
# JMMC 2007 BOOK OF ABSTRACTS



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### *Editorial*

*The Book of Abstracts of the Austrian-German-Hungarian-Italian-Polish-Slovenian Joint Meeting on Medicinal Chemistry held from 17 to 21 June 2007 in Portorož, Slovenia under the auspices of the Eurpoean Federation of Medicinal Chemistry contains the abstracts of 7 plenary lectures, 20 keynote lectures, 15 oral and 172 poster presentations. They were contributed by medicinal chemists and other scientists involved in the drug discovery and development process from 26 countries which gives this Meeting a really international character. Over 300 Meeting participants are a guarantee for creative scientific interactions and wide dissemination of new ideas.*

*The 2007 Austrian-German-Hungarian-Italian-Polish-Slovenian Joint Meeting on Medicinal Chemistry is the 5th meeting in a series initiated in Taormina (Italy) in 1999 and succesfully continued by Budapest (2001), Krakow (2003) and Vienna (2005) meetings. It is dealing with important new aspects of drug discovery and development process with focus on antiinfectives, drugs for cardiovscular and metabolic disorders, enzymes and receptors as targets for new drugs, computer-aided drug design and discovery, emerging strategies in drug discovery and medicinal chemistry case studies.*

*The growing need to develop new better and safer drugs is a big challenge to medicinal chemists and we believe that JMMC 2007 will contribute to this exciting task.*

*Danijel Kikelj - Symposium chairman*

*Lucija Peterlin-Mašič - Head of the Organising Committee*

*Aleš Obreza - Guest editor-in-chief*

IPL

# Proteins and their structures in basic science and medicine

**Robert Huber**

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Protein crystallography experienced in the last twenty years a rapid development in methods and instrumentation, allowing the determination of very large and complex protein structures, particularly when combined with electron microscopy. These structures document an unlimited versatility and adaptability of the proteins´ architecture, but reveal also unexpected relationships. The structures are a basis for understanding their binding specificities and catalytic properties (chemistry), their spectral and electron transfer properties (physics), and their roles in physiological systems (biology and medicine). They allow design and development of specific ligands of target proteins opening novel ways for therapeutic intervention and for plant protection.

PL001

# Drug proteomics

**Giulio Superti-Furga,** Uwe Rix, Oliver Hantschel, Gregor Schütze, Nora Fernbach, Gerhard Dürnberger, Marc Brehme, Tilmann Bürckstümmer, Thomas Köcher, Jacques Colinge and Keiryn Bennett

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Biology relies on the concerted action of a number of molecular interactions of gene products and metabolites operationally organized in so-called pathways and in yet larger molecular networks. Impairment of pathway flow or connection can lead to pathological states. The majority of targets of current therapeutics cluster in a limited number of these cellular pathways. However, current appreciation of the "wiring diagram" or "molecular maps" of these pathways is scanty. Through integrated approaches using proteomics as central "glue" it is possible to obtain physical, functional and knowledge maps of entire human disease pathways. Moreover, it is feasible to map active chemical compounds back on the pathways by identifying the protein interactors of the immobilized compounds. The mode-of-action of novel and existing clinical drugs, natural products and metabolites can be determined, linked to biological processes by positioning on molecular networks and implemented into novel therapeutic and diagnostic approaches. Second-generation tyrosine kinase inhibitors developed against the Bcr-Abl target, dasatinib and nilotinib are analyzed and compared to imatinib to reveal dramatically different specificities and impact on molecular networks. Such a "systems biology" approach promises to create important synergies between the different research avenues and may inaugurate a truly "postgenomic" molecular medicine era.

PL002

# Ligand design using crystallography, data mining and virtual screening to develop novel leads and to probe structure-activity relationships

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Structure-based drug design tries to mutually map pharmacological space, populated by putative target proteins, onto chemical space, comprising possible small molecule drug candidates. Both spaces intersect where proteins and ligands recognize each other: in the binding pockets. We therefore developed a new method to detect, characterize and compare binding pockets in terms of functional relationships and exposed physicochemical binding properties shared in common among proteins independent of sequence or fold homology. The new method has strong potential to suggest alternative molecular skeletons for ligand design. The retrieval of molecular building blocks accommodated in particular sub-pockets that shares similarity with the pocket of the target protein under investigation can inspire the discovery of novel ligands.

Our approach of designing novel leads starts with a privileged ligand scaffold well-suited to address the key interactions of the conserved recognition pattern of the respective protein target family. Through specific decoration with appropriate side chains individual library members can be tailored with respect to selectivity towards particular family members. The selection of appropriate side chains also takes feasible synthetic strategies into account giving rise to a subset of putative building blocks useful for decoration of the core scaffold. The obtained building blocks are docked as virtual library into the target protein and the most promising library members are determined. To verify this knowleged-based design approach, we selected serine and aspartic proteases as well studied model cases.

For both protease families, we investigated five-membered azacycles as privileged structural elements for the design and synthesis of selective inhibitors. In case of the aspartic proteases, the azacycle-scaffold addresses via its basic nitrogen the conserved catalytic dyad as revealed by X-ray crystallography. With respect to the serine proteases, the S2-pocket is addressed, in this case by a non-basic heterocycle. Both core-structures can easily be modified by means of standard synthetic chemistry guided by an iterative cycle of design, synthesis, testing and crystal structure analysis.

In due course of drug development prospective lead structures are optimized by systematically exchanging ligand functional groups at a given core skeleton. Medicinal chemists follow some basic rules collected for intrinsic functional group contributions that allow estimating by how much the binding affinity is expected to improve for functional group replacements. E.g. growing a ligand by one methylene group should augment binding affinity by ca. 3-4 kJ/mol. However, even very similar ligands can exhibit very different binding properties that destroy a simple structure-activity relationship. We studied the thrombin binding of two derivatives only differing by a cyclopentyl and cyclohexyl ring planned to accommodated the enzyme's S3/S4 pocket. The cyclopentyl derivative shows well defined electron density in the crystal structure thus experiencing good enthalpic interactions with the protein. Accordingly, an increase of its hydrophobic contact interface is expected to enhance binding. Nevertheless, the cyclohexyl derivative shows a high residual mobility in this part of the molecule. It cannot establish comparably strong contacts with the S3/S4 pocket. Likely, with these binding properties it will not profit from a further expansion of its hydrophobic surface. Yet, its binding free energy matches with that of the cyclopentyl derivative as the loss of good enthalpic contacts is compensated by an entropic advantage. This example of two closely related ligands shows that binding properties are a complex interplay of structure and dynamics. It also shows that some, on first glance trivial and "obvious" structure-activity relationships do not necessarily display the anticipated straightforward correlation.

PL003

# New antimicrobial agents: Why we need them and what molecular targets should we choose?

**Ian Chopra**

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The modern era of antibacterial chemotherapy began in 1935 with the discovery of prontosil and over the next three decades virtually all the major classes of antibiotics and synthetic anti-bacterial agents in current use were discovered. The widespread introduction of these antimicrobials into clinical practice has made possible the treatment of previously life-threatening illnesses and has enabled advances in surgical techniques by permitting the prophylaxis and treatment of surgery-related infections.

However, resistance to antibacterial drugs is now becoming an increasingly difficult problem in the management of bacterial infections. The challenge which resistance poses for health care requires a number of initiatives including improved infection control measures to prevent transmission of resistance and resistant organisms, modification of prescribing practices to achieve more rational use of antimicrobials, and the development of new vaccines and other means of strengthening immune responses.

The discovery of new therapeutic agents with novel modes of action will also be vital to meet the threats created by the emergence of bacteria resistant to current drugs. Unfortunately, since the 1980s the introduction of new agents for clinical use has diminished, reflecting both the challenge of identifying new drug classes and a declining commitment to antibacterial drug discovery by major pharmaceutical companies. This underscores the urgent need to select suitable molecular targets for high throughput screening and rational design of new inhibitors.

Bacterial genomics has identified numerous potential drug targets, but this knowledge has not yet resulted in the delivery of a novel antibacterial drug to the clinic. Consequently it may be prudent to exploit opportunities for drug discovery by targeting biochemical pathways, or processes, inhibited by existing drugs, but seeking different points for molecular intervention [1]. A major benefit of employing pathways or processes that are already known to contain drug targets is that proof of principle for drug intervention is already established. This approach to drug discovery will be illustrated by reviewing the possibilities for discovery of new agents inhibiting RNA polymerase in bacteria.

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PL004

# Sigma ligands and neurodegenerative diseases.

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Neurodegenerative diseases are usually associated with the presence of insoluble proteins or with neuronal death. Several immunohistochemical studies showed that in Alzheimer's disease or in Huntington's disease is evident an improper activation of tTG-2, a calcium-dependent protein, member of the family of transglutaminase enzymes, implicated in several CNS processes.
We evaluated weather tTG expression is linked with the sigma receptor activation in primary astroglial cell culture. We also studied tTG reaction, caspase-3-like proteases cleavage, and DNA fragmentation by using the TUNEL test analysis in the presence of sigma ligands with different pharmacological profile.
Our studies strongly support the hypothesis that tTG plays an important protective role against apoptosis [1]. Selective sigma ligands are an important tool to modulate intracellular calcium levels and consequently the up-regulation of tTG typical of several neurodegenerative diseases.

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PL005

# Indole alkaloids in medicinal chemistry

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Rye is sometimes infected by a fungus called *Claviceps purpurea.* The term ergot designates the dark, brown, tuberous bodies which can be collected before or during harvesting and represent one of the most remarkable drugs of our terapeutic arsenal. Actually, the most significant are the metabolic products of the fungi. We elaborated three alternative total synthetic pathways to construct the ergoline skeleton, one of which finally resulted in (+)-lysergic acid and α-ergocryptine.
Synthesis of vindoline derivatives with remarkable antitumor effects will also be presented.

PL006

# Genome as a source for the discovery of new peptide hormones. Synthesis and pharmacological characterization of new active peptides.

Puget Karine, Carnazzi Eric, Le Gallic Lionel, Valeau Stéphanie, Clavier Karine, Subra Gilles, Gaudry Hervé, Ripoll Jean-Philippe, Bergé Gilbert, and **Martinez Jean**

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We have designed a program for generating active peptides from protein and genomic sequence data. This program is able to search in the available data concerning protein structures and nucleotidic sequences to provide putative natural active peptide sequences ending by a C-terminal amide. Among the peptide sequences that were generated, quite an important number have been synthesized, either in 96-well plates on classical solid supports using an ACT instrument, by the Multipin technology, or using classical peptide synthesis for the longer peptides. So far, more than 2500 peptides of variable lenght were obtained. As a general screening, these peptides were tested for their affinity to Guinea Pig brain membranes and for their activity on a second messenger system (cAMP, IP, Ca++) on transfected cells. Among the tested peptides, two of them showed high and specific affinity for the brain membranes (in the nanomolar range) and a significant activity in stimulating cAMP accumulation in various cells.

The details of the mining program will be presented, as well as the automated synthesis on solid support of libraries of amidated peptides and their general screening. The pharmacology of active peptides that were identified will be presented in more details.

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KL001

# Drug targets in sphingolipid signalling pathways

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Following the discovery of sphingolipids at the end of the 19th century, it was believed for more than one hundred years that they were merely structural components of higher eukaryotic cells. But starting in the late 1980s, it was discovered that sphingolipids can also serve as extra- and intracellular mediators in cell signalling and that specific receptors exist. Today, sphingolipids are an active field of research, and a number of hypotheses for therapeutic opportunities have emerged. With the invention of FTY720, which is currently in Phase III clinical trials for multiple sclerosis, medicinal chemistry proved that sphingolipid-like structures can be successfully developed into drugs [1]. This has further stimulated interest in drug discovery in this field but, overall, medicinal chemistry aspects are still rather unexplored.

This presentation discusses potential drug targets in the sphingolipid metabolism and signalling area for therapeutic intervention with low molecular weight compounds. In general, these targets include receptors for the respective sphingolipid mediators (extracellular function) as well as enzymes involved in their biosynthesis and degradation modulating endogenous levels of the signalling molecules (intracellular function). The latter approach is supported by the rheostat concept which highlights that one particular sphingolipid mediator does not have an assigned function per se but that its balance with respect to other sphingolipids determines effects like cell survival versus cell death [2, 3]. The focus of the presentation is on enzyme targets in the sphingolipid catabolism addressing target validation, drugability and available tool compounds. In addition, contributions of medicinal chemistry enabling more insight and solving biological problems are demonstrated by selected examples, such as the design of an assay substrate for sphingosine kinases.

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KL002

# Oncogenic JAK/STAT signalling pathways: Modelling of inhibitors and simulation studies

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One of the most recently recognized oncogenic signalling pathways involve the signal transducer and activator of transcription (STAT) proteins. This set of proteins comprises seven members: STAT1 - STAT4, STAT6, as well as closely related STAT5a and STAT5b. STATs are activated by janus kinases (JAKs). In diverse human cancers, such like leukemias, lymphomas, breast cancers, melanomas, ovarian cancers, lung cancers, pancreatic cancers or prostate cancers, constitutive activation of STATs has been detected at high frequency. For review see e.g. [1]. In our studies we concentrate on JAK2/STAT3 [2] as well as on JAK3/STAT5 signalling pathways. One hypothesizes that inhibition of JAK2 and/or JAK3 decreases activation of the STAT proteins, and in consequence inhibits the downstream signalling. We found that caffeic acid derivatives are inhibitors of these pathways. Modelling of these inhibitors followed by synthesis [3], as well as experimental studies of the influence of these inhibitors on tumor cell lines [4] will be reported. Preliminary works [5] on the design of the JAK/STAT signalling system using CellDesigner [6]) and Systems Biology Markup Language (SBML) [7] will be outlined.

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KL003

# Modulators of integrin receptors

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Integrins are adhesion receptors that transmit bi-directional signals during many vital physiological processes, for example tissue remodelling, repair, angiogenesis, development, the immune response and haemostasis. They are formed by the non-covalent association of $\alpha$ and $\beta$ subunits. So far 18 $\alpha$ and 8 $\beta$ subunits have been identified that assemble into 24 different receptors (1). Different integrins can recognise the same ligand. However, integrins composed of a common subunit may show different ligand binding specificities.

Ligand binding is not constitutive, but is regulated by the activation state of the cell. All integrins bind their ligands in a divalent cation-dependent manner. Manganese and magnesium are usually promoters of binding in contrast to calcium which has opposite effects based on its concentration and the integrin involved. High concentrations of $Ca^{2+}$ inhibited ligand binding to $\alpha_5\beta_1$, whereas low concentrations promoted binding. In case of $\alpha_4\beta_1$ $Ca^{2+}$ only diminished affinities for ligand binding. On the other hand, integrin $\alpha_{IIb}\beta_3$ binds ligands in $Ca^{2+}$-dependent manner (1).

Ligands are structurally diverse. However, they all contain an acidic residue involved in ligand recognition. Ligand binding can activate the integrin resulting in activation of intracellular signalling pathways (outside-in signalling). Activation of integrins can also come from the cytoplasm - the signals received by other receptors activate intracellular signalling pathways which make the extracellular domain of integrins competent for ligand binding (inside-out signalling). Inside-out and outside-in signalling are associated with distinct tertiary and quaternary conformational changes in the extracellular segment of integrins (2).

Many efforts have been made towards the modulators of integrin receptors. Much research has been directed to the field of $\alpha_{IIb}\beta_3$, $\alpha_V\beta_3$, and $\alpha_4$ integrin antagonists, resulting in several sub-nanomolar antagonists. On the other hand, inhibitors of $\alpha_5\beta_1$ or $\alpha_2\beta_1$ integrins have yet to be described in detail. The most promise ones are antagonists of $\alpha_{IIb}\beta_3$. Development of $\alpha_{IIb}\beta_3$ antagonists has been one of the main focuses in antitrombotic research over the last decade. Orally active, low molecular weight $\alpha_{IIb}\beta_3$ antagonists with 2H-1,4-benzoxazine-3(4H)-one scaffold are therefore still of interests especially because their high affinity towards thrombin (3).

Recently, the concept of targeting only one integrin has shifted to blocking multiple integrins simultaneously to achieve synergistic therapeutic effect. Attachment and migration of malignant astrocytoma cells toward osteopontin, expressed by both the normal brain and the malignant astrocytic tumours, are mediated by integrins $\alpha_V\beta_3$, $\alpha_V\beta_5$ and $\alpha_5\beta_1$. Therapy with molecules directed against these three targets may stop invasion of malignant astrocytoma and improve prognosis for the patients. Several new compounds with 1,2,4-oxadiazole or triazine scaffold have been prepared and tested their activity on $\alpha_V\beta_3$, $\alpha_V\beta_5$ and $\alpha_5\beta_1$ with an *in vitro* solid phase assay against their natural ligands fibrinogen, vitronectin and fibronectin.

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KL004

# NEXAVAR® – a novel inhibitor of signal transduction

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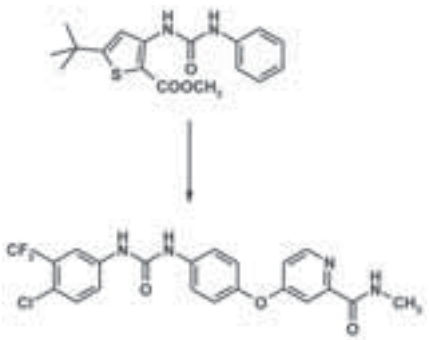
The discovery of Nexavar® (sorafenib tosylate) is the result of a collaboration between Bayer HealthCare and Onyx Pharmaceuticals. Sorafenib belongs to a novel class of kinase inhibitor exhibiting a dual mode of action. This compound inhibits Raf kinase, a key mediator of the MAP kinase pathway, thereby blocking tumor cell proliferation. In addition, sorafenib inhibits a series of receptor tyrosine kinases involved in angiogenesis and stromal activation, notably VEGFR and PDGFR. Therefore, the kinase profile of sorafenib results in inhibition of both tumor growth and tumor angiogenesis in xenograft models.

The medicinal chemistry program started from a lead structure identified via high-throughput screening. The confirmed hit, a thienyl-phenyl-urea ($IC_{50}$ = 17 μM), was optimized using classical medicinal chemistry as well as combinatorial chemistry techniques.



Nexavar® was first approved in the US in late 2005 for the treatment of advanced renal cell carcinoma. Additional Phase III clinical trials are currently ongoing, evaluating its potential in the treatment of hepatocellular carcinoma and non-small cell lung carcinoma, either as a single agent or in combination with cytotoxic therapies.

KL005

# Pattern matching as an alternative approach for pharmacophore modelling and efficient virtual screening

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Chemical-feature based pharmacophore models have been established as state-of-the-art technique for characterizing the interaction between a macromolecule and a potential inhibitor [1]. While in ligand-based drug design, feature-based pharmacophore creation from a set of bio-active molecules is a frequently chosen approach, structure-based pharmacophores models are still lacking the reputation to be an alternative or at least a supplement to docking techniques. Nevertheless, 3D pharmacophore screening bears the advantage of being faster than docking and to transparently provide the user with all the information that is used by the screening algorithms to characterize the ligand-macromolecule interaction.
Based on our structure-based pharmacophore elucidation [2, 3], our novel, rigid 3D pharmacophore super-positioning technique will be applied to several examples (Fig. 1). Geometric fitting of multi-conformational models of small organic molecules to structure-based pharmacophores is compared to docking methods and discussed in terms of conformational coverage, flexibility and eligibility for virtual screening.

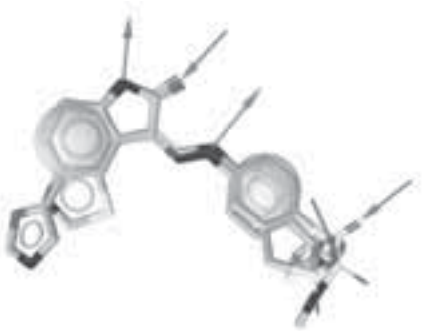

***Fig. 1:*** *Three CDK2 inhibitors in their bio-active conformations aligned to their automatically generated common structure-based 3D pharmacophores using the presented alignment technique (Hydrogen bonds are represented as vectors, hydrophobic areas as spheres)*

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KL006

# Ligand binding modes for serotonin receptors

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The first 3D structure of the G protein-coupled receptor (bovine rhodopsin) was published in 2000 [1]; since then, it has served as a template for the homology modeling of hundreds of other GPCRs, including the numerous subtypes of serotonin receptors. Homology models are tools for the structure-based drug design, virtual screening of compound libraries, and they also enable an investigation of the phenomena involved in receptor activation at the atomic detail level using molecular dynamics simulations.

The lecture focuses on summarizing the available rhodopsin-based serotonin receptors models. First, different approaches used in their construction, in a context of recent advances in modeling methodologies are presented. Next, the experimental site-directed mutagenesis data on serotonin receptors is shortly reviewed and its application in the process of receptor modeling and ligand binding mode prediction is discussed. The differences in amino acid composition of all the binding sites are then emphasized, concentrating on their possible influence on ligands selectivity. Finally, the binding modes proposed by different authors are compared and discussed in details. Special attention is paid to arylpiperazine type of ligands due to their importance, multireceptoral profile and authors own experience [2,3].

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KL007

# Modeling the hERG potassium channel and its interactions with drugs

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The human *ether-à-go-go*-related gene (*HERG*) was isolated in 1994, and shown to encode a six trans-membrane protein that assembles in a tetrameric complex to form the hERG potassium channel. The hERG $K^+$ channel was then demonstrated to conduct the rapid delayed rectifier current ($I_{Kr}$), which is a component of the repolarization phase of the action potential of cardiomyocytes. In recent years, a large amount of evidence has accumulated that drugs belonging to different pharmacological classes may unintentionally block hERG channels, thus causing a prolongation of the action potential duration resulting in the so-called long QT syndrome (LQTS). Drug-induced LQTS predisposes individuals to a potentially lethal form of arrhythmia named *torsades de pointes* (TdP), and it is therefore not surprising that nowadays in the drug design and development process a great emphasis is cast on methods aimed at an early identification of the hERG blockade potential of new molecules [1]. Several *in vitro* and *in vivo* models are currently available for the assessment of the proarrhythmic potential of new chemical entities, but anyway, prediction of drug-induced LQTS is still problematic [2]. On the molecular side, many efforts have been devoted to understanding the determinants of hERG block by drugs, and site-directed mutagenesis combined with the voltage-clamp technique is continuously providing increased and precise information [3].

*In silico* methods have recently been proposed as a means to increase the capability of predicting hERG liability [1, 4], and indeed several ligand-based QSAR models have recently appeared in the literature showing good accuracy in assessing the potential for hERG blockade. On the other hand, a parallel development of target-based studies has been partly hampered by the lack of a sound experimental basis, on which to build a fully reliable model of the channel complex. Nevertheless, in the most recent times, some drug/hERG docking models of increasing quality have started to appear, and despite the limitations imposed by the homology modeling approach, they promise to become useful tools for predicting the hERG binding affinity and interpreting the hERG blockade by small molecules.

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KL008

# New inhibitors of cytoplasmatic steps of bacterial peptidoglycan biosynthesis

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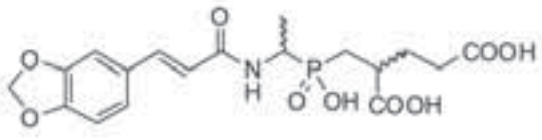
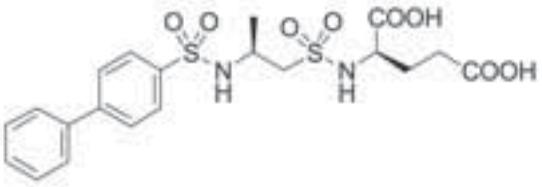
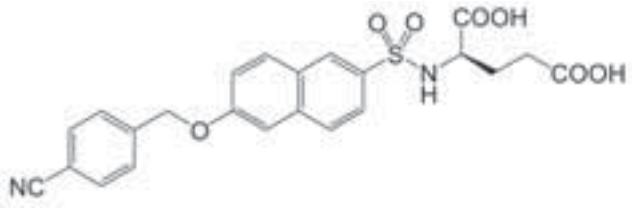
The increasing emergence of pathogenic bacterial strains with high resistance to antibiotic therapy has created an urgent need for the development of new antibacterial agents that are directed towards novel targets. We have focused our attention on the Mur ligases (MurC to MurF) and D-alanine-D-alanine ligase (Ddl). These enzymes catalyze the cytoplasmatic steps of bacterial peptidoglycan biosynthesis and represent under-exploited targets for antibacterial drug design. Recently, we designed and synthesized a series of new phosphinate and sulfonamide compounds as transition-state analogue inhibitors of MurD and substrate analogues of MurE. They were tested against the MurD enzyme from *Escherichia coli,* and MurE from *Staphylococcus aureus,* allowing initial structure-activity relationships to be deduced [1-3]. Some compounds had $IC_{50}$ values below 200 μM and constitute a promising starting point for further development of Mur inhibitors. In addition, we screened our in-house bank of compounds against DdlB from *E. coli* and we found that a series of diazenedicarboxamides inhibit the enzyme more than D-cycloserine. Some diazenedicarboxamides also have promising *in vitro* antibacterial activities [4].



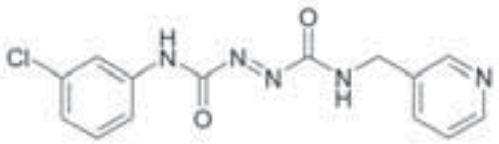
$IC_{50}$=95μM (MurD)



$IC_{50}$=181μM (MurE)



$IC_{50}$=134μM (MurD)



$IC_{50}$=15μM (Ddl)

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KL009

# Multifunctional and DNA-cleaving antibiotic analogs

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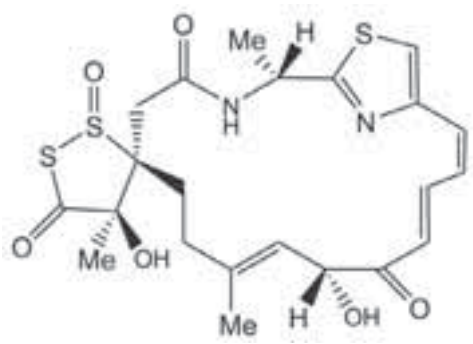
Methods that can be used for the covalent attachment of biologically active molecules to amino group containing polymers have been applied for the preparation of multifunctional antibiotics. This way telechelic polyethylene glycol derivatives of nucleosides, quinolonecarboxylic acids and glycopeptide antibiotics have been prepared and their aggregation and antimicrobial activity were also studied.

A new method for the synthesis of the "warhead" of the DNA cleaving antibiotic leinamycin has been elaborated. Using this protocol several simple analogs of leinamycin have been prepared from nucleosides.




*Leinamycin*

KL010

# 4-Substituted Trinems: Broad Spectrum β-Lactamase Inhibitors.

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A wide variety of pathogens have acquired antimicrobial resistances as an inevitable evolutionary response to the extensive use of antibacterial agents. In particular, one of the widely used antibiotics structural classes is the β-lactams, in which the most common and the most efficient mechanism of bacterial resistance is the synthesis of β-lactamases. Class C β-lactamase enzymes are primarily cephalosporinases, mostly chromosomally encoded, and are inducible by exposure to some β-lactam agents and resistant to inhibition by marketed β-lactamase inhibitors. In an ongoing effort to alleviate this problem a series of novel 4-substituted trinems was designed and synthesized. Significant *in vitro* inhibitory activity was measured against the bacterial β-lactamases of Class C and additionally against Class A. The lead compound LK-157 was shown to be a potent mechanism-based inactivator. Acylation of the active site Ser 64 of the Class C enzyme β-lactamase was observed in the solved crystal structure of the inhibitor complex to AmpC enzyme from *E. cloacae 908R*. Structure-activity relationships in the series reveal the importance of the trinem scaffold for inhibitory activity and the interesting potential of the series for further development.

KL011

# 6-Vinylpyrimidines: Non nucleotide inhibitors of HIV-1 reverse transcriptase competing with the nucleotide substrate

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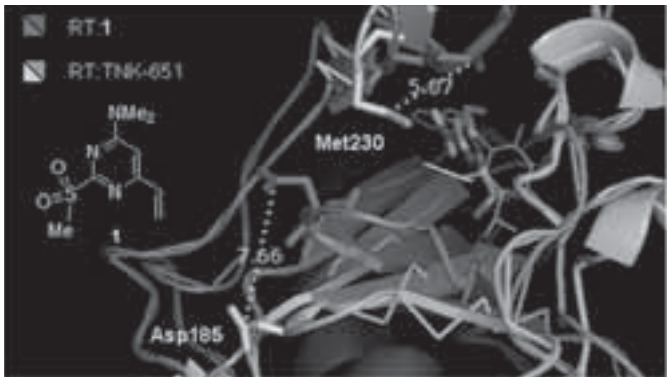
In the fight against AIDS, first and second generation non-nucleoside reverse transcriptase inhibitors (NNRTIs) are now established as part of highly active antiretroviral therapy (HAART) for treating HIV infection. However, because of the rapid selection of mutants, further new drugs with activity against the clinically relevant drug-resistant viruses are need. As an extension of our ongoing efforts toward the identification of novel NNRTIs capable to overcome the effects of drug resistance mutations,[1],[2],[3] we have recently reported the development of a straightforward combinatorial approach for the synthesis of 6-vinylpyrimidine derivatives whose structure may be related to that of TNK-651 more than to any other known NNRTI.[4] Preliminary biological screening data have been used to focus the synthetic efforts on the development of better inhibitors, a selection of which (**1-5**) has been described in the present paper (Chart 1).[5]

We report here a new class of NNRTIs with a 6-vinylpyrimidine scaffold found to exhibit a peculiar behavior: contrary to the NNRTIs reported to date, enzymological studies revealed that such compounds inhibit HIV-1 reverse transcriptase (RT) by a competitive mechanism with the nucleotide substrate after binding to the non-nucleoside inhibitors binding pocket (NNIBP) of the free enzyme. The most potent analogue, 2-methylsulfonyl-4-dimethylamino-6-vinylpyrimidine (**1**), is endowed with high activity toward both wild type (wt) RT and drug-resistant mutants. Molecular docking and dynamics simulations have been finally performed to hypothesize the interaction mode of these ligands with the NNIBP (both of wt and mutated RT) and to suggest a possible explanation for their unique mechanism of action.

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KL012

# Serotoninergic oxindoles

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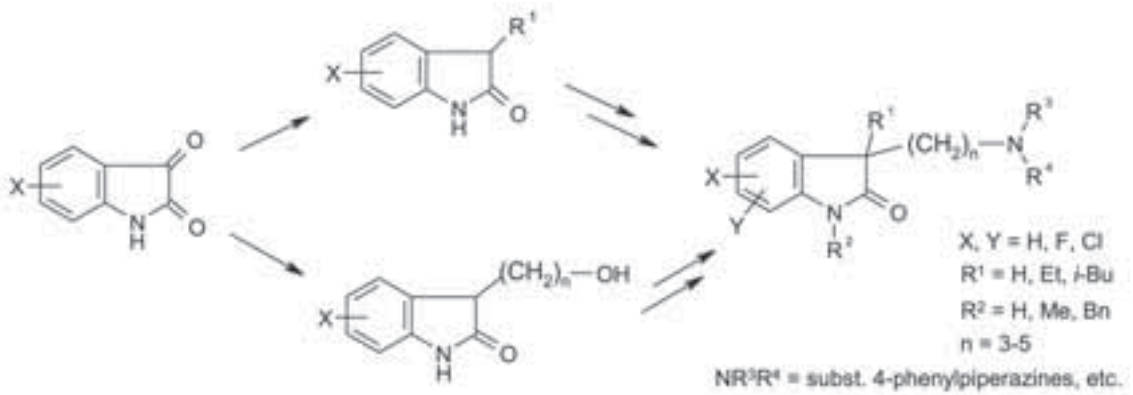
A series of potent 5-hydroxytryptamine$_7$ (5-HT$_7$) ligands have been synthesised, which contain an oxindole skeleton. A new, high-yielding one-pot method has been elaborated for the key step of the synthesis, *i.e.* for the selective 3-alkylation or 3-(ω-hydroxyalkylation) of oxindoles [1] or isatins [2].




The binding of the products was tested on the 5-HT$_7$ and 5-HT$_{1A}$ receptors. Despite the structural similarity of these two serotonin receptor subtypes, several derivatives exhibited high selectivity to 5-HT$_7$. Based on the almost 300 analogues synthesised, systematic SAR studies have been performed [3]. Compounds unsubstituted at the oxindole nitrogen atom ($R^2$=H) and containing a tetramethylene spacer (n=4) were the most potent ligands, with outstanding 5-HT$_7$ affinities ($K_i$ < 0.5 nM for several derivatives). Concerning the basic group, beside the pharmacophores of 4-phenylpiperazine type, certain halophenyl-1,2,3,6-tetrahydropyridines proved to be efficacious, as well. Due to halogen substitution at the aromatic rings, a good metabolic stability could also be achieved. Some representatives of this family have been investigated on their effect on cAMP levels of CHO cells stably expressing the human 5-HT$_7$ receptor, in comparison with the reference compound SB-269970. The EGIS compounds exhibited a strong 5-HT$_7$ antagonist activity and also a good selectivity against a series of other receptors. The enantiomeric separation and the *in vivo* anxiolytic and antidepressant tests of the above compounds are in progress.

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KL013

# Possible therapeutic use of tocopherols in cardiovascular and neurodegenerative disorders

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Vitamin E consists of a group of eight isomers, four tocopherols (α, β, γ and δ) and four tocotrienols (α, β, γ and δ). The group of tocopherols represents the most important lipid-soluble antioxidant. Since its discovery, mainly antioxidant and recently also cell signalling aspects of tocopherols and tocotrienols have been studied. Tocopherols and tocotrienols are part of an interlinking set of antioxidant cycles.

In cultured cells has been demonstrated that vitamin E inhibits inflammation, cell adhesion, platelet aggregation and smooth muscle proliferation. Recent advances in molecular biology and genomic techniques have led to the discovery of novel vitamin-E sensitive genes and signal transduction pathways. Thus, the possibility was discussed that many of the effect previously attributed to the antioxidant functions can also be explained by non-antioxidant mechanisms.

Tocopherols ought to have protective effects in diseases coupled to oxidative stress, e.g. atherosclerosis and neurodegeneration in Alzheimer's disease.

Laboratory and observational studies suggest that antioxidant supplementation may prevent atherosclerosis as well as Alzheimer's disease. However, clinical trials provided controversial results. The most recent randomized control trials could not prove significant evidence of a protective effect of tocopherols neither on the progression of atherosclerosis nor of Alzheimer's disease. The obtained results suggest that supplementation of antioxidants cannot be recommended for the normal population.

The negative results of clinical trials my have different reasons. The bioavailability and absorption of tocopherols from the gut of the elderly population is decreasing in correlation with the age. Furthermore, tocotrienols and γ-tocopherol are more effective in signalization processes than α-tocopherol. Tocopherols can hardly penetrate across the blood-brain-barrier (BBB) but their presence would be necessary for the local neuroprotection against β-amyloid in Alzheimer's disease.

We performed a series of in vitro and in vivo experiments for working out a standard protocol for the application of tocopherols as preventive agents. Tocopherols were administered per os in composits with lecithin or β-cyclodextrine in rat experiments with positive results: absorption of tocopherols from the gut was increased. Tocopherols showed a protecting effect on endothel cells in brain capillaries against β-amyloid. However, penetration of tocopherols across the BBB remained very low up to 50 mg tocopherol per kilogram of animal. A new BBB model consisting of endothel cells was used for in vitro experiments using novel carrier molecules for tocopherols. New composits of tocopherols seem to be putative drugs for atherosclerosis and Alzheimer's disease.

KL014

# Structure based design of coagulation inhibitors

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Thrombo-embolic disorders are the leading cause of cardiovascular disease in industrialised countries. Thrombosis results from unwanted blood clot formation leading to the reduction of blood flow. Serine proteases hereby play an important role in the process of thrombogenesis. Therefore, tremendous efforts in pharmaceutical industry have been assigned for the discovery of inhibitors of the key enzymes that are part of the coagulation cascade, especially thrombin and FXa. This contribution will focus on the impact of structure-based design on our lead optimization process. Especially, X-ray analysis of inhibitor-protease cocrystals helped broadening the structural basis of our leads and accelerated the search for potential new anticoagulants. A historical perspective starting from our selective thrombin inhibitor dabigatran **A** to selective FXa inhibitors e.g. **B** will be given. [1,2,3].

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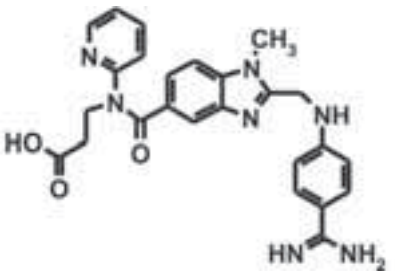
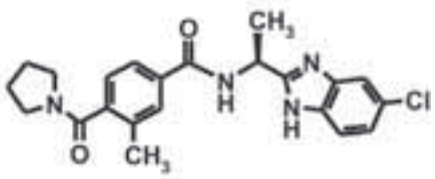



**A**




**B**

KL015

# Medicinal chemistry strategies to minimize hERG channel blockade

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The withdrawal of several marketed drugs causing QT prolongation in humans, a potentially lethal cardiac event linked to inhibition of the hERG potassium channel, led to a worldwide quest for medicinal chemistry concepts that both explain the molecular mode of action of hERG inhibition and result in strategies to minimize channel blockade. In order to develop intelligent strategies, it is imperative to use predictive in vitro assays and to strive for a good correlation with appropriate in vivo and ex vivo animal models. Many categorizations based on past achievements are applied to new molecules with varying success. On the other hand, very discrete modifications of molecular structure can make a huge difference, posing a particular challenge to ligand-based models. This presentation reviews the usefulness of computational models as developed at Novartis [1], and critically appraises medicinal chemistry approaches towards hERG inactive drug candidates [2]. Some in-house examples, conclusions and recommendations will be given.

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KL016

# NO-Donor hybrid drugs: searching for new NO-donor aspirin-like agents

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Aspirin (acetylsalycilic acid, ASA) is a well-established nonsteroidal anti-inflammatory drug (NSAID) widely used as an anti-inflammatory, analgesic, antipyretic as well as antithrombotic agent. The pharmacological basis of these effects largely relies on the ability of this drug to covalently modify COX-1 and COX-2 resulting in reduced production of prostanoids. Due to the permanent inhibition of the platelet cyclooxygenase, which prevents the generation of tromboxane $A_2$, aspirin is unique among NSAIDs approved for the treatment of acute coronary heart disease and it is recommended as a chronic treatment for secondary cardiovascular prevention in patients at risk. Unfortunately, even low-dose aspirin induces gastrointestinal disorders, including ulceration and bleeding, due to the reduced production of sodium bicarbonate and mucus lining the stomach walls and, partly, to the local irritation from contact with gastric mucosa. Nitric oxide (NO) is an important physiological messenger which displays a wide spectrum of effects on different biological systems, including protective action on the gastrointestinal tract [1]. On this basis, in the past few years a number of *NO-donor aspirins*, namely molecules obtained by joining NO-donor moieties (*i.e.* nitrooxy, furoxan, *N*-diazeniumdiolate) with ASA, have been developed with the aim of reducing gastrointestinal side effects. These compounds have been obtained linking the NO-releasing moiety to the aspirin carboxylic function through an ester bridge. As a whole, these new agents proved to be successful both in vitro and in vivo in reducing gastrotoxicity, while retaining the antiinflammatory and antithrombotic effects of the parent drug. Interestingly, in addition to the expected effects, there is increasing evidence that their pharmacological profile involves other therapeutic areas, including cancer and chronic inflammation, making such compounds very promising in the field of multitarget therapy [2]. However, their exact mechanism of action is still in need of investigation, also in the light of the fact that a number of NO-donor aspirins are not “true” aspirin pro-drugs [3]. In this communication a new class of *NO-donor aspirin-like* molecules is described in which the acetyl group in ASA is substituted by an NO-donating acyl substructure. This structural modification implies the variation of their chemical and/or metabolic behaviour. Their preliminary pharmacodynamic/pharmacokinetic profile will be discussed in this respect.

