easy. In this letter, we report a new and efficient synthetic method of (*R*)-α-alkylcysteines and (*S*)-α-alkylcysteines via phase-transfer catalytic ?-alkylation[1,2] of thiazoline carboxylates, which could be applied to industrial process.

P353

Lithiation of substituted acetophenone ketals

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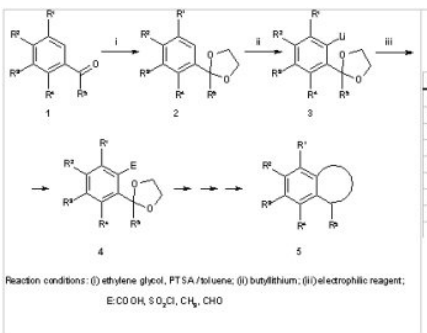
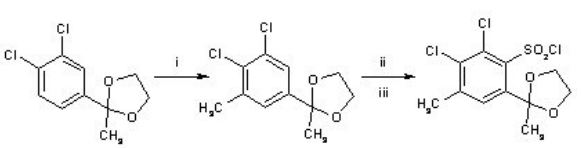
Several 2-aryl-2-alkyl-1,3-dioxolanes **2** were lithiated *ortho* to the ketal group by treatment with butyllithium in THF between –78 °C and 0 °C. The lithio species **3** thus formed were treated with various electrophiles to give *ortho*-functionalized acetophenone derivatives **4** [1], precursors of a series of benzannelated heterocyclic systems **5**. The site selectivities of some deprotonations have been rationalized by the long-range effect of the 4-chloro substituent.



1-5	R[1]	R[2]	R[3]	R[4]	R[5]
a	H	Cl	H	H	Me
b	H	Cl	H	H	Et
c	Cl	H	H	H	Me
d	Cl	Cl	H	H	Me
e	Cl	Cl	H	H	Et
f	Cl	Cl	H	Me	Me
g	Cl	OMe	H	H	Me
h	OMe	H	H	H	Me
i	OMe	OMe	H	H	Me

Modifying the reagent of lithiation, a different stereoselectivity was achieved, thus generating new acetophenone derivatives, intermediates for several new polifunctionalized condensed heterocycles.



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P354

A new chemoselective-oxidizing reagent for oxidation of organic substrates and antiseptics alcohols

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Department of Chemistry, Faculty of Pharmacy, Mazandaran University of Medical Sciences, Sari, Iran

A new chemoselective-oxidizing reagent for oxidation of organic substrates and antiseptics alcoholsAhmad Reza BekhradniaDepartment of Chemistry, Faculty of Pharmacy, Mazandaran University of Medical Sciences, Sari, IranA new and selective chromium (VI) oxidizing reagent, pyridinium-1-sulfonate flourochromate, 1, is synthesized [1]. Its C,H,N; 1H-NMR; FTIR and UV-Vis are reported. Reactions of 1 with primary, secondary, benzylic and allylic alcohols, under very mild conditions give the corresponding aldehydes with relatively short reaction times. The extend of this oxidation is determined via 1H-NMR (Table).In comparison to GC and TLC, 1H-NMR is faster, more convenient and economically sound method. This proves as accurate as GC but more precise than TLC.

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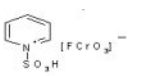

(1)

Entry	Substrates	Products	Yield% (by ^{1}HNMR and GC)
1	Ethyl alcohol	Acetaldehyde	61%
2	Anthracene	9,10-Anthraquinone	53%
3	*p*-methoxybenzyl alcohol	*p*-methoxybenzaldehyde	91%
4	Benzyl alcohol	Benzaldehyde	90%
5	Furfuryl alcohol	Furfural	56%
6	1-pentanol	1-pentanal	69%
7	Isopropyl alcohol	Acetone	61%
8	1-Butanol	1-butanal	58%
9	Ethylene glycol	Glyoxal	52%
10	Isobutanol	2-methylpropanal	68%
11	Allyl alcohol	Acrolein	78%

^{1}HNMR and GC analyzes of oxidation of organic substrates with pyridinium-1- sulfonate flourochromate

P355

Synthesis of homogeneous and heterogeneous divalent carbohydrate systems

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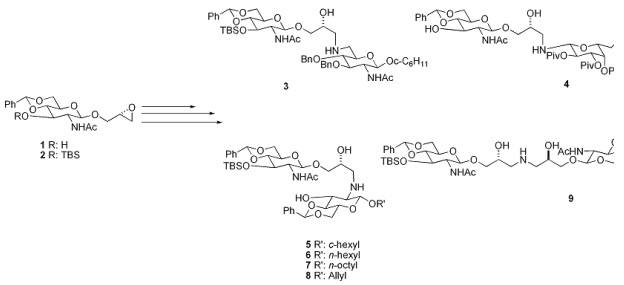
Universidad de Sevilla, Facultad de Farmacia, Dpto Química Orgánica y Farmacéutica, Sevilla, Spain.

The emergence necessity of synthesizing multivalent carbohydrates systems to enhance binding affinity over different drug targets by targeting multiple binding sites

has been widely demonstrated over recent years [[1]]; allowing a better understanding of the key roles that carbohydrates play in vital biological recognition processes and in development of diseases, which remain still poorly understood.

The outstanding utility of multivalent carbohydrates and our strategy developed for accessing a wide range of stereochemical pure glycosyl glycidol derivatives [[2]] *via* stereoselective oxidation of alkenyl glycosides prompted us to the development of an easy and mild methodology for the synthesis of divalent carbohydrate systems by means of the coupling of monosaccharides through a hydrophilic linker (Scheme 1).



Acknowledgment. We thank the Ministerio de Educación y Ciencia (Spain) and the FEDER program for financial support (CTQ2004-1057).

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P356

Synthesis and catalytic hydrogenation of substituted imidazo[1,2-a]pyrazines

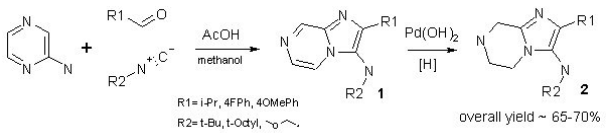
A. Zagorodnikov, M. Kopyshev, V. Kochubey, O. Rodin

ASINEX Ltd , Moscow, Russia,

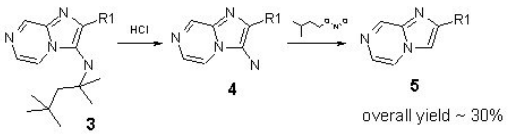
The fragment imidazo[1,2-a]pyrazine is presented in many different bio-active molecules, also could be an elemental part of some natural compounds. For synthesis of imidazo[1,2-a]pyrazines are mainly used reactions of á-halogencarbonyles with Aminopyrazine (Chichibabin's reaction) and α-halogencarbonyles with imidazole-2-carboxamides [1]. But low yield of final compounds is a disadvantage of these methods.

A number of imidazo[1,2-a]pyrazine's derivatives **1** have been synthesized in our laboratory with good yields by reaction Ugi [2]. Then these imidazo[1,2-a]pyrazines have been reduced to imidazo[1,2-a]piperazines by hydrogenation with $Pd(OH)_2$.

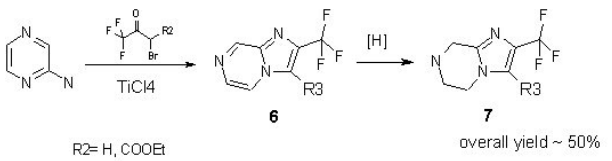
It is to be noted that descriptions of pyrazine's cycle reduction is almost absent in scientific literature.



Treatment of **3** with 5N HCl in methanol resulted in clean dealkylation, giving primary amine **4**, which further reacted with isoamilnitrile to archive compound **5**.



Compound **6** was synthesized by different method; after this, **6** was reduced to target product **7**.



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P357

Kinetic Study of Oxidative-Addition Reactions of some Alkyl Halides on PtMe2(Ph2Phen) complex

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Razi University, Kermanshah, IRAN

In this study the oxidative-addition reactions of reagents,1-Bromobutane (n-BuBr) and 2-Bromo-2-methylbutane (t-BuBr) with [PtMe2(Ph2phen)](?) were studied in acetone and benzene.