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KL017

# Small-molecule based delivery systems for alkylating antineoplastic compounds.

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Cytotoxic agents that act by covalent modification of DNA were the first modern anticancer chemotherapeutics and remain components of combination chemotherapy regimens. The alkylating antitumor drugs are effective but relatively non-selective because they target fundamental biochemical processes, such as DNA and protein production. This indiscriminate action is one of the reasons for their severe toxic side effects. In cancer research, many approaches have been dedicated to increase the selectivity of antineoplastic agents by associating them to a carrier that could guide them to the target cells. Among them are amidine analogues of alkylating drugs. We describe our attempts to rationalise their mode of actions and to further improve the efficacy of this class of compounds [1]. The nitrogen mustards have been reported to have limited sequence specificity with alkylation occurring preferentially in the middle guanine-N7 position of runs of guanines. A therapeutic advantage might therefore be gained if the bifunctional alkylating agents could be more precisely targeted to defined genomic sites. Recent work on the targeting of nitrogen mustard alkylating agents to DNA by the use of DNA minor groove-binding ligands has shown that this strategy can greatly enhance both the in vitro cytotoxicity and the in vivo antitumor activity of the mustard moiety, when compared with untargeted mustards of similar reactivity. Several structural results and their implication with the properties and function of these compounds will be discussed [2].

In recent years, there has been significant progress in the identification of cancer-specific cellular drug targets and in the design of drugs that selectively target cancer-specific cellular processes. These treatments should exploit the biochemical differences between normal and cancerous cells, resulting in drugs with greater potency and less toxic side effects. Enzymes that are differentially expressed in disease states are possible targets since enzymes comprise 28% of all drug targets. Of several possible enzymes that were so identified, prolidase was found to be overexpressed at least some tumour tissues and was selected as the most desirable enzyme target. The primary biological function of prolidase involves the metabolism of proline-containing protein degradation products and the recycling of proline from imidodipeptides for proline containing protein resynthesis, mainly collagen. We describe the synthesis of proline prodrugs of established antineoplastic agents, and demonstrate the feasibility of prolidase-specific targeting with cell proliferation studies in breast cancer cells. The cytostatic character of the most active compounds will be discussed in the light of identified biological pathways [3, 4]. We believe that the outlined prodrug strategy is an attractive approach of altering the pharmacokinetic profile of established anticancer drugs and increasing their therapeutic index.

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KL018

# Diazenes as potential anticancer agents

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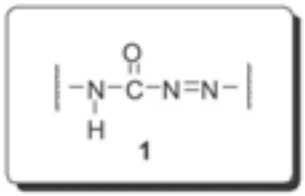
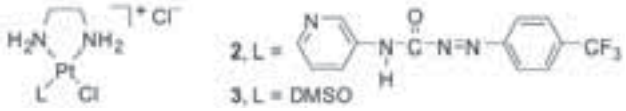
Platinum complexes, particularly cisplatin, are among the most widely used drugs for the treatment of tumors. Unfortunately, the success of chemotherapy in cancer patients is impeded by the problem of platinum-drug resistance. Although it is probably multifactorial, one form of the acquired platinum-drug resistance originates in an elevated intracellular glutathione (GSH) concentration. This type of resistance can be reduced by GSH-depleting agents.

We have recently addressed the challenge of the oxidation of a thiol to a disulfide *in vitro* by diazenecarboxamides (shortly *diazenes*, **1**) [1]. In the biological screening experiments we have found that several *diazenes* decreased intracellular GSH concentration and inhibited growth of different tumor cell lines in as low as nanomolar concentrations. More than 50 *diazenes* have been tested *in vitro* on human tumor cell lines including cervical carcinoma cells (HeLa), glioblastoma cells (A1235), laryngeal carcinoma cells (HEp2), mammary carcinoma cells (MCF-7), breast adenocarcinoma cells (SK-BR-3), non-small cell lung cancer (NCI-H460), and others [2].



**1**

*Diazenes* also reduced the survival of the cisplatin resistant sub-lines, as well as exhibited synergistic effect with cisplatin when applied together, breaking new grounds of a combined treatment of cancer [2]. To prove the concept, we designed and evaluated new cisplatin analogues. An example is [PtCl(en)(L-*N1*)]Cl (**2**), in which L is *diazene* ligand coordinated to Pt(II). The *diazene* in **2** retained its oxidative properties to GSH, and in comparison to the parent [PtCl(dmso)(en)]Cl (**3**) it exhibited higher cytotoxicity against T24 human bladder carcinoma cells [3].



**2**, L = **3**, L = DMSO

*Syntheses, chemical, and biochemical properties of **diazenes** will be discussed.*

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KL019

# Insights into the CDK project at BSP

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Loss of cell cycle control and tumor induced neovascularization represent two major hallmarks of cancer. Cyclin-dependent kinases (CDKs), a family of Ser/Thr kinases which requires association with a cyclin regulatory subunit for activation, are required for the correct timing and order of the events of the cell division cycle. Aberrant CDK control and consequent loss of cell cycle checkpoint function have been directly linked to the molecular pathology of cancer. Tumor induced neoangiogenesis was identified as a crucial mechanism to achieve blood supply for tumor growth, invasion and metastasis. The endothelial cell specific vascular endothelial growth factor (VEGF) / VEGF-receptor tyrosine kinase (VEGF-RTK) system has been validated as a main signalling pathway in tumor angiogenesis.

The presentation describes the identification and optimisation of a novel class of potent CDK and VEGF-R inhibitors, the macrocyclic aminopyrimidines, by a collaborative effort of Medicinal Chemistry, Structural Biology and Computational Chemistry.

X-ray structures from CDK2 / aminopyrimidine inhibitor complexes led to the idea to stabilise the bioactive conformation of this inhibitor class by incorporating the recognition site into a macrocyclic framework. A modular synthesis approach relying on a new, late stage macrocyclization protocol that enables fast, efficient synthesis of macrocyclic aminopyrimidines was developed. A set of structurally diverse derivatives was prepared. Macrocyclic aminopyrimidines were shown to be potent inhibitors of CDK 1/2 and VEGF-RTKs. Potent anti-proliferative activities at various human tumor cells and in human tumor xenograft models were demonstrated.

Further on, the presentation gives an impression of ZK 304709, a pyrimidine-based Multi-target Tumor Growth Inhibitor™ which has been undergoing phase I clinical trials. ZK 304709 is a first-in-class oral Multi-target Tumor Growth Inhibitor™ (MTGI™), that blocks tumor proliferation and induces apoptosis by inhibiting a unique combination of tumorigenic mechanisms. This is accomplished by potent inhibition of: serine/threonine kinases CDK 1, 2 & 4 leading to inhibition of cell cycle progression, serine/threonine kinases CDK 7 & 9 leading to apoptosis in resting tumor cells, receptor tyrosine kinases VEGF-R1, R2 & R3, and PDGF-Rβ inhibiting tumor angiogenesis. The compound potently inhibits proliferation of various human tumor cells in the nanomolar range. Upon oral dosing of ZK 304709 significant tumor growth inhibition was observed in a variety of human tumor xenograft models.

The multi-targeted mechanism of action of ZK 304709 results in highly efficacious inhibition of growth of human tumor xenografts.

KL020

# Nitroxides in medicinal chemistry

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In last sixty years stable free radicals of nitroxide type appear in a different fields of science and technology. Their unique structure where the unpaired electron, located between nitrogen and oxygen, is stericaly shielded makes these radicals very stable. Great chemical stability of nitroxide radical enables a lot of chemical transformation of parent moiety without participation of radical.

At the beginning, nitroxides were mostly used in electron spin resonance (ESR) spectroscopy as spin labells or probes. One of the most important success of spin labelling method was its contribution to the explanation of membrane structure and dynamics. The synthesis of spin labelled analogs of biologically relevant molecules caused a significant progress in molecular biochemistry and furthermore, by spin labelled biologically active compounds and drugs the use of nitroxides in pharmacology and medicinal chemistry began.

Many sterical shapes of enzyme's active sites were described by spin labeled substrates or inhibitors before the crystal structure was determined by X-ray. Spin labelled drugs gave an important contribution to the understanding of drug action, to their interaction with different targets and with biological membranes. A step forward was done by combination of paramagnetic (spin) and fluorescence or radioactive isotope labelling.

In last two decades nitroxides are used in EPR imaging and they are probing as contrast agents in NMR imaging but more important and promising are their redox properties. They are intensively studied as SOD mimetic drugs and as potent antioxidants.

The role of nitroxides in medicinal chemistry will be illustrated by some selected examples of our recent results. Design and synthesis of:

-some amphiphilic nitroxides where nitroxide group is located in a polar region of the amphiphilic molecule. These compounds will be used as molecular tools to study motional changes in water region close to the membrane surface;

-spin labelled ligands for adenosine receptor. Their biological activity is presented and their potential for study adenosine receptors is discussed;

-spin labelled alkylphospholipids with antitumor activity and their interaction with lipid bilayer (molecular dynamics simulation), liposomes and cell membranes have been presented.

OP001

# Target vs. spectrum selectivity: Differential target profiles of the clinical Bcr-Abl inhibitors in chronic myeloid leukemia by chemical proteomics

**Uwe Rix,** Oliver Hantschel, Gerhard Durnberger, Lily L.R. Rix, Melanie Planyavski, Gregor Schutze, Nora V. Fernbach, Jacques Colinge, Thomas Kocher, and Giulio Superti-Furga

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The chimeric tyrosine kinase Bcr-Abl, which derives from the t(9;22) chromosomal translocation (Philadelphia chromosome), is causatively involved in the development of Chronic Myeloid Leukemia (CML). The current frontline therapy for CML, the tyrosine kinase inhibitor imatinib (Gleevec, STI-571), effectively and relatively specifically blocks Bcr-Abl kinase activity and is considered the paradigm for targeted therapy. Since many patients develop resistance to long-term imatinib therapy, two second-generation drugs that display increased potency, nilotinib (Tasigna, AMN107) and dasatinib (Sprycel, BMS-354825), have been developed. We set out to determine the respective target profiles in a disease-relevant cellular context. Using immobilized derivatives of imatinib, nilotinib and dasatinib, we performed a set of coherent chemical proteomics parallel screens using lysates from the Bcr-Abl positive cell line K562, as well as in patient-derived CML cells. We identified potential novel kinase and non-kinase interaction partners for each of the three drugs and characterized their target profiles. Informatic analysis of the collective drug-binder interaction networks of the three drugs displayed dramatic differences and a surprisingly low degree target overlap. Whereas nilotinib appears to bind only one other protein kinase beyond the Abl family, and is therefore judged to be quite target-selective, a total of 24 kinases are found in the dasatinib network. Clustering of the targets in pathway analysis gave insight into the biological processes likely to be affected by the rather multitargeted drug dasatinib, some of which were verified experimentally, suggesting possible side effects and/or second medical uses of the drug.

OP002

# Design, selection and biological evaluation of kinase focused libraries

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The human kinome comprises about 500 different kinases. Kinases play fundamental roles in many intracellular pathways (e.g. in cytokinesis, cell proliferation, differentiation, and apoptosis), therefore, they are involved in various diseases. There are significant efforts to identify selective inhibitors, thus, libraries focused towards particular kinases in the target family have become very attractive starting points in the screening campaigns [1].

In our focused library design approach first we extracted structures that inhibit various kinases with an activity threshold of 150 nM from publicly available databases and publications. In parallel, kinase-biased privileged structures were collected from the literature and the list was complemented with additional recurring substructures obtained after clustering and analyzing in-house the kinase inhibitor chemical space.

Since the central privileged cores interact with the hinge-region (ATP-binding domain) and also direct the selectivity mediating groups towards the front and back pocket, we extended the chemical space around the cores and developed a multistep procedure combining various 2D approaches to generate focused libraries.

First, applying the previously identified recurring structures we carried out an extended substructure search on our 260,000 non-exclusive repository containing 248 libraries with Markush structures allowing any kind of substituent and fused ring system variations. As a result, we selected more than 100 libraries. In the second step, this subset was applied in a series of similarity searches using subtype specific inhibitors collected (Tanimoto similarity limit was 0.7). The resulting libraries were between 300-2000 in size depending on the similarity threshold applied, and the purity limit was 85 % LC-MS.

In this way several kinase focused sets were generated including p38 MAP kinase, CDK2, PI3$\gamma$ kinase, Abl kinase, Akt kinase, Raf kinase, GSK$\beta$ kinase, JNK, IKK and various protein tyrosine kinases (PKA, PKB, PKC).

In our presentation we describe the major steps of our focused library generation together with the preliminary biological evaluation using low- and medium-throughput assays.

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OP003

# Protein-protein binding sites prediction by protein surface structure conservation

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A new method to predict protein-protein binding sites using conservation of both protein surface structure and physical-chemical properties in structurally similar proteins is developed [1]. Binding site residues in proteins are known to be more conserved than the rest of the surface and finding local surface similarities by comparing a protein to its structural neighbors can potentially reveal the location of binding sites on this protein. This approach, which has previously been used to predict binding sites for small ligands [2], is now extended to predict protein-protein binding sites. Examples of binding site predictions for a set of proteins, which have previously been studied for sequence conservation in protein-protein interfaces, are given. The predicted binding sites and the actual binding sites are in good agreement. Our method for finding conserved surface structures in a set of similar proteins is a useful tool for prediction of protein-protein binding sites and may be used in the design of new therapeuticals interfering with protein-protein interactions [3].

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OP004

# Comparative analysis of two different classifiers for predicting P-glycoportein substrates

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The human membrane-embedded ABC-transporter P-glycoportein (P-gp), which is a key player in the mediation of multi-drug resistance, represents an oncologically relevant anti-target. The multi-facetted recognition of structurally and functionally diverse drugs by this protein highlights the importance for the development of computer-assisted tools to predict P-gp substrates in order to lower the risk of failures in clinical trials.
In this study Binary QSAR (BQSAR), which is implemented in the MOE software package, is applied as a linear classification method and compared to Kohonen`s self-organizing maps (SOM) as implemented in the SONNIA neural network software. Two different data sets with equal representation of substrates and non-substrates have been generated. One consists of 258 compounds collected from the literature, the other embodies 240 compounds derived from the NCI60 cancer screen [1]. For both datasets 2D-van-der-Waals surface area (VSA) descriptors [2] and 2D-property weighted autocorrelation descriptors (2D-autocorr) [3] were calculated.

The cross-validated BQSAR models show in all cases a higher accuracy for prediction of non-substrates (96,2%) than for substrates (70,0%). In contrast, precision is higher for actives (93,9%) than for inactives (79,1%). The classification performed by the SOM shows almost equal performance, whereby the differences between accuracy on actives and accuracy on inactives are smaller. Interestingly, using SOMs as classifier, the NCI60 dataset performs better in classifying non-substrates, whereas it is the other way round for the literature dataset.
Supported by grant # B1-812074 from the FFG, Austria

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OP005

# Combatting the challenge of community-acquired methicillin-resistant staphylococcus aureus (CA-MRSA) from a topical racemic entity to an oral chiral prodrug

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The enormity of the challenge is succinctly communicated in the following editorial quote on the report of G.J. Moran [1] by M. L.Grayson [2]…"a landmark study that defines the amazing extent to which CA-MRSA…has spread through the US population." The spread and rise of outbreaks of MRSA outside of nosocomial settings is to be found not alone in the US, but world-wide, cutting across diverse populations and all ages, including previously healthy neonates and postpartum mastitis [3, 4]. CA-MRSA, the most common cause of skin and soft tissue infections, also tends to produce the Panton Valentine Leukocidin (PVL)-producing *S. aureus* strains, which are much nastier and more aggressive than other strains and can cause the highly lethal condition of necrotizing pneumonia [5, 6]. Necrotizing fasciitis, known as 'flesh-eating bacteria', is also associated with CA-MRSA [7].
The current therapy for CA-MRSA is antibiotic treatment. A continuing effort is on to improve its clinical management [8]. There is an urgent need for novel antibacterial agents for CA-MRSA, even as there is for hospital MRSA infections, especially those caused by multidrug-resistant pathogens.
This presentation will review the strategy the author, and the team he directed, put in place between 1997-2004 to discover novel drug entities against multidrug-resistant Gram-positive pathogens and how it sucessfully identified and validated (a) a topical racemic entity,named nadifloxacin, in a product for successful market introduction, (b) the arginine salt of its more potent chiral isomer as an agent active against Vancomycin-resistant *S. aureus* (VRSA) for use as an injectable, especially in a hospital setting [9] and (c) a prodrug of the active chiral isomer for use in a solid oral dosage form. The injectable has successfully cleared Phase II clinical trials. The oral chiral prodrug form has entered Phase I / early Phase II trials. All the three agents are highly valuable for the potential treatment of CA-MRSA. The presentation will especially highlight the unusual structural/physicochemical features of the racemic/chiral so-called "floxacin" in regard to its primary bacterial target, its propensity to resist selection of resistant mutants in view of its dual target properties, the correlation of its MPC/MIC ratios with the frequency of resistance emergence for MRSA, & its ability to withstand efflux by the NorA-mediated efflux in staphylococci.

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OP006

# Rational approaches to discover novel antimicrobial RNA ligands

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The bacterial ribosomal RNA (rRNA) is the main target of a chemically diverse group of substances, which are acting as translation inhibiting molecules [1]. To discover novel potential rRNA ligands with antimicrobial activity, we used different approaches to generate and characterise a pool of chemically diverse molecules.

A computer-assisted ligand-based virtual screening approach was carried out to obtain a set of 36 potential RNA ligands out of large compound libraries of approximately 430,000 substances.

Furthermore we accomplished a combinatorial chemistry approach to gain a sample pool of 26 basic peptides with different natural and non-natural amino acids [2]. Finally crude bacterial supernatants of different *Bacillus* strains, which are known to produce antimicrobial substances were isolated and screened for their transcription/translation inhibition potential. The activity of all generated samples was determined under cell-free conditions and against bacterial whole cells by different biochemical assays. Cell-free activity was determined via a batch *in-vitro* coupled transcription/translation inhibition assay based on an *E. coli* S30 extract. MIC values were obtained by fluorescence measurements of the reporter green fluorescent protein (GFP). The whole cell activity was determined in a microtitre broth dilution method with the gram negative *E. coli* A19 and the gram positive *B. subtilis* 168 model strains.

Most potent identified inhibitors showed an MIC in the lower μM range against whole cells and in the cell-free coupled transcription/translation experiment.

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OP007

# De novo molecular design: A powerful strategy for the discovery of new potential antimalarials

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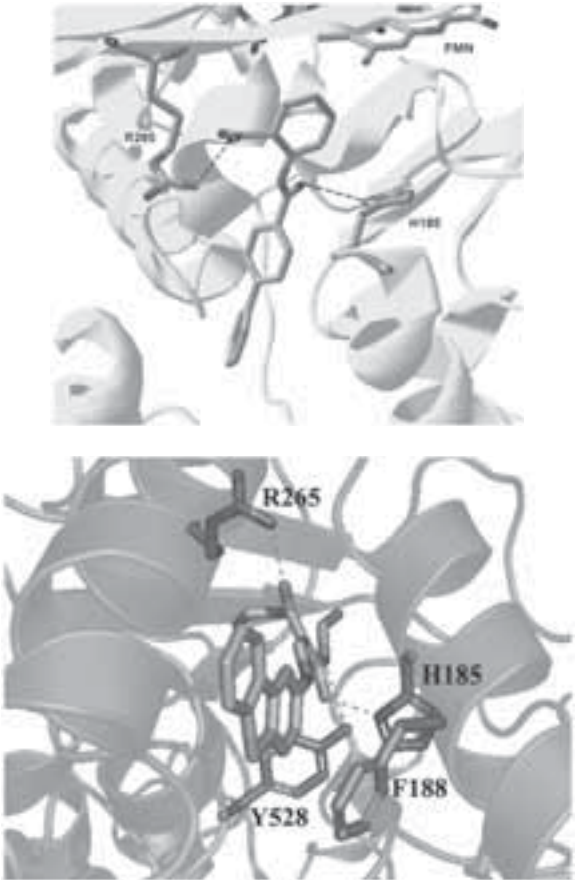
In silico molecular docking techniques such as virtual high-throughput screening (VHTS), is a powerful approach to the discovery of new enzyme inhibitors. However, this technique is limited by the size and diversity of the small-molecule databases used which, even for databases consisting of millions of compounds, can only sample a fraction of the available 'diversity space'. *De novo* design is a powerful complementary strategy for inhibitor discovery. Here, by using the structural features present within the enzyme only, new inhibitor designs are built-up sequentially according to the requirements of the targeted binding site. Therefore, *de novo* design is an important technique to use in parallel with VHTS in a particular hit identification campaign, as a good *de novo* design program will examine structure space larger by many orders of magnitude than that of most virtual libraries currently used for this purpose.

We have recently applied the *de novo* molecular design computer program SPROUT, developed at Leeds, to the rational design of inhibitors of dihydroorotate dehydrogenase (DHODH) from *plasmodium falciparum* [1, 2]. A particular feature of this work has been to develop inhibitors that are selective for either human or plasmodium DHODH. Two distinct classes of inhibitors have been produced (see Scheme below) and their efficacy for either human or plasmodium DHODH established using competition assays. The co-crystal structures of a number of inhibitors with DHODH have been obtained and confirm that these inhibitors closely obey the SPROUT design criteria when complexing with DHODH. Additionally, a number of inhibitors display useful anti-plasmodium activity and may represent promising starting points for the development of new antimalarials.

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OP008

# Ethenylbenzofuroxans actives against t.cruzi: in vitro and in vivo studies

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Chagas disease cause by *T. cruzi* affects to mostly 18 millions people in South America. Drugs currently used in the treatment of Chagas' disease are Nifurtimox and Benznidazole Both are active in the acute phase of the disease but efficacy is very low in the established chronic phase. What is more, differences in drug susceptibility among different *T. cruzi* strains lead to varied parasitological cure rates according to the geographical area. We have demonstrated that 5-ethenyl benzofuroxan derivatives possess high anti-*T. cruzi in vitro* activity against Tulahuen 2 strain. [1] Among all assayed benzofuroxans (Bfx) we selected three derivatives for further in vitro and in vivo studies (Figure 1). Because of the different strains susceptibility we evaluated the Bfx **1**, **2**, and **3** against another susceptible strain, Brener strain and a partially Nifurtimox and Benznidazole resistant strain, Y strain. We did not observe significant differences between different strains. Unspecific cytotoxicity against human macrophages was evaluated to investigate selectivity.
*In vivo* assays were done using also different strains, Tulahuen 2, Colombiana (a resistant to Nifurtimox and Benznidazole strain), wild strain isolated from weasel and from a Uruguayan patient. No signs of *in vivo* toxicity are observed with the benzofuroxans orally administered. Compound **1*E*** and the mixture of isomers **1*E*:*Z*** are the best for treating infection with Tulahuen 2, with wild strain isolate from weasel and from Uruguayan patient and with Colombiana strain. Compound **2*Z*** shows good behaviour against Colombiana strain. The mixture **3*E:Z(1:1)*** shows good behaviour against Tulahuen 2 and against Colombiana strains. After 65 days post-inoculation with Colombiana strain, animals treated with Benznidazole increase trypomastigote levels in blood. However, any of the animals treated with benzofuroxans (**1*E***, **2*Z***, **2*E***, and mixtures of **1*E:Z(1:1)*** and **3*E:Z(1:1)***) showed this behaviour.

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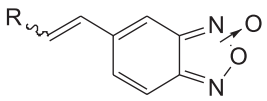


| R | Ref. | *In vitro* anti-*T. cruzi* activity ($IC_{50}$, μM) | | | $IC_{50}$ - THP-1 (μM) | TI [1] | | |
|---|---|---|---|---|---|---|---|---|
| | | Tulahuen | Brener | Y | | | | |
| **-Ph** | 1*Z* | **10.8** | **15.7** | **9.0** | **62.6** | **5.8** | **4.0** | **7.0** |
| | 1*E* | **11.2** | **7.5** | **6.2** | **109.9** | **9.8** | **14.7** | **17.7** |
| **3,4-$OCH_2O$-** | 2*Z* | **5.3** | **9.5** | **8.5** | **120.0** | **22.6** | **12.6** | **14.1** |
| | 2*E* | **2.9** | **9.5** | **7.3** | **220.0** | **75.9** | **23.2** | **30.1** |
| **-*p*-Cl-Ph** | 3*E:Z(1:1)* | **3.6** | **7.5** | **9.0** | **66.7** | **18.5** | **8.9** | **7.4** |

[1] Therapeutic Index = $IC_{50}$ mammal / $IC_{50}$ *T. cruzi*.

**Figure 1**

OP009

# Conceptual models of Presenilin-1 based on patterns of Alzheimer's disease mutations

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Presenilins (PS-1 and PS-2) are highly evolutionary conserved integral membrane proteins that – as a part of a large, multiprotein γ-secretase complex - cleave other transmembrane proteins, such as Notch receptor or β-amyloid precursor protein. Presenilins contain ten identified hydrophobic regions (HRs) in their primary structure and nearly all of them, except HR-7, were suggested to form transmembrane helices leading to many topological models of these proteins. Proteolytic processing of APP by the sequential action of β-secretase and γ-secretase releases amyloid-β peptides (Aβ) – highly aggregative components of senile plaques. Presently, more than 160 Alzheimer's Disease (AD) mutations is in the *PSEN1* gene.

We performed the analysis of mutation patterns [1] in all ten HRs using the most up-to-date information about AD mutations and we have built a conceptual model of PS-1 based on the distribution of these mutations. Linear patterns of mutations along each α-helix were reported [2], and nearly all novel mutations fulfilled this pattern so far. We tried to explain the reason why AD mutations fall into lines or more extended structures by building an appropriate model of PS-1 (Fig. 1). The model properly distinguishes residues belonging to AD-affected sites and non-pathogenic areas and may be used for classification purposes. It also complies with experimental results such as different accessibilities of the catalytic residues in PS-1 and binding of PEN-2 by the PS-1 HR-4 NF motif.

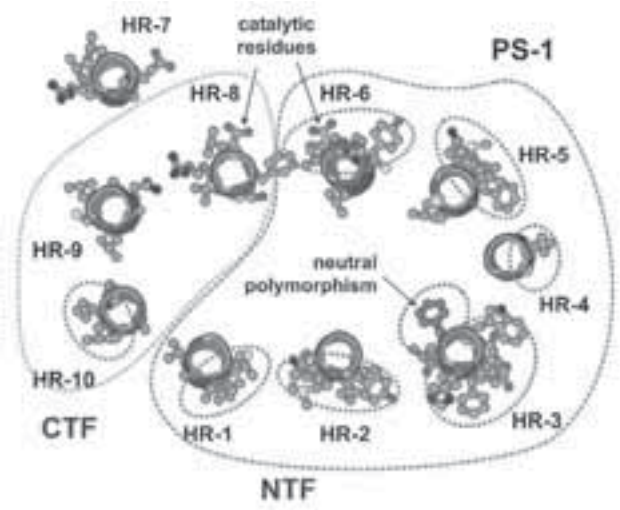


Fig. 1. The conceptual model of PS-1. Dashed red ellipses indicate concentrations of AD mutations on particular faces of helices. Dashed green ellipse denotes region containing neutral polymorphism.

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OP010

# Discovery of new pyrrolidone derivative with antiepileptic properties

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(S)-α-ethyl-2-oxo-pyrrolidine acetamide (Levetiracetam, Keppra®, UCB S.A.), a structural analogue of piracetam, has recently been approved as add-on treatment of refractory partial onset seizures in adults. This drug combines significant efficacy and high tolerability with unique mechanism(s) of action. A brain-specific binding site for Levetiracetam (LBS for **L**evetiracetam **B**inding **S**ite) has been reported and this probably exerts a major role in its antiepileptic properties. Using this novel molecular target we initiated a drug discovery program targeting the identification of ligands with significant affinity to LBS with the aim to characterize their therapeutic potential in epilepsy and other CNS diseases. [1]

Various positions of the pyrrolidone acetamide scaffold were systematically investigated. This revealed that: (i) the carboxamide moiety on Levetiracetam is essential for affinity, (ii) among 100 different side chains, the preferred substitution alpha to the carboxamide is an ethyl group with the (S)-configuration, (iii) the 2-oxo-pyrrolidine ring is preferred over piperidine analogues or acyclic compounds, (iv) substitution of positions 3 or 5 of the lactam ring decreases the LBS affinity and (v) 4-substitution of the lactam ring by small hydrophobic groups improves the *in vitro* and *in vivo* potency. Six interesting candidates substituted in the 4-position have been shown to be more potent anti-seizure agents *in vivo* than Levetiracetam.

Further pharmacological studies from our group led to the selection of (2S)-2-[(4R)-2-oxo-4-propylpyrrolidin-1-yl] butanamide (Brivaracetam) as the most interesting candidate. It is approximately 10 times more potent than Levetiracetam as an anti-seizure agent in audiogenic seizure-prone mice. A clinical Phase I program has successfully been terminated and Phase II trials are ongoing.

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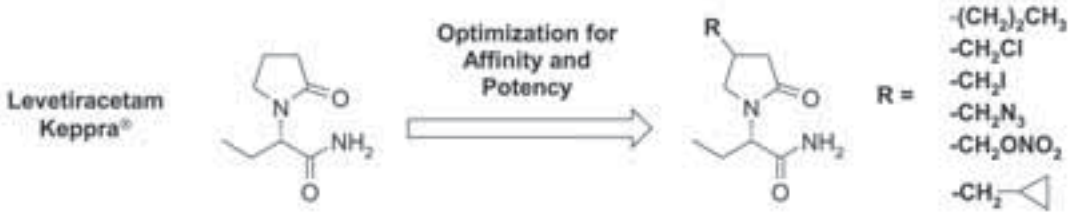

OP011

# (-)-HIP-A and (+)-HIP-B: investigation of the pharmacological profile and potential therapeutic applications

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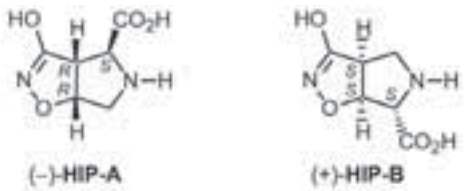
Glutamatergic synapses are involved in a large number of brain functions and an imbalance of the reuptake mechanisms operated by specific excitatory amino acid transporters (EAATs) could cause serious neurological and mental disorders (e.g. schizophrenia, epilepsy, ALS). Moreover, in pathological conditions in which energy levels fall and $Na^+$ transmembrane gradient collapses (e.g. ischemia, neurotrauma), EAATs release additional glutamate through the reversed mode of operation, thus contributing to neuronal cell death. This scenario candidates EAATs as molecular targets for the development of new CNS drugs [1-2].

The two conformationally constrained aspartate and glutamate analogues (±)-**HIP-A** and (±)-**HIP-B** were characterized as potent inhibitors of synaptosomal excitatory amino acid transporters (EAATs) [3]. Recently, we synthesized the two couples of enantiomers [(+)- and (–)-**HIP-A**, (+)- and (–)-**HIP-B**], and investigated their interaction with the different human EAATs subtypes expressed in HEK-293 cells as well as with native EAATs localized in rat brain synaptosomes. In both assays, the two stereoisomers having (*S*) configuration at the amino acidic center, i.e. (–)-**HIP-A** and (+)-**HIP-B**, turned out to be more potent EAAT inhibitors than their (*R*) counterpart.




Accordingly, (–)-**HIP-A** and (+)-**HIP-B** were selected for further pharmacological investigations to evaluate their potential therapeutic applications for the treatment of neurodegenerative and neurological disorders. In particular, experiments aimed at testing their neuroprotective activity in an in vitro model of cerebral ischemia, and their anticonvulsant properties in DBA/2 mice are in progress. In addition, following the recent publication of the crystal structure of a glutamate transporter from *Pyrococcus horikoshii* [4], we are at present building up homology models of rat EAATs aimed at understanding the molecular interactions of our ligands with relevant amino acid residues localized in the transporter binding pocket. The pharmacological results as well as the molecular modeling studies will be presented and discussed.

## References

[1] Mennini, T. et al. Eur. J. Pharmacol. 2003, 479, 291-296.
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OP012

# Endomorphin analogs with alicyclic β-amino acids

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Endomorphin (H-Tyr-Pro-Trp-Phe-$NH_2$, H-Tyr-Pro-Phe-Phe-$NH_2$) [1] analogues have been engineered to possess proteolyticly stable character with the retention of mu-opioid receptor agonist property. In this study, we aimed to synthesize and pharmacologically characterize numerous endomorphin analogs incorporated racemic 2-aminocyclopentane- (ACPC) and 2-aminocyclohexane-(ACHC) carboxylic acids in the position 2. Peptide surrogates were synthesized manually using Boc-chemistry. Diastereomer peptides were identified and purified by RP-HPLC. The potencies of analogs were tested by competitive displacements against the highly selective [$^3$H]DAMGO for mu-receptor and the delta-specific [$^3$H]Ile$^{5,6}$-deltorphin-2 using rat brain membrane homogenate. Data are summarized below:

| Peptide sequence | $K_i^{\mu}$ (nM) | $K_i^{\delta}$ (nM) | $K_i^{\delta}/K_i^{\mu}$ (nM) |
|---|---|---|---|
| H-Tyr-Pro-Trp-Phe-$NH_2$ | 0.74 ± 0.03 | 1909.3 ± 232.6 | 2579 |
| H-Tyr-Pro-Phe-Phe-$NH_2$ | 1.3 ± 0.2 | 5651.7 ± 202.1 | 4346 |
| H-Tyr-(1S,2R)ACPC-Trp-Phe-$NH_2$ | 3.6 ± 0.3 | 1341.3 ± 122.8 | 372 |
| H-Tyr-(1S,2R)ACHC-Phe-Phe-$NH_2$ | 2.4 ± 0.17 | 812.1 ± 17.7 | 338 |
| H-Tyr-(1S,2R)ACPC-Phe-Phe-$NH_2$ | 2.4 ± 0.09 | 4798 ± 310.6 | 1999 |
| H-Tyr-(1R,2S)ACPC-Trp-Phe-$NH_2$ | 274.5 ± 41.3 | 17700 ± 14610 | 64 |
| H-Tyr-(1R,2S)ACPC-Phe-Phe-$NH_2$ | 4435.5 ± 645 | 40693 ± 12390 | 9 |
| H-Tyr-(1R,2S)ACHC-Trp-Phe-$NH_2$ | 741.6 ± 61 | 48460 ± 16645 | 65 |
| H-Tyr-(1R,2S)ACHC-Phe-Phe-$NH_2$ | 1984 ± 193.6 | 17200 ± 11179 | 8 |

Results showed that *cis*-(1S,2R) analogs retained mu-receptor affinity but their receptor selectivities got worse compared with their parent ligands. It was interesting that H-Tyr-(1S,2R)ACHC-Phe-Phe-$NH_2$ and H-Tyr-(1S,2R) ACPC-Phe-Phe-$NH_2$ displayed similar affinities for labeling mu-receptor though they disposed with diverse selectivities for delta-binding sites. In addition, *cis*-(1R,2S) analogs presented worse affinities for both mu- and delta-binding sites with decreased selectivities. [$^{35}$S]GTPγS experiments uniformly proved the agonist character of some of these peptides [2]. The most promising analogs were investigated in their proteolytic stability in rat brain homogenate and these peptides were practically enzyme-resistant analogs (half life:>12 hours).

## References:

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OP013

# Enantioselective interactions of stereoisomers of fenoterol derivatives with the β2 adrenergic receptor

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Stereoisomers of fenoterol and six fenoterol derivatives (Fig.1.) have been synthesized and their binding affinities for the $\beta_2$ adrenergic receptor ($\beta_1$–AR) and the subtype selectivity relative to the $\beta_1$–AR were determined. Of the 26 compounds synthesized in the study, sub-micromolar binding affinities were observed for (R,R)-fenoterol, the (R,R) isomer of the *p*-methoxy, and (R,R) and (R,S) isomers of 1-naphthyl.

The binding data was analyzed using comparative molecular field analysis (CoMFA) and the resulting pseudoreceptor model identified three separate binding fields, two of them were responsible for asymmetric recognition of chiral centers. The steric restriction on the second chiral center is responsible for observed $\beta_2/\beta_1$ selectivity [1]. To complement the pseudoreceptor model, the analysis of the ligand-receptor interactions was performed using *de novo* model of the $\beta_2$-AR and $\beta_1$-AR [2]. Docking simulations revealed that the following residues form the network of hydrogen bonds interacting with the catechol moiety: S204, S207 and T118. Residues N293 and D113 are mainly affecting the asymmetric recognition of diastereoisomers. On the other hand, T308 and H296 are responsible for $\beta_2/\beta_1$ subtype selectivity profile of studied compounds. The role of these residues was probed and evaluated in molecular dynamics simulations of ligand – receptor complexes.

The aim of the project is to develop an approach, which could be used to guide the design of new selective agents for use in the treatment of astma and congestive heart failure.

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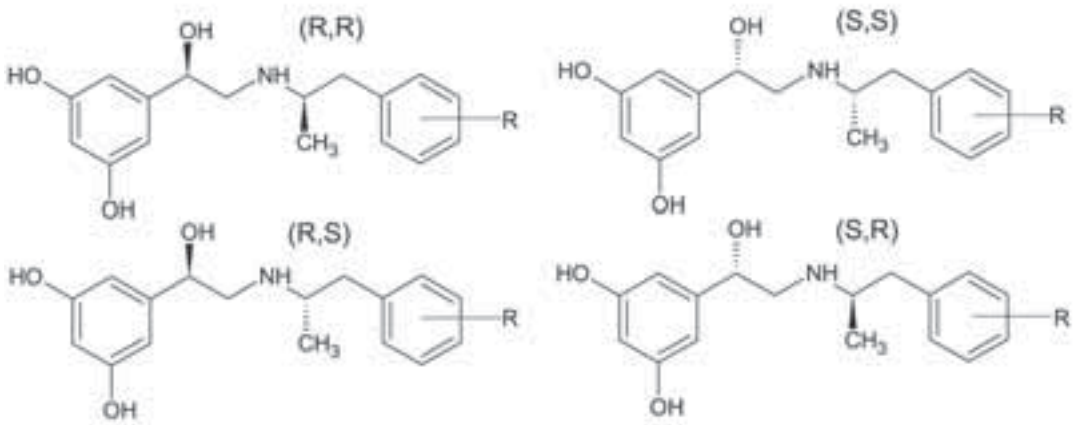


Fig. 1. Structures of studied compounds.

OP014

# Novel aldose reductase acetic acid inhibitors bearing a five membered heterocyclic core

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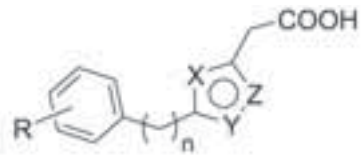
Diabetes is a metabolic disorder that affects a significant portion of the population worldwide and it is nowadays recognized as a public health problem. It seriously affects a person's quality of life and, even if it can be successfully controlled by the administration of insulin and/or potent oral hypoglicemics, it still remains the cause of significant morbidity and mortality, due to a progressive development of disabling complications regarding nervous, renal, cardiovascular and visual sistems. Experimental and clinical evidence demonstrates that the pathogenic mechanism leading to these complications is causally linked to an increased activity of the enzyme Aldose Reductase (alditol:NADP+ oxidoreductase, EC 1.1.1.21, ALR2). Therefore, inhibition of ALR2 is a useful therapeutic strategy to prevent the onset or at least delay the progression and the severity of diabetic complications. Many compounds have been shown to inhibit the enzyme with various degrees of efficacy and specificity. However, promising products during in vitro studies or in trials with animal models often fail to proceed any further, due to low efficacy or adverse side effects mainly attributed to pharmacokinetic problems and lack of specificity for the target enzyme. The attention of the medicinal chemistry is therefore continuously directed towards the discovery of more specific and clinically effective inhibitors. Our well documented interest in the ALR2 inhibitors field [1-3] led us to develop a series of five membered heterocyclic acetic acid derivatives as potential active compounds. Here we present the synthesis, the structure-activity relationships and an extensive biological evaluation of a number of oxazole, [1,2,4]oxadiazole and [1,2,4]triazole derivatives, which proved to be effective inhibitors of ALR2 showing $IC_{50}$ values in the micromolar/submicromolar range.




n : 0, 1
X, Y, Z : N, O, CH
R : H, $CH_3$, $OCH_3$, $NO_2$, F, Br, $CF_3$

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[1] Da Settimo, F.; Primofiore, G.; La Motta, C.; Sartini, S.; Taliani, S.; Simorini, F.; Marini, A.M.; Lavecchia, A.; Novellino, E.; Boldrini, E. Journal of Medicinal Chemistry, 2005, 48, 6897-6907. [2] Da Settimo, F.; Primofiore, G.; Da Settimo, A.; La Motta, C.; Simorini, F.; Novellino, E.; Greco, G.; Lavecchia, A.; Boldrini, E. Journal of Medicinal Chemistry, 2003, 46, 1419-1428. [3] Da Settimo, F.; Primofiore, G.; Da Settimo, A.; La Motta, C.; Taliani, S.; Simorini, F.; Novellino, E.; Greco, G.; Lavecchia, A.; Boldrini, E. Journal of Medicinal Chemistry, 2001, 44, 4359-4369.

OP015

# An approach to construction of diagnostic chips for thyroid cancer.

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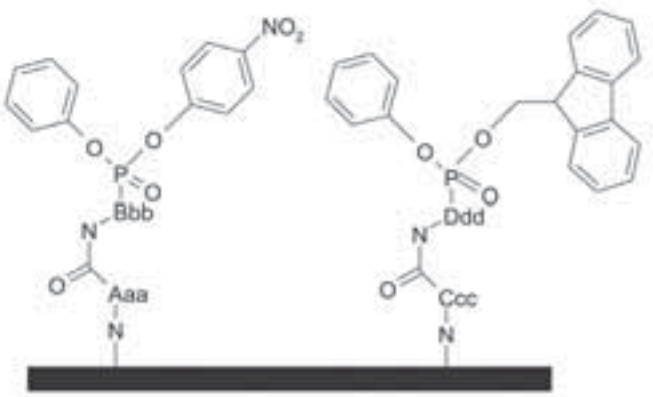
After diabetes, thyroid disease is the most common glandular disorder with thyroid cancer, albeit relatively rare, progressively increasing each year. Thyroid cancer can occur in any age group, although it is most common after age of 30 and its aggressiveness increases significantly in older patients, with females being more likely to have this cancer at a ratio three to one. Diagnostics of types of thyroid cancer relays on histological analysis done after surgeries of invaded tissue and therefore it is rather postoperative than preoperative.

During cancer development significant biochemical imbalance of the cells is observed, which gives rise in the level of various enzymes and metabolites. Our approach for construction of diagnostic chips for evaluation of cancerous state of thyroid cells bases on the use of fine-needle biopsy to study the profile of proteolytic enzymes present in diagnosed tissues. For this purpose libraries of potential phosphonate and phosphono peptides inhibitors carrying out various fluorescent groups were synthesized and arranged onto solid supports (Scheme) or applied in small microreactors, thus forming precursors of diagnostic tools for thyroid cancer evaluation. Preliminary results indicate that the observed pattern of fluorescence might be characteristic to certain types of cancer.



PO001

# The discovery of 2-amino-3,5-diarylbenzamide inhibitors of IKK-α and IKK-β kinases

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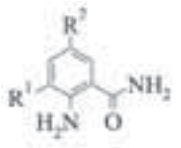
The IκB (IKK) family of kinases represent an area of intense research since they are central regulators of NF-κB transcription factors, controlling gene expression in innate and adaptive immune responses. The most widely studied family member to date is IKK-β which is ubiquitously expressed and mediates activation of NF-κB p50/RelA in response to pro-inflammatory stimuli such as tumour necrosis factor-α (TNF-α) and lipopolysaccharide (LPS). The role of this canonical NF-κB pathway is well documented in chronic inflammatory disease. More recently a non-redundant role for IKK-α has been elucidated in mediating signal transduction from TNFR family members, such as co-stimulation receptors CD40 and BAFFR on B lymphocytes. Several observations also suggest that IKK-ε might be involved in the regulation of transcription factors, such as NFκB, IRF3 and C/EBP, all of which are known to be involved in the regulation of pro-inflammatory cytokines.

The identification of isoform selective IKK inhibitors will provide pharmacological reagents to further address the differential role of these kinases in health and disease. The discovery of 2-amino-3,5-diarylbenzamide inhibitors of IKK-α and IKK-β kinases is presented.



PO002

# Fluorinated 2-phenylbenzoxazoles as potent and selective antitumour agents.

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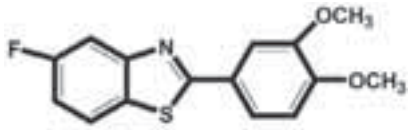
GW 610 {2-(3,4-dimethoxyphenyl)-5-fluorobenzothiazole} is a simple planar 2-phenylbenzothiazole derivative with remarkable in vitro antitumour properties.[1]
GW 610 was found to possess exquisitely potent antiproliferative activity in certain human cancer cell lines (e.g. $GI_{50}$ <0.1 nM for MCF-7 and MDA 468 breast cell lines), and potent and selective activity in the NCI 60 human cancer cell panel.




**GW610**

In order to generate more water soluble analogues of GW 610 (LogP 4.21) for in vitro and in vivo antitumour studies, we have synthesised the benzoxazole version of GW 610 (logP 2.84) plus a range of 2-arylbenzoxazole analogues, using the methodology developed by Evindar and Batey[2], and illustrated below.
These compounds were tested *in vitro* against cancer breast, colon and lung cell lines MCF-7, MDA 468, HCT 116, HT29, A549 and showed impressive results.
The synthesis will be extended to incorporate solution-phase parallel synthesis methods and synthesis of an analogue library to establish the viability and scope of the synthetic approach.

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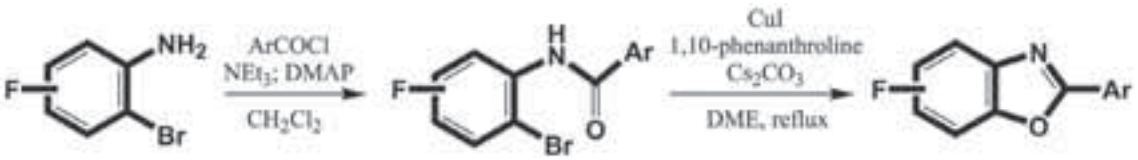


**Ar**=(methoxy)phenyl

PO003

# Diarylureas as Maxi-K potassium channel openers.

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The k channel superfamily is divided into a number of subfamilies based on molecular structure and function. An important subfamily is a group of K channels regulating by the intracellular concentration of the calcium ion. On the base of single channel conductance, the K channels are again subdivided into three types: BK (Maxi), IK (Intermediate), SK (Small). The BK are ubiquitously expressed and they are the target of different molecules [1]. Activation of BK channels with small molecules can provide the opportunity for therapeutic intervention for conditions such as stroke, brain injury, urinary incontinence, epilepsy etc..In the last years part of our studies concerned on different substituted 1,2,3 triazoles and series of benzanilides [2,3] [fig.A, B], from which a pharmacophoric model was optimised [fig.C]. Regarding the importance of a H bond donor group in the linker area and of a symmetric structure of the molecules [4], we considered a large series of benzanilides [fig.B] synthetized in our lab. and we introduced another –NH group to give a series of symmetric and asymmetric diarylureas [fig.D], which were tested in vitro on endothelium-denuded rat aortic rings. The first pharmacological results were more interesting for the activity on BK channels.

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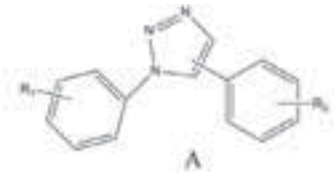
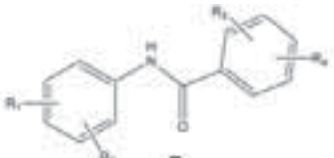


A



B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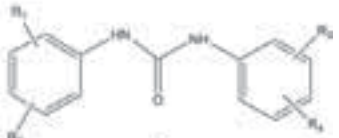

D

PO004

# Restorative effects of sigma1 receptor ligands in a rat model of cholinergic loss, amyloid deposition and cognitive dysfunction

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Sigma-1 agonists have recently been reported to improve cognitive abilities in experimental animals with Alzheimer's Disease (AD)-like symptoms induced by either neurotoxic lesions, cholinergic receptor blockade or intraventricular injections of amyloid peptides. Although extensively used, however, none of these approaches recapitulate the critical features of AD (i.e. amyloid and tau pathology, cholinergic neuronal degeneration and cognitive deficits), nor do they allow to address the possible relationships between the various hallmarks of the disease. In the present study, we have introduced a novel murine model of Alzheimer disease based on the i.c.v infusion of a powerful and selective cholinergic immunotoxin, 192 IgG-Saporin, coupled with the intrahippocampal injection of aggregated $ß_{25-35}$ amyloid peptide. The treatment induces a specific decline in spatial memory associated to a dramatic cholinergic neuronal and terminal fiber loss and histopathological changes reminiscent of the extracellular deposits of amyloid plaques in hippocampal and neocortical areas of AD patients.

The sigma1 ligand methyl (1S,2R)-2-{[1-adamantyl-(methyl)amino]-methyl-1-phenilcyclopropanecarboxylate [(-)MR22] is a newly synthesised agonist selective for the sigma1 receptor that has been observed to protect cortical neurons from amyloid-induced toxicity *in vitro*, and whose functional efficiency *in vivo* has never been tested so far. In preliminary investigations, we have observed (-)MR22 (1 mg/kg i.p.) able to efficiently reverse the severe 192 IgG-saporin- or atropine-induced deficits in spatial learning and memory, an effect which was abolished by treatment with the sigma receptor antagonist BD1047. These initial findings confirm the memory-enhancer role proposed for the sigma1 agonists on animals with learning impairments. Further analyses are presently underway. Data will be presented concerning the possible protective action of the (-)MR22 ligand on 192 IgG-saporin- and $ß_{25-35}$ amyloid-induced anatomical and functional impairments, so as to better define the therapeutic potential of such compound.

PO005

# Computational approaches to synthetic design of new neuroactive glutamate receptor ligands

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Specific blockade of calcium ions influx via activated NMDA-specific subtype of glutamate receptor (NMDAR) provides efficient protection against diverse group of neurological disorders related to glutamate excitotoxicity. From the other hand, an activation of another subtype of glutamate receptor – AMPA-receptors (AMPAR) can improve deficit of cognition functions typical to different neurodegenerative diseases, in particular, Alzheimer's disease (AD). Earlier it was revealed that model NMDAR antagonist agent MK-801 shows strong neuroprotective properties in cell culture experiments, but it therapeutic potential is totally diminished by remarkable side psychotomimetic effects, associated with its high-affinity interaction with the intra-channel binding site of NMDAR. In contrast to MK-801 the low-affinity open-channel blockers, such as memantine show better therapeutic indices due to their rapid re-binding kinetics and strong voltage dependency. The goal of the present study was to provide: 1) computational design and synthesis of novel bioisosteric analogs of model neuroprotector MK-801 more flexible towards NMDAR; 2) functionalization of developed structures by farmacophores possessing AMPAR-potentiating activity; 3) comparative docking of MK-801 and its bioisosteric analogs binding to NMDA- and AMPA-receptors 4) study of behavioral, in particular, cognition-enhancing properties in series of novel original compounds. As a result of this study novel group of neuroactive compounds with strong cognition-enhancing properties in series aryl-substituted isothiuroniun derivatives was developed. The lead-compound exhibited very promising pharmacological profile, in particular: anti-NMDA activity (ED50 ≈10 mg/kg); positive modulation of AMPA receptors (ED300 ≈ 20 μM); cognition enhancer properties in neurotoxicological animal models of AD (ED50 ≈ 1.0 mg/kg); no MK-801-like side psychotomimetic effects in parallel to low acute toxicity (LD50 >1g/kg).

PO006

# Synthesis of 3-[1-(3-pyridazinyl)-5-phenyl-pyrazole-3-yl]propanoic acids towards dual inhibition of 5-LOX/COXs

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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. As a safer approach, dual inhibition of cyclooxygenases (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 [1]. Licofelone is the only molecule of this new class in the clinical trials and preliminary data seem to be promising. In addition, there is an evidence that 5-LOX and COX-2 were shown to be involved in cellular proliferation processes and indicated that dual inhibition of these enzymes opens up new doors for treatment of certain types of cancers [2].

These finding have stimulated us to develop a project for designing dual COX and 5-LOX inhibitors which might 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-inflammatory agents, we required the synthesis of 3-[1-(6-substituted-pyridazin-3-yl)-5-phenyl-pyrazole-3-yl]propanoic acids (Figure 1). Among the synthesized compounds, TEP-122 showed nonselective COX inhibition with a selectivity index of 0.93 ($IC_{50}$COX1=1,5 μM, $IC_{50}$COX2=1,6 μM), whereas compounds TEP-42 and TEP-72 resulted in the inhibition of 5-LOX at 14 μM and 12 μM $IC_{50}$ values, respectively.

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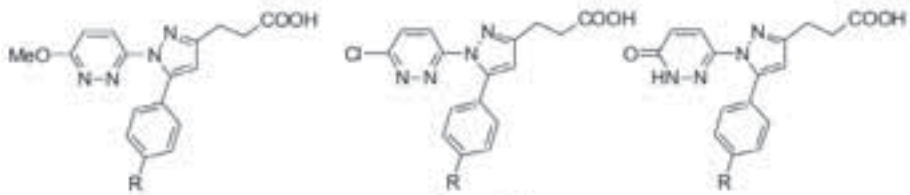


Figure 1

PO007

# Phosphinic and phosphonic peptides – novel inhibitors of urease

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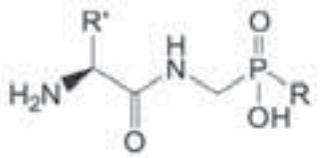
Urease (E.C. 3.5.1.5) is an enzyme which catalyses hydrolysis of urea into ammonia and carbamate [1]. This protein is crucial for existence of *Helicobater pylori* bacteria in urinal and digestion tracts [2], as urea hydrolysis causes the increase of local pH and forms microenviroment suitable for bacterial survival. This pathogen causes stomach ulcers and finally stomach cancer. Thus, inhibitors of urease are potential drugs against these diseases. Among several known inhibitors of urease, phosphoramidates are the most potent. Phosphorus acid diamide — enzymatic reaction transition state analogue is the simplest example of this group of compounds. The main disadvantages of this class of compounds are their low stability in aqueous solutions and their low selectivity. In this paper we present novel class of urease inhibitors — phosphonic and phosphinic peptides. These compounds are hydrolytically stable analogues of phosphoramidates. Moreover, their structure allows several modifications in order to enhance both inhibitory activity and selectivity towards pathogen protein. Several different peptidic structures were synthesized and evaluated towards bacterial urease showing their interesting inhibitory activities.

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PO008

# Proline-linked nitrosoureas as prolidase-convertible prodrugs in human breast cancer cells.

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2-Chloroethylnitrosoureas belong to the alkylating antitumour agents and some of them have found application for the treatment of human cancer, mainly lymphomas, gliomas, a few solid tumours and melanomas. More recently, they have been incorporated into multiagent high-dose chemotheraphy regimens with stem cell support in patients with breast cancer, neuroblastoma, glioma, melanoma and sarcomas. Despite their broad antitumor activity, the clinical usefulness of nitrosoureas has been limited by delayed-onset, cumulative myelosuppression and pulmonary toxicity. Therefore, although the most widely used compound in this class, carmustine, was approved for clinical use in the mid 1960s, efforts to develop new analogues with a better therapeutic index have continued to the present day.

In the present study, we report the synthesis and biological evaluations of four new alkylating agents utilizing L-proline derivatives as carriers of the nitrosourea cytotoxic group. We hypothesized that coupling of L-proline through imido-bond to anticancer drugs might create prodrugs which would be locally activated by tumor-associated prolidase and consequently would be less toxic to normal cells that evoke lower prolidase activity. This strategy should be of benefit particularly in case of antineoplastic prodrugs, since at least some tumor tissues evoke increased prolidase activity compared to normal tissues. A number of novel proline-linked nitrosoureas (**1-4**) were synthesized and examined for cytotoxicity, DNA and collagen biosynthesis in MDA-MB-231 and MCF-7 human breast cancer cells. Evaluation of the cytotoxicity of these compounds employing a MTT assay and inhibition of [$^3$H]thymidine incorporation into DNA in both MDA-MB-231 and MCF-7 breast cancer cells demonstrated that compound **2,** the most active of the series, proved to be only slightly less potent than carmustine. It has also been found that carmustine no inhibited MCF-7 cells prolidase activity, while compounds **1-4** significantly increase the activity, when were used at 50-250 μM concentrations. Proline-linked nitrosoureas (**1-4**) also evoked lower ability to inhibit collagen biosynthesis in MCF-7 cells, compared to carmustine. The expression of $\beta_1$-integrin receptor, as well as phosphorylated MAPK, $ERK_1$ and $ERK_2$ were significantly decreased in MCF-7 cells incubated for 24 h with 60 μM of **2** and **4** compared to the control, not treated cells, whereas in the same conditions carmustine did not evoke any changes in expression of all these signaling proteins, as shown by Western immunoblot analysis. Treatment of the cells with proline-linked nitrosoureas decreased expression of IGF-I receptor in MCF-7 lines. IGF-I receptor is involved in cellular transformation, mitogenesis and inhibition of apoptosis. Therefore inhibition of the receptor may represent approach to the inhibition of tumor growth. Blockade of the receptor or down regulation of its expression reduces cancer proliferation and induces apoptosis.These results indicate the proline-linked nitrosoureas, represent multifunctional inhibitor of breast cancer cells growth and metabolism.

PO009

# Chemoenzymatic synthesis of the nucleotide substrates of the mur ligases

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The Mur ligases catalyze the synthesis of the peptide moiety of bacterial peptidoglycan by the successive attachments to UDP-*N*-acetylmuramic acid (UDP-MurNAc) of L-Ala (MurC), D-Glu (MurD), *meso*-$A_2$pm or L-Lys (MurE of Gram-negative or Gram-positive bacteria, respectively), and D-Ala-D-Ala (MurF). Owing to the essential and specific character of this macromolecule for the bacterial cell, they constitute promising targets for the design and synthesis of new antibacterial compounds.

The Mur ligases have three substrates: i) ATP, ii) an amino acid or dipeptide, and iii) a nucleotide precursor consisting of UDP-MurNAc linked or not to an amino acid or a peptide. The study of the Mur ligases is hampered by the fact that the nucleotide precursors are not commercially available. In this communication, we wish to present an improved chemical synthesis of UDP-MurNAc and the enzymatic synthesis of the other nucleotide precursors.

UDP-MurNAc was synthesized at the gram scale by the procedure of Dini *et al.* [1] with modifications. The synthesis started from D-glucosamine which was transformed into protected muramic acid. The use of other protecting groups for muramic acid improved the yields of crucial reaction steps and made the total synthesis more straightforward. The purification of UDP-MurNAc was achieved by gel filtration.

Starting from synthetic UDP-MurNAc, the other nucleotide precursors were synthesized at 50-mg scale using purified Mur ligases [2]: UDP-MurNAc-L-Ala (*E. coli* MurC), UDP-MurNAc-L-Ala-D-Glu (*E. coli* MurC + MurD), UDP-MurNAc-L-Ala-γ-D-Glu-*meso*-$A_2$pm (*E. coli* MurC + MurD + MurE) and UDP-MurNAc-L-Ala-γ-D-Glu-L-Lys (*E. coli* MurC + MurD + *T. maritima* MurE). They were purified from the reaction mixtures in one step by gel filtration on Sephadex G-25 in water [3] ; yields were >80% in all cases.

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PO010

# Structural and Docking studies of new cytisine derivatives.

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Neuronal nicotinic acetylcholine receptors (nAChRs) form a family of pentameric ACh-gated ion channel, made up of different subtypes ($\alpha_1$-$\alpha_{10}$, $\beta_1$-$\beta_4$, $\delta$, $\gamma$, $\varepsilon$) each of which has a specific pharmacology, physiology and anatomical distribution in brain and ganglia [1,2]. An homology model of the human $(\alpha_4)_2(\beta_2)_3$ ligand-binding domain (LBD), in its agonist-bound conformation, has been built up based on the X-ray structure of the acetylcholine binding-protein (AChBP) [3] allowing the docking of numerous cytisine derivatives (in their neutral and protonated forms) into the binding pockets of this structural model [4]. For N-[(3-benzoyl)-ethyl]cytisine and N-Phenylethylcytisine their X-ray structures have been used. The docking results allowed the detection of a cluster of aromatic amino-acid residues belonging to the alpha and beta subunits essential for the pharmacological selectivity of nAChR. This novel structural framework could be useful for the development of new $(\alpha_4)_2(\beta_2)_3$ selective ligands.

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PO011

# Versatile prediction of selectivity profiles using Bioprint® QSAR models

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Reducing the attrition rates of the NCEs all along the multiple stages of a drug development process remains one of the main challenges for the drug discovery community. In this context, starting with a clean selectivity profile at the very beginning of the R&D phases is one additional guarantee to further get safer compounds.
Bioprint® is a unique collection of about 2400 compounds – marketed drugs, failed compounds, reference and discovery compounds - and more than two million individual data points obtained at CEREP on a set of binding, enzymatic, cellular and *in vitro* ADME-Tox assays. These data were used to build a series of highly reliable QSAR models. [1, 2]

We describe herein the use of several QSAR Bioprint® models during the early optimisation stages of a privileged structure, applied to the selection of the most promising compounds from the standpoint of their selectivity profile. The *in silico* predicted selectivity profile was compared to the *in vitro* experimentally assessed one. The predictions were in excellent agreement with the experimental data and the QSAR Bioprint® models were used as a powerful selection tool for further optimisation programs.

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[2] Gozalbes et al QSAR Comb. Sci., 2005, 24, 508-516

PO012

# Chemical composition and antioxidant potential of ruta montana Essential Oil from Algeria

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**Key Word** - *Ruta montana* L., Essential oil, Undecan-2-one, Antioxidant, DPPH radical, cytométry.

The essential oil of aerial parts of *Ruta montana* growing in the Oran region in the west of Algeria was obtained by hydrodistillation with a 1.63% yield on a dry weight basis. The GC and GC–MS analyses were carried out to identify the chemical composition of *R. montana* essential oil. Moreover, we have studied the antioxydant activity of the Essential oil by the technique of the flow cytometry and the spectrophotometric analyses using the DPPH test. Twenty compounds were identified by GC and CG–MS analyses and the main of compounds of the oil were undecan-2- one (32.81%), nonan-2-one (29.54%), nonanol-2- acetate (18.20%) and psoralen (3.52%). The results obtained using the DPPH test show that *R. montana* essential oil possess antiradical activity in a concentration-dependent manner. Thus, a linear correlation (correlation coefficient $R^2$=0.971, $p<0.001$) was found between the reduction of 2,2-diphenyl-1-picrylhydrazyl (DPPH) stable free radical and the concentration of *R. montana* essential oil.. Reactive oxygen species (ROS) can be generated during diverse biological and cellular reactions either positively for biological activities or negatively resulting in toxicity.

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PO013

# Constrained peptide inhibitors of cysteine cathepsins

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Bacteriophage display library [1] of cyclic random nonapeptides (type $CX_7C$, where the two bordering cysteines form an intramolecular disulphide bond) was affinity selected against plant protease papain. Selections resulted in enrichment of closely related peptides with the general motif CW(T/S)L(G/A/I/V/L)GY(K/H)C [2].

Some of the peptides recovered from affinity selections were synthesized and determined to inhibit model target enzyme papain as well as human cathepsins L, H, and K with the $K_i$ values in the low to mid micromolar range. Reduced (acyclic) forms of peptides had markedly lower inhibitory activity. Additionally, an undecapeptide analog GNWTLGGYKGG based on the common motif with tryptophan preceded by asparagine (a feature of a number of selected peptides) and cyclized head-to-tail was synthesized and found to selectively inhibit cathepsins L and K with the $K_i$ values in the mid nanomolar and low micromolar range, respectively.

Finally, amino acid sequence NWTLGGYK with N- and C-terminal alanyl or glycylalanyl spacers was grafted to a loop of small but extremely stable cysteine-free helical protein (IgG-binding domain B of staphylococcal protein A (SpA) (Figure 1)) using recombinant DNA technology [3]. The two recombinant constructs bound to and selectively inhibited cathepsin L with $K_i$ of 14 and 6 μM, respectively, while retaining the ability to bind the Fc-region of IgG, indicating that SpA IgG-binding domains with engineered additional functionalities, such as the ones reported here, might find use in *in vitro* diagnostic immunological assays.

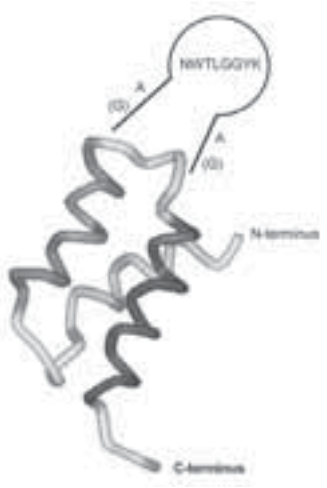


**Figure 1**: Schematically depicted structure of recombinant fusion proteins with peptide sequence (G)ANWTLGGYKA(G) replacing four amino acids forming the second loop of domain B. Seven N-terminal amino acids were excised from the scaffold protein in order to allow solvent exposed display of fused peptide loop.

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PO014

# New inhibitors of human hydroxysteroid dehydrogenase AKR1C1

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The hydroxysteroid dehydrogenases (HSDs) are enzymes implicated in regulation of the local concentrations of the active steroid hormones at the pre-receptor level. They act as molecular switches, which interconvert active forms of steroid hormones with high affinities and inactive forms of steroid hormones with very low affinities towards their corresponding receptors [1, 2]. HSDs belong either to the short-chain dehydrogenase/ reductase (SDR) superfamily or to the aldo-keto reductase (AKR) superfamily [2]. Four human AKRs, AKR1C1-AKR1C4, can function in vitro as 3-keto-, 17-keto- and 20-ketosteroid reductases, and as 3α-, 3γ-, 17β- and 20α-hydroxysteroid oxidases to varying degrees. In intact cells, all of these AKR1C isozymes preferentially work as reductases and regulate the occupancy and *trans*-activation of the androgen, estrogen and progesterone receptors [3]. We have focused our attention to AKR1C1, which acts preferentially as a 20α-HSD and inactivates progesterone by converting it to 20α-hydroxyprogesterone. It also converts 5α-tetrahydroprogesterone (5α-THP) into 5 -pregnane-3α,20α-diol, which has a weak affinity for the gamma aminobutyric acid $(GABA)_A$ receptor. AKR1C1 thus diminishes the levels of progesterone and 5α-THP in peripheral tissue. It can therefore have an important role in the development of breast and endometrial cancers, as well as in conditions such as premenstrual syndrome, catamenial epilepsy, depressive disorders and it thus represents interesting therapeutic target for the treatment of these conditions [4].

In this work, we examined the inhibitory potencies of structurally different derivates of anthranillic acid, pyrimidine and phtalimide. Inhibition was determined on recombinant human AKR1C1, which was overexpressed in *E. coli* and purified to homogeneity. Catalytic activity of AKR1C1 was determined by the spectrophotometric assay at 340 nm following the oxidation of a common AKR substrate 1-acenaphthenol in the presence of $NAD^+$ without and in the presence of inhibitor. Percentages of inhibition were determined at 30 μM substrate and 100 μM inhibitor concentrations, and $IC_{50}$ values were calculated for compounds that showed more than 50% inhibition. The most potent inhibitors of AKR1C1 were pyrimidine derivative *N*-benzyl-2-(2-(4-methoxybenzyl)-6-oxo-1,6-dihydropyrimidin-4-yl)acetamide ($IC_{50}$ = 18 μM) and anthranillic acid derivative 2-(((2',3-dichlorobiphenyl-4-yl)-carbonyl)(methyl)amino)benzoic acid ($IC_{50}$ = 35 μM). Tested compounds with $IC_{50}$ in micromolar range represent promising starting points for further structural modifications in the search for more potent inhibitors of AKR1C1.

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PO015

# Inhibitors of trihydroxynaphthalene reductase from fungus *curvularia lunata* as potential antimicotic drugs

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Fungal melanins are dark-brown to black pigments that protect fungi against enviromental stress, radiation, heat or cold and hydrolitic enzymes [1,2]. They are considered important virulence factors for certain plant and animal pathogenic fungi [2]. *Curvularia lunata* is a dematiaceous fungus found ubiquitously in soil. It is known human pathogen that causes disseminated phaeohyphomycosis with clinical manifestations including allergic sinusitis, keratitis, pneumonia, brain abscess and disseminated infections [3,4,5]. In this fungus melanin is produced by a pentaketide pathway from 1,8-dihydroxynaphthalene [6]. One of the enzymes in the biosynthesis of melanin is 1,3,8-trihydroxynaphthalene reductase (THNR), which catalyses the conversion of 1,3,8-trihydroxynaphthalene to vermelon [1]. THNR is particularly attractive target for design of antimicotic drugs because the fungal melanin biosynthetic pathway does not exist in off-target organisms.

In our research a series of structurally different compounds were evaluated as potential inhibitors of THNR from fungus *Curvularia lunata*. Inhibitory potential of compounds was screened on recombinant THNR, that was isolated from *Escherichia coli,* transformed with plasmid pGex-THNR, that contains a genetic code for fusion protein between THNR and glutathione transferase. THNR catalyses oxidation of nonphysiological substrate 2,3-dihydro-2,5-dihydroxy-4H-benzopyran-4-one to 4,5-dihydroxy-2H-benzopyran-2-one with concomitant reduction of $NADP^+$ to NADPH [7]. The reaction was followed spectrophotometrically by measuring the difference in NADPH absorbance at 340 nm. Inhibitory activities were measured at 100 μM substrate and 100 μM inhibitor concentrations. $IC_{50}$ values were determined for compounds that showed at least 50% inhibition.

Hits from different structural classes were identified. A common feature of all active compounds was aromatic ring with functional groups which enable H-bonds with the enzyme. The most potent inhibitors were etodolac and 1,3-indandione with $IC_{50}$ of 17 μM and 66 μM, respectively. These compounds represent promising starting points for the development of new and more efficient inhibitors of THNR with the potential use in prevention and treatment of disseminated phaeohyphomycosis, allergic sinusitis, keratitis and other diseases involving melanized fungi.

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PO016

# Synthesis of quinazolinocarboline alkaloid analogues

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Policyclic heterocondensed compounds, including natural alkaloids exhibit potent antitumor activity. Among them the pentacyclic quinazolinocarboline alkaloids (rutaecarpine, evodiamine) isolated from different species of *Rutaceae* posses excellent antineoplastic properties on several malignant cell cultures[1] by inducing apoptosis via activation of caspase cascade. Rutaecarpine is structurally related to the quinazolinoquinoline alkaloids (luotonin A, B, E) isolated from *Peganum nigellastrum* which have camptothecin-like antitumor activity as potent topoisomerase I. inhibitors[2].

The high structural similarity and the possible bioisosterism of these ring systems prompted us to realize the synthetic combinations of their structural features leading to hybrid alkaloid analogues.

The pyrroloquinazoline alkaloid deoxyvasicinone was formylated in Vilsmeier-Haack reaction. Japp-Klingemann reaction of the dimethylaminomethylene-deoxyvasicinone led to the phenylhydrazone derivative, which was afforded to pentacyclic indolopyrroloquinazolone by Fisher indolization. This new ring system is the nor-derivative of natural alkaloids.

The second approach for synthesis of structural variations of the alkaloid analogues based on synthesis and substitution of 2-indolyl-quinazolone. The latter compound was prepared from 2-ethyl-quinazolone by bromination, following nucleophil substitution of the bromo-compound with phenylhydrazine, then Fischer-indolization of phenylhydrazone. Indolil-quinazolone is readily available on an alternative way by condensation of indole-2-carboxylic acid and anthranilamide.

We studied the electrophilic substitution of indolyl-quinazolone on indole in 3-position. Vilsmeier-Haack formylation provided a natural alkaloid, bouchardatine (*Bouchardatia neurococca)*[3], which was closured to norluotonin B by acid catalysis. Aclyation, bromination and azo-coupling reactions of indolyl-quinazolone were performed with high selectivity. The ring closure reactions of 3-substituted indole derivatives were also studied to perform the synthesis of heterocyclic C-ring analogues of rutaecarpine.

The synthesized compounds will characterize in detail by spectroscopic methods and molecular modeling experiment.

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PO017

# New derivatives of arylsulfonylpiperidines – The influence of structural modifications on 5-HT$_7$ and 5-HT$_{1A}$ receptor affinity

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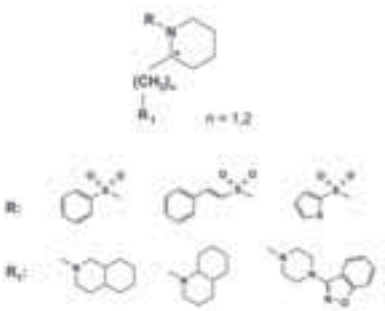
In recent years among 5-HT receptors much attention has been focused on the newest member of serotoninergic receptors family – 5-HT$_7$. The numerous papers that have been published successively provided evidences that 5-HT$_7$ receptor ligands can find potential applications in therapy of different mental disorders i.e. anxiety and depression.[1]

Inspired appearing reports discovering new potent and selective 5-HT$_7$ receptor ligands[2] we designed and synthesized a new series of arylsulfonamide derivatives. The structural modifications included replacement of amine fragment, changes of aromatic substituent in arylsulfonylpiperidine moiety and length of alkyl chain.

Radioligand binding study showed that the investigated compounds reveal diverse affinity for 5-HT$_7$ receptor and selectivity over 5-HT$_{1A}$ receptors. The structure-affinity relationships for all the new derivatives are discussed.



This study was partly supported by the Ministry of Science and Higher Education (MNiSW), Grant No. N405 026 32/1743

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PO018

# Design, synthesis, 3D QSAR and biological evaluation of novel CYP19 (aromatase) inhibitors

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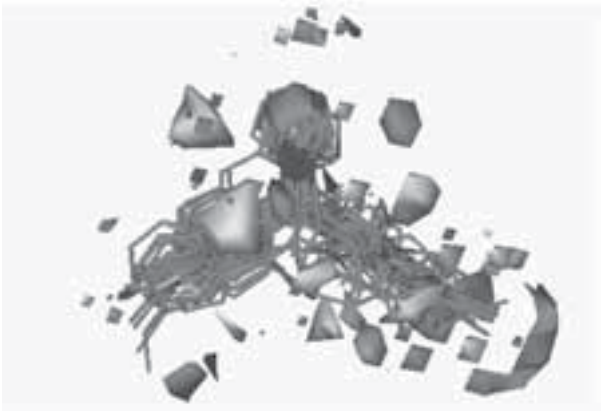

Breast cancer is the most commonly diagnosed cancer among women and continues to be a major cause of cancer deaths [1] As a high proportion of breast tumors in postmenopausal women are estrogen dependent, the two major therapeutic approaches consist in blocking estrogen receptors by selective estrogen receptor modulators or blocking estrogen biosynthesis using aromatase (CYP19) inhibitors [3]. Clinical trials showed advantages of nonsteroidal aromatase inhibitors (NSAIs), particularly if containing imidazole or triazole ring, over tamoxifen in adjuvant treatment and proposed them as an interesting alternative in the first-line therapy [4].

Starting from a library of azole-containing derivatives previously synthesized by us, we have developed a 3-D QSAR model and took advantage of it to design novel potent CYP19 inhibitors.

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PO019

# Quinazoline-based kinase inhibitors: New synthetic approach and biological evaluation

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In the last few years, particular attention was given to 4-anilinoquinazoline derivates for their highly specific and potent antitumoral activity as tyrosine kinase inhibitors [1]. This significant interest for this class of compounds leads our research group to perform a new strategy for the synthesis of the quinazolinic scaffold [2,3] (Figure 1), with the aim to going over some disadvantages of the already known synthetic routes, in particular the lack of commercially available starting products.


**Figure 1.** New synthetic route to quinazolines.

Reaction has been further performed and improved using microwave-assisted technique [4], leading both to an increase in total yields and to a reduction of reaction time and of work-up.

The prepared quinazoline scaffolds are suitable to be easily functionalized in 4-position with various amino substituents, to obtain potential ATP analogues, as schematically shown in the Figure 2.

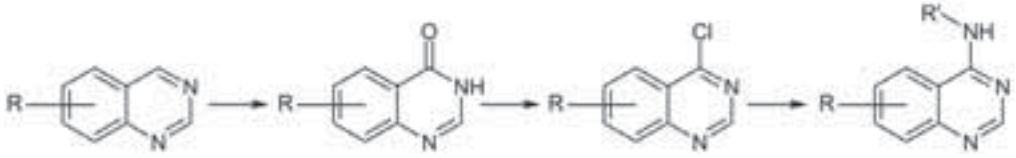
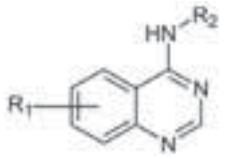



**Figure 2.** Synthetic strategy to 4-aminoquinazolines.

Structures of the final compounds are shown in the Figure 3.




$R_1$ = one or two *O*-alkyl, *N*-acyl or condensed heterocycles

$R_2$ = substituted phenyls, substituted heteroaryles (in particular picolines), alkyls or cycloalkyls

**Figure 3.** Structures of synthesized quinazolines.

The functionalized quinazolines have been submitted to biological assay on several kinase to evaluate their inhibitor activity.

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PO020

# New method for monitoring different polymorphs during precipitation

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Polymorphs are compounds with identical chemical composition but different physical and chemical properties. These differences can significantly influence the way such a compound interacts with biological targets. Finding the polymorph changes in a drug-like molecule is very important and can have serious consequences on the drug's absorption and stability.

This study explains how a new method for solubility measurements, CheqSol (Chasing Equilibrium Solubility, patent pending) can be used to monitor polymorph changes during precipitation. The method uses potentiometric titration and a pH cycle to precipitate or dissolve an ionisable compound. It also generates the most stable polymorph in solution, which may not necessarily be the first form precipitating. Examples of drug compound undergoing polymorph changes are examined in this study.

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PO021

# Measuring solubility of ionisable compounds by a novel pH-metric approach: CheqSol (Chasing Equilibrium Solubility)

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The primary objective of this study was to distinguish between two different behaviors of ionisable molecules when precipitating. Chasing Equilibrium Solubility (CheqSol, patent pending) is a new pH-metric approach for the measurement of solubility of ionizable drug molecules. Equilibrium and kinetic values are obtained in the same measurement. A small quantity of solid sample is dissolved in an aqueous solution by adjusting the pH such that the compound exists mainly in its ionized form. The sample is then titrated to a pH where the neutral species begin to precipitate. The concentration of sample at the point of initial precipitation, the kinetic solubility, is recorded.

The rate of change of pH is then monitored, whilst strong acid and strong base are added alternately to force the sample to fluctuate between a supersaturated and "subsaturated" state. By careful monitoring of the rate of pH change, the equilibrium conditions can be determined and an intrinsic solubility can be measured. This process is called chasing equilibrium. While many samples chase equilibrium; some samples don't, and the result is calculated differently.

Most samples can be analyzed in 20 to 60 minutes. The results are in excellent correlation with published values. Besides its speed and accuracy, this method confirms the result several times within the same experiment, and measures solubility in the presence of solid material without separation.

The kinetic and equilibrium solubility values were measured for compounds with well-known pharmaceutical activity (e.g. ibuprofen, diclofenac, sulfamerazine, pindolol, lidocaine, propranolol, famotidine etc) and compared with the values reported in the literature.

## References:

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PO022

# Molecular modeling on $\alpha_2$-adrenoceptors

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$\alpha_2$-Adrenergic receptors belong to the family of seven transmembrane-spanning G-protein-linked receptors. The $\alpha_2$ receptors can be grouped into three highly homologous subtypes ($\alpha_{2A}$, $\alpha_{2B}$, and $\alpha_{2C}$) which may be involved in a number of patho-physiological events.

The identification of subtype-specific functions from pharmacological experiments is currently not possible because of the lack of subtype-specific ligands and the possible cross-reactivity with imidazoline receptors. The rational, structure-based design of subtype-selective agonists may therefore contribute to better understanding of the precise pathophysiological roles of these receptors, moreover, it may lead to new drug candidates.

In this presentation, molecular modeling studies on $\alpha_2$-receptors are described.

Two different approaches have been applied.

In the first method, an atomic-resolution model of the $\alpha_{2A}$-adrenoceptor has been constructed through use of its amino acid sequence and the crystallographic bovine rhodopsin structure as a template. The receptor was generated by MODELLER, subsequently ligands were docked into the receptor by AUTODOCK, and finally a QSAR model with acceptable predictability for binding energies was developed by using the CODESSA program.

In the second method, 3DQSAR calculations, DISCOtech and CoMFA, were carried out by SYBYL software package, using a set of known agonists with well-defined binding data.

The CoMFA procedure, utilizing the pharmacophore model developed by DISCOtech for the alignment, resulted in a model with fairly good cross-validated $R^2$, suggesting an acceptable performance for predictivity of new compounds.