Oxidative-Addition reactions of [PtMe2(Ph2phen)] with (n-BuBr) and (t-BuBr)

produced the [PtMe2n-Bu(Ph2phen)Br] (??) and [PtMe2t-Bu(Ph2phen)Br] (???). These complexes were characterized by nuclear magnetic resonance of 1H, 13C-NMR and UV-Vis spectroscopy and elemental analysis.

The kinetic study of these reactions was preceded by UV-Vis spectrosphotometry.

The decrease in MLCT absorbtion band of starting Pt(??) complexes during the conversion to Pt(?V) products were selected as a criterion for the reaction rate. The reactions were monitored at different concentrations of excess amounts of reagents in each temperature.

These reactions were performed in acetone and benzene, the reaction rate was determined and the effect of

solvent and reagent on reaction rate has been discussed. The activation parameters were calculated by Arrhenius and Eyring equations and compared with other data previously reported on analogous systems.

With the aid of the kinetic and other collected results an SN2 mechanism was suggested for oxidative-addition reactions.

It has been concluded that the mechanism is strongly dependent on the solvent and kind of reagent.

P358

Synthesis of D-ring fused P-heterocyclic estrone derivatives

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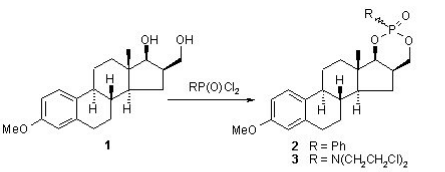
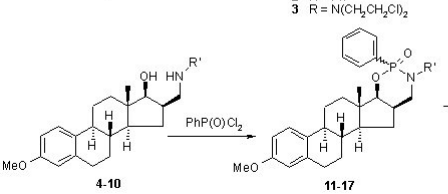
The efficient syntheses of some novel D-ring fused dioxa- and oxazaphosphorinane ring systems were carried out *via* the reactions of estrone precursors with phenylphosphonic dichloride and *bis*(2-chloroethyl) phosphoramide dichloride in the presence of triethylamine.

The reaction of 17b-hydroxy-16b-hydroxymethylestrone 3-methyl ether (**1**) with $PhP(O)Cl_2$ resulted in the corresponding epimeric oxazaphosphorinanes **2a** and **2b** in good yield, in a nearly 1:1 ratio. However, the same transformation with the other reagent furnished a single product (**3**) in low yield. The 1,3-amino-alcohol (**4**) and its *N*- substituted derivatives (**5-10**) were converted to the corresponding oxaza-phosphorinanes (**11-17**) in altering yields, giving mostly diastereomeric pairs. The nature of the substituent at the aromatic ring of **7-10** affected the epimeric ratios of the cyclic products **14-17**.



	R'
4, 11	H
5, 12	*n*-Pr
6, 13	Bn
7, 14	*p*-CH_3-Bn
8, 15	*p*-NO_2-Bn
9, 16	*p*-F-Bn
10, 17	*p*-Cl-Bn



	Epimeric ratio a β(P=O)	b α(P=O)
12	3	2
13	3	2
14	2	1
15	1	2
16	1	2
17	1	2

[1] Frank É, Kazi B, Ludányi K, Keglevich Gy. Tetrahedron Lett 2006;47:1105-1108.

P359

Thermal fragmentation of benzoin arylsulfonylhydrazone derivatives

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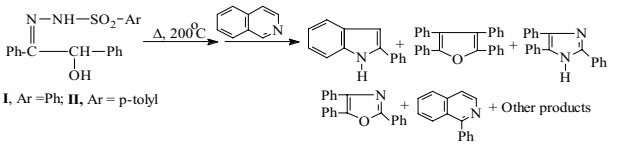
In continuation of synthesis of heterocyclic and organic compounds through thermolysis and photolysis [1,2]. In this paper, thermolysis of benzoin benzene-p-sulfonylhydrazone **I** leads to the formation of benzoic acid, benzophenone, benzil, aniline, diphenyl acetylene, deoxybenzoin, N-benzylbenzamide, diphenyldisulfide, diphenylthiosulfone, S-phenylthiobenzoate, 2-phenylindole, 2,3,4,5-tetraphenylfuran, 2,4,5-triphenylimid- azole, and 2,4,5-triphenyloxazole. Similarly, thermolysis of **I** in the presence of isoquinoline as a radical trap gave 1-phenylisoquioline in addition to the previously mentioned products. The formation of such products which implies the homolysis of different bonds in the benzoin derivatives followed by reaction of the so formed radicals.



[1] M.Z.A. Badr, A. M. Gaber, O.S. Moustafa, J. Anal. Appl. Pyrolysis, 71, 2**004**, 445.

[2] A. M. Gaber, O. S. Mohamed, M. M. Aly, J. Anal. Appl. Pyrolysis, 73, **2005**, 53.

Prodrugs

P360

Synthesis and biological evaluation of SN-38 disulfide prodrug vectorised by the B-subunit of Shiga Toxin

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The ultimate goal of cancer therapy is to develop agents that will selectively destroy cancer cells, sparing the normal tissues of the patient.

A promising approach to overcome sides effects and to achieve a more tumor selective cancer treatment is a targeted prodrug therapy.

Shiga toxin is a bacterial toxin composed of two parts : one pathogenic part (A), and one part that binds to the cell surface (STxB). The receptor for STxB is a globotriaosyl ceramide (Gb3 or CD77) that is overexpressed in different cancer cells and more particulary in intestinal and colorectal tumors.

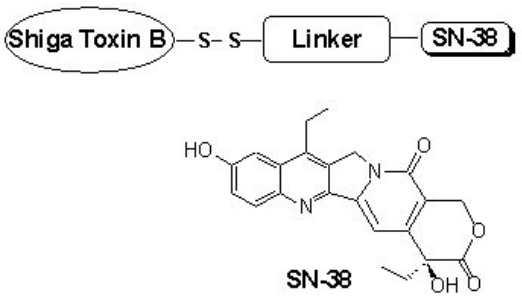
In this work, we used the non-toxic B subunit of Shiga toxin as a cancer cell vector. Mutants of STxB possess-

ing a free sulfhydryl groups can be used to link prodrugs or drugs.

Indeed, the concept is the following : once at the tumor site, the conjugate (produg-STxB) binds to the surface of tumor cells, is internalised and then is cleaved by the intracellular glutathione in order to liberate the drug.

In our study we have chosen the SN-38 as chemotherapeutic agent based on Topoisomerase I inhibition.

Thereby we synthetisized different SN-38 disulfide prodrugs that differentiate by the linker. These linkers are coupled via disulfide bond to STxB to provide the drug-conjugates.



We will present the synthesis of these conjugates and the first biological results obtained which furnish proof of concept of this approach.

P361

Development of an albumin-binding prodrug that is cleaved by urokinase-type-plasminogen activator (uPA)

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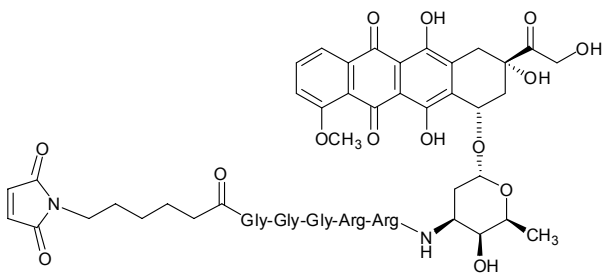
In-situ coupling of prodrugs to circulating albumin is a promising approach for anticancer drug delivery [1,2]. Recently, we set out to develop albumin-binding prodrugs of doxorubicin that are cleaved by urokinase-type plasminogen activator (uPA). uPA is an extracellular serine protease that is especially over-expressed in breast, ovarian and cervical carcinoma and represents a molecular target that hitherto has not been exploited for selectively releasing an anticancer agent from a prodrug formulation. Thus, we synthesized a water-soluble albumin-binding doxorubicin prodrug that incorporated the peptide sequence Gly-Gly-Gly-Arg as the substrate for uPA:



Since direct C-terminal coupling of the protease substrate to the 3´-amino position of doxorubicin impairs cleavage by proteases, we introduced a further amino acid that could act as a spacer and subsequently be cleaved in tumor tissue. Among a spectrum of amino acid derivatives of doxorubicin, we discovered that Arg-DOXO was the only derivative which was further degraded to doxorubicin in tumor homogenates at physiological pH.