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We thank National Office for Research and Technology (NKTH), Hungary for a support provided via Szentágothai Knowledge Center.
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PO023

# Synthesis and in vitro antiinflammatory profile of novel 5-arylpyrimido[4,5-d]pyridazin-4,8-dione derivatives.

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Tumor necrosis factor alpha (TNFα), interleukin-1 beta (IL-1 β) and monocyte chemoattractant protein-1 (MCP-1) are inflammatory mediators involved in a variety of pathologies like rheumatoid arthritis (RA), inflammatory bowel disease (IBD), multiple sclerosis (MS), atopic dermatitis (AD) and so on.
Despite the success in the treatment of some of these pathologies with protein therapies which are able to control the level or activity of proinflammatory cytokines [1], there is still an unmet need of novel classes of small molecules of modulators.
Our interest in this field prompted us to design and synthesize a series of pyrimido[4,5-d]pyridazin-4,8-diones of general structure **1** as possible inhibitors of the synthesis and release of TNFα, IL-1 β, and MCP-1.
Evaluation of the novel compounds in a cellular model generating the above proinflammatory cytokines allowed us to identify some interesting agents. Among them compound with Ar = 3-chlorophenyl, $R_1$=H and $R_2$= cyclohexylmethyl emerged as the most potent inhibitor.
Biological data and structure-activity relationships (SAR) studies will be presented in the occasion of the meeting.

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PO024

# Distribution aspects of trace elements in some north african wild medicinal herbs

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A rather broad survey of trace elements was carried out in anumber of wild medicinal herbs known to grow wildly in North Africa wilderness and claimed by general public to be of therapeutic importance. The herbs and their plant families were positively identified by University Botany Department.
Representative samples of the herbs were carefully collected from Libyan wilderness at the end of spring 2006, stored then analyzed for their contents of macro, micronutrients and toxic trace elements.
The concentration pattern of the determined elements were carefully studied regarding the concentrations of different chemical elements in the herbs, plant family, environmental aspects and the claimed therapeutic values. Interestingly, different families showed different chemical interactions and concentration trends.

PO025

# Synthesis, study and comparison of antitumor activity of pyrazole derivatives – pyrazolo[1,5-a]pyrimidines and of their acyclic analogs

**Gevorg Danagulyan**[a, b], Armen Nersesyan[c], Aida Boyakhchyan[a], Anna Danagulyan[a], Vigen Topuzyan[d], Marina Mkrtchyan[d], Rafael Muradyan[d], Flora Arsenyan[d]

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[b]*Russian-Armenian (Slavonic) State University, 123 H.Emin Street, 375051 Yerevan,*
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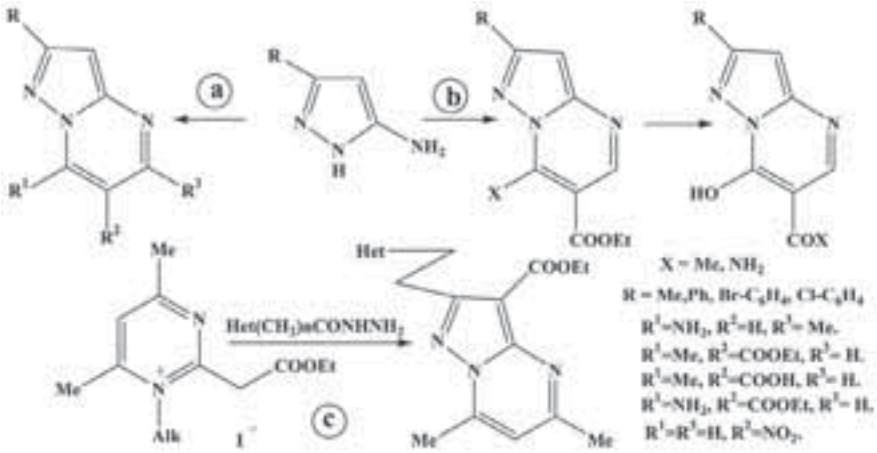
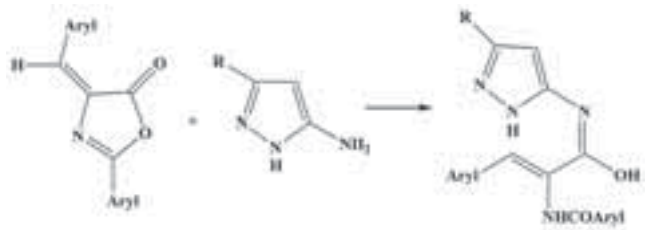
To study antitumor properties and compare biological activity of two series of compounds synthesis of about 40 new pyrazolo[1,5-a]pyrimidine derivatives and their acyclic analogs – pyrazole derivatives has been carried out.
Synthesis of polysubstituted derivatives of pyrazolo[1,5-a]pyrimidine has been carried out by various routes: **a)** by condensation of aminoazoles with the derivatives of β-di- and those of tricarbonyl compounds; **b)** by C-C recyclization of 6-ethoxycarbonyl-7-amino(7-methyl)pyrazolo[1,5-a]pyrimidines into new 2-substituted-6-acetyl (6-carbamoyl)-7-hydroxy pyrazolo[1,5-a]pyrimidines; **c)** by the unusual recyclization of 1-alkyl-2-(ethoxycarbonyl)methylpyrimidinium iodide under the action of carboxylic acid hydrazides.




Acyclic analogs - [3-methyl(aryl)pyrazol-5-yl]amides of 2-arylacylamino-β-arylacrilyc acid derivatives were synthesized by the reaction of 3-amino-5-methyl(aryl)pyrazoles with 4-arylidene-2-aryloxazol-5-ones.




Some of the synthesized acyclic derivatives of the studied compounds are noticed to show significant antitumor activity.

*This work was fulfilled owing to the financial support provided by the US Civilian Research and Development Foundation (US CRDF, grant ARB2-2640-YE-05) and by the Ministry of Science and Education of the Republic of Armenia within the framework of theme 0543.*

PO026

# Introduction of pharmacophore groups into pyridines and pyrazolo[1,5-a]pyrimidine derivatives via pyrimidines rearrangement

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In extension of studies of pyrimidinium salts interaction with nucleophiles [1, 2], rearrangements of 1,4,6-trimethyl-2-(ethoxycarbonyl/carbamoyl)methylpyrimidinium iodides under the action of biogenic amines (amino acids, triptamine, octopamine, aminoheterocycles) have been investigated. As a result of these transformations were synthesized new derivatives of nicotinic acid and nicotinamide containing in position 2 of the forming pyridine fragments of the corresponding biogenic amines.

Upon interaction of 1,4,6-trimethyl-2-ethoxycarbonylpyrimidinium iodide with carboxylic acid hydrazides inordinary rearrangement to pyrazolo[1,5-a]pyrimidine derivatives occurs.

The composition of compounds was proved by X-ray structural investigation as well as mass- and NMR spectra.

*The work includes the stuff obtained during fulfillment of the awards ARB2-2640-YE-05 (US CRDF), CH 090-02/21040 (NFSAT RA – US CRDF), and within the framework of the theme 0543 of the Ministry of Education and Science of Armenia.*

The author thanks Prof. A.R. Katritzky (Florida, USA) for support and collaboration.

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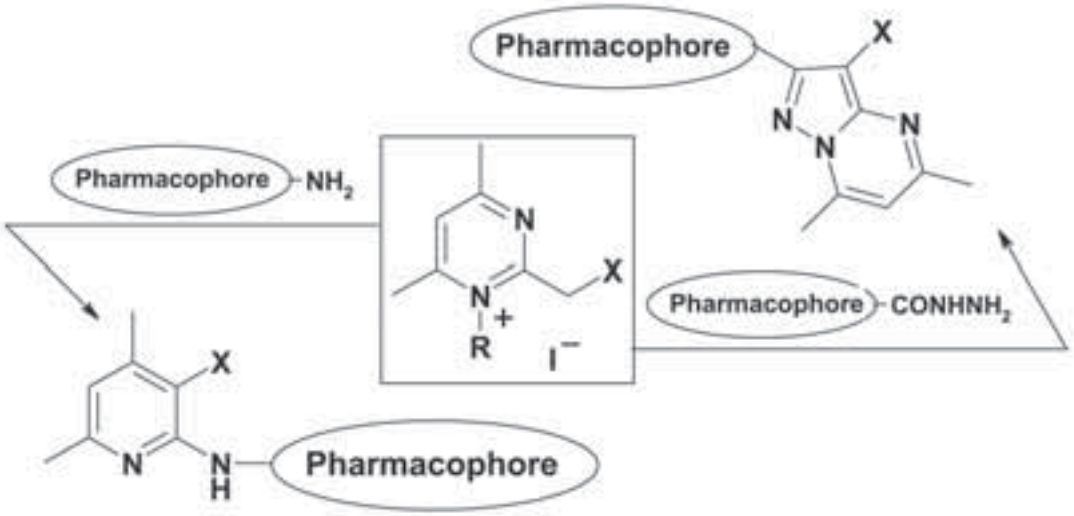

PO027

# Molecular modeling studies: discovery of novel potent non-nucleoside RT inhibitors

**Laura De Luca[a,*],** Maria Letizia Barreca[a], Nunzio Iraci[a], Angela Rao[a], Anna-Maria Monforte[a], Giovanni Maga[c], Erik De Clercq[c], and Alba Chimirri[a]

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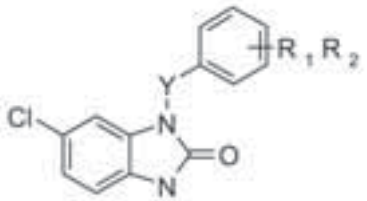
The non-nucleoside HIV-1 reverse transcriptase inhibitors (NNRTIs) are an important class of anti-AIDS drugs increasingly used in combination therapy with nucleoside/nucleotide RTI, protease and fusion inhibitors.
NNRTIs bind in a noncompetitive manner to a specific allosteric non-substrate binding pocket site of the enzyme, altering its ability to function and achieving a highly selective suppression of HIV-1 replication with little cytotoxicity.
After having focused our researches on the identification of new molecules with anti-RT activity, [1] we have recently used different computational methods in attempts to gather additional information for rational design and discover of new classes of NNRTIs. At first, a combined ligand- and structure-based molecular modeling approach led a new class of NNRTIs, having the benzimidazol-2-one system as a scaffold [2].




Afterwards, a lead optimization strategy highlighted $N_1$-arylsulfonyl-1,3-dihydro-2H-benzimidazol-2-one as a novel template for the design of new NNRTIs that are highly active against wild-type and mutant strains of HIV-1 [3].
In particular, it was found that some compounds showed antiretroviral activity similar to that of efavirenz and greater than that of nevirapine, two of the three NNRTIs currently available in antiretroviral therapy [3].
These encouraging results support our strategy and diverse chemical modifications will be introduce on the skeleton of these molecules to further optimize their antiviral potency.

Research supported by TRIoH project (LSHB-CT-2003-503480)

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PO028

# Synthesis and biological evaluation of new thyrotropin-releasing hormone (TRH) analogues containing unnatural amino acids

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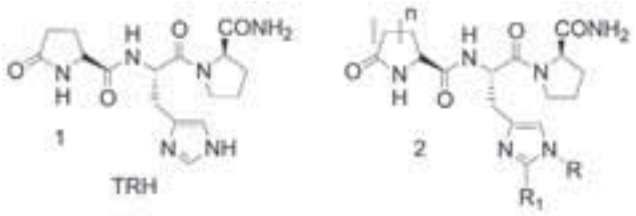
Thyrotropin releasing hormone (TRH, L-pGlu-L-His-L-Pro-$NH_2$), a tripeptide synthesized in the hypothalamus, operates in the anterior pituitary to control levels of TSH (thyroid-stimulating hormone) and prolactin. The thyrotropin-releasing hormone (TRH) receptor (TRH-R) belongs to the rhodopsin/β-adrenergic receptor subfamily of seven transmembrane (TM)-spanning, G protein-coupled receptors (GPCRs). The two G-protein-coupled receptors for TRH, TRH receptor type 1 (TRH-R1) and TRH receptor type 2 (TRH-R2), have been cloned from mammals and are distributed differently in the brain and peripheral tissues. The TRH receptor subtype-1 appears to mediate the hormonal and visceral effects, whereas TRH receptor subtype-2 has been implicated in its central stimulatory actions. Identification of critical features of the TRH, separation of its multiple activities through design of selective analogues and affinity labels have been elusive and unfulfilled goals for more then 30 years.

This presentation will highlight our studies on effect of the biological activity of TRH with the introduction of alkyl groups of varying sizes at the N-1 and C-2 position of the centrally placed histidine residue and modifying the pyroglutamic acid residue of TRH peptide[1]. The requisite building scaffolds have been synthesized via multistep synthesis from L-histidine methyl ester dihydrochloride and used for the synthesis of various TRH analogues.




The results of receptor binding studies of synthesized analogues indicate them to show selectivity to TRH-R2 subtype. In addition, all synthesized TRH analogues were biologically evaluated for their effect on pentobarbitone induced sleeping time duration. Some of the reported analogues displayed activity superior to that of TRH in vivo.

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PO029

# Synthesis of N-(3,5-difluoro-4-hydroxyphenyl)-4-(1*H*-pyrrol-1-yl) benzenesulfonamide as a putative aldose reductase inhibitor

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In the last years increasing interest is shown towards the discovery of putative inhibitors of the enzyme aldose reductase (ALR2) since the interference in the polyol metabolic pathway comprises a much promising therapeutic approach not only for the treatment of the long term diabetic complications but for other pathological conditions too.

An effort [1] was made in order to improve the inhibitory activity towards ALR2 of a series of compounds, which are aroyl-sulfonyl-glycine derivatives [2]. Taking into consideration the fact that the replacement of the acetic acid functionality with that of difluorophenol (more lipophilic and less acidic, non-classical bioisosteric moiety) [3] can improve the above biological response, N-(3,5-difluoro-4-hydroxy-phenyl)benzenesulfonamide was initially synthesized and tested in vitro. It was found that this compound was approximately three times more active against ALR2 than the respective glycine derivative [4]. In the present study, in order to further improve the inhibitory activity we increased the aromaticity by synthesizing N-(3,5-difluoro-4-hydroxyphenyl)-4-(1*H*-pyrrol-1-yl)benzenesulfonamide.

For the formation of the aroyl-sulfonamide moiety in the aminophenol ring a one-pot reaction has taken place with a good yield, followed by the reduction of the nitro-group under hydrogenation conditions of hydrogen transfer and formation of the pyrrole ring under conditions of a Clauson-Kaas type reaction.

In order to support the theoretical improvement of the inhibitory activity against ALR2 the compound was tested in vitro. In addition the antioxidant activity was studied in the homogeneous system of DPPH according to which the compound's scavenging activity against the free radical 1,1-diphelyl-2-picrylhydrazine (DPPH) was tested. The results showed high antioxidant activity and this implies possible activity of the compound to prevent the formation of the Advanced Glycation End Products (AGEs), which are also responsible for the pathogenesis of the long-term diabetic complications.

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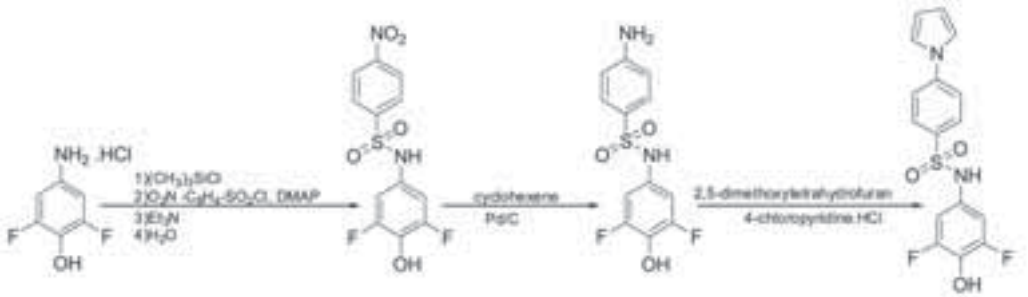

PO030

# Leelamine, a novel diterpene, exhibits potent cannabinoid-like effects *in vivo*

**William A. Devane**[a], David Stevens[b], Ntsang Nebane[c], Zhao-Hui Song[c], David Peters[d], Duncan Crawford[d], J. Michael Walker[e], Benjamin Applegate[f], John Kennish[f], Michael P. Cassidy[b], Dana E. Selley[b], and William L. Dewey[b]

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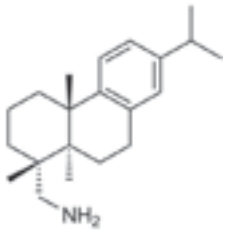
While searching for new ligands for the cannabinoid receptor system, we discovered that leelamine displayed a weak affinity for the the rat CB1 receptor. We tested leelamine and several analogues for cannabinoid-like effects in biochemical and behavioural paradigms.

Leelamine displayed a Ki of ~ 5 μM at the transfected human CB1 receptor using [3H]SR141716A. Leelamine is the primary amine analogue of dehydroabietic acid, a component of pulp mill effluent. Using simple chemical syntheses, the carboxylic acid moiety of dehydroabietic acid was converted into the ethanolamide, the carbamide, and the primary alcohol. These closely-related compounds displayed meagre affinities for the rat CB1 receptor. Unlike typical cannabinoid agonists, leelamine failed to stimulate G-protein activity as measured by the binding of GTPγS to rat cerebellar and striatal homogenates

Leelamine (3-125 mg/kg) was tested in the cannabinoid tetrad tests using C57 mice (25-40g). Leelamine was more potent and efficacious than Δ-9-THC at inhibiting spontaneous motor activity and decreasing rectal temperature, but less potent and efficacious than THC in the ring immobility and tail flick tests. The CB1 antagonist SR141716A was able to reverse the behavioural effects of leelamine, but not as potently or efficaciously as its reversal of THC s effects. When the tetrad tests were performed using C57-CB1 $^{-/-}$ knockout mice, leelamine was almost as efficacious in all tests as in wild-type C57 mice; THC was relatively inactive in knockout mice, except for inhibiting spontaneous activity.




It was investigated whether leelamine is present in brain using LC-MS technology. Leelamine was not found in rat or porcine brain using assays with detection limits of 0.5-2 fmol.

Leelamine binds weakly to the CB1 receptor and does not stimulate G-protein activity *in vitro*. Leelamine exhibits potent behavioural effects reminiscent of THC which are reversed by the CB1 antagonist, SR141716A, but it is also active in CB1 knockout mice. The biochemical substrates manifesting the actions of leelamine are currently a conundrum. Leelamine was coined from the Sanskrit word "lıla", which means play, or game, or sport.

PO031

# Synthesis, antifungal and antimycobacterial properties of pyrazinamide analogues

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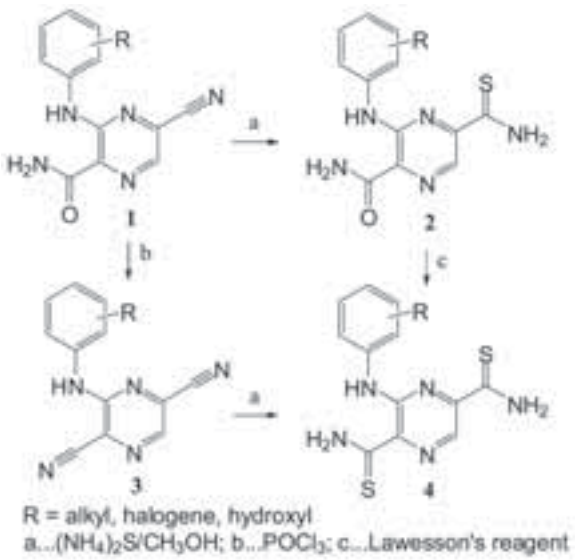
Tuberculosis (tbc) is an infectious disease that is still on the increase. Tbc has resurfaced as a public health concern in the industrialized world. Mycoses are also on the rise, the incidence of bloodstream infection by *Candida albicans* has increased by almost 50% in the last decade, and other fungal species are gaining ground as well. We have previously reported the synthesis of a series of *N*-substituted 3-amino-5-cyanopyrazine-2-carboxamides with high antimycobaterial and/or antifungal activity [1-3]. The continuation of our SAR studies among pyrazine derivatives finalised in substituted 3-arylaminopyrazine-2,5-dicarbonitriles and corresponding carbothioamides. Strong effects against selected pathogenic fungi and some effect on mycobacteria were discovered in the series of compounds **1**-**4**:

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*Study was supported by the Ministry of Education of the Czech Republic (MSM0021620822) and by the Contact-Slovenia program Nr. 06/2006-2007.*

PO032

# Synthesis and pharmacokinetic study of PEG-dexamethasone

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Corticosteroids are frequently used for their immunosuppressive and anti-inflammatory actions. Most glucocorticoids are administrated repeatedly because of their half-life and used for continuous as well as pulse treatment.

Dexamethasone (DXM) is a corticosteroid with mainly glucocorticoid activity; 750 μg of DXM is equivalent in anti-inflammatory activity to about 5 mg of prednisolone. However, even at moderate doses, the systemic administration of DXM causes many severe side effects, especially in case of long term therapy.

The DXM is practically insoluble in water. It is readily adsorbed from the gastrointestinal tract and its biological half-life is about 190 min [1].

Poly(ethylene glycol) (PEG) conjugation has been proposed as a new delivery system to increase solubility and bioavailability of many drugs [2].

In this study, dexamethasone was covalently attached to an amino mPEG ($M_w$=10000) by use of a succinate linker. A new PEG prodrug system has been designed using ester derivatives. The PEG-dexamethasone conjugate (PEG-DXM) was highly water soluble at room temperature. The hydrolytic stability of the PEG-DXM, investigated *in vitro* at physiological pH, was confirmed, but *in vivo* release studies demonstrated a good release of parent drug. We have investigated the pharmacokinetic of the new prodrug after its intravenous administration in rabbit: the area under the concentration-time curve (*AUC*) of PEGylated drug was larger than parent drug. The blood profiles of drug after the administration of PEG-DXM were analyzed according to a two-compartment model.

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PO033

# Solid phase synthesis and DNA topoisomerases activity of new netropsin analogues

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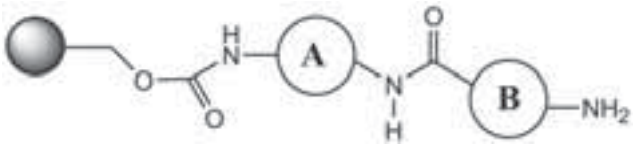
My investigations concentrate on carbocyclic analogues of netropsin and distamycin, with benzene in place N-methylpyrrole rings. The carbocyclic analogues of distamycin with unsubstituted N-terminal group $NH_2$ inhibited *in vitro* activity of topoisomerase I and II [1], similarly like derivatives of netropsin with aliphatic linker (4 and 6 groups $CH_2$) [2]. Derivatives with N-terminal chlorambucyl group exhibited activity in cultured breast cancer MCF-7 [3], and with 5-[N,N-bis(2-chlorethyl)amine]-2,4-dinitrobenzoyl group - in the face of hepatoma HEP G2 in hypoxic conditions [4]. The study of interaction of compounds to DNA by the *ethidium displacement assay* it confirmed that they had the larger specificity to A-T in comparison to G-C rich regions, similarly how model netropsin and distamycin [5, 6].

In this communication I present solid-phase synthesis of netropsin analogues. It was started by connecting five amine-nitro compounds $\mathbf{A_1}$-$\mathbf{A_5}$ to polystyrene grains with Wang linker. Received in this way, immobilizing on grains, compounds **A** with nitro aromatic group were reduced giving in this way free amine group. The next steps were reactions of acylation by acid chlorides $\mathbf{B_1}$-$\mathbf{B_4}$ with nitro groups. These one were also reduced. This procedure led to obtainment 20 dimmers **AB,** analogues of netropsin, after using of solution of trifluoroacetic acid to separate these compounds from grains.

The biological activity obtained compounds against DNA topoisomerases was investigated.

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PO034

# Synthesis of 4,5-diaryloxazolone derivatives for selective inhibition of COX-2

**Yasemin Dündar[a],** Serdar Unlü[a], Erden Banoglu[a], Antonio Entrana[b], Maria-Teresa Nunez[c], Luis Labeaga[c], Aurelio Orjales[c], Ningur Noyanalpan[a]

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Nonsteroidal anti-inflammatory drugs (NSAIDs) act through the inhibition of cyclooxygenases (COX-1 and COX-2) and show some side-effects including gastric toxicity [1]. After the discovery of current NSAIDs exhibit their side-effects through the inhibition of COX-1 isoform, development of selective COX-2 inhibitors has gained a lot of attention since the COX-2 isoform plays an important role for the inflammatory response while the COX-1 enzyme responsible for gastroprotection. In addition, there is evidence that COX-2 was shown to be involved in cellular proliferation processes and indicated that selective inhibition of this enzyme opens up new doors for treatment of certain types of cancers [2].

These findings have stimulated us to develop a project for designing novel selective COX-2 inhibitors which might maintain the high anti-inflammatory activities without common side-effects. In our continuing efforts towards the synthesis of diarylheterocycle anti-inflammatory agents, we required the preparation of 4,5-diaryloxazolone derivatives (Figure 1). Among the synthesized compounds, TYD-48 showed selective COX-2 inhibition with a selectivity index of >50 ($IC_{50}$COX1= >100 μM, $IC_{50}$COX2= 2 μM). Here, we present the screening results of the synthesized compounds and also docking studies of TYD-48 showing the selective binding mode of these derivatives to the COX-2 active site.

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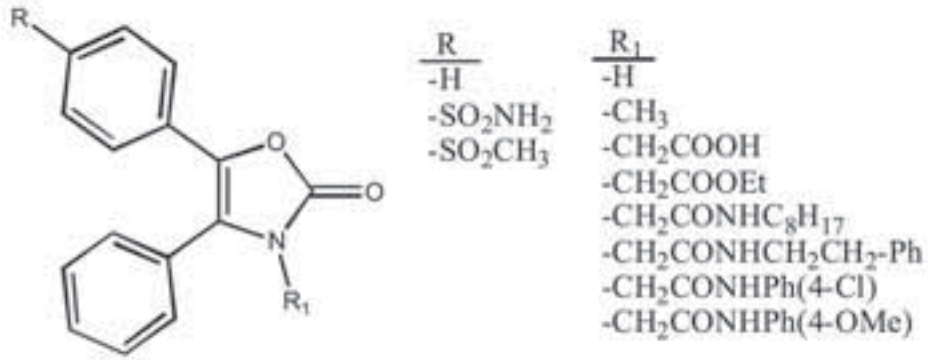


Figure 1

PO035

# Approach to anticarcinogenic agents based on paclitaxel

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Paclitaxel is a nongenotoxic cytostatic agent which is now utilized in a treatment of human cancer, especially ovarian, breast [1] and lung [2] cancer. Paclitaxel exerts its antitumor activity through the stabilization of microtubule assemblies, thus interupting mitosis and cell division [3]. However, paclitaxel has very low water solubility, which leads to difficulties in its administration. Therefore, it has been of special interest to synthesize paclitaxel derivatives with improved water solubility, carrying potential targeting moieties.

We have prepared a set of 2'-acyl paclitaxel analogues (examples in Scheme 1) with various amino acids and biologically active peptides, bound to paclitaxel both directly and through linkers. Peptides were chosen with the aim to target paclitaxel towards receptors in special tissues (breast and ovary). As linkers, succinyl and chloracetyl derivatives were used. Prepared analogues were tested *in vitro* for cytotoxic activity and they were slightly less potent than paclitaxel. However, analogues should not only to enhance cytotoxic activity but mainly to improve water solubility.

It is known that 2'-OH group of paclitaxel is essencial for inhibition of microtubule disassembly [4]. On the other hand, it is assumed that 2'-acyl group is hydrolysed in cells. Therefore, we have prepared such analogues which we expect to be hydrolysable at acidic conditions (pH inside cell) to release free paclitaxel, but stable enough at neutral pH to target paclitaxel to tumors through blood vessels.

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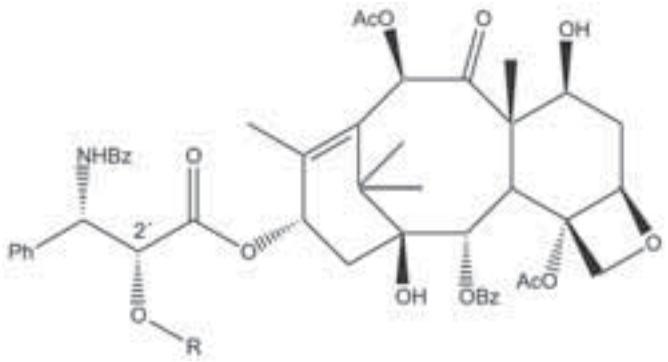
## Acknowledgement

The work was supported by grant 1P04OC926.001 from MYC CR.




1, R= succinyl
2, R= chloracetyl
3, R= succinyl-triethyleneglykol
4, R= succinyl-PhePhe
5, R= Z-Glycyl
6, R= succinyl-triethyleneglykol-succinyl-Paclitaxel
7, R= succinyl-hexapeptide

PO036

# Alkylphospholipids in oncology

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The first development of alkylphospholipids (APL) goes back to the seventies, when APLs were synthesized for immunomodulating applications. Soon afterwards, their activity against tumor cells was discovered. A derivate without glycerol backbone, miltefosine, exhibited a higher metabolic stability and was successfully translated into clinics [1]. However, dose limiting toxicity prevented the development of miltefosine as an oral anti-tumor agent and the use of miltefosine in the anti-tumor indication is limited to a topical formulation against malignant skin leasions (Miltex®).

The third generation APL perifosine is characterized by the substitution of the choline headgroup by a cyclic aliphatic piperidyl residue and a slightly increased alkyl chain length in comparison to miltefosine. Perifosine targets cancer cells at different sites. After active uptake, perifosine interferes and blocks central, membrane located, signalling pathways including the Ras-Raf-Mek-Erk and the PI3K-Akt cascade [2,3]. In addition, perifosine and other APLs directly induce apoptosis via several mechanisms involving the mitochondrial pathway. In vivo, dose limiting gastrointestinal toxicity occurred within the pharmacological relevant range. Therefore, oral tumor therapy with perifosine is feasible and assessed in a multitude of phase I and II studies against different tumors.

However, gastrointestinal toxicity remains dose limiting for the whole class of APL which would make i.v. injections an attractive alternative for application. Unfortunately, APLs including miltefosine and perifosine have haemolytic activity making i.v. applications impossible.

To allow intravenous administration, several derivates were synthesized and evaluated. Erucyl-phosphocholine (Erucyl-PC) turned out to be the most potent candidate without haemolytic activity. Structurally, Eurcyl-PC is closely related to miltefosine and differs only by the longer chain length ($C_{16}$ ➔ $C_{22}$) and the introduction of a cis-double bound. Despite these small changes, Erucyl-PC shows marked differences in the case of i.v. administration in comparison to other APLs. The increased chain length increased hydrophobicity leading to the formation of lamellar membranous Erucyl-PC structures preventing haemolytic toxicity.

Erucyl-PC showed efficacy against a variety of cancer cell lines including cells resistant to chemotherapy and radiation [4]. In vivo, Erucyl-PC showed promising efficacy in an autochthonous rat mammary carcinoma model and a glioblastoma model. Currently, formal preclinical pharmacology and safety studies for the preparation of a Phase I clinical study have been initiated.

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PO037

# Synthesis of salicylanilide esters prodrug forms

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Salicylanilides are antibacterial active compounds with high activity against *Mycobacterium tuberculosis* $H_{37}Rv$ and some atypical strains, against which the generally used antituberculotics are less active or inactive [1,2]. The presence of phenolic hydroxyls in salicylanilides seems to be necessary for the activity and is probably responsible for oxidative phosphorylation cleavage [3] but also adds irritative properties to the compounds. The goal of this work was the preparation of esters of amino acids with highly active salicylanilides as a new group of prodrugs with high activity, improved solubility and low toxicity.

The starting salicylanilides were prepared by the reaction of substituted salicylic acids with the appropriate anilines in chlorobenzene with $PCl_3$ in micro-wave reactor. The optimal method found for the esterification was the reaction of *N*-CBZ- -amino acid with the appropriate salicylanilide with activation by *N,N´*-dicyclohexylcarbodiimide (DCC) in DMF. The expected esters **1** were obtained by the reaction of *N*-CBZ-Val and *N*-CBZ-Phe. However, when it was used *N*-CBZ-L-Ala or *N*-CBZ-Gly, a 7-exo-trig cyclization process took place, originating an unexpected benzoxazepine [4] unluckily without antitubercular activity.

*N*-deprotection of esters **1** by acidolysis (33% HBr in anhydrous acetic acid) gave hydrobromide amino salts **2**. Subsequent amino group liberation by triethylamine under anhydrous conditions yielded unexpected product **3** (determined like “diamide”). The structures were characterized by IR, mass spectroscopy and 2D NMR analysis.

The synthesis, general characteristics, physical and antimycobacterial activity evaluation will be presented.

Authors would like to thank the grant FRVŠ 231/2007/G6 and MSM 0021620822 for financial support.

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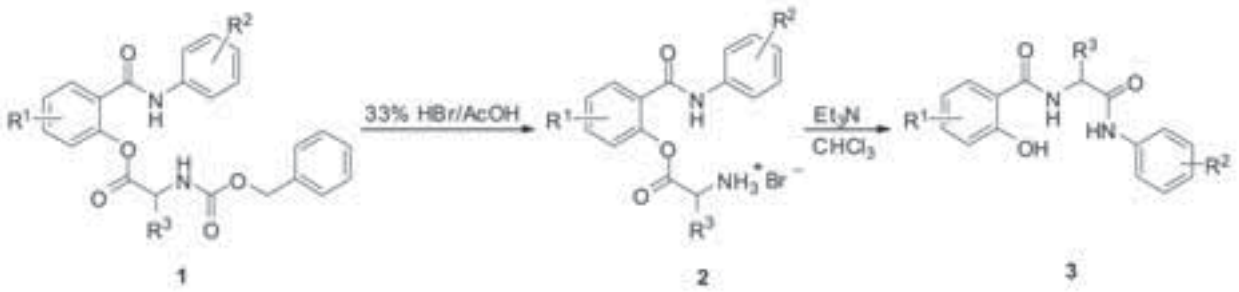

PO038

# HIV-1 Integrase strand transfer inhibitors (INSTIs): design, synthesis and biological evaluation

**Stefania Ferro[a,*],** Maria Letizia Barreca[a], Laura De Luca[a], Angela Rao[a], Anna-Maria Monforte[a], Zeger Debyser[b], Myriam Witvrouw[b] and Alba Chimirri[a]

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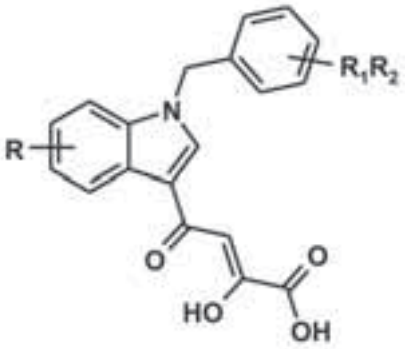
FDA-approved therapies for the treatment of AIDS target three steps of the HIV life cycle: reverse transcription, proteolytic maturation and fusion. However, the poor tolerability and development of multidrug resistance of the current therapy, commonly referred as HAART (highly active antiretroviral therapy), emphasize the demand of new anti-HIV agents for anti-HIV drug combinations. Hence, one additional therapeutic approach might be to target different stages of viral life cycle such as integration, catalyzed by Integrase (IN) enzyme.




In recent years our molecular modeling studies and a highly predictive 3-D QSAR model allowed the rational design of potent integrase strand-transfer inhibitors (INSTIs) characterized by the presence of benzylindoles as substituents at β-hydroxyketoacid moiety [1-3].
However, even if the 1,3-diketo acid portion seems particularly important to capture bivalent cations for the IN inhibitory activity, it would affect the activity of other specific enzymes such as Recombinase Gene Activator (RGA). On this basis and in the search for more potent and less toxic IN inhibitors, we rationally designed and synthesized novel benzylindole derivatives characterized by the presence of new functionalities able to mimic the interaction with the enzyme in a more selective way [4].

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PO039

# Searching for new CB1 antagonists, analogues of rimonabant

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Rimonabant, 5-(4-chlorophenyl)-1-(2,4-dichloro-phenyl)-4-methyl-*N*-(piperidin-1-yl)-1*H*-pyrazole-3-carboxamide, SR141716, Acomplia® is the most up-to-date cannabinoid receptor type 1 (CB1) antagonist, widely used as a tool to evaluate the mechanisms by which cannabinoid antagonists produce their pharmacological effects and to elucidate the physiological and the pathophysiological roles deriving from the inhibition of the CB1 receptors. Among the results of these studies, data obtained from different clinical trials clearly indicate that rimonabant may have benefits in the treatment of metabolic and cardiovascular disorders associated with overweight and obesity [1, 2]. In addition, other studies have pointed out its efficacy in reducing tobacco and drug dependence, thus suggesting that CB1 receptor antagonists might be of potential interest in these fields [3, 4].

On the basis of these experimental results, a rational approach to the synthesis of new 1,5-diarylpyrazole analogues of rimonabant has been taken. Using the 3D model of the human CB1 receptor, a data set of different antagonists selected from literature were docked inside the protein, thus exploring at a molecular level the nature of their interaction with the CB1 receptor. The resulting binding poses of the different structures were superimposed so as to derive a common pharmacophoric map which was used as a "filter" to identify potentially CB1 ligands from an in-house library of diarylpyrazoles. Then, the synthesis of a series of new 1,5-diarypyrazoles was performed. The binding affinity data toward human CB1 receptor confirmed the validity of the *in silico* approach. Chemical and biological as well as structure-activity relationships will be presented and discussed.

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PO040

# Facile synthesis of mercaptobenzothiazoles and their antimicrobial activity screening

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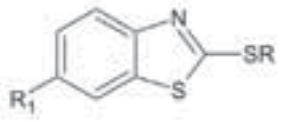
As reported in the literature, there are a large number of benzothiazole derivatives which showed potential antibacterial activity.[1] In order to find a lead compound for developing novel antimicrobial molecules, we have synthesized a large new series of 2-mercaptobenzothiazole derivatives by simple high yielding routes (**Figure I**).
The aims of this project are the investigation of: a) steric and electronic effects of different substituents at position 6 of the heterocyclic moiety;
b) the role of lipophilicity by introducing a benzyl group on the thiomethyl bridge at position 2;
c) antimicrobial potency and selectivity against Gram negative and Gram positive bacteria.
Actually, microbiological results indicated that the synthesized compounds have shown a significant broad spectrum of activity against the bacteria strains exhibiting a MIC values between 100 and 12.5 μg/ml. The most active derivatives have been also subjected to the cytotoxic effect assay against the HeLa cells, to establish the Safety Index.[2] Further details about the synthetic procedure and the biological results will be discussed in the poster session.




R= H, Bn
$R_1$= H, Cl, $CH_3$, $CF_3$, $(CH_3)_2$-CH, $NH_2$, $NO_2$, OH, CN, F, $SO_2NH_2$, $CH_3O$, $CH_3CH_2O$

**Figure I**

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PO041

# Stereoselective approach to some new androstene-fused pyrazolines and isoxazolidines by 1,3-dipolar cycloaddition

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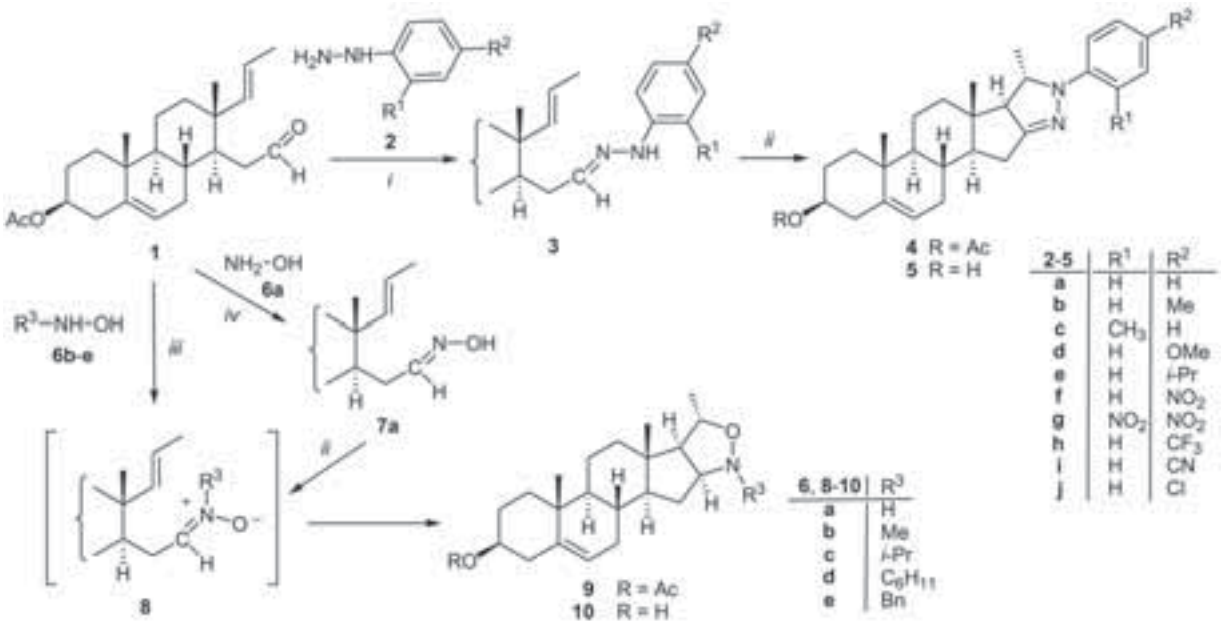
The thermally or acid-induced 1,3-dipolar cycloaddition of azomethine imine and nitrone 1,3-dipoles with multiple-bond systems is a generally used and extensively studied method for the formation of five-membered heterocycles. A rate acceleration and a diastereoselectivity improvement associated with the intramolecular cycloaddition of olefinic dipoles are mostly observed in the presence of Lewis acid catalysts. One of the possible methods for the synthesis of novel steroid heterocycles is the ring-closure reaction of *seco*-steroids with different reagents under certain conditions. Accordingly, D-*seco*-pregnene aldehyde **1**, containing a propenyl side-chain, can be a suitable precursor for internal 1,3-cycloaddition. The reactions of compound **1** with phenylhydrazine (**2a**) or its Ph-substituted derivatives (**2b**-**j**) yielded the corresponding phenylhydrazones **3a**-**j**, which readily cyclized in the presence of a catalytic amount of $BF_3{\cdot}OEt_2$ to afford androstene-fused pyrazolines **4a**-**j** stereoselectively. The yields of the products were strongly influenced by the electronic effects of the Ph substituents and the reaction conditions. Oximation of the aldehyde **1** with hydroxylamine hydrochloride **6a** gave the corresponding aldoxime **7a**, which was catalytically tautomerized by $BF_3{\cdot}OEt_2$ into its nitrone form **8a**, followed by intramolecular cyclization to give **9a**. The cyclization could also be carried out as a one-pot reaction via the nitrone intermediates **8b**-**e** formed *in situ* by heating aldehyde **1** with the *N*-substituted hydroxylamines **6b**-**e**, respectively, to furnish **9b**-**e**. Deacylation of the heterocyclic derivatives **4a**-**j** and **9a**-**e** afforded compounds **5a**-**j** and **10a**-**e**, which may be of value from a pharmacological aspect.




(i) MeOH, rt; (ii) $BF_3{\cdot}OEt_2$ (catalytic amount), $CH_2Cl_2$, $N_2$ atm; (iii) MeOH, NaOAc; (iv) *i*-PrOH, NaOAc

PO042

# The design and synthesis of arylsulfonohydrazide inhibitors of bacterial cell-wall

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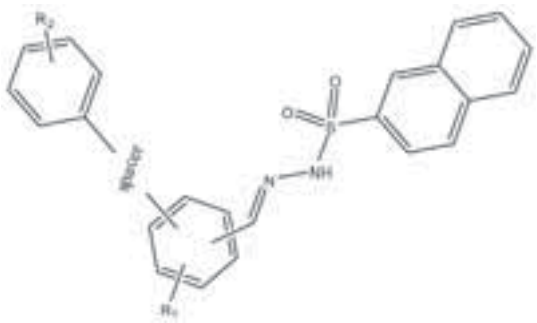
One of the most attractive sites for novel antibacterial targets is the bacterial peptidoglycan biosynthetic pathway. Peptidoglycan is an essential component of the bacterial cell wall, which is responsible for a defined cell shape and preserves cell integrity by withstanding internal osmotic pressure. Disruption of peptidoglycan biosynthesis leads to cell lysis. Furthermore, peptidoglycan is a unique bacterial structure, absent from eukaryontic cells. Peptidoglycan is formed as linear repeating *N*-acetylglucosamine (Glc*N*Ac) and *N*-acetylmuramic acid (Mur-*N*Ac) units, interconnected by a short peptide moiety [1,2].

We designed, synthesised and biologically evaluated a series of novel MurC and MurD inhibitors. For this purpose, benzylidene rodanines which possess MurC inhibitory activity in low micromolar range were used as leads. Leads were then further modified on thioxothiazolidin-4-one ring, which was exchanged with arylsulfonohydrazone moiety. The aim of this structural modification was to maintain the acidity of thioxothiazolidin-4-one. Hydrazone functionality was also used by other authors in the design of potent novel antibacterial compunds [3,4].



The activity of all compounds against MurC and MurD from *E.coli* was tested for their ability to inhibit the addition of D-Glu to UDP-Mur*N*Ac-L-Ala. Most of the synthesized and evaluated compounds possessed inhibitory against both MurC and MurD in micromolar range.

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PO043

# Full Automation for Microwave Synthesis Workflows

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Since microwaves have been introduced as an alternative source of heating in organic chemistry laboratories in the late 90's, the number of publications on Microwave Assisted Organic Synthesis (MAOS) exponentially has been growing. One of the most eye catching advantages of MAOS compared to classical methods are reaction times significantly being reduced from typically several hours to just minutes. Although, this achievement was resolving a very important bottleneck in the discovery research, other tedious steps of the workflow now became the "rate determining step":

- dispensing of solid and liquid chemicals
- automated capping and crimping
- transfer of vials, from and to the Microwave syntheziser
- sampling and work-up of the reaction mixture

Based on selected applications examples from worldwide renowned "CombiChem" labs we will show in this presentation, how the Chemspeed SWAVE Microwave synthesis platform, not only accelerates the reaction step, but also dramatically increases the productivity for all pre- and postsynthetic steps of a typical Microwave chemistry workflow.

PO44

# New antimuscarinic ligands designed on the imidazoline template.

Cristian Vesprini, Claudia Cardinaletti, Fabio Del Bello, Francesco Gentili, Francesca Ghelfi, Alessandro Piergentili, Maria Pigini, Wilma Quaglia, **Mario Giannella.**

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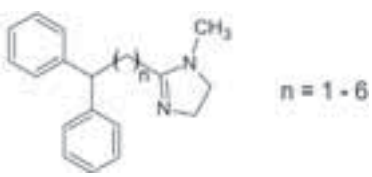
The muscarinic receptors, expressed in large amount in the parasympathetic nervous system, and belonging to the superfamily of G-protein-coupled receptors (GPCRs), have been divided into five subtypes, $M_1$-$M_5$. These govern specific physiological functions. It has been suggested that (i) the blockade of central $M_1$ and $M_4$ receptors might be of therapeutic benefit in Parkinson's disease, (ii) the $M_2$ receptor antagonism is a therapeutic approach in Alzheimer's disease, (iii) the $M_3$ receptor blockade alone is useful for treating urinary tract disorders, (iiii) the selective blockade of central $M_5$ receptors might represent a novel strategy for the treatment of addiction to drugs of abuse [1, 2]. This assumption and the current resurgence of interest in muscarinic receptor physiology and pharmacology prompted us to design novel potential selective muscarinic subtype antagonists. Several studies of ours demonstrated the versatility of the imidazoline ring. In fact, depending on the particular kind of substituent inserted in position 2, it was possible to modulate the ligand profile, both with regard to different systems ($\alpha_2$-Adrenergic Receptors [3], Imidazoline Binding Sites [4], Nicotinic Receptors [5]) and inside the same system, with resultant enhanced subtype selectivity. Therefore, in the new muscarinic antagonists of this study the basic function, that drives the primary interaction to receptor site, was represented by the imidazoline ring. The diphenylmethane moiety, an analogously versatile substructure, common to several efficacious drugs, (e.g. tolterodine, darifenacin, vamicamide), characterized all the substituents in position 2. This terminal scaffold was differently spaced from the imidazoline ring.

The encouraging preliminary results showed that the alkylic spacer length significantly affected potency and $M_1$-$M_5$ subtype selectivity.

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PO045

# AMPA receptor antagonists: synthesis and pharmacological evaluation

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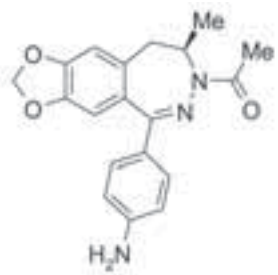
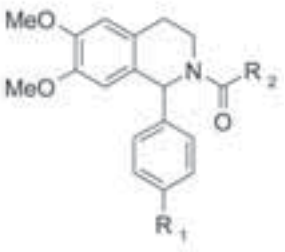
AMPA receptors are ligand-gated ion channels belonging to the glutamate receptor (iGluR) family. They are involved in many physiological processes such as neuronal development, learning and memory but also in acute and chronic neuropathologies. For this reason AMPAR antagonists can be considered useful therapeutic agents for these disorders. Moreover, the unwanted side effects of competitive AMPAR antagonists steered the search for new agents, such as talampanel (**1**), that inhibit AMPARs in a noncompetitive fashion [1]. Following our previous studies that led to the discovery of potent antagonists containing the tetrahydroisoquinoline scaffold, we planned some structural modifications in order to explore structure-activity relationships for this class of compounds [2-3].

We now report new 1-aryl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolines (**2**) designed according our molecular modelling studies [4] and synthesized employing innovative methodologies. The novel compounds were evaluated against audiogenic seizures in DBA/2 mice whereas their noncompetitive AMPA receptor antagonism was estimated through electrophysiological studies performed on hippocampal slices using the patch clamp technique. The results of these studies will be reported and discussed.




**1, talampanel**




**2**

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PO046

# Preclinical development of antichagasic benzofuroxans.

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Chagas' disease or American trypanosomiasis is an important health problem that affects around twenty million people in Central and South America. The causative agent of this disease is the haemoflagellate protozoan *Trypanosoma cruzi* (*T. cruzi*). We have shown that benzofuroxan derivatives posses high anti-*T. cruzi* activity *in vitro* against Tulahuen 2 strain. From these previous biological results we identified the 5-ethenyl derivatives as the most actives. [1,2] According to its unspecific cytotoxicity in macrophages, we selected derivatives **1**, **2**, and **3** (Figure 1) to further development of preclinical studies.

A complete metabolic study was performed using microsomal and cytosolic fractions, from rat hepatocytes. Besides, the same studies were done using whole *T. cruzi* cells. Mammal metabolic profiles showed two main patterns: *Z* isomers were metabolized to two main products, M1 (major) and M2 (minor), while *E* isomers remained almost unmetabolized. M1 were identified as both *o*-nitroaniline positional isomers. M2 corresponded to *o*-hydroxylaminoaniline. Benzofuroxans metabolism by *T. cruzi* required shorter incubation time than mammal one. Again differences were seen between *Z* and *E*–isomers, the formers were higher metabolized than the latter. However, only one metabolic product was observed (M1).

*In vitro* toxicity was studied by mutagenic and Clastogenic effect of the compounds and potential metabolites. Mutagenic studies: The number of $His^+$ revertants of *Salmonella typhimurium* (lineage TA98) (Ames test) with and without metabolic activation (S9 mix) was determined. Benzofuroxans presented different mutagenic characteristic, i.e. **1*Z*** is not mutagenic in all conditions. As a first study of the chromosomal aberrations promoted by the compounds we have studied the DNA damage by the alkaline comet assay. Again the same profile was observed.

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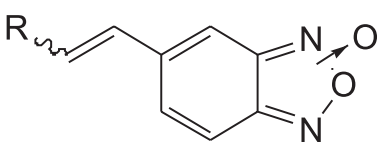


| Ref. | -R | Isomeric geometry | $IC_{50}$ (μM) [a] |
|---|---|---|---|
| 1 | -Ph | *E* | 10.8 |
| | | *Z* | 7.0 |
| 2 | -*p*-Cl-Ph | *E:Z* (1:1) | 4.3 |
| 3 | 3,4-$OCH_2O$- | *E* | 5.3 |
| | | *Z* | 2.9 |

[a] ***In vitro* activity on *T. cruzi* (Tulahuen 2 strain).**

Figure 1

PO047

# Enzymatic and biological activity of angiogenin and its tryptic peptides

**Grandi Claudio[1],** Finezzo Maria Letizia[2], Di Liddo Rosa[2], Conconi Maria Teresa[1], Dettin Monica[3], Dalzoppo Daniele[1] and Parnigotto Pierpaolo[1]

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**INTRODUCTION**: Angiogenesis is the process of generating new blood vessels derived as extensions from the existing vasculature. Among angiogenic molecules Angiogenin is also a ribonuclease. Angiogenin is a 14.1 kDa single-chain polypeptide and it has been isolated from different sources. The aim of the present research is to study the relationship between chemical structure and biological effects of bovine angiogenin (ANG-1) on endothelial cells in *in vitro* models.

**METHODS**: ANG-1 was isolated from fresh cow milk following the method of caseins precipitation by milk acidification and subsequent fractionation with ammonium sulphate. The angiogenin has then been purified to homogeneity using ion exchange chromatographic steps on Sepharose CM Fast Flow and Mono-S followed by RP-HPLC on Vyadc C4. ANG-1 was then treated with immobilized trypsin in order to obtain its tryptic peptides, which were purified through RP-HPLC on C18 Vydac column. Each peptide was characterized by aminoacid analysis and mass spectrometry. To test ribonucleolytic activity of ANG-1, macromolecular rRNA and tRNA and dinucleotide substrates (CpG and UpG) were used. Viability of HUVEC treated with ANG-1 or its tryptic peptides was determined by MTS assay on the contrary cell proliferation was assessed by quantification of cells BrdU incorporation. Biological activity was evaluated by means of Matrigel *in vitro* assay.

**RESULTS**: ANG-1 presents a modest ribonucleolytic activity; by means of tRNA, protein specific activity resulted $5.4 \pm 0.5$ AU $mg^{-1}*min^{-1}$, as already reported for human Angiogenin. ANG-1 tested on small dinucleotides like UpG and CpG allowed the quantitative determination of specificity constants. The tryptic peptides do not present intrinsic ribonucleolytic activity. The cellular mechanisms by which ANG-1 induces its effects is not yet fully elucidated. HUVEC viability is not affected by treatment with ANG-1 or its tryptic peptides. HUVEC treated with ANG-1 for 24 hours were induced to proliferate and form capillary-like network formation after seeding on Matrigel. Fragment 1-6 (N-terminal, termed P1) causes a cellular proliferation comparable to the native protein. Fragment 108-123 of human Angiogenin (termed C-terminal-hu) inhibits cell proliferation while a C-terminal fragment of ANG-1 103-124 (termed P9) is less inhibitory. P9 contains also a disulfide bond and fragment 56-61 wich could be probably involved in receptor binding. On the contrary fragment 62-67 (termed P2) have no significant effects on HUVEC proliferation. Fragments P1, P9 and C-terminal-hu show a positive results in matrigel assay as resulted in branch points formation.

**CONCLUSIONS:** In the present research we have shown that ANG-1 exhibits a low ribonucleolytic activity while its tryptic peptides have completely abolished any catalytic function. In the presence of ANG-1, HUVEC were induced to proliferate (20 % more vs control) and form a capillary-like network formation on Matrigel. Among fragments only P1 induces a proliferative effects while the others are inhibitory, in particular, P9 and C-terminal-hu. Fragments P1, P9 and C-terminal-hu display similar angiogenic activity to the native protein.

PO048

# Structural and biological comparison of RNase-A, RNase-4 and angiogenin-1 as therapeutical proteins

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**INTRODUCTION:** Ribonucleases (RNases) play a critical role in many biological processes. Studies on RNase-A folding led to assume that the particular amino-acid sequence of a protein predisposes it to fold into its native conformation. The objective of this research is the comparison between three bovine proteins with ribonucleolytic activities such as RNase-A, RNase-4 (also called Lactogenin) and Angiogenin-1 (ANG-1). These proteins are all present in cow milk. Three-dimensional structure is known for RNase-A and ANG-1 while not for bovine RNase-4.

**METHODS:** RNase-A was purchased from Fluka. ANG-1 and RNase-4 were isolated from dairy milk using the method of casein precipitation step by milk acidification followed by fractionation with ammonium sulphate. Then the two proteins have been purified using ion exchange chromatography and RP-HPLC and characterized by mass spectrometry and circular dichroism. Ribonucleolytic activities of three proteins were tested on tRNA, CpG and UpG as substrates. Cell proliferation on HUVEC was assessed by quantification of cells incorporating BrdU and biological activity was evaluated using Matrigel *in vitro* assay. Microbicidal activities were assessed on *E.coli* B48. *In vitro* antitumour properties were evaluated by MTT test against different human tumour cell lines that are among the 60 cell lines screened by the NCI in search of novel cancer chemotherapeutic agents. Cytokine testing has been performed by means of Bioplex suspension system in order to analyse the different profile of cytokines expressed by samples treated with each of the three proteins.

**RESULTS:** The great homology existing between primary structures of human RNase-A and bovine RNase-4 suggested an hypothetical 3D-model for RNase-4. The three proteins have a similar secondary structure because they show a far UV CD spectrum similar in shape and intensity. On the contrary CD spectrum in the near UV is unique for RNase-4 because of a single residue of triptofan which is quite rare in other ribonucleases. Ribonucleolytic activity against tRNA is in order RNaseA>RNase-4>ANG-1. Tests on CpG and UpG as substrates allow the quantitative determination of specificity constants. ANG-1 and RNase-4 induce HUVEC proliferation about 20% more vs control while RNase-A has not significant effects on cellular proliferation. ANG-1 stimulates HUVEC to form capillary-like structure after seeding on Matrigel. RNase-4 seems to have similar effects; results are well observable at concentration $10^{-8}$M. Weak effects are observed for RNAse-A treated cultures. Tests performed on tumour cell lines show that these proteins exert their cytotoxic activity on cancer cells quite selectively, even if ANG-1 elicited the most relevant cytotoxic potential. RNase-4 and RNase-A exhibit an antimicrobial activity against *E.coli.* ANG-1 show less microbicidal activity than the other two proteins.

PO049

# Synthesis of coumarinic compounds as CK2 inhibitors

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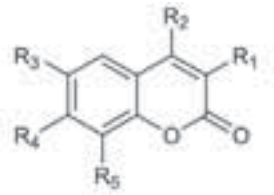
Protein kinase CK2 is ubiquitous, essential and highly pleiotropic kinase whose abnormally high constitutive activity is suspected to underlie its pathogenic potential in neoplasia and possible implication in viral infections. CK2 is a messenger-independent protein serine/threonine kinase with hundreds of protein substrates and is implicated in many cellular functions [1, 2]. Recently a number of halogen-substituted 4-methylcoumarins has shown a good inhibitory activity on proteinkinase CK2. In this work the synthesis of new substituted coumarins is described and a preliminary structure-activity relationship through comparison with literature data was made [3]. Two goals were pursued: to verify the importance of hydroxy group in 7 position of the 3,8-dibromo-7-hydroxy-4-methylcoumarin (DBC) [3] to maintain biological activities; to study how various substituent in 4-position can influence the inhibitory activity.

The structures of novel coumarinic compounds as CK2 inhibitors are summarized in the Figure.




**Structure of synthesized coumarinic compounds.**
$R_1$ = H, Br; $R_2$ = H, OH, $CH_2CH_3$, $CH_2Br$, $CH_2Cl$, $CF_3$; $R_3$ = H, Br; $R_4$ = OH, $NH_2$, $OCH_3$, $OCOCH_3$, SH; $R_5$ = H, Br.

Preliminary results obtained with some prepared compounds demonstrated the relevant role of hydroxy group in 7 position to maintain a good inhibitory activity. Further biological assays are in progress.

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PO050

# Microwave-assisted synthesis of new derivatives of khellinone under phase-transfer catalytic conditions

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Benzofuran derivatives of plant origin have been the subject of increased interest in recent years due to their diverse biological activities such as anticancer, antioxidant, antimicrobial and antifungal properties [1].
Khellinone derivatives: dimers linked via the alkylation of the 6-hydroxy groups and chalcone derivatives discovered by Baell and coworkers are blockers of the voltage-gated potassium channel Kv1.3. In particular several chalcone derivatives of khellinone represent attractive lead compounds for the development of more potent and selective Kv1.3 blocking immunosuppressants [2].
We report the synthesis, preliminary cytotoxicity screening results, $^{13}C$ CP/MAS NMR spectral data of symmetrical and unsymmetrical derivatives of khellinone. An exemplary structure is given in Figure 1.

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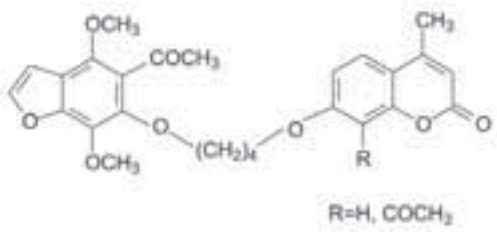


**Figure 1**

PO051

# Synthesis, functional and structural characterization of glutamic acid derivatives as inhibitors of MurD from *Escherichia coli*

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Mur ligases (MurC - MurF) play an essential role in the intracellular biosynthesis pathway of the bacterial peptidoglycan, and therefore represent attractive targets for the design of novel antibacterial agents [1]. Recently, we focused our attention to D-glutamic acid-adding enzyme (UDP-*N*-acetylmuramoyl-L-alanine:D-glutamate ligase or MurD), which catalyzes the addition of D-Glu to the growing UDP-Mur*N*Ac-pentapeptide [2].

During the course of our work, D-Glu derivatives containing sulfonamide functionality were found active against *E. coli* MurD, with $IC_{50}$ values in the range from 100 to 300 μM (e.g. Compound **1**, Fig.1). To check the importance of the stereochemistry of glutamic acid, we also synthesized an L-isomer containing analogue of compound **1**. Surprisingly, compound **2** was also endowed with inhibitory activity, although to a lesser extent ($IC_{50}$ value of 800 μM). In addition, the results of a kinetic study with inhibitors **1** and **2** (performed on MurD from *E. coli*) revealed their competitive mode of inhibition with respect to D-Glu. These results were explained by the crystal structures of the complexes, which unambiguously demonstrated that the glutamate moiety of both stereoisomeric inhibitors binds within the D-Glu binding pocket. The observed binding modes represent an excellent starting point for further development of novel inhibitors of this enzyme.

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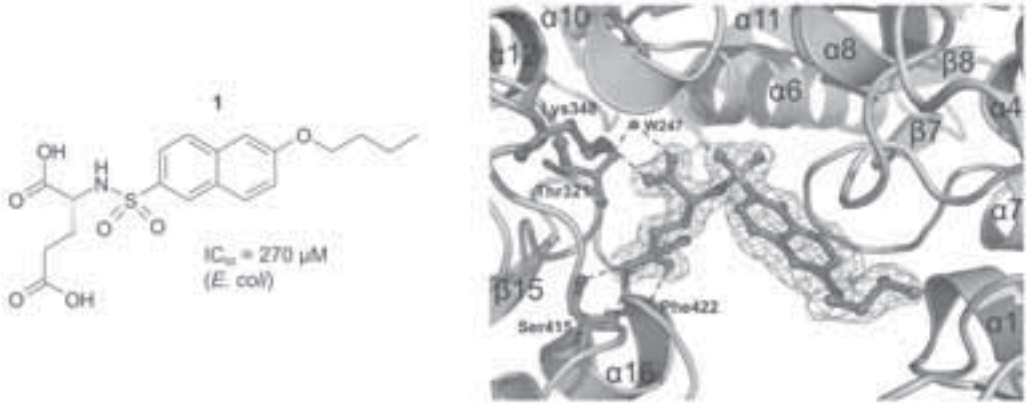


Figure 1: Details of the ligand binding site in the MurD complex with compound **1**. The Fo-Fc residual map is contoured at 3σ.

PO052

# 2-[3-(Biphenyl-4-yl)propan-3-one]-5-(substituted phenyl)-1,3,4-oxadiazoles

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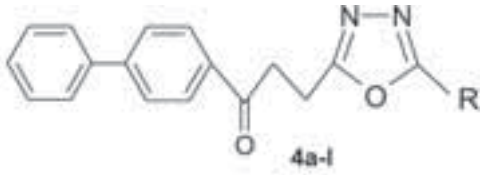
Non-steroidal anti-inflammatory drugs (NSAIDs) are the most commonly prescribed medications in the world. 3-(4-Phenyl-benzoyl)propionic acid is an example of well known aroylpropionic acid class of anti-inflammatory drugs and available in the market under the name fenbufen [1]. Fenbufen is a good anti-inflammatory agent associated with gastrointestinal side effects, which is a common feature with NSAIDs [2, 3]. In our attempt to discover safer agents for treatment of inflammatory conditions, we have replaced the carboxylic acid group of 3-(4-Phenyl-benzoyl)propionic acid with an additional heterocycle i.e. 1,3,4-oxadiazole.




4a-l

Reaction between fenbufen and aryl acid hydrazides in phosphorous oxychloride (reaction time varies from 2 to 5 h) afforded title compounds **4a-l** in 52-71% yield after recrystallization with methanol. Both analytical and spectral data ($^1$H NMR, IR, Mass) of all the synthesized compounds were in full agreement with the proposed structures. The synthesized compounds **4a-l** were evaluated for in-vivo anti-inflammatory activity at a dose level of 20 mg/kg b.w. in rats [4] and showed the activity ranging from 41.08% to 63.28%. Two compounds, 2-[3-(biphenyl-4-yl)propan-3-one]-5-(4-methoxyphenyl)-1,3,4-oxadiazole **4h** and 2-[3-(biphenyl-4-yl)propan-3-one]-5-(3,4-dimethoxyphenyl)-1,3,4-oxadiazole **4i** with anti-inflammatory activity 60.76 and 63.28 respectively, were found to be more active than the standard drug fenbufen which showed 58.46% activity. The compound, 2-[3-(biphenyl-4-yl)propan-3-one]-5-(4-nitro phenyl)-1,3,4-oxadiazole **4e** showed equipotent activity (58.46%) with the parent drug fenbufen. The ulcerogenic liability of the synthesized compounds and the parent drug fenbufen was evaluated at the dose level of 60 mg/kg in rats [5]. The tested compounds showed significant reduction in ulcerogenic activity (severity index) ranging from 0.417 to 0.583, whereas the standard drug fenbufen showed high severity index of 2.516.

Thus it is concluded that cyclization of carboxylic group of fenbufen into oxadiazole nucleus resulted in the significant decrease of ulcerogenic activity while retaining their high anti-inflammatory activity.

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PO053

# Synthesis and cytotoxicity of heterocyclic aldimines

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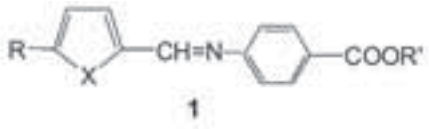
Derivatives of furan and thiophene aldehydes are potential synthones for the preparation of biologically active compounds [1]. Introduction of silyl or germyl group into the heterocycle improves the lipophilicity of the compound and in many cases increases the biological activity [2, 3].

We have studied the reactions of 2-furaldehyde, 2-thiophenecarbaldehyde, and their 5-methyl, 5-trialkylsilyl, 5-dimethylphenylsilyl and 5-trialkylgermyl derivatives with *p*-(alkoxycarbonyl)anilines in dry benzene at room temperature (16-20 hrs) in the presence of molecular sieves (1g per mmole reagent) as dehydrating agent and acid catalyst. A series of new heterocyclic aldimines **1** was synthesized in good yields.




**1**

X = O,S; R = H, Me, $Me_3Si$, $Me_2PhSi$, $Et_3Si$, $Me_3Ge$, $Et_3Ge$; R'= Me, Et, Bu

The cytotoxicity of synthesized compounds was studied *in vitro* on tumour cell lines HT-1080 (human fibrosarcoma), MG-22A (mouse hepatoma) and normal fibroblasts NIH 3T3. All studied furan and thiophene aldimines were compounds with moderate or low toxicity ($LD_{50}$ in range 314-2085 mg $kg^{-1}$). The influence of heterocycle and substituent R on cytotoxic activity of studied compounds was not significant. Only in the case of 5-trimethylsilylderivatives **1** (R' = Me, Et, Bu) the thiophene-containing aldimines exhibited higher cytotoxic activity than furan analogues on HT1080 cell line ($LC_{50}$ 2-9 μg $ml^{-1}$), and at the same time these compounds exhibited low cytotoxicity and toxicity for normal fibroblasts NIH 3T3 ($LC_{50}$ 125-396 μg $ml^{-1}$).

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PO054

# Peptide conjugates possessing thrombin inhibition activity and fibrinogen receptor antagonism

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Thromboembolic diseases are a leading cause of mortality and morbidity in the developed world [1]. Huge development has been achieved in last decades in treating of thromboembolic diseases; many new compounds were introduced to the market, however lacking satisfying result. To achieve a therapeutically efficient antithrombotic effect, a combination of various antiaggregatory and anticoagulant agents is used in clinical practice nowadays.

We explored the possibilities of a single drug [2] possessing thrombin inhibitory activity and fibrinogen receptor antagonism. Ac-*D*-Phe-Pro-Arg and H-Arg-Gly-Asp-OH are peptide lead compounds for thrombin inhibitory activity and fibrinogen receptor antagonism respectively. Both moieties were used in a single peptide linked with a glycine linker. Compounds were synthesized using solid phase approach and solution phase condensation. Comparison of activity of all three compounds will show the successfulness of proposed concept. As an addition to peptide conjugate, fused multiple ligands were also prepared – tripeptides, modified tripeptides and pseudopeptides as compounds capable of acting on both targets.

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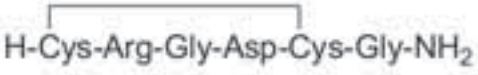


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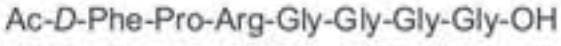


[3]

PO055

# Antithrombotic compounds with dual activity- thrombin inhibitory activity and fibrinogen receptor antagonism

**Janez Ilaš,** Danijel Kikelj, Žiga Jakopin, Tihomir Tomašič, Tina Borštnar, Tilen Matjaž

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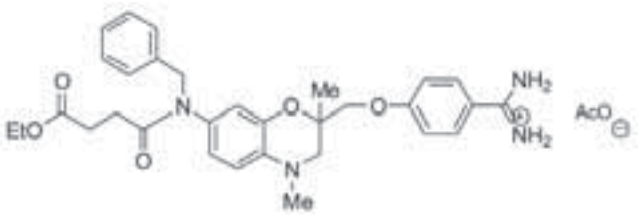
Rational design of compounds with designed multiple mode of action towards multiple targets is becoming a widely used approach in drug design [1]. We have continued our work in the development of substituted 1,4-benzoxazines with thrombin inhibitory activity and fibrinogen receptor antagonism as novel antithrombotic drugs [2]. Benzamidine moiety was used for the P1 part of the molecule, methyleneoxy spacer between benzoxazine and benzamidine moiety was shown to be very effective for the thrombin inhibitory activity and good fibrinogen receptor antagonistic activity, compounds with methylene spacer between benzoxazine and benzamidine moiety showed even superior effect on thrombin inhibition, however with reduced fibrinogen receptor antagonistic activity. Main focus was pointed towards optimization of P3 moiety, with different combination of aromatic and carboxyl group moieties.

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PO056

# Synthesis and evaluation of novel propafenone analogs as potential antiarrhythmic agent

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Propafenone is a class Ic antiarrythmic drug widely used for conversion of atrial fibrilation to sinus rhythm [1,2], to prevent recurrences in patients with atrial fibrillation. As such it is able to depress intracardiac conduction velocity as a consequence of its binding to the open state of cardiac $Na^+$ channels [3]. However, propafenone at the same range of concentrations inhibits several $K^+$ currents: $I_{TO,}$ $I_{KUR,}$ $I_{Kr}$, $I_{Ks}$ and $I_{K1}$ and at higher concentration the L-typ $Ca^{2+}$ current ($I_{Ca}$).

A range of compounds analoges of propafenone designd to study the centr aromatic ring and the amino substituent has been reported in the literature [4].

In order to investigate this class of compounds further of serial of compounds with modifications in the benzyl moiety have been designed on the basis of pre QSAR and pharmacophoric studies.

The proposed derivatives were prepared in four steps. The first step in the synthetic route consisted in the condensation of acetophenone with *o*-, *m*- or *p*- mono substituted benzaldehyde to give an α,β-unsaturated ketone. The reduction of α,β-unsaturated ketone with triethylammonium formate produced saturated ketone. The addition of phenolic nucleophiles to epichlorohydrin in basic media afforded aryloxyepoxipropane derivatives that was subjected to aminolysis with propylamine to give aryloxypropanolamine derivatives.

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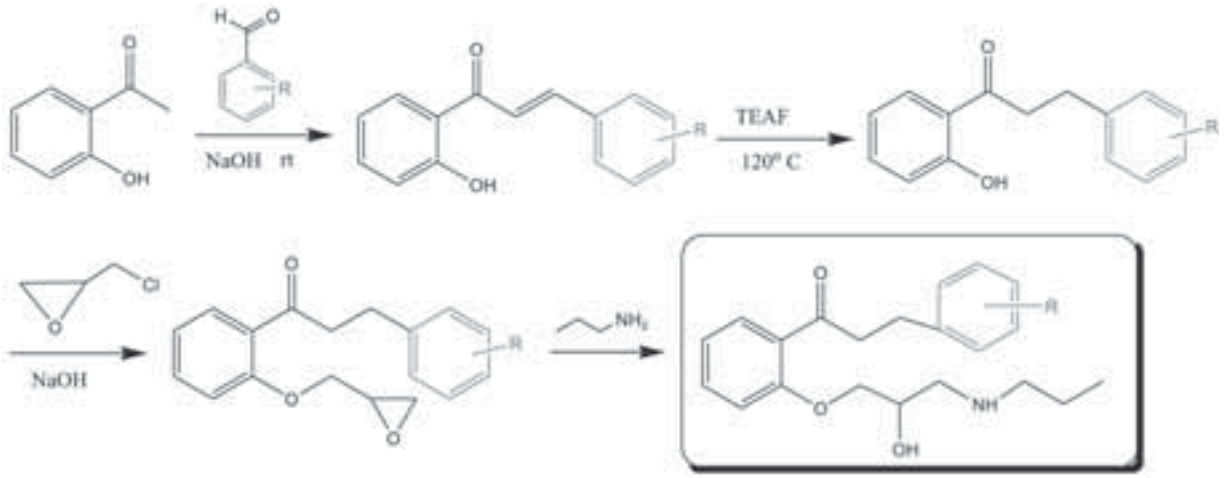


Physicochemical properties of the proposed compounds have been showed as well.

PO057

# Novel 4-aryl-2H-pyrido[1,2-c]pyrimidines with 5-HT-transporter and 5-HT$_{1A}$ affinity

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Depression is a mood disorder, which affects an estimated number of more then 100 million people worldwide. The treatment of depression has been revolutionized by the introduction of selective serotonin reuptake site inhibitors (SSRIs) that possess fewer side effects than tricyclic antidepressants. The major drawback with the current line of SSRIs is that they have a delayed onset before the beneficial therapeutic effect is observed. One approach to create a fast-acting SSRI is to combine, in a single molecule, an agent with an SSRI activity and an 5-HT$_{1A}$ antagonist activity [1-5].

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

The structure of new compounds was confirmed by $^{1}$H and $^{13}$C NMR spectral data as well as by C, H, N analysis.

The target compounds were tested for their affinity for 5-HT$_{1A}$ receptor and 5-HT reuptake inhibition using radioligand binding assay.

The tested compounds showed relatively high affinity for the 5-HT$_{1A}$ receptor and serotonin reuptake inhibition at nanomolar concentration for dual activities ($K_i$ = 4,8 – 10,9 nM and $K_i$ = 15,3 – 35,2 nM respectively). The compounds with the highest affinity for 5-HT$_{1A}$ receptor and serotonin reuptake inhibition are now tested for their antidepressant activity.

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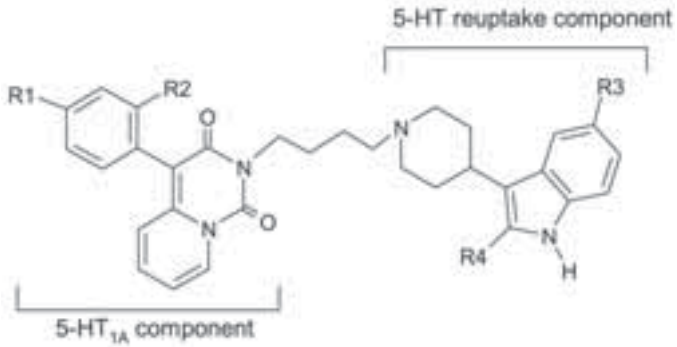


R1 = H, $CH_3$, $OCH_3$, F, Cl
R2 = H, $CH_3$, $OCH_3$, F, Cl
R3 = H, $OCH_3$
R4 = H, $CH_3$

PO058

# Synthesis of 3,5-disubstituted 1,2,4-oxodiazoles as peptidomimetic building blocks

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The 1,2,4-oxadiazole nucleus is often used as a classical bioisosteric replacement for an amide or ester functionality [1,2], because of its electronic properties. Its derivatives can be found in a vast number of compounds exerting biological activity. 1,2,4-Oxadiazole moieties have already been used in the design of dipeptidomimetics as peptide building blocks [3,4]. The compounds described have an ester functionality attached directly to the heterocycle at the 3-position, which had to be converted to free acids when used in the following reactions [3,4,5]. It is a well known fact that 1,2,4-oxadiazole-3-carboxylic acids are readily decarboxylated [5]. In an attempt to overcome this problem, we describe the synthesis of 12 new 3,5-disubstituted 1,2,4-oxadiazoles, bearing in their structure a Boc-protected amine and a carboxylic acid or an ester functionality, which makes them useful as peptidomimetic building blocks [6]. One, two and three methylene groups have been incorporated between the heterocycle and the carboxylic acid group to increase the stability of the final compounds. The synthetic steps are simple and require only readily accessible chemicals (the amidoxime precursors are derived from α-amino acids [7]) thus affording the products in good overall yields. The mild reaction conditions allow the chiral configuration of the starting amino acids to be retained, thus affording enantiopure products. The synthesized compounds were fully characterized by spectroscopic data whereas the enantiomeric purity of these compounds was evaluated by HPLC using a chiral stationary phase.

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PO059

# Structure-lipophilicity relationships in series of methyl moiety ring-substituted pyrazinecarboxanilides as new antituberculotics

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One of the major prerequisites for pharmacological screening and drug development is the prediction of absorption, e.g. the transport of a molecule through cellular membranes. The drugs cross biological barriers most frequently through passive transport, which strongly depends on their lipophilicity. Therefore hydrophobicity is one of the most important physical properties of biologically active compounds.

Various ring-substituted pyrazinecarboxamides have been prepared and evaluated for their biological activity. Twenty-six anilides of pyrazine-2-carboxylic acid substituted with methyl moiety in anilide part of the molecule [1-5] were analysed using the RP-HPLC method for the lipophilicity measurement. The procedure was performed under isocratic conditions with methanol as an organic modifier in the mobile phase using end-capped non-polar $C_{18}$ stationary RP column.

In the present study the correlation between RP-HPLC retention parameter log *K* and log *P* data calculated in various ways is discussed as well as the relationships between the lipophilicity and the chemical structure of the studied compounds.

The study was supported by MSM 0021620822 and by the Contact-Slovenia program Nr. 06/2006-2007.

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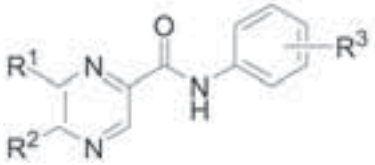

$R^1$ = H, Cl
$R^2$ = H, *tert*-Butyl
$R^3$ = H, 2-$CH_3$, 3-$CH_3$, 4-$CH_3$, 2,6-$CH_3$, 2,4,6-$CH_3$

PO060

# Improved o-acylcarnitine derivates.

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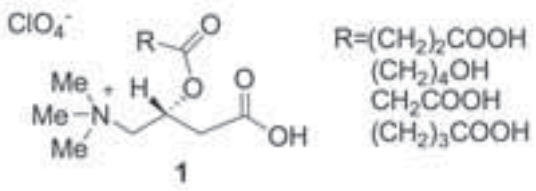
O-Acylcarnitines (1) are diagnostic metabolites for certain inherited diseases [1].
Published syntheses of O-acylcarnitines are neither efficient nor do they lead to pure products [2].
In order to supply reference materials for the unequivocal identification of O-acylcarnitines in the context of a program to screen for metabolic disorders in newborns we have developed improved methods for compounds **1** including the use of solid supports.

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PO061

# The Synthesis of deuterated tolperisone and tolperisone metabolites

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Certain 2-methyl-3-aminopropiophenones exhibit muscle relaxant activity and are being developed for the treatment of back pain [1]. Among these, tolperisone is of particular interest because L-tolperisone exhibits bronchodilatory and peripheral vasodilatory activity, while the D-tolperisone acts as a muscle relaxant [2]. The metabolic pathway of tolperisone is known and eleven of its metabolites have been identified in human urine [3]. Additionally, it has been claimed [4], that deuterated analogs may exhibit improved pharmacokinetics and pharmacodynamic properties.