The novel prodrug bound rapidly to the cysteine-34 position of albumin and was efficiently cleaved by uPA releasing Arg-DOXO thus representing, to the best of our knowledge, the first prodrug of a cytostatic agent that is cleaved by this tumor-associated protease.

[1] Kratz et al.: Probing the cysteine-34 position of endogenous serum albumin with thiol-binding doxorubicin derivatives: Improved efficacy of an acid-sensitive doxorubicin derivative with specific albumin-binding properties compared to the parent compound, J Med Chem 2002;45:5523-5533.

[2] Mansour et al.: A new approach for the treatment of malignant melanoma: Enhanced antitumor efficacy of an albumin-binding doxorubicin prodrug that is cleaved by matrix metalloproteinase 2, Cancer Res 2003;63:4062-4066.

P362

Enhanced antitumor efficacy of an albumin-binding prodrug of the anticancer agent methotrexate that is cleaved by both cathepsin B and plasmin

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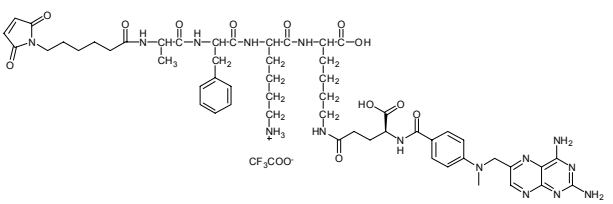
Cathepsin B and plasmin are intra- or extracelullar proteases that are over-expressed by several solid tumors [1, 2]. In order to exploit both proteases as molecular targets for tumorspecific cleavage of prodrugs, we developed an albumin-binding prodrug of methotrexate that incorporated the peptide sequence D-Ala-Phe-Lys as the protease substrate. Albumin is a suitable carrier of cytostatic agents due to passive accumulation in solid tumors. Synthesis was performed by coupling the peptide linker EMC-D-Ala-Phe-Lys(Boc)-Lys-OH (EMC = ε-maleimidocaproic acid) to the γ-COOH group of methotrexate, cleaving the Boc-group with CF_3COOH and isolating the prodrug with reverse phase HPLC:



The albumin-bound form of the prodrug was efficiently cleaved by cathepsin B and plasmin as well as in homogenates of human tumor xenografts liberating a

methotrexate-lysine derivative. In an ovarian carcinoma xenograft model (OVCAR-3), the prodrug at a dose of 4 x 15 mg/kg methotrexate equivalents demonstrated distinctly superior antitumor efficacy compared to free methotrexate at a dose of 4 x 100 mg/kg [T/C(%) for MTX = 69; T/C(%) for MTX prodrug = 29].

[1] Sloane et al. Cathepsin B and tumor proteolysis: contribution of the tumor microenvironment. Seminars in Cancer Biology. 15:149-57, 2005.

[2] Renard et al. The plasminogen activation system in tumor growth, invasion, and metastasis. Cellular & Molecular Life Sciences. 57:25-40, 2000.

P363

Development of albumin-binding camptothecin and doxorubicin prodrugs that are cleaved by cathepsin B

Da-Eun Chung[1], Björn Schmid[1], André Warnecke[1], Iduna Fichtner[2], Felix Kratz[1]

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We have recently validated a macromolecular prodrug strategy for improved cancer chemotherapy based on two features: (a) rapid and selective binding of thiol-reactive prodrugs to the cysteine-34 position of endogenous albumin and (b) acid-sensitive or enzymatic release of the albumin-bound drug at the tumor site [1,2]. In the present work, we developed water-soluble camptothecin (CPT) and doxorubicin (DOXO) prodrugs that incorporated a plasma stable peptide linker and served as a substrate for a tumor-associated protease, cathepsin B, which is overexpressed in several solid tumors. Two albumin-binding prodrugs containing the peptide sequence Ala-Leu-Ala-Leu as the protease substrate were synthesized [EMC-Arg-Arg-Ala-Leu-Ala-Leu-Ala-CPT and EMC-Arg-Arg-Ala-Leu-Ala-Leu-DOXO (EMC = 6-Maleimidocaproic acid)]. Both prodrugs exhibited excellent water-solubility and bound rapidly and selectively to the cysteine-34 position of exogenous and endogenous albumin. Further in vitro studies showed the albumin-bound form of the prodrugs was cleaved by cathepsin B as well as in human tumor homogenates. Major cleavage products were CPT-peptide derivatives and CPT for the CPT-prodrug and DOXO-Leu and DOXO for the doxorubicin prodrug. In vivo studies in human tumor xenografts with the prodrugs compared to the free drug are underway.

[1] Kratz et al.: Probing the cysteine-34 position of endogenous serum albumin with thiol-binding doxorubicin derivatives: Improved efficacy of an acid-sensitive doxorubicin derivative with specific albumin-binding properties compared to the parent compound, J Med Chem 2002;45:5523-5533.

[2] Mansour et al.: A new approach for the treatment of malignant melanoma: Enhanced antitumor efficacy of an albumin-binding doxorubicin prodrug that is cleaved by matrix metalloproteinase 2, Cancer Res 2003;63:4062-4066.

P364

Development of Camptothecin Prodrugs Using a Peptide Positional-Scanning Library

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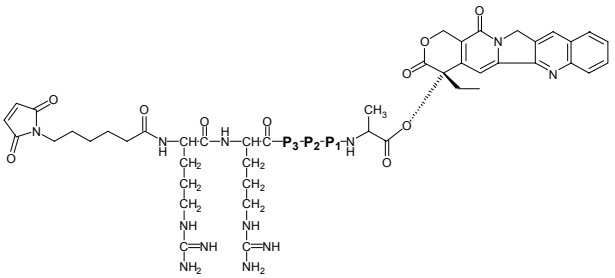
Designing truly tumor-specific prodrugs remains a challenge in the field of cancer chemotherapy. As a new strategy, we incubated homogenates of a spectrum of human colon tumor xenografts with a tetrapeptide positional-scanning library [ACC-P_1-P_2-P_3-P_4 (ACC = 7-aminocoumarin acetic acid)] in order to identify peptide sequences that were preferentially cleaved by colon tumors. Our screening experiments revealed that Met >> Lys, Leu were preferred amino acids in the P_1-position, Met >Tyr > Phe in the P_2-position and Ala, Tyr, Phe in the P_3-position.

Based on these results and our synthetic experience with camptothecin (CPT) prodrugs [1], we developed albumin-binding camptothecin prodrugs of the general formula (figure): that incorporated two plasma stable peptide linkers: Met-Phe-Ala and Met-Tyr-Phe (P_1-P_2-P_3). Albumin is a potential drug carrier for antitumor agents due to passive targeting to solid tumors [2].

Due to the incorporation of two arginine residues, the prodrugs were water-soluble (> 6 mg/mL) and bound rapidly and selectively to the cysteine-34 position of endogenous albumin. Incubation studies with HT29 colon tumor homogenates demonstrated cleavage of the peptide linker with CPT-peptide derivatives and CPT being the major cleavage products. *In vivo* studies in the HT29-xenograft model with both prodrugs are underway.



[1] Warnecke A, Kratz F. Maleimide-Oligo(ethylene glycol) derivatives of camptothecin as albumin-binding prodrugs: Synthesis and antitumor efficacy. Bioconjugate Chemistry 2003;14:377-387.

[2] Kratz F, Beyer U. Serum proteins as drug carriers of anticancer agents, a review. Drug Delivery 1998;5:1-19.