We have synthesized deuterated tolperisone (1), as well as deuterated tolperisone metabolites (2, 3, 4, 5, 6). These deuterated analogs will aid in the analysis of low concentrations of **1** and its metabolites in body fluids.

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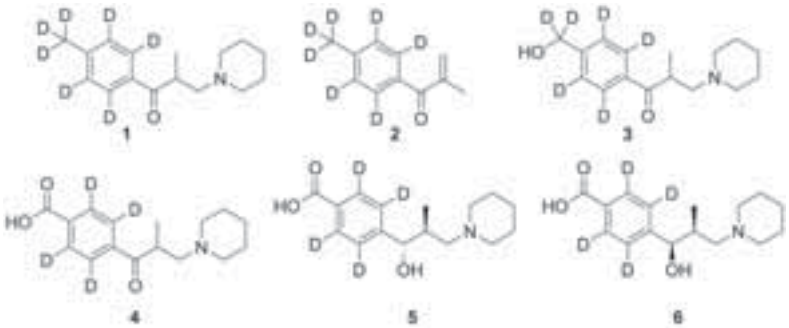

PO062

# Effect of polymyxin B on human erythrocytes with membrane-incorporated lipopolysaccharide from *Rhodobacter capsulatus*

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Lipopolysaccharides (LPSs, endotoxins) of Gram-negative bacteria stimulate the release of inflammatory mediators which are responsible for clinical manifestation of the septic (endotoxic) shock [1]. One of the perspective approaches of septic shock prevention is an application of relatively nontoxic LPSs from photosynthetic bacteria *Rhodobacter capsulatus* ($LPS_{Rb.caps}$) [2], *Rhodobacter sphaeroides* [3], as well as lipid A synthetic analogues such as E5564 [4]. These compounds are relatively nonagonistic and can effectively block some of the *in vitro* and *in vivo* effects of agonistic LPSs like these from *Escherichia coli*. Now, it is known, that $LPS_{Rb.caps}$ is capable to insert into human erythrocyte membranes *in vitro* [5] and upon long time injection into bloodstream may interact with these cells *in vivo*. It remains to be determined whether cationic antibiotics like polymyxin B (PMB), which specifically interacts with Re form of LPSs on the cell wall surface and lyses the bacterial cell, may cause identical effect on human erythrocytes with membrane-intercalated $LPS_{Rb.caps}$.

$LPS_{Rb.caps}$ was extracted according to [6] and characterized on the presence of contaminations. The isolated erythrocytes (E) were washed three times with phosphate buffered saline (PBS), pH 7.4 at 4°C. The E ($5\times10^6$/ml) were incubated in PBS with $LPS_{Rb.caps}$ concentrations 10 or 30 μg/ml for 5′, 15′, 30′ and 60′ at 37°C. The cells were washed further three times with PBS and incubated in PBS-PMB (20 and 60 μg/ml respectively) solution for 15′ at 37°C [7]. The absorbance of supernatants was measured with the "Hitachi" spectrophotometer at 540 nm [8].

Our findings suggest that PMB interacts with E membrane-intercalated $LPS_{Rb.caps}$ and causes hemoglobin release. Hemoglobin release was time- and dose-dependent and detected after 60′ of incubation at $LPS_{Rb.caps}$ concentration of 10 μg/ml, reaching maximal level at 30′ of incubation at $LPS_{Rb.caps}$ concentration of 30 μg/ml. The same result of PMB-induced hemolysis was obtained with rabbit E after its incubation with Re-LPS from *Salmonella minnesota* R595, but the high level of E lysis was achieved after 5′ of incubation [7, 9]. The most likely explanations of the differences observed are as follows: (i) $LPS_{S.minnes}$ possesses more-hydrophobic lipid A [10] and may interact with E more rapidly in comparison with $LPS_{Rb.caps}$; (ii) $LPS_{Rb.caps}$ has decreased molecular charge and completed core region [11] that may influence on its interaction with PMB; (iii) the differences in molecular conformations of lipid $A_{Rb.caps}$ and lipid $A_{S.minnes}$ [10] may lead to different molecular configurations of the corresponding LPSs in E membranes and different PMB effects. The obtained results in the present work suppose the necessity to exclude administration of specific to LPS cationic antibiotics at endotoxic shock therapy by nontoxic LPSs.

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PO063

# Modeling of GluR5 kainate ion channel in the closed form

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The glutamate and aspartate amino acids, together with a few their analogs, mediate most of the excitatory synaptic transmission in the brain. The glutamatergic pathways are involved in such diverse processes and diseases as epilepsy, ischemic brain damage, learning, Parkinson's disease, Alzheimer's disease, Huntington's disease, alcoholism, schizophrenia, mechanism of stress, regulation of mood and nociception process. Among all the glutamatergic receptors, ionotropic kainate receptors are the least known. However, it is proved, that thay play an important role in the process of epileptogenesis and that they are engaged in inducing of synaptic plasticity. Although the therapeutic potential of kainate receptors is underestimated, the ligands of kainate receptors seem to lack undesirable psychotropic activity, which was demonstrated for many high-affinity NMDA receptor antagonists. The aim of this work was to model kainate GluR5 ion channel in the closed form. The whole subunits of glutamatergic receptors, having about 1000 amino acids, and, as a consequence, the full glutamatergic channels, are rarely modeled, first because of the lack of templates, second – because of difficulties emerging at joining specific domains of a subunit. Thus, either a pore domain or a binding site, rarely ANF receptor region have been modeled up to know. Recently, binding site domain have been solved for many glutamate receptors. Little is known, however, about detailed 3D structure of remaining domains and about the whole receptor protein.

To overcome above difficulties, homology modeling, threading and Rosetta, Robetta and Protinfo methodologies were applied to build a model of closed-form GluR5 ion chanel. The following templates were selected: bacterial periplasmic substrate-biding proteins (1pea and 2liv) for the ANF receptor domain, the experimental structure of GluR5 and GluR6 binding sites as well as potassium channel 1lnq, due to the recent reports about remote homology between glutamatergic and potassium channels. The potassium channel coordinates were used both to model a transmembrane part and to produce the tetrameric channel. All the linkers were modeled with Rosetta, Robetta, Protinfo and HHPred.

The multiple alignment was produced using MAFFT and refined manually. Modeller 9v1 was applied to obtain the population of GluR5 closed-form ion channel. The raw models were minimized with Sybyl7.3 software. The stereochemical structure of the developed models was verified with the Procheck software.

The obtained models are in good agreement with all the available experimental data. S1 and S2 in the bindig site are correctly situated, as well as the transmembrane region with transmembrane helices and a transmembrane loop form a pore as expected. However, further studies are necessary to estimate the quality of models in the docking procedure.

This work is funded by Polish Ministry of Science, grant number 405 021 31/1121. Calculations were performed in the framework of computational grant by Interdisciplinary Centre for Mathematical and Computational Modelling, grant number G30-18.

PO064

# On the way to "bio-chip" for non-invasive lung disease diagnostics

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The lung disease diagnostic methods, utilized in the contemporary medical practice, consist so far of a combination of invasive and *semi*-invasive interventions. These, however, require a significant patient's endurance or even worse, especially in the case of children, are often burdened with a traumatic experience. The analysis of the breath condensate is a relatively novel method, which represents an alternative way that can be characterized as an entirely non-invasive and comfortable diagnostic method even to infant patients. The principle of this method is based on the quantification of substances specific for the given disease – "biomarkers" that are contained in body fluids and whose concentration levels are significantly elevated as a consequence of specific processes proceeding in airways and lungs. To analyze a breath condensate, biochemical methods are used, in particular, methods utilizing highly sensitive and specific techniques based on mass spectrometry. The current state-of-the-art of analyzing the breath condensate from exhaled air, used as a source of a broad scale of biomarkers indicating lung disorders, encompasses a significant series of methods applied for their determination. However, up to now, the published data exhibit a rather eminent variance. The cause seems to be a negligence of a sequence of specific factors that are essential for the successful processing and consecutive analysis of this liquid fluid. The submitted work had aspired to review and quantify key parameters that in authors' opinion led to the contradictory results published in the professional literature and establish a protocol for an effective processing of breath condensate and determination of specific biomarkers by the method of mass spectrometry. The outcome of this experimental pursuit for this field is the development of a genuine method of a highly specific separation of biomarkers – immunoaffinity extraction, which is based on the reaction of a monoclonal antibody with a relevant marker, combined with a sufficiently sensitive and selective detection method – mass spectrometry analysis, allowing the quantification of these specific substances. For the developed method, a high sensitivity, a low determination error (< 8 %) and a high reproducibility are typical characteristics. The presented method is suitable and recommendable for a further expansion by a parallel determination of a wide spectrum of biomarkers applicable for various lung disorders in just one analysis. The last but not the least, this invention is well-suggestive of "bio-chip" applications for routine, time-undemanding determinations of concrete biomarkers implemented to the practice as e.g. rapid and on-time/early diagnoses for a varied number of lung disorders.

Acknowledgement Ministry of Health of Czech Republic (Grant 8108-3/2004), Ministry of Education of Czech Republic (Grant CEZ: MSM 223 100001).

PO065

# The effect of the glutathione and its analogues on activity of Na,K-ATPase of human brain in vitro

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In present study we have examined the effect of the antioxidant tripeptide GSH and its synthetic analogues UPF1 [1] and UPF17 to Na,K-ATPase of the *post mortem* human brain *in vitro* reactions.

Both analogues are tetrapeptides, where on to glutathione has been compound methylated tyrosin. In UPF17 structure glutamates carboxyl group and cysteines amino group are bonded by $\alpha$-peptical linkage, which is less stabile than $\gamma$-peptical linkage.

Earlier researches with UPF1 and UPF17 have shown their potent effects as antioxidants. Thus it is important to research these substances as possible prodrugs to be used in future.

The present findings indicate that GSH and synthetic analogues are able to affect activity of sodium pump. Effect of analogues depends of their chemical structures. Synthetic peptides compared with natural GSH have much powerful inhibiting effect, thus it would be practical output for using them to affect function of Na,K-ATPase.

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PO066

# The structural study of (4-arylpiperazin-1-methyl)-isothiazolo [5,4-b]pyridines as analgesic agents.

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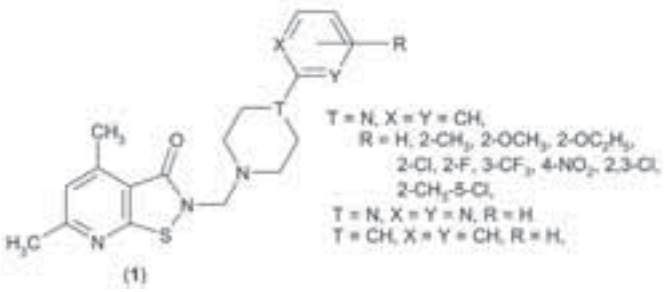
Arylpiperazine derivatives of isothiazolopyridine of Mannich base type of general structure (**1**) exhibited differentiated analgesic activity in pharmacological screening [1].




(1)

To explain the observed discrepancy of biological effects within series (**1**), we studied structure/analgesic activity relationship with a view of determining which geometry and electronic parameters of the molecules are important in concerning of the analgesia. At our SAR study we found that the conformation of arylpiperazine substructure, steric and substituent effects, molecular electrostatic potential and charge distributions within arylpiperazine part may exert influence on the pharmacological activity of compounds (**1**). Basic structural and conformational information were obtained from X-ray investigations [2]. Electronic parameters were calculated using semiempirical (AM1) and *ab-initio* (RHF SCF, 6-31G** basis set) quantum chemical methods.

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PO067

# Impact of chloro-substituents on the structures of N-(chloro)-benzyl-3-pirocycloalkylopyrrolidine-2,5-diones

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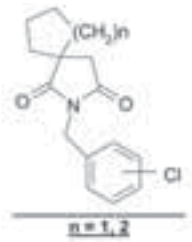
In our research program concerning compounds with confirmed biological activity, we have focused our attention on H-bond net in new class compound N-(chloro)-benzyl-3-pirocycloalkylo-pyrrolidine-2,5-diones (see scheme).




It is well known that hydrogen bonds are the most important among all intermolecular interactions in any environment. H-bonds take part in the formation of complexes between biological receptor and particular ligand [1-3]. But on the other hand H-bonds are responsible for packing motifs formation in nearly each crystal of organic substances and in most cases direct information about the H-bonds geometrical arrangements is taken directly from crystallographic data. Based on the six structures, it was established that the strongly electron-withdrawing Cl-substitunt(s) has/have not destroyed general supramolecular synthons formation of other spiro-succinimides [4, 5]. Furthermore, such substituent has become the origin of non-conventional interactions of Cl…H-C classified as specific weak H-bonds. Geometrical and topological details for weak Cl…H-C interaction have been determined based also on the data from CSD [6].

This study was supported partly by the KBN grand Nr 3P05F 024 25.

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PO068

# Investigation of membrane interactions of pharmacologically active compounds by a novel biomimetic colorimetric sensor

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Elucidation of interactions between pharmacological compounds with the cell membrane plays major roles in determination of the mechanisms of action of membrane-active compounds. We demonstrate applications of a new biomimetic membrane sensor composed of conjugated polydiacetylene with synthetic or/and natural lipids or fragments of nature membrane which undergoes visible and quantifiable blue-to-red color transitions upon cell membrane perturbation. Data obtained by the colorimetric assay sheds light on mechanisms of drug-membrane interaction, degree of bilayer insertion, pore formation and transport through the cell membrane. It can provide useful information that can further help in diagnosis of different diseases, such as cancers, AIDS, central nervous system (CNS) disorders, Alzheimer disease and psychiatric disorders.

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PO069

# Syntesis of pentabromobenzylisothiourea derivatives and their proapoptotic activity against malignant glioma cells

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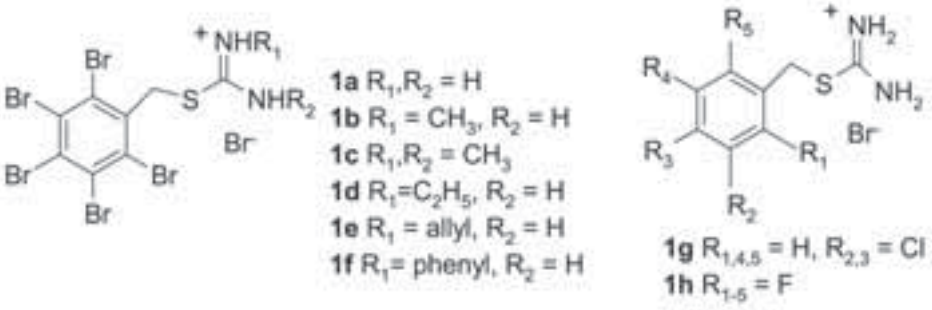
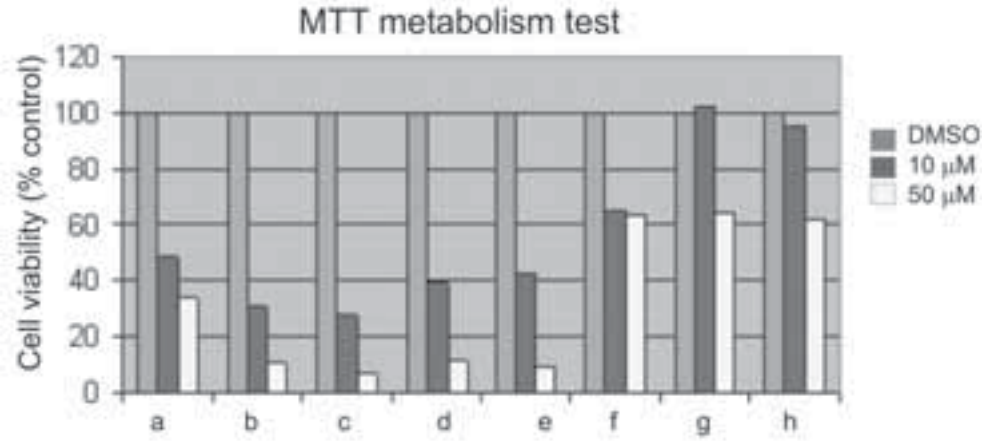
Isothioureas are known as iNOS inhibitors and some of them showed a limited antibacterial activity. Here we report a cytotoxic and proapoptotic activities of new pentabromobenzylisothioureas. The isothioureas **1a-f** were obtained in the reaction of bentabromobenzylbromide with thiourea derivatives. Additionally as comparison 3,4-dichlorobenzyl- (**1g**) and pentafluorobenzyl-(**1h**) isothioureas were used in this study




Cytotoxic activity was assessed by exposing the C6 rat glioma cells *in vitro* to 10 and 50 μM of aforementioned compounds for 24 and 48 h and evaluation cell viability using MTT metabolism test. The most active compounds were **1a-e**. The more bulky phenyl substituent in thiourea fragment (**1f**) of the molecule reduces anti-glioma activity in pentabromobenzyl-series. It is worth to note, that compounds **1g** and **1h** carrying Cl or F substituents showed much lower activity. The isothioureas induce apoptosis in glioma cells evidenced by high expression of active caspases 3 and 7. The detailed mechanism of action is not clear. We assume that amphiphilic isothioureas probably interact with cell membrane inducing cascade reactions inside the cell.




The study is supported by Ministry of Education and Science grant PBZ-MIN 014/P05/2004 (Poland) and Foundation for the Development diagnostic and Therapy (Warsaw, Poland).

PO070

# Predictive QSAR modeling for $A_1$ and $A_{2A}$ adenosine receptors' ligands.

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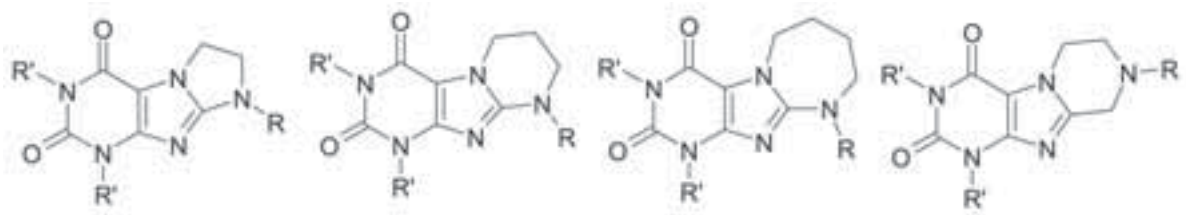
Adenosine receptors (AR) play an important role in regulation of the many processes in the body. ARs selective antagonists are developed for the treatment of such neurodegenerative disorders as Alzheimer, Parkinson and Huntington diseases. Xanthine derivatives have been described to posses high ARs antagonistic activity.
Potent ligands among tricyclic ligands have been synthesised in the Department of Technology and Biotechnology of Drugs [1-2].

As a continuation of this research, more than 100 new xanthine derivatives have been obtained recently and studied for their $A_1$ and $A_{2A}$ ARs binding affinity:
A large number of various antagonist derivatives have been tested at the adenosine receptors binding but only several research groups are developing QSAR models that allow prediction of the active compounds and design of new potent antagonists. In this investigation QSAR studies have been performed based on the ligands bio-



logical activity and physicochemical properties. Molecular descriptors have been calculated. The best QSAR equation was obtained on the base of 4 descriptors to predict the $A_{2A}$ AR's affinity values (Ki) using multiple linear regression:

$$K_i = -k_1 * LogP - k_2 * E_{HOMO} + k_3 * E_{LUMO} + k_4 * E_{steric} - k_0$$

where $k_{0\text{-}4}$ are constants, $E_{HOMO}$, $E_{LUMO}$ and $E_{steric}$ are HOMO, LUMO and steric energy values. The model derived gave a correlation with a cross-validated coefficient (predictive power) of $\mathbf{r^2_{CV}=0.847}$ and square regression coefficient $\mathbf{r^2=0.923}$.
The linear equation that best represents the dependence between experimental and predicted $K_i$ values is:

$$K_{iexp} = 1.063 * K_{ipred} - 0.793, \quad r^2 = 0.943$$

## Acknowledgment

This work was supported by the Polish Committee of Scientific Research (Grants No 2 PO5F 014 28, No 2 PO5F 022 26)

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PO071

# Theoretical studies on lipophilicity and selected structural parametres of novel derivatives of 3,5-dioxo-4-azatricyclo[5.2.2.0$^{2,6}$]undecanes

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The aim of this work was determination of the selected structural parameters of 12 novel N-substituted 3,5-dioxo-4-azatricyclo[5.2.2.0$^{2,6}$]undecanes which are potent antagonists of 5-HT$_{1A}$ receptor. These structural parameters are responsible for weak correlation of lipophilicity parameter logP$_{exp}$, determined using reverse phase thin layer chromatography (RP-TLC) with logP values calculated with application of suitable algorithms.

Taking into account use of the ion-pair agent in the experimental procedure, the HYBOT PLUS software was used to calculate the following structural parameters: sum of all overall H-bond acceptor factor, van der Waals' acceptor surface area which is proportional to H-bond overall free energy factors (CaO) of acceptor atoms, surface area around a molecule where interactions of acceptor atoms of a molecule with a H-bond donor probe have been optimally placed and which is proportional to product of H-bond enthalpy factor absolute values of those atoms, surface integral for enthalpy values of interactions between acceptor atoms of a molecule and a donor probe on the surface (OEASA). The obtained results of calculations indicate that factors connected with formation of acceptor hydrogen bond are the most probable reason of a discrepancy between experimental and theoretical logP values.

The next stage of research was conformational analysis for the considered ligands with simulation of a solvent by introduction of distance-dependent dielectric constant. The conformational analysis was performed for both neutral and protonated molecules in vacuum, n-octanol and water with application of Sybyl7.3 software.

The conformational analysis allowed to conclude that for all the ligands, including compounds with 3-carbon chain, the "non-linear" form is energetically favored. However, there are considerable differences within the group concerning a degree of exposure for hydrogen bond of N2 nitrogen atom of the piperazine moiety. It affects binding of the ion-pair agent during lipophilicity determination, and – as a consequence – the experimental value of lipophilicity parameter LogP$_{exp}$.

The results of calculations are in agreement with experiment. They prove the importance and necessity of studying of inter- and intramolecular structural factors in the process of lipophilicity determination. Moreover, the data resulted from conformational analysis, will be further applied in the procedure of docking of novel ligands to 5-HT$_{1A}$ receptor, because the N2 nitrogen atom of piperazine moiety is responsible for key interaction with Asp(3.32). Furthermore, the results of conformational analysis may also explain the differences in affinity of considered ligands to 5-HT$_{1A}$ receptor.

This work is performed in the framework of computational grant by Interdisciplinary Centre for Mathematical and Computational Modelling, grant number G30-18.

PO072

# Homology modeling and binding site analysis of the human histamine H4 receptor

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

Three dimensional models of the human histamine H4 receptor (hH4R) were developed by means of homology modeling. The binding site of hH4R was mapped, and several interaction points were explored. Histamine and other known H4 agonists and antagonists were docked successfully into the homology model with FlexX.

To evaluate whether any of our models are suitable for selecting hH4R active compounds from a dataset containing known actives and inactives, enrichment studies were carried out with diverse and druglike sets of molecules. Five different scoring functions were used in this study. We have also analyzed the influence of using different sets of inactives. Pharmacophoric constraints were also applied. The results showed that some of our models could achieve reasonably high enrichment factors that makes it possible to use them for virtual screening. One of these models were chosen for screening about 9 millions of compounds by FlexX. The in vitro screening of the most interesting compounds is currently investigated.

PO073

# Site-specific protonation study on tenoxicam using a novel deductive approach

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Tenoxicam, 4-hydroxy-2-methyl-*N*-(pyridin-2-yl)-2H-thieno[2,3-*e*]-1,2-thiazine-3-carboxamide 1,1-dioxide, the potent NSAID drug, acts by blocking the formation of prostaglandins and has had a significance roll in the treatment of rheumatic diseases over the last decades. It has a bifunctional amphoteric structure: the enolic acid and the pyridin-2-yl moieties represent the acidic and basic sites, respectively. This result in the presence of four individual microspecies in protic solvents (e.g. in tissue fluids): anionic, zwitterionic, uncharged and cationic. The fused-ring thienothiazine system and the conjugative enolic-carboxylic acid amide structure of the molecule manifest in dynamic structural properties that change significantly in terms of protonating states, e.g. the UV spectrum is strongly dependent on pH.

Tenoxicam is structurally related to piroxicam, i.e. the benzene ring of piroxicam is replaced by the bioisostere thiophene heterocycle in tenoxicam. The microspeciation of piroxicam was resolved through the O-methyl-piroxicam molecule that could be regarded as a model compound for the protonation of the pyridin-2-yl group while the protonation state of the enolic functional group remains protonated [1]. The same method could not be used for tenoxicam as the sulphur atom of the thiophene ring destabilise the ether bond of O-methyl-tenoxicam resulting in rapid hydrolytic cleavage [2].

Here we report a novel deductive approach for the determination of the site-specific ionisation properties of tenoxicam. To describe the influence of the pyridin-2-yl group to the acidity of the enolic hydroxyl group we synthesised a series of variously substituted anilides of both oxicams and determined their acidities spectrophotometrically. From the Hammett plot of piroxicam derivatives the 2-aza-$\sigma^{o}_{R}$ value of the unprotonated pyridyl substituent was identified as 0.29. It designated the site-specific protonation constant for the enolic group of the unprotonated tenoxicam from the Hammett plot of tenoxicam analogues. All the other microconstants were also calculated. The Hammett approach as a deductive method was applied at first in the determination of the microconstants of drug molecules and proven to be a helpful tool to find the ideal electronwithdrawing substituent in aromatic ring replacements.

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PO074

# Novel cholesterol biosynthesis inhibitors targeting the human lanosterol 14α-demethylase (CYP51)

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**These authors contributed equally to the content of this work*
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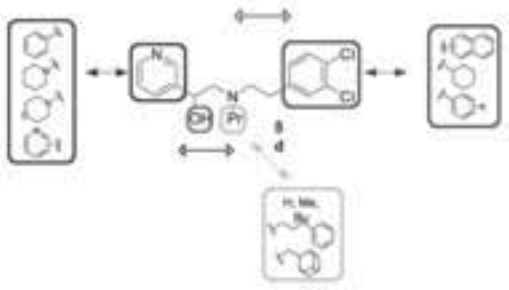
Coronary heart disease is one of the leading cause of mortality and morbidity in Europe, United States and Australia. Treatment of hyperlipidemia is one of the main prevention measures for the development of atherosclerosis that may result in coronary heart disease.

Several different lipid-lowering therapies exist. Cholesterol biosynthesis inhibition has been proven as the most effective approach in lipid lowering therapy. Statins, the most potent and widely used cholesterol-lowering drugs, function as competitive inhibitors of HMG-CoA reductase. This enzyme is involved in cell homeostasis. We expect that post-squalene cholesterol biosynthesis inhibition will have less adverse effects. Non-responsiveness to statin therapy is another important reason, which makes the searching for novel hypolipidemic drugs remains a very important task

We have discovered a novel group of cholesterol biosynthesis inhibitors, pyridylethanol(phenylethyl)amines, that target the human lanosterol 14α-demethylase *(CYP51).* The lead compound is 2-{[2-(3,4-dichlorophenyl)ethyl](propyl)amino}-1-pyridin-3-ylethanol dihydrobromide (**5d**). Structure activity relationship (SAR) studies by sterol profiling and the overexpressed human CYP51 revealed subgroups of strong CYP51 (Kd 0.34 – 3.3μM) and weak CYP51 inhibitors (Kd 27 – 35 μM). A subgroup of inhibitors targeting sterol Δ14-reductase *(DHCR14)* has also been identified.

**5d** lead optimization was directed towards the following modifications:



PO075

# Diazenedicarboxamides as inhibitors of D-alanine-D-alanine ligase (Ddl)

**Andreja Kovač**[a], Vita Majce[b], Roman Lenaršič[b], Sergeja Bombek[b], Julieanne M. Bostock[c], **Ian Chopra**[c], **Slovenko Polanc**[b], and **Stanislav Gobec**[a]

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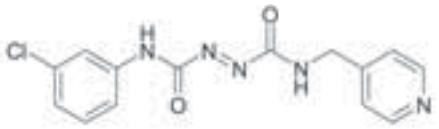
The emergence of bacterial resistance to antibiotic therapy has become a global health threat. To overcome this problem, new antibacterial agents directed toward novel targets have to be developed. The best known and most validated target for antibacterial therapy is the system of enzymes responsible for the construction of peptidoglycan. D-Alanine-D-alanine ligase (Ddl) catalyzes the biosynthesis of an essential bacterial peptidoglycan precursor D-alanyl-D-alanine and since it has no human counterpart it represents an important target for development of new antibacterial drugs. The most important inhibitor of Ddl is no doubt a structural analog of D-alanine, the antitubercular agent D-cycloserine.

We synthesized series of semicarbazides, aminocarbonyldiazenecarboxylates, diazenedicarboxamides, and hydrazinedicarboxamides and screened them for inhibition of DdlB from *Escherichia coli* [1]. In a series of semicarbazides, aminocarbonyldiazenecarboxylates and hydrazinedicarboxamides, only some aminocarbonyldiazenecarboxylates displayed moderate inhibition at 500 μM, while the remainder were inactive. In contrast, very potent inhibitors were obtained in the series of diazenedicarboxamides. Compounds that exhibited DdlB inhibitory activities were further tested for their antimicrobial activities. The minimal inhibitory concentrations (MICs) of each compound were determined against *E. coli* 1411, and SM1411, an acrAB deficient derivative of 1411, and *S. aureus* 8325-4. Thirteen compounds were better inhibitors than D-cycloserine, and five of them had $IC_{50}$ values below 50 μM and MIC values as low as 64 μg/mL against *E. coli* and *S. aureus*. These inhibitors are structurally distinct from both ADP and D-alanine, so we expect that they inhibit Ddl by a novel binding mode.



$IC_{50}$ (DdlB) = 33 μM
MIC (*E. coli* 1411, *E. coli* 1411 AcrAB-, *S. aureus*) = 64 μg/ml

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PO076

# Bicyclic-heterocyclic-derivatives as cannabinoid $CB_1$ receptor ligands

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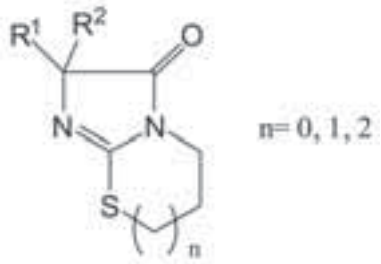
The cloning of cannabinoid $CB_1$ receptor in the early 90s [1], followed by the discovery of it's endogenous ligands, i.e. Anandamide, caused the researchers to focus on its ligands. It has been shown, that the cannabinoid $CB_1$ receptor antagonists or inverse agonists, while being devoid of the psychotropic side effects may have the influence on memory extinction [2]. The other proposed applications are: obesity, smoking cessation, alcohol/drug dependence treatment, and brain injury caused by focal ischaemia [3].

In this study we focused on the synthesis and the pharmacological tests evaluation of the series of diphenyl- or benzylidene derivatives of imidazothiazole-, -thiazine, and -thiazepines according the scheme. The mentioned compounds were evaluated for cannabinoid $CB_1$ receptor antagonist activity.




$R^1$, $R^2$ = Ph, Ph;  $R^1$, $R^2$ = (un) substituted benzylidene

In the aim to evaluate the structure - bioactivity relationships we calculated their lipophilicity, by the means of logP using computer programs: ChemDraw, HyperChem, PALLAS, CAChe Project Leader [4]. The correlation between parameters of lipophilicity (logP) calculated by various programs, as well as between calculated logP and activity ($K_i$) was examined.

**Acknowledgement**:

This work was partly supported by grant No BBN501/P/185/F from Polish Ministry of Education

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[4] Chem Draw Ultra 9.0 by CambridgeSoft, HyperChem 6.01 by Hypercube Inc., PALLAS 3.2.2.1 (demo version) by CompuDrug, CAChe Project Leader 6.1 by Fujitsu Ltd.

PO077

# Affinity of vitamin D analogs to VD-receptor and DB-proteins

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Calcitriol, [1$\alpha$,25-dihydroxyvitamin $D_3$], the seco-steroid hormone, has proven to be a potent antiproliferative agent against various normal and neoplastic cells. Moreover, calcitriol and other vitamin D analogs revealed the ability to induce differentiation of many human cancer cells. Biological activity of these compounds is mediated by the nuclear vitamin D receptor (*n*VDR). Such biological properties suggest the potential therapeutic application for these agents, including antitumor therapy. The undesired hypercalcemia after calcitriol application which is a serious limitation to their clinical use, explains the motivation to develop new analogs. Three of the promising analogs: PRI-2191, PRI-1906 and PRI-1907, have been the objects of our intensive studies.

In this work, we report the docking of vitamin D analogs into VDR (Vitamin D Receptor) and DBP (Vitamin D Binding protein) by using the Molecular Operating Environment (MOE) program. All of the tested ligands exhibited similar high docking potential to the VDR model. However, the vitamin D analogs affinity to DBP is various in this group. Calcitriol and PRI-2191 exhibited high affinity, but PRI-1906 and PRI-1907 low affinity. In conclusion, we could hypothesize that antiproliferative activity of vitamin D analogs is parallel to its high VDR affinity. Affinity for DBP could depend on the accessibility of particular vitamin D analogs as well as on the lability of the bonds formed between ligand and protein.

**Acknowledgments**:
This work was partly supported by grant PBZ-KBN-091/P05/2003 from Ministry of Informatics and Science, Poland. The authors are grateful to Chemical Computing Group Inc for making the MOE program available for free testing work.

PO078

# High–Performance Capillary Electrophoresis and chiral HPLC analytics in the biosynthesis of D-amino acids enantiomers

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D-amino acids are important chiral building blocks for biologically active compounds including: pesticides, peptidomimetics, semisynthetic antibiotics [1]. Several enantiomers hydroxy- or carboxy- derivatives of phenylglycine or phenylalanine have been described as potent agonists or antagonists at glutamate receptors of the central nervous system [2,3].

We applied two-steps enzymatic hydantoinase method to obtain optically pure D-phenylalanine derivatives. The first step involved a stereoselective hydrolysis of racemic phenyl ring-substituted 5-benzylhydantoin derivatives to optically pure N-carbamoyl-D-amino acids by commercially available immobilized, cloned and expressed in *Escherichia coli* hydantoinase. In the second step N-carbamoyl-D-amino acids were converted into the corresponding D-amino acids by diazotization [2].

We used high-performance capillary electrophoresis (HPCE) as a rapid and highly efficient method to monitor the biosynthesis of D-amino acids. In our study samples were analysed after a second step of hydantoinase method. Electropherograms showed not only the presence of the obtained D-amino acids but also unreacted substrates and one or two more by-products in the reaction mixtures. All data were collected using P/ACE 500 Beckmann® instrument equipped with an UV detector (sample detection at 200 nm). Separations were performed at 25 kV constant voltage, current 100 mA, 25 degrees in borate buffer pH = 8,9 as a background electrolyte.

Chiral HPLC was applied to measure enantiomeric purity of the obtained D-phenylalanine derivatives. Before analysis amino acids were deposited on Amberlite® IR 120 cation exchange column to remove contaminations and unreacted substrates. Seperations were run on a Chirosil® (RCA+) column at room temperature. The mobile phase was methanol:10 mmol $CH_3COOH$ water solution (70:30) at flow rate of 1,5 ml $min^{-1}$ with UV detection at 210 nm. Enantiomeric excess (ee%) for each D-phenylalanine derivative was determinated.

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## Acknowledgment

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PO079

# Synthesis of new pyridocarbazoles as anticancer compounds

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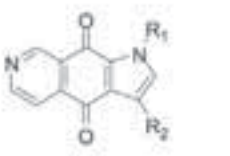
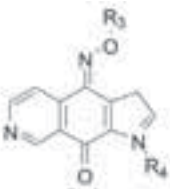
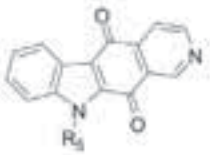
In continuation of our studies we would like to report the synthesis of a series of novel pyridocarbazoles (**3**, **4**). As compounds **1a-l** [1] showed anticancer activity, the aim was to extend the aromatic main frame and additionally to vary the substituted side chains in order to improve the biological activity. The pyridocarbazole main core was synthesized in a multistep procedure as described by Ketcha and Gribble [2] with minor modifications. Referring to the side chains, the focus was put on basic and alkylating groups. Oximes of type **4** were also prepared, as compounds **2a-b** [3] have shown quite promising cytostatic activities. The results of the antiproliferative experiments will be discussed; anticancer activities have been evaluated on four different cell lines: KB/HeLa, NCIH460, SKOV3 and SF268.



**1a-l**



**2a-b**



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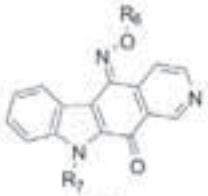

**4**

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PO080

# Synthesis and activity of novel TRH analogues targetes to TRH-$R_2$

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The hypothalamic thyrotropin-releasing hormone **1** (TRH, L-pyroglutamyl-L-histidyl-L-prolinamide) has been long recognized a role in modulation of sensory, locomotor and cognitive pathways in the CNS, apart from its endocrine TSH and prolactin releasing activity [1]. Accordingly, TRH cellular signalling is mediated by two G-protein-coupled receptor subtypes, TRH-$R_1$ and TRH-$R_2$, the latter being highly expressed in brain areas that are involved in CNS higher functions. Thus a variety of TRH analogues has been investigated to get insights into the structure-activity relationship of receptor subtype 2-targeted ligands, in an effort to develop centrally active drugs to be used in both brain and spinal cord maladies. TRH analogs modified in the central position with hydrophobic moieties have been shown to display unusual high activity in the CNS, due to ameliorated ability to penetrate the blood-brain barrier and intrinsic affinity for TRH-$R_2$. A convergent approach relies on structural (chemical and/or conformational) modification at tripeptide cleavable sites, to obtain metabolically stable analogs with prolonged activity at the desidered receptor [2].

As a prosecution of our studies in this field, two novel TRH analogues containing Leu (analog **2**) and *t*Leu (analog **3**) as hydrophobic amino acids in place of central His have been synthesized and preliminarly tested *in vitro* for hormonal and CNS activity.

Mimetic **2** is complemented by the replacement of the endocyclic pGlu CONH bond by its sulphonamido counterpart. The interest inborn in these new derivatives is thoroughly discussed.

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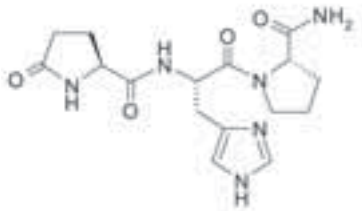
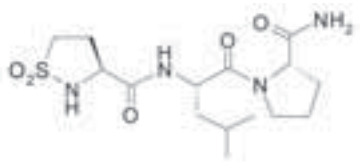
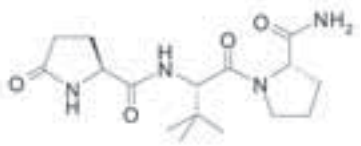


TRH (1)



dual-substituted TRH analog (2)



TRH analog (3)

PO081

# Synthesis and cytotoxicity of di(8-quinolyl)disulfides

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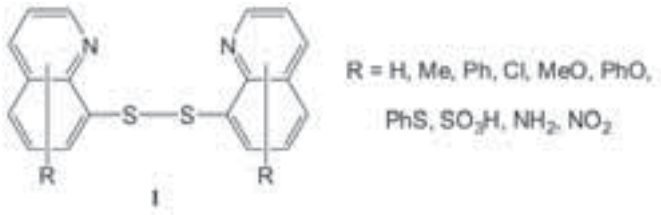
We have found that di(8-quinolyl)disulfide (**1**, R=H) exhibits a high cytotoxicity ($LC_{50}$ 0.3-0.4 μg $ml^{-1}$) against tumour cell lines HT-1080 (human fibrosarcoma) and MG-22A (mouse hepatoma) [1]. But this compound was also highly toxic for normal fibroblasts NIH 3T3 ($LC_{50}$ 0.7 μg $ml^{-1}$). With the aim to increase the selectivity against the tumour cells we have synthesized and tested a series of monosubstituted di(8-quinolyl)disulfides (**1**) with different substituents in the 2-, 3-, 4-,5-,6-, and 7-position of the quinoline ring.