P365

Synthesis of Stabilized Ketomethylene Carrier Tripeptides targeted PepT1 applying the Tandem-Chain-Extension-Aldol Reaction

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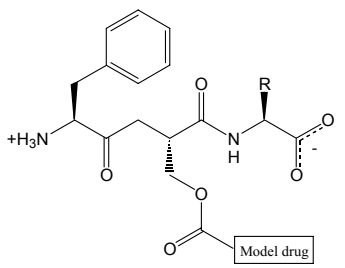
The membrane transport protein PepT1 is an excellent target for drug delivery systems. A strategy for enhancing the oral bioavailability of drug substances using PepT1 as a vehicle is promising due to the high transport capability and the broad substrate affinity of PepT1 [1,2]. Design and synthesis of di- and tripeptides which can transport attached drug molecules across the intestinal membrane to the blood stream may raise biologically active substances to potential drugs. Here serine, containing a hydroxy group, has been incorporated into the peptides to link the drug molecule with a hydrolysable ester linkage.

To avoid enzymatic and hydrolytic degradation of the carrier peptide in the intestinal lumen, peptidomimetic alterations as amide bond bioisosteres and unnatural amino acids have been applied. A synthetic approach for ketomethylene tripeptides has been developed using the tandem-chain-extension-aldol reaction [3]. Protected amino acid derived β-keto imides are stereoselectively converted to the γ-keto imides with incorporation of an α-side chain mimicking the natural peptides.



H-Phe-ø[COCH2]-Ser(Model drug)-UAA-OH

The synthesis and pharmacological data of a series of stabilized ketomethylene tripeptides containing various unnatural amino acids in the C-terminal will be presented.

[1] Steffansen B. et al, *Eur. J. Pharm. Sci.* **2004**, *21*, 3-16.

[2] Nielsen C.U. et al, *Exp. Opin. Ther. Patents* **2005**, *15*, 153-166.

[3] Lai S. et al, *Org. Lett.* **2001**, 3, 4169-4171.

P366

Dendrimers as prodrugs

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Highly branched, globular, and mono disperse macromolecules are promising platforms for drug delivery. Due to their properties, multifunctional dendrimers have a high potential for use in drug delivery systems as they accommodate a wide variety of functional groups on their surface. These surface groups can be prodrug molecules as well as targeting groups. Such a dual design will allow the plasma level of the drug to stay at the desired level for a longer duration, and thus increase its pharmaceutical efficiency. Direct application of drug molecules to the diseased tissue or organ increases the effect of the therapy and decreases the side effects.[1]

For evaluation of dendrimers as drug and targeting group carriers, the development of efficient methods for block dendrimer synthesis is necessary. Two widely used approaches for synthesis of dendrimers are divergent and convergent methods.[2] The convergent methodology involves coupling of dendron units to afford dendrimers and is prefered since it provides pure and defect-free dendrimers. Synthesis of 'segment block' dendrimers was realized in high yield and purity utilizing a variety of cycloaddition reactions between two different dendrons. Application of these coupling methodologies in the presence of targeting groups and effect of generation and constitution of branching units of the dendrons on the cycloaddition was investigated.

[1] Torchilin, V.P. *Eur. J. Pharm. Sci*. (Suppl. 2), 2000, *11,* S81–S91.

[2] J.Frechet *et al*., *Chem.Rev*., 2001, 101, 3819.

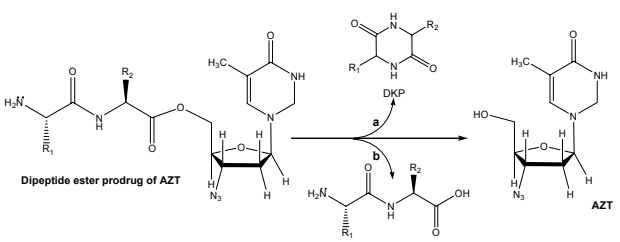
P367

Dipeptide ester prodrugs of AZT: Synthesis and *in vitro* stability

Cledir R. Santos[1], Rui Moreira[2] and Paula Gomes[1]

[1]CIQUP, Faculdade de Ciências, Universidade do Porto, Portugal. [2]CECF, Faculdade de Farmácia, Universidade de Lisboa, Portugal.

Reduced oral bio-availability is a major limiting factor for the efficacy of many drugs. This can be obviated by the development of prodrugs, which can delivery the parent drug through chemical or enzymatic pathways *in vivo*. Ester prodrugs of hydroxyl compounds have been described [1,2] and dipeptides are attractive acyl donors in these esters as they can release the drug by intramolecular cyclization to a 2,5-diketopiperazine (Scheme 1, path a). In comparison to ester bond hydrolysis (Scheme 1, path b) [1], which is mediated by esterases, the chemical cyclization pathway is not subject to biological diversity that is reflected on enzyme levels among different individuals or species.



Scheme 1

Thus, we have developed dipeptide ester derivatives of AZT (Scheme 1) as a possible approach to suppress the bioavailability problems associated to this drug [2]. We have synthesized eight AZT dipeptide esters, which have already been tested for their interactions with the intestinal oligopeptide transporter hPEPT1 [3]. The *in vitro* stability of these esters in buffer, at physiological pH and T, has been assayed in order to evaluate relationships between amino acid side chains and drug release rates, as well as to establish whether intramolecular cyclization or direct hydrolysis of the ester bond has the prominent role as the AZT release pathway [1].

[1] Larsen, S.W.; Andersen, M. and Larsen, C.; Kinetics of degradation and oil solubility of ester prodrugs of a model dipeptide (Gly-Phe). *J. Pharm. Sci.* **2004***, 22, 399-408.*

[2] Santos, C.; Mateus, M. L.; Santos, A. P.; Moreira, R., Oliveira, E. and Gomes, P. Cyclization-activated prodrugs: synthesis, reactivity and toxicity of dipeptide esters of paracetamol. *Bioorg. Med. Chem. Lett.* **1990**, *33*, 1505-1510.

[3] Santos C., Moreira R., Steffansen B., Nielsen B.U., Gomes P. Synthesis of dipeptide esters of AZT and their interaction with the hPEPT1 peptide transporter. *Sci. Pharm*. **2005**, 73, S134.

* We thank FCT (Portugal) for financial support to CECF (RM), CIQUP (PG) and through grant SFRH/BD/9272/2002 (CS). We also thank LAFEPE (PE, Brazil) and FARMANGUINHOS (RJ, Brazil) for their kind gift of AZT.

P368

The Synthesis of 3-Substituted-5-(2-Benzimidazoly)-Methyl-Tetrahidro-2H-1,3,5-Thiadiazine-2-Thione Derivates

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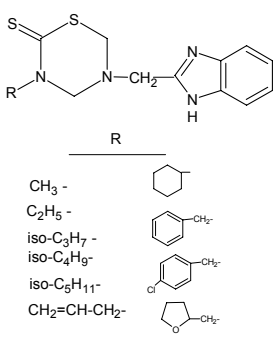
[1]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Gazi University, Etiler, Ankara, Turkey. [2]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Hacettepe University, Sıhhıye, Ankara, Turkey

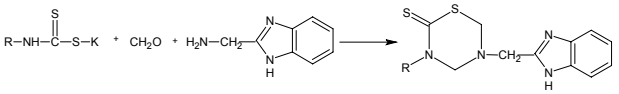
It is known that; substituted tetrahydro-1,3,5-thiadiazine2-thione derivates have several biological activities such as antibacterial[1-2], antifungal[3-4], antiviral[5], antihelmentic[6] and antifibrinolytic[7] activity. In this study, ten new derivates were synthesised. Their structures have been elucidated by spectral methods.

Structures of synthesized compounds are given below:



The aim of this study was to prepare some new compounds to be used as prodrugs, in which amino grup of 2-aminomethylbenzimidazole is a member of tetrahydro-2H-1,3,5-thiadiazine-2-thione ring.

3-substituted-5-(2-benzimidazoly)methyl-tetrahydro-2H-1,3,5-thiadiazine derivates were synthesised by the reaction of dithiocarbamic acid salts prepared from primary amines, with formaldehyde and 2-aminomethylbenzimidazole.



Scheme 1 ; The process of the synthesis.