I

It has been found that the position of the substituent essentially influenced the cytotoxicity and toxicity of the compound **1**. For methyl derivatives (R = Me) the 7-, 6- and 3-isomers exhibited the highest cytotoxicity ($LC_{50}$ < 1 μg $ml^{-1}$), the 4- and 5-isomers were less cytotoxic while 2-isomer was not cytotoxic for HT-1080 and MG-22A cells. High activity ($LC_{50}$ < 1 μg $ml^{-1}$) was observed also for other 7-substituted (Cl, PhO, PhS) quinolines but their toxicity was still high. The same was true for some 5-substituted compounds (Cl, MeO, $NH_2$, $NO_2$). The compounds containing PhO, $SO_3H$, PhS and Ph groups were less toxic but their cytotoxicity was not sufficient.

The best selectivity was found for 6-substituted di(8-quinolyl)disulfides, e.g. the Meo derivative had $LC_{50}$ < 1 μg $ml^{-1}$ for tumour cells and 100 μg $ml^{-1}$ for normal fibroblasts ($LD_{50}$ 874 mg $kg^{-1}$).

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PO082

# Synthesis, structural analysis and cytotoxicity of some bis-indoles

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Recently, many investigations have focused on an attempt to define antiproliferative signaling pathway of indole-3-carbinol (a natural component of cruciferous vegetables) and its major indole metabolite 3,3'-diindolylmethane. It seems, that among their derivatives we could find new potent chemotherapeutics.

In the course of our studies on *bis*-indoles [1,2] we have focused on optimization of the synthetic procedure leading to substituted indoles and subsequently to new 3,3'-diindolylmethanes, which could inhibit human cancer cell growth stronger than normal one. The biological evaluation was made on the following cells: WS1 – human embryonic skin fibroblasts, ME18 – human melanoma cells and ME18/R – human melanoma subline resistant to doxorubicin. Cytotoxic activity ($IC_{50}$), was obtain accordingly to MTT assay as given in [3].

For disubstituted-3,3'-methanediyl-bis-indoles we observed a diversified resonance pattern in solid state $^{13}C$ CP/MAS NMR spectra, which could be an indication of different intermolecular interactions and different structural alignment in solid state, which could be responsible for their biological function.

In the poster we report our novel protocol of the synthesis of *bis*-indoles, spectral data for new derivatives, as well as their cytotoxicity.

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PO083

# Searching for new immunomodulators in a group of isoxazole derivatives

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For many years isoxazoles have been of interest as a class of heteroaromatic compounds with pharmacological activity. Some of the compounds have found broad application in medicine. Looking for active immunomodulators we synthesized two groups of monocyclic and two groups of bicyclic derivatives, deriving from 5-amino-3-methyl-4-isoxazolecarboxylic acid: N'-substituted hydrazides of 5-amino-3-methyl-4-isoxazolecarboxylic acid (1-10), semicarbazides and semithiocarbazides of 5-amino-3-methyl-4-isoxazolecarboxylic acid (11-20) [1], 5-substituted 3-methylisoxazole[5,4-d]-pyrimidin-4-ones (21-30) [2] and 5-substituted 3-methylisoxazole[5,4-d]-1,2,3-triazin-4-ones (31- 40) [3,4].

The obtained 5-amino-3-methyl-4-isoxazolecarboxylic acid hydrazide, which in a reaction with carbonyl compounds gave N'-substituted hydrazides (1-10), in the course of a reaction with isocyanate and isothiocyanate produced semicarbazides and semithiocarbazides (11-20). N'-substituted hydrazides (1-10) were applied for the synthesis of 3-methylisoxazole[5,4-d]-pyrimidin-4-ones (21-30) and 3-methylisoxazole[5,4-d]-1,2,3-triazin-4-ones (31-40), respectively. Compounds (21-30) were obtained by cyclization of derivatives (1-10) with ethyl ortho-formate while the series of (31-40) were prepared by diazotization of compounds (1-10). Our latest research on isoxazole derivatives showed significant immunological activity of these compounds, which were tested in several *in vitro* and *in vivo* assays in mice and human models. Most compounds (1-30) exhibited generally inhibitory activities whereas derivatives (31-40) demonstrated differential activities in the studied models. Some of them were stimulators and the others were regulators of the immune response. The studies were supported by the Wrocław Medical University, grant 1312.

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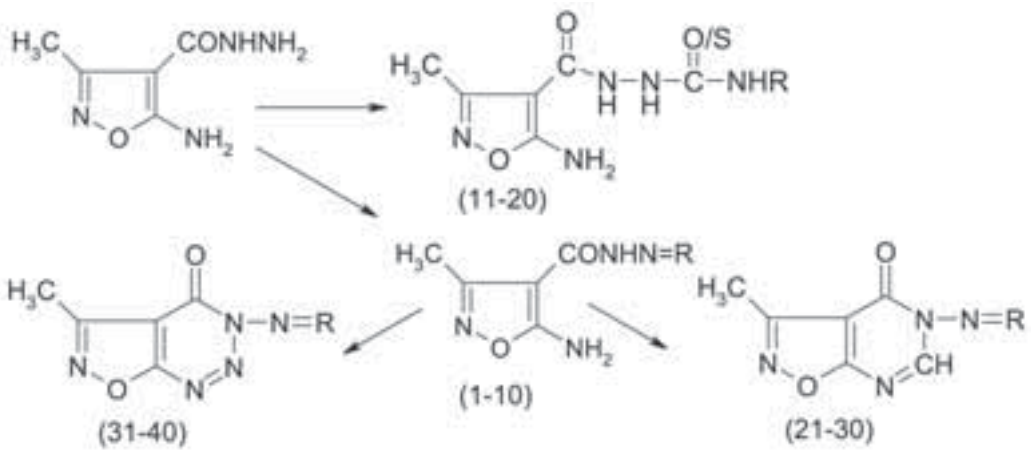

PO084

# Novel UPF analogues affect glutathione peroxidase and glutathione reductase activity

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Tripeptide glutathione (GSH, L-γ-Glu-L-Cys-Gly) is the most abundant low molecular weight thiol that is present in most cells in millimolar amounts. In a reduced form GSH is a major intracellular antioxidant. The molecules containing a cysteine residue (the sulfhydryl group) easily participate in the thiol-disulfide exchange. In different conditions GSH is oxidized to glutathione disulfide (GSSG).

GSH is a co-factor in enzymatic reactions. Endogenously produced radicals are effectively reduced by the glutathione peroxidase (GPx), in the presence of GSH as a substrate. During this reaction, known as a redox cycle, GSH is converted to GSSG, which is recycled back to 2 GSH by glutathione reductase.

In present study we designed and synthesized new effective glutathione analogues, tetrapeptides UPF1, UPF6 and UPF17, whose antioxidative qualities are superior to the GSH [1]. The peptides were synthesized using *t*-Boc or Fmoc solid phase peptide synthesis chemistry. UPF17 was synthesized so that original γ-glutamate was changed to the α-glutamate. The aim of this study was to determine whether and how UPFs have an effect on the activity of GSH bound enzymes. The results demonstrate that the natural GSH and it`s analogues UPFs have an effect to glutathione peroxidase. GSH activates GPx and UPFs inhibit it concentration dependent. Present study has high impact on working out novel GSH analogues, which can be used in oxidative stress treatment [1,2]. We have shown that UPF1 is an effective potential agent diminishing neuronal injury in global cerebral ischemia.

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PO085

# The study of the lipophilicity of α-(4-phenyl-piperazin-1-yl)-γ-phthalimido-butyramides using chromatographic and calculation methods

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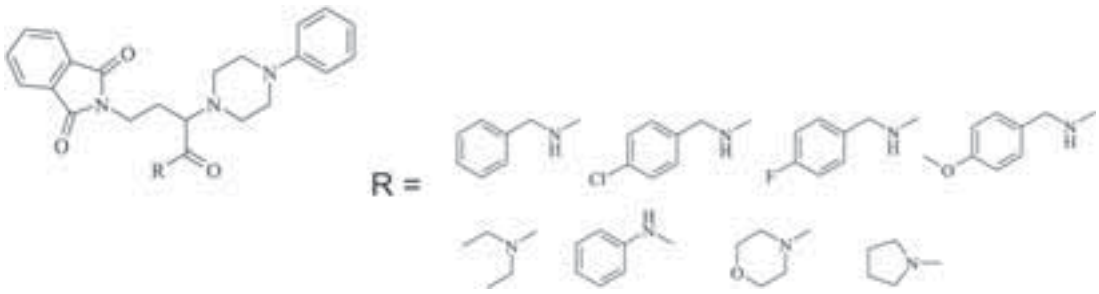


This work is a part of our physicochemical and pharmacological studies in a group of anticonvulsant compounds, derivatives of two endogenous acids: α-amino- and γ-hydroxybutyric acid, which are important neurotransmitters in the central nervous system [1, 2]. The aim of the study was determination of the lipophilicity and phospholipophilicity of a series of α-(4-phenyl-piperazin-1-yl)-γ-phthalimido-butyramides, by chromatographic and computational methods.

Parameters of the relative lipophilicity ($R_{MO}$ and log $k$') of the tested compounds were determined experimentally both by reverse-phase thin layer chromatography (RP-TLC), and high-performance liquid chromatographic methods (RP-HPLC, LiChrospher® 100 RP-18 column). Phospholipophilicity (log $k'_{IAM}$) was determined using immobilized artificial membrane HPLC (IAM·PC DD2 Regis column). Mixtures of acetonitrile or acetone and water in different proportions were used as mobile phases. Acetonitrile/water or acetone/water 50-95% were used in RP-TLC, acetonitrile/0.01 % TFA 45-65% in RP-HPLC and acetonitrile/water 35-55% in IAM-HPLC. Retention parameters ($R_M$) and capacity factors (log $k$ and log $k_{IAM}$) determined by applied methods were linearly dependent on organic modifier concentration in mobile phases and enabled us to estimate the relative lipophilicity factors: $R_{MO}$, log $k$' and log $k'_{IAM}$. Their partition coefficients (clog $P$) were also calculated with the Pallas and CAChe programs. According to the research among the experimental methods, both RP-TLC and RP-HPLC gave similar results. The IAM-HPLC technique might give more effective data than others for prediction of partition between biological membranes and trans-membrane transport of tested compounds.

## References

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PO086

# Synthesis and antifungal activity of new N-[1-aryl-2-(1*H*-imidazol-1-yl and 1*H*-1,2,4-triazol-1-yl)-1-ethylidene]-N'-phenylhydrazine derivatives.

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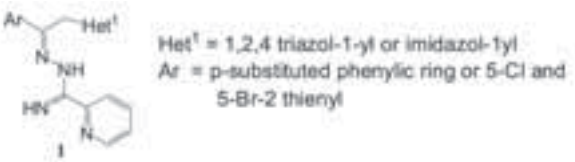
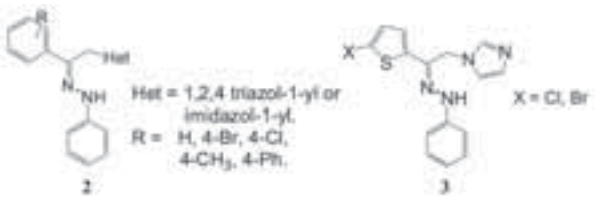
Systemic fungal infections have emerged as important causes of morbidity and mortality in immunocompromised patients (*e.g.*, AIDS, cancer chemotherapy, organ or bone marrow transplantation). In addition, hospital-related infections in patients not previously considered at risk (*e.g.* patients on an intensive care unit) have become a cause of major health concern. On the other hand the increased incidence of severe opportunistic fungal infection together with the rise up of resistance to many antifungal drugs bring to need to development of new antifungal compounds. The azole compounds interact at the target enzyme cytochrome P450-dependent lanosterol 14α-demethylase in the ergosterol-biosynthesis pathway. Our search of new antifungal compounds was performed through a preliminary computer modeling of drug/enzyme-complexes beginning from $N^1$-[1-aryl-2-(1*H*-imidazol-1-yl and 1*H*-1,2,4-triazol-1-yl)ethylidene]-pyridine-2-carboxamidrazone derivatives **1**[1]. Some of these compounds exhibited a remarkable antifungal activity.




With the aim to enhance the antifungal activity of compounds **1** we synthesized a new N-[1-aryl-2-(1*H*-imidazol-1-yl and 1*H*-1,2,4-triazol-1-yl)-1-ethylidene]-N -phenyl-hydrazine derivatives **2** and **3**, characterized by the presence of a phenylhydrazine moiety.




All compounds have been tested against a strain of *Candida Albicans* 3038 and a strain of *Mycobacterium tuberculosis* $H_{37}Rv$ to evaluate the antifungal and the antimycobacterial activity respectively. The phenylhydrazine derivatives demonstrated a very moderate antimycobacterial activity. Only the 5-chloro-2-thienyl derivative is gifted with a significant activity (MIC = 8 μg/ml after 24h). On the other hand, all compounds showed a very interesting activity against a fungal strain of *Candida* (all MIC<32 μg/ml). Particularly, both the 5-chloro and 5-bromo-2-thienyl derivatives, structurally related to the antifungal drug zinoconazole, exhibited a MIC = 2 μg/ml after 24 h.

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PO087

# Synthesis and antimycobacterial activity of 1,3,4-oxadiazol-2-one derivatives

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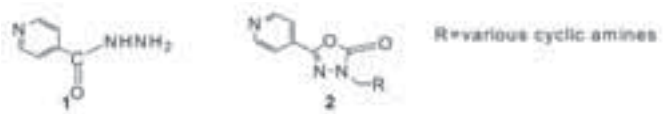
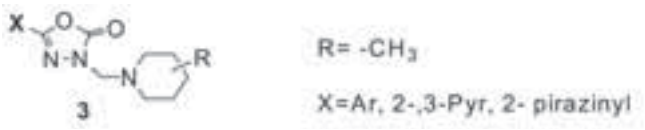
Tuberculosis is a contagious disease with high mortality worldwide. The recent emergence of cases of multidrug resistant tuberculosis (MDR-TB) becomes a serious problem to the treatment of the disease. The disease resurgence in most countries is due to the human immunodeficiency virus (HIV) epidemic, in addition to the emergence of drug-resistant strains and immigration from high-prevalence countries.

Moreover, species of mycobacteria other than *M. tuberculosis* (MOTT) are able to cause a wide range of infections. Among these bacteria, the most dangerous for humans are *M. avium*, *M. fortuitum*, *M. kansasii*, *M. chelonei* and the *M. avium-intracellulare* complex (MAC). Therefore, new drugs for the treatment of infection sustained by MOTT and strains of MDR mycobacteria are indispensable.

Our search consists on the design, synthesis and *in vitro* evaluation of antimycobacterial activity of new isoniazid analogues. We observed[1] that the conversion of isoniazid **1** in 3-substituted 5-(pyridin-4-yl)-3*H*-1,3,4-oxadiazol-2-one derivatives **2** gave compounds with interesting antimycobacterial activity**:**




Trying to increase the antimycobacterial activity of the compounds **2**, we synthesized a series of 3-(2, 3, and 4-methyl-piperidin-1-ylmethyl)-5-heteroaryl-3*H*-1,3,4-oxadiazol-2-one derivatives **3**, characterized by the presence of piperidine moiety, which gave the most active compounds of series **2**.




All compounds have been tested to evaluate their *in vitro* antifungal against a strain of *Candida Albicans* 685 and antimycobacterial activity against a strain of *Mycobacterium Tuberculosis* $H_{37}Rv$. Though the antifungal activity resulted of few interest (all MIC>32 μg/ml after 24h), the 1,3,4-oxadiazol-2-one derivatives proved good antimycobacterial activity. Particularly the 2-pyridinyl substituted compounds are gifted with the highest activity against mycobacterial strain (MIC=4 μg/ml) in agreement with our previously synthesized 1,3,4-oxadiazol-2-one derivatives.

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PO088

# Synthesis and biological evaluation of 1,8-naphthyridine and quinoline derivatives as CB2 selective agonists

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Cannabinoids exhibit a complex array of pharmacological effects that are generally considered to be mediated through at least two G-protein-coupled (GPCR) seven-transmembrane receptors. One of these receptors, designated CB1, is found principally in the central nervous system while the other, designated CB2, is expressed primarily in the periphery. Because of the virtually exclusive peripheral expression of CB2 and its presence only in microglial cells in the central nervous system, selective CB2 ligands should be devoid of the undesired central nervous system effects typical of (-)-*trans*-$\Delta^9$-tetrahydrocannabinol, the major psychoactive component of *Cannabis sativa* L. We have previously reported the binding results of a series of 1,8-naphthyridin- and quinolin-4(1H)-on-3-carboxamide derivatives.[1,2] These compounds generally exhibit a remarkable affinity, with a $K_i$ value <20 nM, which was also accompanied by a high selectivity for the CB2 receptor.

In the present study, new 1,8-naphthyridine and quinoline derivatives of structure **A** and **B** and tricyclic analogs of structure **C** were synthesized and tested on membranes prepared from HEK cells expressing the hCB1 and hCB2 receptors, to determine their affinities towards both cannabinoid receptors. Furthermore in order to obtain further information about SAR of these compounds we docked them into the three-dimensional model of CB2 receptor that was recently constructed. [3]

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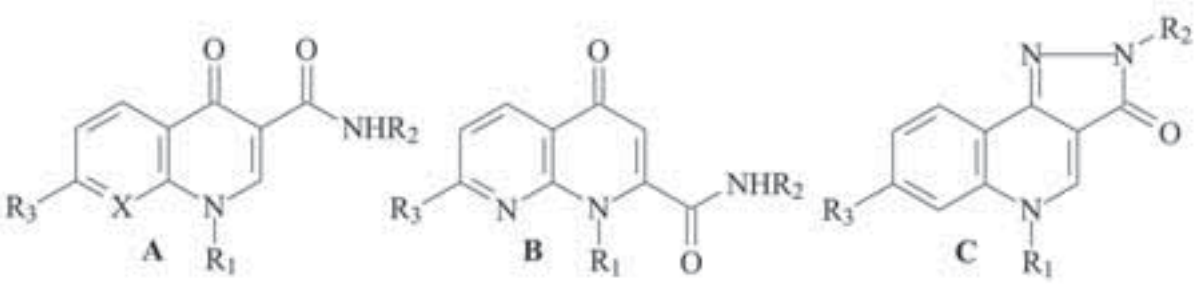

PO089

# Synthesis and antitumor evaluation of 1,8-naphtyridine derivatives

**Elisabetta Mantuano**[a], Giuseppe Saccomanni[a], Clementina Manera[a], Andrea Chicca[b], Enrica Martinotti[b], Roberta Misasi[c], Pier Luigi Ferrarini[a]

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Microtubules show highly dynamic instability and play an important role in mitosis, in fact inhibition of their functions appears to be the mechanistic basis of most antimitotic compounds as Vinca alkaloids[1] and taxoids[2]. However, classical approaches involving the discovery of cytotoxic agents interfering with DNA, either directly or inhibiting DNA-binding enzymes, have led to the identification of new promising anticancer agents[3]. Quinolinones and their isosteric counterpart, naphthyridinones are known for their antimicrobial and antitumor activity[4]. Nalidixic acid, the first prototypic “quinolinone” and its modified analogue ofloxacin were believed to exert their activities through DNA intercalation and alteration of the normal functions of the human enzyme, topoisomerase II. Quinolinones and naphthyridinones were also found to inhibit tubulin polymerization resulting in the disruption or the suppression of both microtubule structure and physiological functions of the eukaryotic cells with consequent mitosis arrest [4,5]. On the basis of these considerations a large series of substituted 1,8-naphthyridine derivatives of general formula A-E were synthesized and tested for potential anticancer activity.

Antitumour activity was evaluated *in vitro* by growth inhibition assay, apoptosis assays and microtubule polymerization assay. A trypan blue assay was performed to determine human lynphoblastoid CEM cell viability and cytotoxic effect of each compound. Apoptosis assays were carried out throuhgt DNA fragmentation measurement, flow cytometric analysis, and nuclear morphological modification. Microtubule polymerization was induced by taxol, in the presence of different 1,8-naphthyridine derivatives and analyzed by both Western blotting and immunofluorescence assays. Several tested 1,8-naphthyridine derivatives elicited antiproliferative effect with $IC_{50}$ values comparable to the reference drug Paclitaxel (21,0 $\pm$ 0.3 nM). Apoptosis evaluation indicated some compounds as marked programmed cell death inducers. Moreover, a strict structure-activity relationship was found through the correlation between compound cytotoxicity and inhibition of tubulin polymerization. Three of the analyzed series of compounds showed a potent antimitotic and antitumor *in vitro* activity, in the nanomolar range, indicating these molecules as suitable drugs for a further development.

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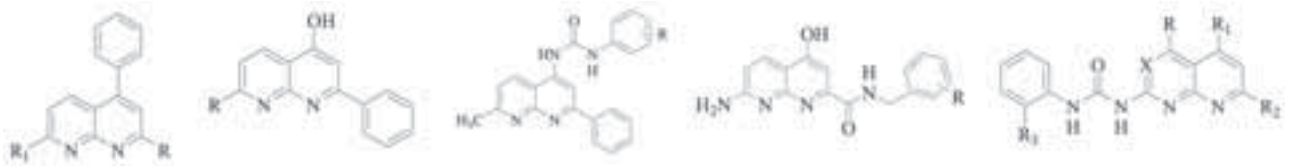

PO090

# Vinyl ester cyclopeptides: New proteasome inhibitors

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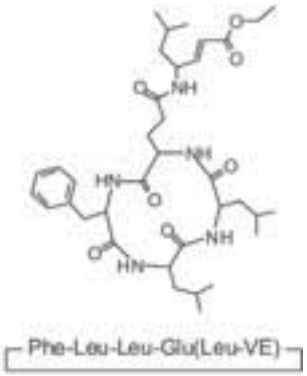
The proteasome is a multicatalytic protease complex, which represents the central enzyme of intracellular protein degradation in prokaryotes and eukaryotes [1]. The development of proteasome inhibitors into novel therapeutic agents represents a stimulating approach in the treatment of many disease states including inflammation and cancer, and for the modulation of immune responses. Once synthesized and tested peptide-based derivatives showed good enzymatic inhibition and favourable pharmacokinetic properties [2, 3].
Now we report the synthesis and biological activities of new cyclic peptides able to interact with catalytic subunits of the proteasic complex.

The more effective inhibitors of the series showed inhibition and selectivity for the chymotrypsin-like (β5) subunit of the proteasome. Furthermore these new vinyl ester cyclopeptides have good enzymatic stability in human plasma.

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PO091

# (+)-MR200 derivatives as potential new selective sigma ligands

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Recently, we reported the synthesis and pharmacological evaluation of **(+)-MR200** [(+)-methyl (1R,2S)-2-{[4-(4-chlorophenyl)-4-hydroxypiperidin-1-yl]methyl}-1-phenylcyclopropanecarboxylate] (Fig. 1) as a new selective sigma ligand with increased affinity and selectivity compared to the structurally related ligand haloperidol [1-3].
With the aim to improve the sigma-1 selectivity with respect to sigma-2 subtype, a new series of 1-phenyl-2-[(4-phenylpiperidin-1-yl)methyl]cyclopropanecarboxylate derivatives were synthesized.
Moreover, in order to evaluate the stereoselectivity, all single *cis* and *trans* enantiomers were obtained performing a stereoselective reaction.

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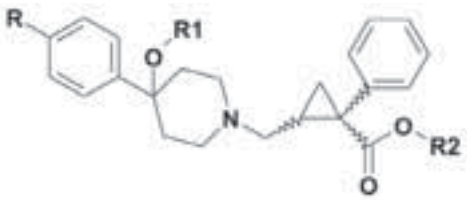
**Fig.1**




**(+)-MR200** R = Cl, R1 = H, R2 = $CH_3$

PO092

# Synthesis of new diastereoisomer esters of antiinflammatory steroids

**Bojan Marković,** Sote Vladimirov, Vladimir Savić

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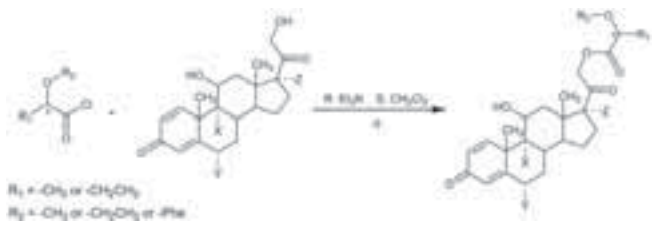
Glucocorticoids (GC) are widely used in therapy for their many pharmacological properties including antiinflammatory and immunosuppressive actions. Topical GC creams and ointments are applied to the skin to reduce inflammation in conditions such as eczema, dermatitis and psoriasis. GC are used in the treatment of ophthalmic disorders and asthma as well. Use of GC in therapy is limited by their side effects, which depend on the dose used and duration of treatment. For a useful topical agent it is desirable for the compound to remain in the epidermis and to migrate only slowly into the dermis. This property is fostered by the presence of lipophilic groups and by the absence of hydroxy groups in the steroid. The conversion of one or two hydroxyl groups in antiinflammatory steroids to lipophilic derivatives, such as esters or ketals, is an effective way to locally active antiinflammatory agents. Thus, fluocinonide, the 21-acetate of fluocinolone acetonide, is about five times as active as the latter compound. It was described a parabolic relationship between log permeability and the octanol/water partition coefficient with an optimum at $\log P_0$ of 2.9 [1]. It was shown that GC esters with ω-alkoxyacids exhibited a strong antiinflamatory activity upon topical administration and relatively weak activity upon systemic administration [2].
Therefore, new diastereoisomer esters of corticosteroids were synthesized. Chlorids of α-alkoxyacids have been used for esterification. These acids have been prepared in reaction of α-halogenacid with corresponding sodium alkoxyde [3]. An object of this invention is to provide new derivatives with good local antiinflammatory activity and with low skin permeability coefficient. Synthesis of new derivatives of GC is shown on the following scheme:



Skin permeability coefficients ($\log K_p$) for these new esters have been calculated and determinated using PAMPA test (membrane has been impregnated with 70% silicone oil and 30 % isopropyl myristate) [4] and these results will be discussed.

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PO093

# Imidazolin-2-yl-urea derivatives, new antinociceptive agents with affinity to opioid receptors.

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Antinociceptive activity of various chain and cyclic carbonyl derivatives of 1-aryl-2-aminoimidazoline-2 is known for some time already [1-4]. It was confirmed that in some cases their activity can be correlated with affinity to the opioid receptors.

In behavioural tests (e.g. "writhing test") their activity was at the doses of 0.05 to 0.0125 of $LD_{50}$. In binding affinity test to the MOP opioid receptor with $^3$H-Naloxon activity ($EC_{50}$) of the most active compounds was at the level of 1-1.5 μM (in comparison to 0.2 μM for morphine in the same test).

Affinity to respective types of opioid receptors was also confirmed by their effect on the threshold doses of morphine and reversion of the antinociceptive action by non-selective or selective antagonists of respective receptors (naloxone, naltrindole, cyprodime and binaltorphimine).

Elucidation of the crystal structure of some investigated compounds, their comparison to the modelled structures and finally virtual docking experiments allowed describing pattern of the ligand-receptor interaction and structural prerequisites of the selectivity of their action.

This work was performed in the framework of computational grant by Interdisciplinary Centre for Mathematical and Computational Modelling of Warsaw University, grant number G30-18.

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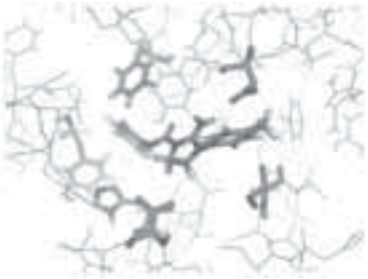

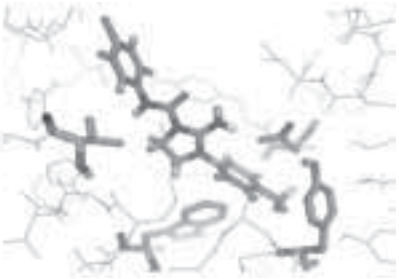

PO094

# Anti-flaviviridae activity of Mannich bases of coumarins

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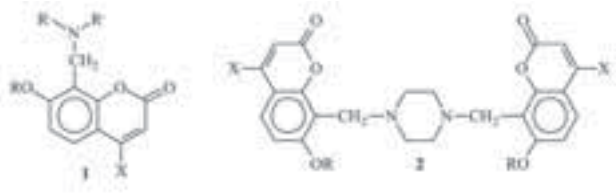
Flaviviridae are enveloped, positive single-stranded RNA viruses. This virus family contains three genera: *Hepacivirus* (e.g., *Hepatitis C virus* [HCV]), *Flavivirus* (e.g., *Yellow fever virus* [YFV]), and *Pestivirus* (e.g., *Bovine viral diarrhea virus* [BVDV]). Currently, more that 70 *Flaviviruses* have been discovered, and many of them cause important human diseases. In particular, HCV has infected an estimated 1-3% of the world population, which means that more than 170 million chronic carriers are at risk of developing cirrhosis and/or liver cancer. HCV infections are now treated with therapies based on combinations of pegylated interferons and ribavirin, which are ineffective in a significant proportion of cases, and are associated with the incidence of severe side effects. Thus, there is an urgent need for a more effective treatment and/or immunoprophylaxis for this viral disease [1].

After the unveiling of HCV genoma, several sites of it have been identified as possible targets for HCV drugs. The major targets are IRES (Internal Ribosome Entry Site), non-structural proteins NS3 (with protease-elicase activity) and NS5B (with RNA-dependent RNA polymerase activity) [2].

As we are interested to develop non-nucleoside drugs for treating Flaviviridae-related pathologies, in this work we desire to present some results coming from biological tests against BVDV (generally accepted as surrogate virus for HCV) of Mannich bases of 7-hydroxycoumarin derivatives, linked with computational studies on HCV.



Different 7-hydroxycoumarins were treated with suitable secondary amines to yield the corresponding Mannich bases 1 and 2. The synthesized compounds showed an interesting activity in counteracting the viral multiplication in BVDV infected cells ($IC_{50}$ = 0.3-1.0 μM). Moreover, by Molecular Modeling, compounds 1 and 2 were proved to bind NS5B of HCV. In this regard, we found an excellent correspondence between experimental and calculated data of binding. These results are encouraging in the hope of finding new non-nucleoside drugs to treat diseases caused by Flaviviridae, especially hepatitis C infection.

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PO095

# Native mass spectrometric studies on human hydroxylases

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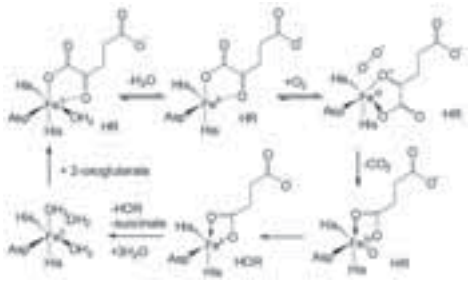
Hypoxia-inducible factor (HIF) is α,β-heterodimeric transcriptional factor present in higher organisms [1]. Although the HIF-β subunit is constitutively present, only HIF-α subunit is oxygen-dependent. Under normoxic conditions the hydroxylation of specific amino acid residues by HIF hydroxylases (Fe(II) and 2-oxoglutarate dependent oxygenases) lead to HIF-α inactivation, whereas under hypoxia, accumulation of HIF-α subunit allows its dimerisation with HIF-β and initiate the expression of an array of genes, including those encoding for vascular endothelial growth factor and erythropoietin. Three hydroxylation sites of HIF-α have been reported [2,3,4]. Hydroxylation at the 4-position of either Pro402 or Pro564, reaction catalysed by prolyl hydroxylase domain containing enzymes (PHDs), results in HIF-α ubiquitinylation and subsequent proteasomal degradation. Hydroxylation of Asn803 located in C-terminal activation domain (CAD) of HIF-α is catalysed by Factor Inhibiting HIF (FIH). This modification blocks the interaction between HIF-α and transcriptional co-activator CBP/p300 so disabling HIF mediated transcription. Consequently, manipulation of HIF pathway could potentially be used for treatment of ischemic disease, anemia and cancer.

The use of electrospray soft ionization mass spectrometry (ESI-MS) has been shown to be useful for the study of noncovalent interactions between enzymes and small organic molecules and ions [5]. Native ESI-MS was used for mechanistic studies on PHD2, including experiments conducted on apo-PHD2, metallated PHD2 and a series of mutants of PHD2. The results were consistent with PHD2 catalysis proceeding via an enzyme/Fe(II)/2OG/-substrate complex. Native ESI-MS provides evidence for competitive or noncompetitive inhibition as well as covalent or noncovalent inhibition. The ESI-MS technique was found to be useful for binding studies with small molecules and could potentially be adopted for screening. A recent crystal structure with small molecule [6] revealed insights into interactions between small molecules and the enzyme and has opened the way for design and synthesis of new PHD2 inhibitors. The structure revealed that small molecules chelate iron(II) in a bidentate manner, which is consistent with 2-oxoglutarate binding mode in the proposed mechanism (see Figure).



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PO096

# Synthesis and anticancer activity of 3-amino-N-phenyl-1H-indazole-1-carboxamide, N-phenyl-3-(phenylureido)-1H-indazole-1-carboxamide and 1-(1H)indazol-3-yl-3-phenylureido derivatives

D. Raffa[a], B. Maggio[a], S. Cascioferro[a], V. Raimondi[a], D. Schillaci[a], G. Gallo[a], G. Daidon[e], S. Plescia[a], **F. Meneghetti[b],** G. Bombieri[b], M. Tolomeo[c], A. Di Cristina[c], S. Grimaudo[c]

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Deepen in our research on the chemistry and pharmacology of 4(3H)-benzotriazinones [1], a large number of derivatives bearing a heterocyclic nucleus in the 3 position have been synthesized. The obtained compounds drew our attention as potential CDK inhibitors [2]. The inhibitory activity of 2-iodobenzamides against CDK1 has been preliminarily assessed and among these the 5-chloro-2-iodo-N-(indazol-3-yl)-benzamide has an $IC_{50}$ of 6.6 μM and it was therefore considered as lead compound. With the aim to improve its inhibitory activity [3], 1-(1H)indazol-3-yl-3-phenylureidic derivatives **5**, 3-amino-N-phenyl-1H-indazole-1-carboxamide **3** and N-phenyl-3-(phenylureido)-1H-indazole-1-carboxamides **4** derivatives have been synthesized (scheme). These compounds present a more evident block in G0-G1 phase and a decrease of phosphorilated Rb in leukemic cell lines. Structure of type **3** was confirmed by X-ray studies.

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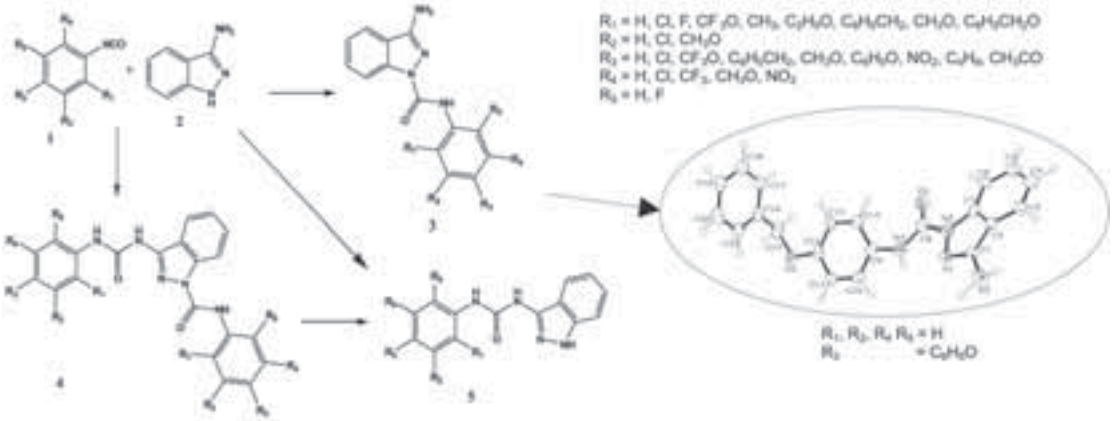

PO097

# Synthesis of aza-D-homo-estrone derivatives: the first bis-oxazepine-derivative in the estrone series

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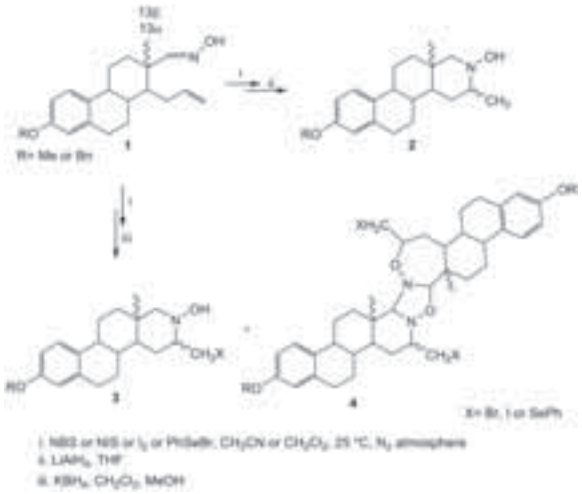
Steroidal oximes (**1**) possessing δ-alkenyl substituents are stereoselectively cyclised by PhSeBr or NBS or NIS or $I_2$ to the corresponding *N*-hydroxypiperidino derivatives (**2**, **3**). Depending on the conditions of the reduction, 16-methyl (**2**) or 16-halomethyl (**3**) derivatives are formed. Using PhSeBr as electrophile, formation of a *bis*-steroid (**4**) was observed, which can be explained by the 1,3-dipolar cycloaddition of a six-membered cyclic nitrone onto the oxazepine moiety. To our knowledge this is the first evidence for the formation of seven-membered ring in the electrophile induced cyclisation of δ-alkenyl-oximes.



We thank for the support of the Hungarian Scientific Research Fund (OTKA D 048294 and T 049366). Erzsébet Mernyák's work was supported by the award of a János Bolyai Research Fellowship.

PO098

# A Binary QSAR Model for classification of hERG potassium channel blockers & SAR study on hERG liability of propafenone derivatives by experimental and docking approaches

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Acquired long QT syndrome caused by drugs that block the human ether-a-go-go-related-gene (hERG) $K^+$ channel causes severe side effects and thus represents a major problem in clinical studies of drug candidates. Therefore, early prediction of hERG K+ channel affinity of drug candidates is becoming increasingly important in the drug discovery process.

Based on a dataset of 313 compounds, a binary QSAR models with threshold values at $IC_{50}$ = 1 μM and 10 μM, respectively, were generated using two different sets of descriptors: 32 P_VSA descriptors and a set of 18 descriptors retrieved by feature selection from 182 2D molecular descriptors in MOE [1]. In LOO cross-validation runs, a total accuracy of 0.79-0.83 was obtained with a classification power for hERG-blockers of 83-86 %. The BQSAR models were successfully used to classify an external test set of 58 newly synthesized compounds collected from the literature.

In an attempt to combine ligand-based with structure-based approaches, a total of 10 propafenone derivatives were docked into the individual homology models of the open and closed states of the hERG channel. The binding scores from the docking studies were compared with the experiment $IC_{50}$ values, in order to appraise the suitability of the models as a predictive tool. Furthermore, insights of ligand binding modes into the open and closed states of the hERG channel obtained from docking runs could explain differences in experimental hERG potency and drug trapping.

TKM is grateful for financial support by the ASEA-Uninet and the Austrian Academic Exchange Service (ÖAD).

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PO099

# Arylpiperazinylalkylthio benzothiazole or benzoxazole derivatives as selective 5-HT$_{1A}$ serotonin receptor ligands

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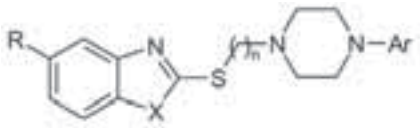
Arylpiperazine derivatives represent one of the most important classes of 5-HT$_{1A}$ serotonin receptor (5-HT$_{1A}$R) ligands. Many of them bind with high affinity to 5-HT$_{1A}$R but often show low selectivity over other G-protein coupled receptors, such as $\alpha_1$ adrenergic and $D_1$ and $D_2$ dopaminergic receptors.

For many years our research group has been interested in the synthesis of arylpiperazine derivatives as 5-HT$_{1A}$ ligands [1-3].

Recently, a new class of arylpiperazinyllkylthio derivatives where 2-nitro or 2-methoxy-phenylpiperazine was linked to benzimidazole, benzothiazole, or benzoxazole ring systems via a flexible thioaliphatic chain of different lenght (n = 2, 3, 4) was investigated. These compounds were characherized by the following general structure:




R = H, Cl
X = NH, S, O
n = 2, 3, 4, 6
Ar = 2-NO2-phenyl, 2-OCH3-phenyl, 2-pyrimidinyl, 2-pyridinyl

Some of them showed very high affinity for 5-HT$_{1A}$R coupled with selectivity over $\alpha_1$ receptors ($K_i$ 5-HT$_{1A}$ = 0.29, 0.27 nM, $K_i$ $\alpha_1$ = 33.25, 16.63 nM, ratio $K_i$ 5-HT$_{1A}$/$K_i$ $\alpha_1$ 114 and 61.59 for more representative compounds).