The reactions were carried out in pH 7.8 phosphate buffer solution to prevent the hydrolysis of the tetrahydro-2H-1,3,5-thiadiazine ring, and the mixture was extracted with the ether to remove isothiocyanate which is formed as a side product.

The chemical structures of the compounds have been elucidated by IR, ^{1}H-NMR and Mass spectral data and elemental analysis.

The antimicrobial activity of these compounds will be tested by further studies and results will be given later on.

[1] Ertan M, Saraç S, Yulug N. Synthesis and Antimicrobial Activities of Some New Tetrahydro-2H-1,3,5-thiadiazine-2-thione Derivates of Amoxicillin. Arzneim.-Forsch/Drug Res.1990; 40(II):790-5.

[2] Balkan A, Ertan M, Saraç S, Yulug N. Synthesis and Antimicrobial Activities of Some New Tetrahydro-2H-1,3,5-thiadiazine-2-thione Derivates of Cephalexin. Arzneim.-Forsch/Drug Res.1990; 40(II):1246-9.

[3] Ertan M, Bilgin A, Palaska E, Yulug N. Synthesis and Antifungal Activities of Some 3-(2-Phenylethyl)-5-substituted-tetrahydro-2H-1,3,5-thiadiazine-2-thiones. Arzneim.-Forsch/Drug Res.1992; 42(I):160-3.

[4] Ertan M, Yıldız HG, Yulug N. Synthesis and Antifungal Activities of Some New Tetrahydro-2H-1,3,5-thiadiazine-2-thiones. Arzneim.-Forsch/Drug Res.1991; 41(II):1182-5.

[5] Rada B, Hanusovska T, Drobnıca, L, Memec P. Inhibition of Virus Multiplication by Selected Isothiocynates. Acta Virol.1971; 15):329-2.

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[7] Würbach G, Klöcking HP. 2-Thiontetrahydro-1,3,5-thiadiazine mit Antifibrinolytishen Eigenschaften,. Pharmazie 1969; 28: 254-7.

Protein Conformational Plasticity

P369

Extracting Essential Motions from Molecular Dynamics Simulations. An Effective Tool to Incorporate Protein Conformational Plasticity in Drug Design.

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The activity of biologically relevant proteins such as enzymes and receptors is very often associated to structural changes, which can vary from subtle to extensive ones, in their tertiary structures. The conformational changes are initiated by ligand binding and are essential to the protein's own functions, such as signal transduction, processing of the substrate, recruitment of other proteins. Thus, the ability to simulate dynamical changes taking place in proteins upon ligand binding is becoming a central issue in the design of bioactive compounds. This task has been facilitated, over the last decade, by the increase in computational power, and today molecular simulation in explicit solvent or membrane environment can be produced approaching the limit of hundreds of nanoseconds. Mathematical approaches able to cope with the huge amounts of data produced by long-time scale molecular dynamics simulations are particularly sought in order to extract the physically and biologically relevant information from complex trajectories. Application of Principal Component Analysis (PCA) to the covariance matrix generated from a MD trajectories is usually referred to as 'essential dynamics' and has been proven to be an effective method to extract the relevant (essential) positional fluctuation from the wide space of physically constrained Gaussian motions. In this work, we apply the essential dynamics technique to explore the conformational changes which take place in the Ligand Binding Domain of the nuclear receptor FXR in response to binding of agonists, partial agonists and antagonists. Thus, MD simulations were carried out for different ligand-bound FXR systems and the obtained trajectories analyzed by essential dynamics. The obtained results demonstrated that the ability of monitoring the differential conformational changes in proteins in response to functionally diverse ligands is a key issue for the understanding of the protein's function and has a great potential in drug design.

Tropical Diseases

P370

Piperidones with activity against plasmodium falciparum

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The increasing resistance of the malaria parasites has enforced new strategies of finding new drug targets. We have isolated two genes involved in spermidine metabolism, encoding deoxyhypusine synthase (*dhs)* and eukaryotic initiation factor 5A *(eIF-5A*) in the malaria parasites *P. falciparum* and *P. vivax.* The 162 amino acid sequence of eIF-5A from *Plasmodium vivax* was functionally analyzed. EIF-5A is activated by the formation of the unusual amino acid hypusine. This process occurs in two steps. DHS transfers an aminobutyl moiety from the triamine spermidine to a specific lysine residue in the eIF-5A precursor protein to form deoxyhypusine. In a second step deoxyhypusine hydroxylase (DHH), completes hypusine biosynthesis.

We used DHH inhibitors which have shown to be effective in mammalian cells to study an antiplasmodial effect in *P. falciparum.* Experiments with the antifungal drug ciclopiroxolamine, an α-hydroxypyridone and the plant amino acid L-mimosine, a 4-pyridone resulted in an antiplasmodial effect *in vitro.* Using mimosine as a new lead structure alkyl piperidone carboxylates were found to have the most efficient antiplasmodial effects *in vitro* and *in vivo*.

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Piperidones with Activity against Plasmodium falciparum: Inhibition of the Hypusine biosynthesis

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We have recently isolated and characterized the 162 amino acid sequence of eukaryotic initiation factor 5A (eIF-5A) from *Plasmodium vivax* [1], being the causative agent of tertiary malaria. EIF-5A is unusually activated by the formation of the unsual amino acid hypusine. This process occurs in a two step mechanism. Deoxyhypusine synthase transfers an aminobutyl moiety from the triamine spermidine to a specific lysine residue in the eIF-5A precursor protein to form deoxyhypusine. In a second step deoxyhypusine hydroxylase (DHH), completes hypusine biosynthesis through hydroxylation.

Hypusine containing mature eIF-5A formation was prevented by inhibition of DHH in *in vitro* cultures of *P. falciparum* and *in vivo* in a rodent malaria model. In a first set of experiments the antifungal drug ciclopiroxolamine, an α-hydroxypyridone and the plant amino acid mimosine, a 4-pyridone resulted in an antiplasmodial effect *in*

vitro with IC_{50} values of 8 µM and 12,4 µM after 24 hours, respectively. However, ciclopiroxolamine was ineffective *in vivo* and mimosine turned out to be toxic. Both drugs served as a lead structure for the development of 4-piperidone esters as active compounds. Among the differently saturated "pyridines" tested the dipyridine substituted 4-piperidone mono- and diesters [2] were found to have the most efficient antiplasmodial effects *in vitro* and *in vivo*. Structure-activity relationships within a library of structural divergent piperidone derivatives will be discussed with regard to activity against malaria, trypanosomes and other microbes.

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P372

Synthesis and biological evaluation of new 3-arylquinoxaline-2-carbonitrile 1,4-di-N-oxides derivatives as antimalarial agents

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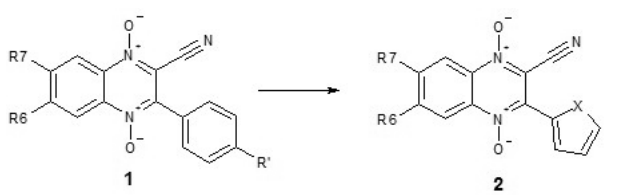
Malaria remains a leading global health problem, especially in tropical and subtropical areas, where every year there are about 500 million clinical cases and more than a million deaths. The spread of drug-resistant parasites, coupled with the absence of an effective vaccine, makes malaria treatment more complicated, and thus, the development of new antimalarial drugs is one of the most urgent tasks in malaria research.

The quinoxaline derivatives show very interesting biological properties (antibacterial, anticancer, antiviral, antifungal, antihelmintic, etc.) [1]. We have recently synthesized new series of 3-arylquinoxaline-2-carbonitrile 1,4-di-N-oxides derivatives **1-2**, in an attempt to optimize the antimalarial activity of other 3-phenylquinoxaline-2-carbonitrile 1,4-di-N-oxides derivatives **1** recently published [2].



In vitro activity against *P. falciparum* was evaluated by a micromethod using the lactate dehydrogenase (LDH) enzyme of *P. falciparum*.

Acknowledgements: We wish to thank the Ministerio de Educación y Ciencia (Project SAF2002-00073 and Grant AP2003-2175 to Esther Vicente) and the Institut de Recherche pour le Développement (IRD) for their financial contribution to this research.

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P373

Synthesis and study of the Fe(II)-induced reduction of labelled antiplasmodial endoperoxides belonging to the G-factor series.

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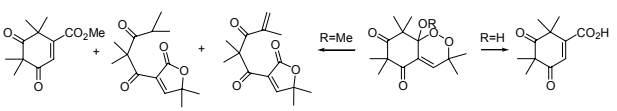
The behaviour of G3 factor and of its methylated analogue G3Me, was studied under Fe(II) conditions. Degradation products were isolated and characterized in each case. The use of labelled compounds allowed us to propose mechanisms in which a tertiary radical is involved. This radical rearranges by *5-exo*-trig cyclisation, or disproportionates in the case of G3Me. A correlation between antiplasmodial activity and stability of this radical is proposed.



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P374

Synthesis and evaluation of transition-state inhibitors of IAG-nucleoside hydrolase

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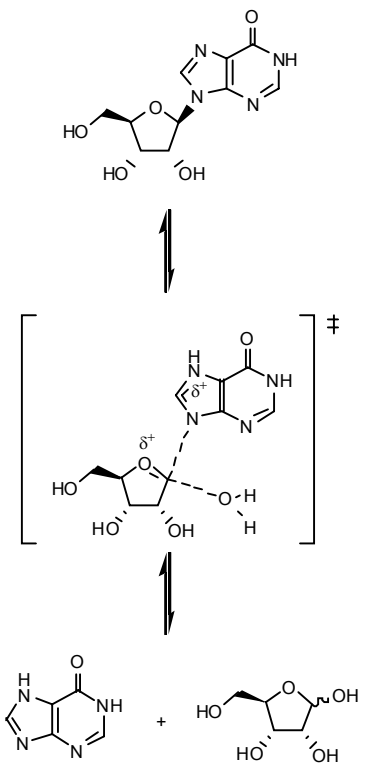
Parasitic infections are a continuing health problem, especially in developing countries. Trypanosomiasis is caused by a unicellular protozoon of the genus *Trypanosoma*. There is an urgent need for new drugs to treat trypanosomiasis; the drugs that are now being used show high host toxicity and there is a growing problem of resistance.

One approach to the development of new anti-trypanosomal compounds is based on the *purine salvage pathway*. Parasitic protozoa lack the ability to synthesise purines *de novo*. Therefore they use this salvage pathway to obtain purine bases from nucleosides of the host. A key enzyme in the purine salvage pathway is *nucleoside hydrolase* (NH). This enzyme hydrolyses nucleosides to ribose and the base, which the parasite can use to synthesise its own nucleosides. Inhibition of NH, which is absent in humans, could be an effective way to kill the parasite.[1]

NHs can be divided into subclasses based on their substrate specificity. Our target enzyme is IAG-NH (inosine-adenosine-guanosine specific NH). A known inhibitor for IAG-NH is 3-deaza-adenosine. Investigation into the binding of this inhibitor in the active site of the enzyme showed π-stacking interactions between the purine-ring of the inhibitor, and two Trp-residues in the active site.[1] These interactions are very important for the affinity of the inhibitor towards the enzyme.



The cleavage of a nucleoside by NH proceeds via an oxocarbenium ion-like transition state.[2] In this transition state, there is a partial positive charge formed on the purine-leaving group and on the ring-oxygen of the ribose. Derivatives that mimick these partial positive charges could act as transition-state inhibitors of NH.

One group of target compounds we developed have a guanidine-group attached on a ribitol or iminoribitol-moiety: molecular modeling studies showed that a guanidine-group can mimick a purine-ring, structurally and electronically. The target compounds were tested on isolated *T. vivax* IAG-NH.

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P375

Synthesis and Evaluation of Triazole-Alkyl-Ribitol Inhibitors of IAG-Nucleoside Hydrolase

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Trypanosomiasis is a parasitic infection that is causing a continuing health problem, especially in developing countries. A unicellular protozoon of the genus *Trypanosoma* gives rise to trypanosomiasis infections. Continually there is a need for new drugs to treat trypanosomiasis with current drugs showing high host toxicity and having a growing problem of resistance.

Nucleoside hydrolase (NH) is an enzyme that is considered as a potential target for the treatment of trypanosomiasis. Parasitic protozoa are unable to synthesise purines *de novo* and are reliant on the purine salvage pathway to provide purinebases obtained from the nucleosides present in the host. NH is an essential enzyme in the purine salvage pathway, hydrolytically cleaving the *N*-glycosidic bond of nucleosides sequested from the host to provide the purinebases which are necessary for the survival of the parasite. NH is absent in humans therefore inhibition of NH has the potential to kill the parasite [1].

Based on its substrate specificity, the NH enzyme we are investigating is known as IAG-NH (inosine-adenosine-guanosine specific NH). From a study of inhibitor binding, using the inhibitor 3-deaza-adenosine, it was discovered that within the active site of the enzyme exist ð-stacking interactions between the purine-ring of the inhibitor, and two Trp-residues in the active site[1]. These interactions are considered as being important for the affinity of the inhibitor towards the enzyme.

We decided to exploit these binding interactions in the development of a novel series of inhibitors. To this

end we synthesised a range of triazole-alkyl-ribitol derivatives which are low micromolar active inhibitors on isolated *T. vivax* IAG-NH. A key step in the construction of the inhibitors was the 1,3-dipolar cycloaddition reaction, which allowed us easy access to a diverse range of inhibitors from simple starting materials.

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Virtual Screening And Data Mining

P376

Concept of Focused Diversity: Application to Development of Specific and Dual Inhibitors of VEGFR-2 with in vivo Activity

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ChemDiv, Inc.

Goal: to develop a robust algorithm for identification of novel specific and dual inhibitors of VEGFR2, the key protein modulating angiogenesis.

In order to achieve our goal, we selected a subset of compounds (Focused Diversity: 3,000 cmpds/275 templates) pre-screened against a number of diverse biological targets. Specifically, this set contained molecules with confirmed *in vitro*/cell-based activities against: i) "traditional" (kinases, GPCR's, ion channels) and ii) "unconventional" targets (Hh/Wnt signaling pathways). Initial assays yielded 11 novel chemotypes, all showing good *in vitro* and cell-based activities (< 1 uM) against VEGFR1/2. MedChem follow-up furnished actives with cell-based potencies (phosphorylation, functional assays) of 25-100 nM. Compounds displayed favorable *in vitro* PK: good absorption (Caco2), hhep stability, water/buffer solubility (> 5 mM) and plasma binding (< 75%). Lead candidates displayed promising oral exposure in murine models (*ca.* 15-20 uM/4 hrs; acute dose of 10 mg/kg, $t_{1/2}$ = 6-9 hrs), MTD (acute/chronic PO's > 250 mg/kg) and efficacy in tumor xenografts to warrant further extended *in vivo* studies.

Conclusion: focused diversity concept has been validated in the identification of novel efficacious inhibitors of VEGFR2s.

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Virtual Screening of Serotonin Antagonist/Serotonin Reuptake Inhibitors (SARI) as Potential Antidepressants

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Serotonin (5-HT), a major neurotransmitter found in the central nervous system (CNS), is also present in many peripheral tissues. Its numerous biological functions are mediated by variety of serotonin receptors. The interaction with these different serotonin receptors constitutes the mechanism of action of many drugs. In particular, type 2 serotonin receptors (5-HT2) mediate the actions of several drugs used in treating diseases such as schizophrenia, feeding disorders, perception, depression, migraines, hypertension, anxiety, hallucinations and gastrointestinal dysfunctions.

Design, synthesis and biological evaluation were performed for developing antagonists active on 5-HT2a with high activity on serotonin transporter at the same time. A dual serotonin antagonist/ serotonin reuptake inhibitor (SARI) is attractive strategy to improve pharmaceutical profile of selective serotonin reuptake inhibitor (SSRI). High-throughput screening to get active 5-HT2A antagonist discovered 13 active hits in the range of 4.5~80nM. Pharmacophore model with most active 6 hits was built and compared with the pharmacophore models for known selective 5-HT2A antagonists, SSRI, and SARI. It suggested that the HTS hits could be a dual serotonin antagonist/ serotonin reuptake inhibitor (SARI). Ligand-based virtual screening was performed for searching SARI and identified the preliminary hits containing large number of false positives. The introduction of 2D structure-based classification resulted in highly active SARI (1.8~80nM for each receptor) with reasonable hit rate. Regardless lack of GPCR structure, our combination of experimental and computational approaches showed successful outcomes. The structural modifications of virtual hits are in progress.

P378

Development of Molecular Fingerprints Derived from Electron Density Critical Points

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The purpose of our work is to build a representation of pharmacological compounds based on the critical points of the Electron Density (ED). It has been showed, for ED maps calculated at a resolution around 2.6 Å on the basis of crystallographic parameters, that more than 91% of the peaks of the ED can be associated to one and only one chemical function [1].

Six peak parameters (ED value and 3 curvatures at peak position, volume, ellipticity) have been computed for 180,000 functional groups extracted from 63,000 organic

compounds taken from the Cambridge Structural Database and belonging to 75 *a priori* different types of functions.

The 6-dimensions problem has then been reduced to a 3D one by Principal Component Analysis (PCA) containing 96.66% of variability. In the PCA space, most of the functions of the same type are regrouped, proving the consistency of the information contained in the critical points.

The most recent part of the work consisted in the development of fingerprints based on the data previously generated. We redefined a set of structural features to be assembled in bits constituting the fingerprint. Starting from the initial 75 functions, similar ones were regrouped and dissimilar ones split, if the ED data defined merging or separations between functions. The position of each cloud of datapoint corresponding to each of the 75 predefined functional groups were reduced to a centroïd and the similarity measure between two functional groups was the Euclidean distance in the 3D PCA space. A Hierarchical Clustering permitted to define how the functions regroup at different value of similarity.

The application of our fingerprints for predicting CYP2D6 and CYP1A2 inhibition is in progress.

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P379

Natural System of Organic Compounds. Possibilities of applications to Drug Design Problems

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Many physical and biological properties of substances are governed by molecular interactions. For these interactions the double complementarity principle is keystone. It extend over (1) shape of molecule features and (2) internal orbital structure of it. [1]. The first three cardinal features of molecule shape are: (1) *N*, the “cyclic order”, *N* = 0, 1, 2,… for acyclic, mono-, bicyclic compounds (C) asf., (2) *n*, number of tertiary atoms which cause the 3D molecule shape and (3) *c*, number of atoms in cycles [1]. The cardinal features of internal structure of C are: *L*, the total number of nonhydrogenous atoms, and numbers σ, π, *h*, i.e. numbers of occupied σ-MO, π-MO and MO of heteroatoms nonshared electron pairs, respectively. The successive ordering of C as vectors in linear spaces displays their natural systematics [2]. The first step of elucidation of “taxonomy” of C is the determination of their position in linear space with linear form $n = 2L + m$, $m = 0,\pm1, \pm2$, The aggregate of C with given *N*, *n* is ordered it it’s turn in linear subspace (σ-set) with linear form $\sigma = 2I + m$, $m = 0, \pm1, \pm2,\ldots$ [2]. In case of necessity of more fine characteristic numbers of shape and MO-structure may be used *c*, *h* asf. [3]. But it is allow by the first two steps to determine a cardinal physical properties of C and their possible affinity to different receptors (targets). Every class of given receptor agonists has appropriate limits *N*, *n*. Thus, for example, GABA-ergics have *N* = 0, *n* = 1, 2, 3; *N* = 1, *n* = 2, 3, 4 and for *N* = 2, *n* = 3, 4, 5. In appropriate σ-sets the C with affinity to given target occupy the homological triangle sectors of affinity [2,3]. Position of C with cross-affinities to two or more targets occupies fields of intersection of relevant affinity sectors. Naturally ordered positions of biologically active substances in proposed version of regular arrangement of C may be useful for many drug design problems. The examples of the search of C with desirable properties and predictions of their biological activity will be discussed.

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P380

Identification of CCR5 non-peptide agonists using structure-based virtual screening

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A three-dimensional model of the chemokine receptor CCR5 has been built to fulfill structural peculiarities of its α-helix bundle and to distinguish known CCR5 antagonists from randomly-chosen drug-like decoys. In silico screening of a library of 1.6 million commercially-available compounds against the CCR5 model by sequential filters (drug-likeness, 2-D pharmacophore, 3-D docking, scaffold clustering) yielded a hit list of 77 compounds, out of which 10 exhibited a detectable binding affinity to the CCR5 receptor. Unexpectedly, all binders tested in a functional assay were shown to be agonists of the CCR5 receptor. Moreover, one of these non-peptide agonists of CCR5 was shown to promote efficient receptor internalization, which is a process therapeutically favorable for protection against HIV-1 infection.

Keywords: HIV, CCR5, GPCR, agonist, docking, virtual screening

P381

The quest for nicotinic receptor ligands by using AChBP as a template

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In our quest for selective ligands for nicotinic receptors (nAChRs), we are using structural information from the homologous acetylcholine binding protein (AChBP)[1]. This homolog has very similar pharmacology as the nAChRs, especially the α7-receptor subtype. We are able to obtain high-quality structural information of AChBPs and co-crystallized ligands using X-ray analysis[2]. The obtained insight in the ligand-binding interactions is used to develop an efficient *in silico* screening protocol which has been applied to our proprietary compound library. The best ranked hits were screened for affinity on AChBP and several structurally new ligands were identified with an affinity similar to or better than acetylcholine. Preliminary results indicate that the ligands are selective for human α7 receptor subtype. Elucidation of the molecular features that induce the subtle differences in selectivity will help structure-based drug design approaches.

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P382

Advanced structural searching using ChemAxon tools

S. Csepregi, S. Dorant, N. Mate, M. Vargyas, P. Kovacs, G. Pirok, F. Csizmadia

ChemAxon

Structural searching techniques are invaluable tools in all cheminformatics systems including but not limited to rational drug design, compound registration systems and laboratory information management systems.

JChem, one of ChemAxon's major suites of programs, provides a very rich set of features related to structural searches. These features are demonstrated by examples. Covered topics are: substructure, exact, superstructure, MCS (maximum common substructure) and similarity searching.

Reaction and R-group searches (including R-logic) are also available, which are complemented by a rich set of query features. SMARTS and query features of the MDL formats are supported. An example of a fast MCS-based clustering is also presented. Finally the recently developed descriptive Chemical Terms Language is demonstrated by powerful structural searches.

P383

Maximum Common Substructure in Molecular Similarity and in Focused Set Profiling

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ChemAxon Ltd. Budapest.

Fingerprints are feasible and efficient tools to solve various problems emerging in computer aided drug design, for instance substructure searching, similarity calculations and similarity based clustering. The success of fingerprint techniques is rooted in their descriptive power, their fast generation, compact storage and simple use.

However, the continuous growth of computational power allows us to use molecular descriptors which are much harder to compute than fingerprints. The maximum common substructure (MCS) provides an example of such descriptor: it requires an exponential computational time to find the MCS of the chemical structures, in the worst case. Yet, it is feasible to compute the MCS of a medium sized set of compounds in a hierarchical manner. Non-hierarchical comparison of millions of compounds against a query structure is also viable.

In this work the use of topological fingerprint in comparing the use of maximum common substructure for various chemo-informatics problems is investigated.

Topic areas include virtual screening, focused set profiling, clustering, total diversity. Results obtained are compared using standard indicators like enrichment ratio, homogeneity and separation. If figures demonstrate that the efficiency of MCS is comparable to the efficiency of fingerprints then drug researchers are provided a novel method applicable in a wide range of chemo-informatics problems.

P384

Drug-membrane interactions from atomistic computer simulations

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To reach their biological targets, drugs have to cross cell membranes. Understanding passive membrane permeation is therefore a critical component of rational drug design. All-atom molecular dynamics simulations offer a powerful way of studying permeation at the atomic level.

They reveal that drugs tend to lie parallel to the membrane normal and, when moving from water solution into biomembranes, permeants lose degrees of freedom. This explains the experimental observation that permeation is highly affected by entropic effects and is size-dependent. Tilted orientations can however occur, when the formation of hydrogen bonds within the mebrane is possible. This helps to understand why hydrogen bonds are often an important parameter in predicting drug absorption. Free energy profiles and diffusion coefficients along the membrane normal can be calculated from simulations. These data allow for the calculation of permeability coefficients, the results for which can be compared with experimental data.

A disadvantage of such simulations is that they are extremely CPU intensive. To address this issue, a coarse-grained biomembrane system has also been constructed by subsuming entire clusters of atoms into rigid bodies of tunable shape. The reduced number of interaction sites results in a much lower computational cost with respect to standard atomistic modelling, and the use of anisotropic potentials still allows the underlying atomistic structure to be captured. With the Gay-Berne potential, each lipid molecule is modelled using ellipsoids with charges or dipoles on their headgroups. Water is modelled explicitly using the single-site Soft Sticky Dipole potential. To simulate the system, a molecular dynamics software has been specifically developed. Our coarse-grained membrane is a hybrid model which is compatible and can be readily mixed with the available atomistic force fields for permeating drug molecules.

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Molecular Similarity values as input vectors for QSAR studies on ABCB1-inhibitors

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The ABC (ATP binding cassette) family of polyspecific membrane transport proteins includes the best-known modulators of the activity of anticancer drugs: ABCB1 (P-gp, MDR1), ABCC1 (MRP1) and ABCG2 (BCRP, MXR). Additionally, ABCB1 is responsible for bad absorption properties of drugs in the gastrointestine as well as improper permeation of the blood-brain barrier. Although considerable efforts have been undertaken to establish *in silico* tools for predicting drug-protein interactions of such multispecific targets, especially in the field of ABC pumps general applicable models are still rare. We recently showed that similarity-based descriptors (SIBAR), developed by our group, are a versatile tool for prediction of ABCB1 inhibitory activity [1]. Our approach is based on calculation of Euclidean distances between a reference compound set and a training set in a n-dimensional descriptor space. These similarity values (SIBAR descriptors) are then used as independent variables for QSAR analyses. Subsequently, 3D shape similarity values based on molecular interaction fields were calculated using the MIMIC software package [2] and further used for QSAR studies. Based on two different reference sets, each providing maximal structural diversity, we performed caculations on our *in house* ABCB1 inhibitor database (292 compounds). For the whole database we obtained moderate q^2-values of 0.51 and 0.61, respectively. Consequently, we diveded the database into subsets according to their chemical structure. Predictive power strongly varied according to their structure and to the reference set used, with benzopyranes (q^2 = 0.84 and 0.86) showing best predictive capacity: q^2 = 0.84 and 0.86. Pyrazolones led to a q^2 of 0.54 and 0.71 and propafenones showed lowest predictivity of q^2 = 0.36 and 0.45.

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Additional Abstracts

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

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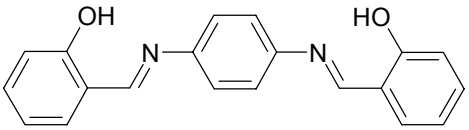
Silent Information Regulator 2 (SIR2) protein is a nicotinamide adenine dinucleotide dependent protein and it belongs to the histone deacetylase class III protein family. SIR2 is widely distributed in organisms [1]. There are seven human sirtuin type (SIRT) homologs. SIRT2 colocalizes with cytoplasmic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

The crystal structure of SIRT2 has been used as a starting point for molecular modeling and virtual screening. A search in the Maybridge database resulted in two hit compounds which showed high inhibitory activity for SIRT2 [3]. These compounds have structural backbones that are new for SIRT2 inhibitors. The new structural

backbones were combined and a series of *N,N'-bis*-benzylidene-benzene-1,4-diamines and *N,N'-bis*-benzylidene-naphthalene-1,4-diamines were synthesized and tested *in vitro* against SIRT2 [4]. *N,N'-bis*-(2-Hydroxybenzylidene)-benzene-1,4-diamine **1** with an IC_{50} value of 58 μM was equipotent with the most potent hit compound and one of the most potent known SIRT2 inhibitors, sirtinol.



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

Quinazolinedione Inhibitors of S-farnesyltransferase: Hit Optimization by Parallel Solid Phase Synthesis

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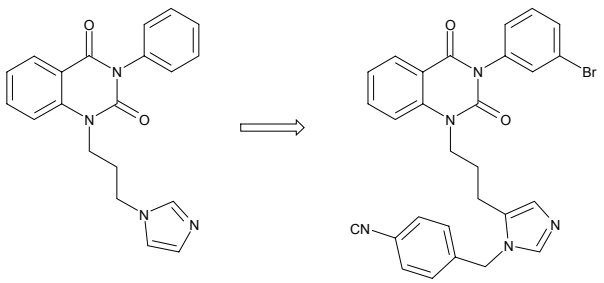
Anticancer drug discovery based on the inhibition of postranslational modification of Ras has been pursued vigourously in recent years. A particular effort has been made to identify inhibitors of S-farnesyltransferase as a main target.

Starting from a weak FTase inhibitor S 35028 (IC_{50} = 22 μM), we were able to improve the potency of the hit by modifying the aryl part of the molecule and incorporating a benzyl substituent on the imidazole.

Hit optimisation was performed in parallel by two automated solid phase approaches using paranitrophenyl carbonate [1] or o-chlorotrityl polystyrene resins [2].

Combination of 4-cyanobenzyl and 3-bromophenyl in S 37987 was found optimal for potent FTase inhibition (IC_{50} = 3 nM).



S 35028 **S 37987**

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The tale of a misunderstood molecule: how a hybrid drug does not function through a hybrid action

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Hybrid drugs are currently subjects of intense study. Their design is based on the assumption that a parent drug's safety can be improved by chemical attachment of a second drug selected for its ability to counter-balance the side-effects of the parent drug. In this presentation, we will discuss how the activity of a known hybrid drug actually does not require the two 'hybrid components' on which its design was based and through which its activity has been explained. Rather, we identified a third pharmacologically active species that had been overlooked so far. We will discuss details on the mechanism of action at the molecular and pharmacological level, and show how our postulated mechanism leads to the inception of molecules that are equally effective but lack both 'hybrid components'.

Evaluation of (–)-MR22 a novel sigma-1 receptor ligand on lesion-induced memory dysfunctions in the rat

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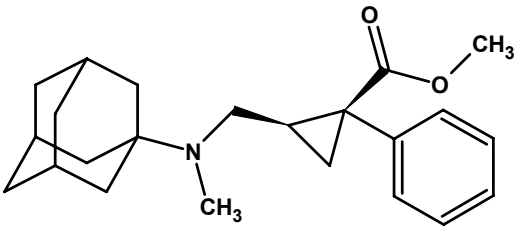
Recent studies have reported that selective sigma-1 agonists may improve cognitive abilities in experimental animals with cholinergic lesion or pharmacological receptor blockade.

(–)-MR22 [(–)-methyl (1*S*,2*R*)-2-{[1-adamantyl-(methyl)amino]methyl}-1-phenylcyclopro-panecarboxylate] is a newly synthesized sigma-1 selective ligand able to protect cultured cortical neurons against β-amyloid toxicity [1-2].

In the present study we sought to investigate the functional effects of **(–)-MR22** on learning and memory abilities in a murine model of Alzheimer disease produced by i.c.v injection of a powerful and selective immunotoxin, 192 IgG-Saporin.

The putative memory-enhancer action proposed for the sigma-1 agonists was evaluated in intact, as well as cognitively impaired animals using the Morris Water Maze spatial navigation task. The results show significant improvements from the lesion-induced deficits in **(–)-MR22**-treated, but not vehicle-treated animals. Further data will be presented about possible differential actions of **(–)-MR22** on lesioned as opposed to normal animals



(–)-MR22

with the aim to better define the therapeutic potential of such compound.

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