On the basis of the above results and in order to extend the study of the structure-activity relationships of these products, in this work we report the synthesis of novel arylpiperazinylalkylthio derivatives with formulae included in the above-mentioned general structure.

All new compounds were tested in binding experiments to evaluate their affinity and selectivity for 5-HT$_{1A}$Rs with respect to $\alpha_1$-adrenoceptors and, in some cases, to $D_1$ and $D_2$ dopaminergic receptors. Selected compounds were also tested in functional assays to evaluate their intrinsic activity as agonists or antagonists.

## References

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PO100

# Discovery of novel and selective beta-secretase inhibitors using *in silico* high-throughput screening

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Aging is a major risk factor for Alzheimer's disease (AD) and the number of AD patients will significantly increase in the next decade. Current therapeutic approaches such as the acetylcholinesterase inhibitors and NMDA receptor antagonists only provide symptomatic relief with no effect in halting disease progression. For this reason, therapeutic interventions targeting the biochemical pathways of AD are urgently needed. The accumulation of amyloid β-peptide (Aβ) into insoluble plaques in the brain is a key event in the pathogenesis of AD. The first step in Aβ formation is the cleavage of the amyloid precursor protein (APP) by the β-site APP cleaving enzyme (BACE1) or beta-secretase. As Aβ appears to initiate the neurodegeneration seen in AD, BACE1 is considered an attractive target for the development of small molecule inhibitors to treat the disease.

With the recent availability of new X-ray crystallographic structures of BACE1 enzyme-inhibitor complexes, *in silico* screening provides an attractive approach to identifying potential BACE1 inhibitors. *In silico* screening of three commercially available libraries containing a total of 300,000 compounds against BACE1 using the docking algorithm eHiTS (Electronic High Throughput Screening) [1] was undertaken and the 100 highest scoring compounds from each library prioritised. Compounds were triaged based upon their predicted binding affinity and physiochemical characteristics. Six compounds were tested in an *in vitro* enzyme activity assay using a quenched fluorescent peptide substrate based on the Swedish mutant APP sequence (SEVNLDAEFK). One compound showed selective inhibition of BACE1 ($IC_{50}$ 2.15 μM) over the close homologue BACE2 ($IC_{50}$ >100 mM). Chemical synthesis of variants of this hit compound has been used to probe the structure-activity relationships of this inhibitor. The combination of an *in silico* screening approach with validation by *in vitro* enzyme assays has led to the identification of a new BACE1 inhibitor scaffold.

## References

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PO101

# In vitro antiproliferative activities and kinases inhibitory potencies of glycosyl-isoindigo derivatives

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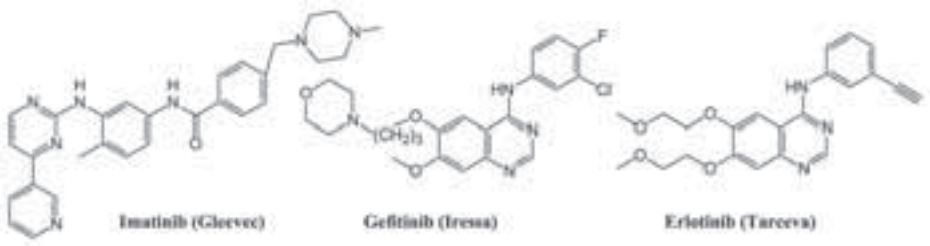
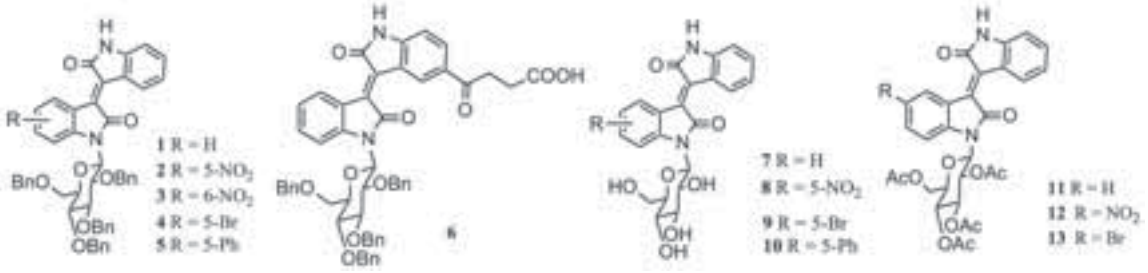
Protein kinases play important roles in regulating most cellular functions such as proliferation/cell cycle, cell metabolism, survival/apoptosis, DNA damage repair, cell motility and response to the microenvironment. Recent investigations reveal that there are over 500 human kinases. Many kinase inhibitors are currently in different phases of clinical development. Moreover some kinase inhibitors are already used for the treatment of different cancers: Gefitinib and Erlotinib are EGFR inhibitors used for the treatment of non-small cell lung cancer (NSCLC) and Imatinib mesylate is a c-Abl inhibitor used for the treatment of chronic myeloid leukaemia (CML).




In the course of studies on the preparation of potential kinase inhibitors, we were interested in the synthesis of indolin-2-ones. These compounds are usually known as ATP competitive inhibitors of kinase. The in vitro antiproliferative activities of thirteen isoindigo derivatives bearing a sugar residue attached to one of the oxindole nitrogens and diversely substituted on the aromatic rings were studied.




Some of these glycosyl-isoindigo derivatives have shown significant cytotoxicities. To get an insight into the best substitution pattern required to get relevant biological activities and to identify the cellular target(s) involved in the in vitro antiproliferative activity of these isoindigo derivatives, their inhibitory potencies toward a panel of ten different kinases are presented. The kinases tested were either receptor tyrosine kinase (RTKs), non receptor tyrosine kinases (CTKs) or serine/threonine kinases (STKs). A binding interactions model between the active site of CDK2 or Src and the most potent glycosyl-isoindigos is also proposed.

PO102

# New 2-aminoethanol derivatives as orally active antiestrogenic agents

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Nonsteroidal antiestrogenic agents represent a major advance in the treatment of hormone-dependent breast cancer in postmenopausal females and in the prevention and therapy of the disease in premenopausal women. The most representative compound of this class is tamoxifen (TAM), widely used as chemotherapeutic agent in the treatment of estrogen (ER) positive breast neoplasm [1], which is a partial agonist of the estrogenic receptor. This partial agonism found in TAM and other antiestrogens, led to an intensive search for new nonsteroidal pure antiestrogenic compounds [2].

There are two common structural features in the nonsteroidal antiestrogens such as TAM, the presence of three aryl rings with a central ethylenic bridge and an alkylaminoethoxy side chain [3].

The present communication refers to the synthesis and biological evaluation of novel 2-aminoethanol derivatives containing only two aryl rings. Oximes of general formula (1) are structurally more similar to TAM than ether compounds of formula (2).

The "*in vivo*" estrogenic and antiestrogenic oral activities of these compounds were determined in immature female mice [4]. All tested drugs administered at 5 mg/kg did not show uterotropic activity (estrogenic activity) but the most active compounds at 0.5 – 5 mg/kg significantly inhibited in a 30-50% the effect of estradiol on uterus weigth (antiestrogenic activity). *In vitro* studies of binding affinity for estrogen receptors ER($\alpha$) and ER($\beta$) are in course.

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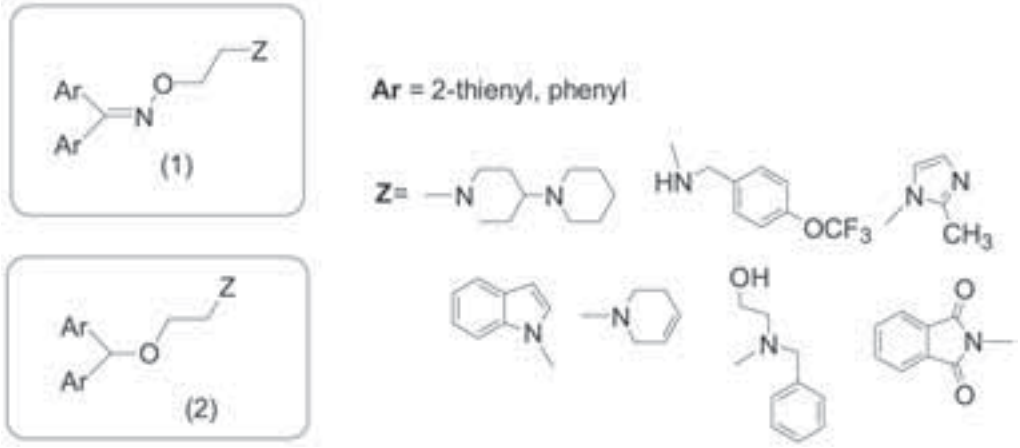
This work was supported in part by the Ministry of Industry, Tourism and Commerce of Spain and the Department of Industry, Commerce and Tourism of the Basque Government.



PO103

# Integrin modulators with a dual/triple action

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Integrins are heterodimeric transmembrane glycoprotein receptors that mediate cell-cell, cell-extracellular matrix, and cell-pathogen interactions. $\alpha_{IIb}\beta_3$ is a long known therapeutic target in thromboembolic disorders [2], while $\alpha_V\beta_3$ is thought to play a role in restenosis [3] tumor cell proliferation [4], adhesion and migration [4,5], and osteoporosis [6]. $\alpha_5\beta_1$ is involved in atherosclerosis [7] and tumor cell invasion [8]. Targeting only one integrin is often inadequate for significant biological effect. Modulators with dual/triple action are especially promising in such cases [9]. Design and development of new integrin modulators is largely based on the Arg-Gly-Asp (RGD) sequence recognized by these four integrins and is common to their natural ligands [1].

We chose a RGD lead from the crystal structure of a $\alpha_V\beta_3$-cycloRGDfV complex [10] and designed several structurally diverse compounds in order to find new leads with a dual or triple action on $\alpha_{IIb}\beta_3$, $\alpha_V\beta_3$, $\alpha_V\beta_5$ and $\alpha_5\beta_1$, and evaluated their biological activity in an *in vitro* solid-phase assay.

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PO104

# Computer model of activation of serotonin receptor 5-HT$_7$ in cellular membrane and its interaction with agonists.

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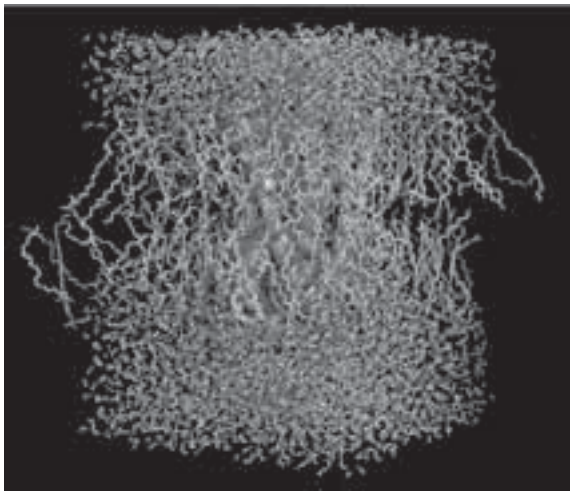

The computer model of activated conformation of serotonin 5-HT$_7$ receptor is presented. It was obtained by means of long molecular dynamics simulation of the receptor with docked agonist (5-HT), embedded in fully hydrated phospholipid bilayer. The activated conformation of the receptor is compared to the results of the similar simulation of "empty" receptor, representing the non-activated form and juxtaposed with the recently published crystal structure of photoactivated bovine rhodopsin [1].

## References

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PO105

# Synthesis and anticonvulsant activity of New N-(4-aryl-piperazin-1-yl)-alkyl-3-(2-trifluoromethyl-phenyl)-pyrrolidine-2,5-diones

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During our previous investigation to search for new anticonvulsant agents on a group of N-substituted 3-fenylpyrrolidine-2,5-diones it has been found that introduction of 4-substituted piperazine at the imide nitrogen atom caused considerable growth of anti-seizure activity. In this series of compounds the most active was N-[4-(3-chloro-phenylpiperazin-1-yl)-methyl]-3-(2-chlorophenyl)-pyrrolidine-2,5-dione with $ED_{50}$= 14.18 mg/kg in the MES test [1, 2]. Carrying on our research we have designed and synthesized some of the analogues of compound mentioned above containing trifluoromethyl group instead of a chloro atom at the 2-position of the phenyl ring. On the other hand, the length of the alkyl spacer between 4-arylpiperazine and imide nitrogen atom has been changed to explain its influence on the activity.

The target compounds **1-5** were prepared by the Mannich-type reaction from the 3-(2-trifluoromethylphenyl)-pyrrolidine-2,5-dione, formaldehyde and the corresponding 4-arylpiperazine. The reaction was carried out in ethanol at a room temperature for ca.6-12h and eventually refluxed for 30min.

The compounds with ethylene (**6-10**) and propylene spacer (**11-15**) were obtained using a one-pot cyclization reaction of the 2-(2-trifluoromethylphenyl)-succinic acid and appropriately substituted 1-aminoalkyl-4-arylpiperazine [3].

The compounds were evaluated for their anticonvulsant and neurotoxic properties through the Antiepileptic Drug Development (ADD) Program, by testing procedures, which have been described earlier [4]. In this series of compounds the most active were derivatives with electron-withdrawing substituents (Cl, $CF_3$) at the 3-position of 4-arylpiperazine moiety.

## References

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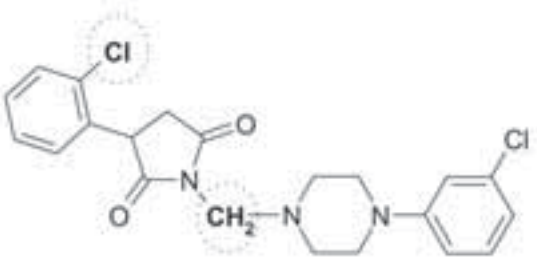
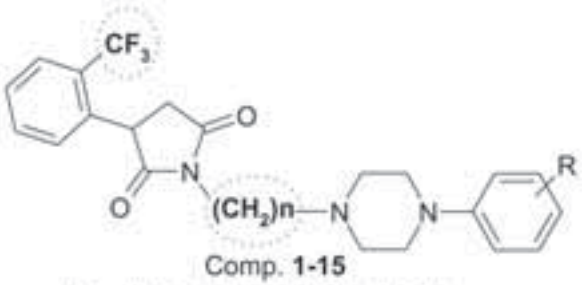



$ED_{50}$ = 14.18 mg/kg




Comp. **1-15**

R = H, 2-F, 3-Cl, 3-$OCH_3$, 3-$CF_3$

n = 1, 2, 3

PO106

# Design, synthesis and in vitro evaluation of 1-amidinopiperidine thrombin inhibitors

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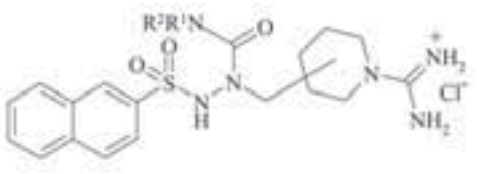
Thrombin is a trypsin-like serine protease that plays a crucial role in the control of blood coagulation. A therapeutically useful inhibitors should be potent, selective and orally bioavailable. Compounds with such properties could be used in the prevention and treatment of several cardiovascular disorders. The derivatives of the amino acid sequence D-Phe-Pro-Arg as in a peptidomimetic argatroban are the most studied group among thrombin inhibitors[1,2].

In our laboratories we prepared analogues in which the aromatic ring in azaphenylalanine part of our previous compounds (rigid mimic of arginine) was replaced by 1-amidinopiperidine[3,4,5]. This modification resulted in the change in overall conformation of molecules and their enhanced basicity. This structural modification would also result in stronger interactions with Asp189 in the active site of an enzyme. Our previous results indicated that proline is not an optimal fragment for binding in proximal pocket of the active site of thrombin, therefore we used a variety of cyclic primary and secondary amines.

The results of in vitro evaluation of newly synthesized molecules has shown a moderate activity against thrombin but high selectivity against trypsin, making these derivatives interesting lead compounds further research in the field of new anticoagulant drugs[6].



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PO107

# Thiazolylbenzo[b]furans with selective inhibition activity of CHO-hBLT$_2$ and their inhibition for MIA PaCa-2 cell

Mari Kuramoto, Yoko Sakata, Kumiko Ando, Mami Yamaguchi and **Yoshitaka Ohishi**

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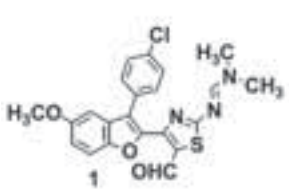
$LTB_4$ receptor inhibition compounds will be useful for treatment several diseases.[1)] We prepared some thiazolylbenzo[*b*]furans to find novel inhibitor for CHO-hBLT$_2$. 2-Thiazolyl-3-phenylbenzo[b]furans (1, 2) showed selective and potential inhibition activity of CHO-hBLT$_2$. 1 and 2 also showed growth inhibitory activity in MIA PaCa-2 cell *in vitro* study.

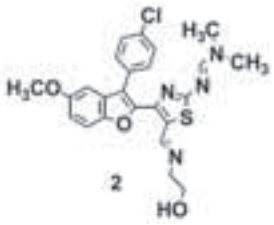
Therefore, several series of thiazolylbenzo[*b*]furans (Type 1, 2, 3, 4) were designed and prepared in our current study.

Thiazolylbenzo[*b*]furans prepared in this study were evaluated their cell viabilities and growth inhibitions of MIA PaCa-2 cell and NHDF. Type 1 compounds among prepared compounds showed the most potent inhibitory activity for MIA PaCa-2 cell and fortunately they had little toxicity for NHDF.

1) W.-G. Tong, et al, Clinical Cancer Research, 2002, 8, 3232.



1



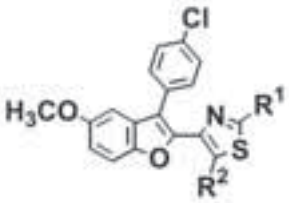
2

Inhibition of calcium mobilization in CHO-hBLT$_1$ and CHO-hBLT$_2$ cells at 300nM $LTB_4$

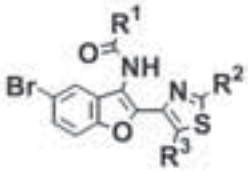
| Compd | % Inhibition (10μM) | | $IC_{50}$ (μM) | |
|---|---|---|---|---|
| | CHO-hBLT$_1$ | CHO-hBLT$_2$ | CHO-hBLT$_1$ | CHO-hBLT$_2$ |
| 1 | 24.4 | 97.9 | 3.55 | 0.19 |
| 2 | 51.3 | >100 | 3.19 | 0.20 |
| ZK-158252 | 97.2 | 96.9 | 1.70 | 1.18 |

In vitro growth inhibitory activity in the MIA PaCa-2

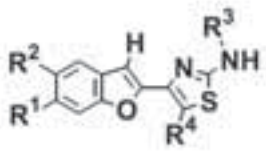
| Compd | $GI_{50}$ (μM) |
|---|---|
| | MIA PaCa-2 |
| 1 | 4.84 |
| 2 | 3.68 |
| 5-FU | >10 |



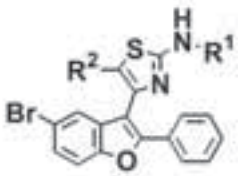
Type 1



Type 2



Type 3



Type 4

PO108

# Stereoselective synthesis of some 17β-dihydrooxazinyl steroids

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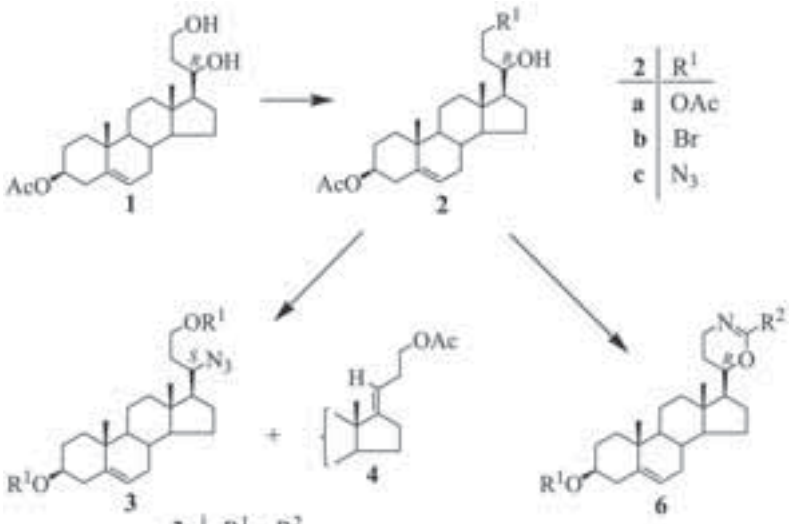
17β-Dihydrooxazinyl steroids **5a l** and **6a l** were synthetized. The acid-catalyzed reactions of 21-azidomethyl-20β-hydroxy- and 21-hydroxymethyl-20α-azidosteroids with substituted aromatic aldehydes led to the formation of androst-5-en-3β-ols substituted in position 17β with dihydrooxazine residues. The inhibitory effects of these compounds on rat testicular $C_{17,20}$-lyase were investigated with an *in vitro* radioincubaton technique.

We thank the Hungarian Scientific Research Fund (OTKA T049366) and the Sigma-Aldrich Kft. (Hungary) for the financial support.



| 2 | $R^1$ |
|---|---|
| a | OAc |
| b | Br |
| c | $N_3$ |

| 3 | $R^1$ | $R^2$ |
|---|---|---|
| a | Ac | Ac |
| b | H | H |

| 5, 6 | $R^1$ | $R^2$ |
|---|---|---|
| a | H | phenyl |
| b | Ac | phenyl |
| c | H | 4-chlorophenyl |
| d | Ac | 4-chlorophenyl |
| e | H | 4-bromophenyl |
| f | Ac | 4-bromophenyl |
| g | H | 4-nitrophenyl |
| h | Ac | 4-nitrophenyl |
| i | H | 4-methoxyphenyl |
| j | Ac | 4-methoxyphenyl |
| k | H | 3,4,5-trimethoxyphenyl |
| l | Ac | 3,4,5-trimethoxyphenyl |

PO109

# Amide derivatives of (6-acyl-2-oxo-3H-benzothiazol-3-yl) aceticacids as potential analgesic and antiinflammatory compounds

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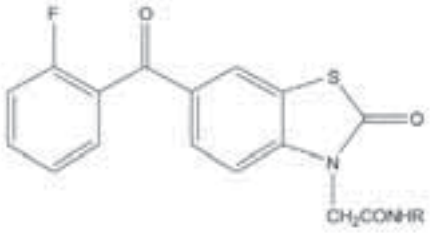
The search for new nonsteroidal antiinflammatory compounds devoid of gastrointestinal side effects has recently been of interest, and 2-oxobenzothiazoline derivatives have been commonly studied for the development of potential drug candidates. Some of the 6-acyl-2-oxobenzothiazoline derivatives have been reported as potent analgesic agents as reported by Ferreira *et al*. indicating that these derivatives might release an endogenous opiod-like substance from the adrenal glands which is responsible for the analgesic activity [1]. Yous *et al*. also reported that 6-benzoyl-2-oxobenzothiazoline represents a new type of analgesic agent acting in periphery by inhibiting the cyclooxygenase pathway and promoting the release of an opioid peptide [2].




**R**= ph (**1a**), 4-Cl-ph (**1b**), 3-Cl-ph (**1c**), 2-pyridinyl (**1d**), 4-Me-2-pyridinyl (**1e**), 6-Me-2-pyridinyl (**1f**)

Therefore, these observations prompted us to prepare the amide derivatives of (6-acyl-2-benzothiazolinon-3-yl)acetic acids as potential analgesic and anti-inflammatory compounds with reduced gastric toxicity. The analgesic and anti-inflammatory activitiy of the compounds were tested by *p*-benzoquinone-induced writhing and carrageenan-induced hind paw edema model, respectively. Compounds **1b** and **1c** showed considerable analgesic and anti-inflammatory activity without any gastric toxicity in the tested animals.

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PO110

# Benzylpiperidin-4-yl-amines: An exploration into the field of antidepressants with dual action on neurokinin-1 receptor and serotonin transporter

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Depression ranks amongst the four leading causes of disability and morbidity throughout the world [1]. Research to identify treatments against this disease has explored the function of the tachykinin substance P and its preferred receptor, neurokinin-1 ($NK_1$), supporting the hypothesis that antagonism of $NK_1$ is a validated and clinically advanced novel approach to treat this disorder [2,3]. Likewise, inhibition of the serotonin transporter (SERT) has provided the rationale for the development of a new generation of faster acting antidepressant drugs with greater efficacy [4].

During the screening in our corporate molecular library, benzylaminopiperidines **1** were identified as a possible scaffold to develop a new series of compounds with affinity for both serotonin transporter and $NK_1$ receptor, with potential antidepressant activity.

On this basis a new series of benzylpiperidin-4-yl-amines of general formula **1** was prepared and biologically evaluated. Derivatives **1** were obtained following a straightforward synthetic pathway starting from *N*-BoC-4-piperidone. Reductive amination with arylmethylamines and subsequent acylation of the exocyclic nitrogen led to the target compounds in a concise way. Alternatively, implementation of a Petasis protocol [5] on intermediate *N*-benzyl-4-aminopiperidines, constituted the key step for the access to benzyl substituted compounds.

Several compounds of this series exhibited activity on hSERT ($K_i$: 78.5-369 nM) and affinity for $NK_1$ ($IC_{50}$: 2.34-4.87μM) together with residual affinity for 5-$HT_{2A}$ receptor ($K_i$: 56.1-260.4 nM).

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## Acknowledgement:

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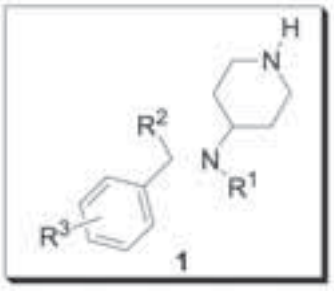


1

$R^1$= Bn, COOAr, $COOCH_2Ar$, $COOCH_2NAlk$
$R^2$= H, $CH_2OH$, $CH_2OBn$
$R^3$= 4-OMe, 2-OMe, 4-F, H

PO111

# SYNTHESIS OF AMPHIPHILIC NITROXIDES

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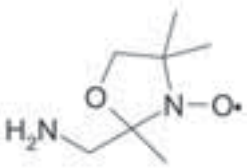
To study the drug - cell membrane interactions and interactions of membranes with drug delivery systems (lyposomes or nanoparticles) using electron spin resonance (ESR) spectroscopy, a special molecular tool would be designed and synthesized.
We present the synthesis of three (**A**, **B** and **C**) types of amphiphilic nitroxides where nitroxide group is located in a polar (water) environment after incorporation of **A**, **B** or **C** into cell membrane.

**A**: Lipophilic moiety – rigid polar group and nitroxide group

**B**: Lipophilic moiety – (ethylene glycol spacer)$_m$ – nitroxide group, m = 1, 2 and 3

**C:** Lipophilic moiety – (-CH(OH)-)$_n$ – nitroxide group, n = 2 and 5

In the case of **A** the possition of nitroxide group is close to the membrane – water interface while in structures **B** and **C** nitroxide group is located between 0,9 to 1,7 nm above membrane surface. Spacer groups in **B** and **C** are flexible, in **B** spacer acts as proton acceptor while in **C** spacer group is proton donor and acceptor.
Due to the ESR reason (orientation of $\pi$ orbital of unpaired electron of the nitroxide group), 2-aminomethyl-3-oxyl-2,4,4-tetramethyl-1,3-oxazolidine was selected as nitroxide group [1]. This amine was *via* amide bond attached to the amphiphilic part of the parent compounds.

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PO112

# Synthesis and evaluation of some novel 2-heteroarylimino-4-thiazolidinones as chondroprotective agents on chondrocytes stimulated by IL-1ß

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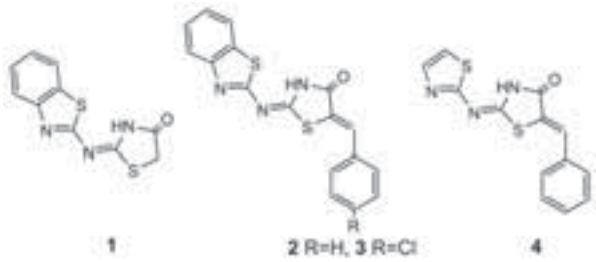
Osteoarthritis (OA) is a joint disease that involves degeneration of articular cartilage, limited intra-articular inflammation manifested by synovitis and changes in the subchondral bone. The etiology of OA is largely unknown, but it may involve multiple factors, including mechanical, biochemical and genetic factors. It has been difficult to identify unique target for therapy since all these processes represent potential fields for therapeutic intervention and drug development. Chondrocytes, which are the unique cellular component of adult articular cartilage, represent a major target for therapeutic intervention.

In this study, we assay the anti-inflammatory/chondroprotective effect of some thiazolidinones, which were predicted by the computer software PASS [1] to be endowed with anti-inflammatory activity, on the production of key molecules released during chronic inflammatory events such as nitric oxide (NO), glycosaminoglycans (GAGs), in human chondrocyte cultures, stimulated with pro-inflammatory cytokine interleukin-1β (IL-1β).

The compounds selected for the present evaluation include the 5-unsubstituted 2-benzo[d]isothiazolyl-imino-4-thiazolidinone 1 and 2-benzo[d]isothiazolyl-, 2-benzo[d]thiazolyl-imino-5-benzylidene-4-thiazolidinones (2,3 and 4).




They were synthetized through the multi-step reaction protocol reported earlier by us for preparing analogous 2-thiazolylimino-4-thiazolidinone derivatives [2]. All the compounds tested in this study showed an interesting profile as active compounds. *In vitro* results pointed out that all molecules were able to contrast the harmful effects of IL-1β on cartilage metabolism.

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PO113

# Hybrid quantum mechanical / molecular mechanical (QM/MM) investigations of the catalytic mechanism of *E. coli* MurD

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MurD (UDP-*N*-acetylmuramoyl-L-alanine:D-glutamate ligase), a three domain bacterial protein, catalyses a highly specific incorporation of D-glutamate to the cytoplasmic intermediate UDP-*N*-acetyl-muramoyl-L-alanine (UMA) utilizing ATP hydrolysis to ADP and $P_i$. This reaction is part of a biosynthetic pathway enabling bacterial peptidoglycan synthesis. Based on the structural studies of *E. Coli* MurD complexes with various ligands a stepwise catalytic mechanism was proposed that commences with the formation of an acyl-phosphate intermediate, followed by the nucleophilic attack of the D-glutamate that through formation of a tetrahedral reaction intermediate yields the final product UDP-*N*-acetyl-muramoyl-L-alanine-D-glutamate (UMAG) [1].

Hybrid quantum mechanical/molecular mechanical (QM/MM) approach was utilized by combining quantum computational package GAMESS at the DFT level of theory with CHARMM empirical force field [2] in order to gain a detailed insight into the mechanism of the amide bond formation catalyzed by MurD. We employed replica path method coupled with the potential of mean force (PMF) analysis as encoded in CHARMM [3]. By considering several hypothetical reaction pathways energy profiles for the most favorable sequences of events along the reaction coordinate have been established. The role of crucial amino acids of the enzyme active site and water molecules involved in the amide bond formation has been assessed and evaluated. Acquired geometries of the transition state structures represent novel and valuable information to assist in the ongoing efforts toward novel transition state mimetics of MurD as potential new antibacterial drugs.

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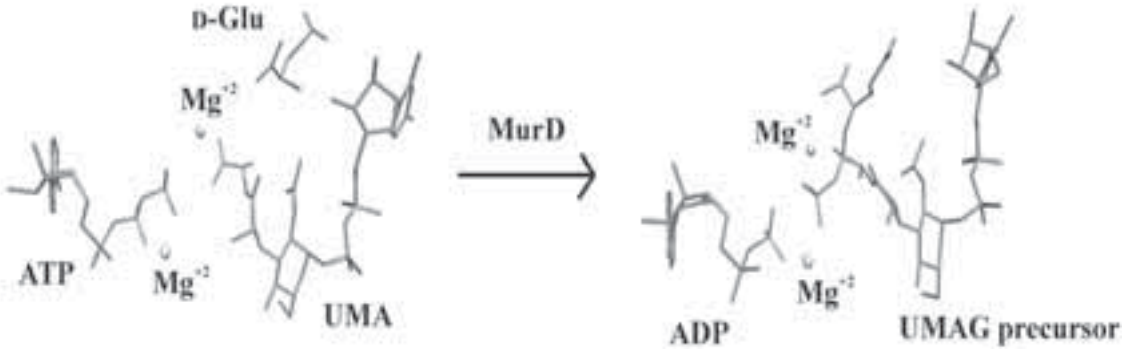


**Figure:** Starting geometries of the ligands (ATP, UMA and D-Glu) and calculated product (UMAG) precursor geometry for the reaction catalyzed by *E.Coli* MurD

PO114

# Design and evaluation of MurC and MurD enzymes inhibitors with a hydrazide scaffold

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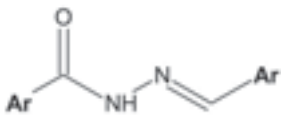
Peptidoglycan is a macromolecule found on the outer face of the cytoplasmic membrane of bacteria and is essential to protect bacteria against the internal osmotic pressure and plays a key role in cell division. Various enzymes involved in its biosynthesis represent potential targets for the search of new antibacterial agents, which has been rendered necessary by the spreading of resistance to existing antibiotics [1].

A series of new compounds with a hydrazide scaffold (Figure) were designed and synthesized as inhibitors of two enzymes, MurC and MurD, involved in peptidoglycan biosynthesis. They were tested against the MurC and MurD enzymes from *Escherichia coli* allowing initial structure-activity relationships to be deduced.

Compounds that exhibited MurC and MurD inhibitory activities were further tested for their antimicrobial activities. The minimal inhibitory concentrations (MICs) of each compound were determined against E. coli 1411 and SM1411 an acrAB deficient derivative of 1411 that exhibits increased susceptibility to a range of antimicrobial agents and S. aureus 8325.

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PO115

# Structure-antibacterial activity of 5-triazolyl oxazolidinones containing long chain acyl groups

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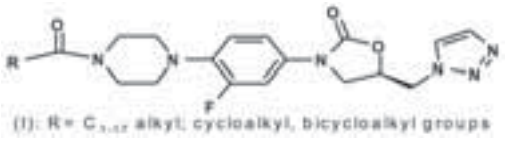
**Objective:** To investigate antibacterial activity of newly synthesized N-4-long chain acylpiperazinyl 5-triazolylmethyl oxazolidinones **(I)** and correlate their activity with selected structural molecular descriptors.

**Materials and Methods**: A series of new 5-triazolylmethyl oxazolidinones were synthesized. Antibacterial activity was evaluated against methicillin-resistant (MRSA) and -susceptible *S. aureus* (MSSA); methicillin-resistant (MR-CNS) and -susceptible coagulase-negative staphylococci (MS-CNS); *Streptococcus pnuemoniae* and vancomycin-resistant (VRE) and -susceptible enterococci (VSE). Minimum inhibitory concentrations (MIC's, μg/ml) were determined by agar dilution method on Mueller-Hinton agar in the presence and absence of 50% human plasma. The structures of the compounds were sketched and the calculated log P values were estimated using ChemDraw Ultra 8.0. The 3D structures and molecular descriptors were derived using Alchemy 2000.

**Results:** Compounds with acyl groups having 2-8 carbon atoms showed weak to strong antibacterial activity against Gram-positive cocci (MIC, 0.25-8 μg/ml). Activity decreased consistently as the number of carbon atoms increased, hence compounds with carbon atoms 9-18 showed reduced antibacterial activity (MIC, 4-≥16 μg/ml) against all bacteria tested. Compounds with 6-8 carbon atoms showed significant increase in MIC values of ≥16 μg/ml in the presence of 50% human plasma, indicative of plasma binding. Nonparametric Spearman's correlation coefficient showed that antibacterial activity correlated strongly (*rho* = 0.846, $p<0.001$) with the heat of formation (HF) of the compounds. On the other hand, activity correlated negatively with Clog P values, surface area (SA), ovality (Ov) and molecular volume (MV), suggesting a consistent decrease in activity with increase in the acyl chain length. However, no significant correlation was observed with activity and $E_{LUMO}$ and $E_{HOMO}$ (energy of lowest-unoccupied and highest-occupied molecular orbitals) and dipole, respectively.

**Conclusion**: Antibacterial activity increased with increase in HF, indicative of conformational stability, which may favor better binding and improved activity at the molecular level. However, activity decreased with increase in carbon chain length, Clog P, SA and MV, respectively. This study highlights structural requirements for antibacterial activity in this class of compounds.



(I): R = $C_{1-17}$ alkyl, cycloalkyl, bicycloalkyl groups

Supported by Research Administration, Kuwait University, Grant PC01/02 (OAP), and Instrument Grants GS01/01 and GS03/01 (Science Analytical Facilities, SAF).

PO116

# New biologically active ebselen analogues from ring-substituted anthranilic acids

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Research of the last twenty years have shown that selenaheterocyclic compounds are active immunostimulants, inhibitors of enzymes, antioxidants, anti-inflammatory, antitumor, antiviral and antimicrobial agents [1-5]. Nowadays, they are considered as new prospective pharmaceuticals.

In this work we report synthesis, chemical properties as well as results of biological activity against three model viruses (HSV-1, EMCV and VSV) of twenty (C-5, N-2)-substituted analogues of benzisoselenazol-3(2*H*)-ones.

The general strategy for synthesis of (C-5, N-2)-substituted benzisoselenazol-3(2*H*)-ones (**3)** is based on the conversion of commercially available ring-substituted anthranilic acids (**1**) into C-5-substituted 2-(chloroseleno)benzoyl chlorides (**2**) and finally on the tandem selenenylation-acylation of primary amines with these reagents.

Biological studies have shown that tested compounds exhibit high level of anti-HSV-1 (1-80 g/ml) and anti-EMCV (4-80 g/ml for alkyl derivatives only) activity, whereas, similarly like ebselen they are inactive against VSV (MIC>1000 g/ml). Based on these results, C-5-substituted benzisoselenazol-3(2*H*)-ones can be considered as new promising antiviral agents.

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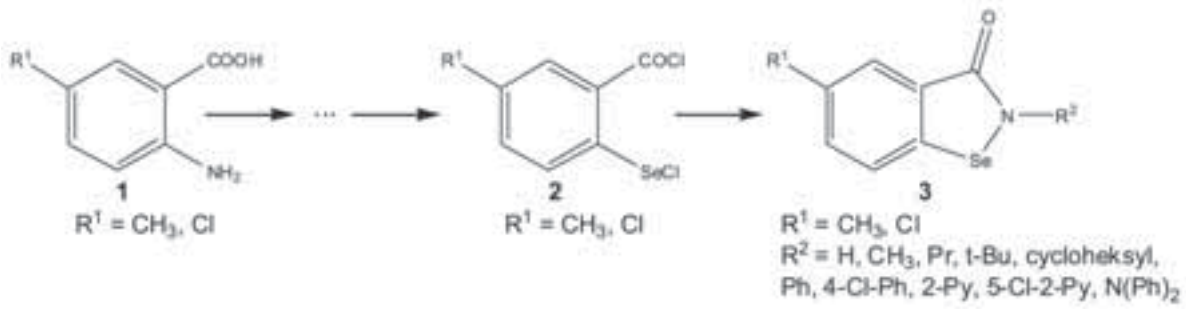
This work was supported by PBZ-Min-015/P05/2004 grant.



PO117

# Azanaphthoquinone annelated pyrrole derivatives as anticancer compounds, part III

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Previous work on the synthesis of pharmacologically active pyrrole and isoindole derivatives led to the discovery of azanaphthoquinone derivatives of type **1** [1] which exhibited remarkable anticancer activities. Dose dependent capase activity was found indicating an apoptosis inducing property of these compounds. The cell cycle was arrested in $G_2$ /M phase.

The present work describes the design and synthesis of analogous isoindole **3**. Azanaphthoquinone annelated pyrrole **2** [2-3] was successfully prepared by four-step reaction. The key transformation is acidic cyclization of 7-aminoisoquinoline-5,8-dione with acetaldehyde diethylacetal to furnish pyrrole system. The Grignard reaction of **2** occurred regioselectively at C-4 to obtain the corresponding carbinol **3** in moderate to good yields. After structure modification, a series of carbinols and carbinol ethers was obtained. The anticancer activities were evaluated on four different cell lines: KB/HeLa, NCIH460, SKOV3 and SF268. The results of biological evaluation are discussed.

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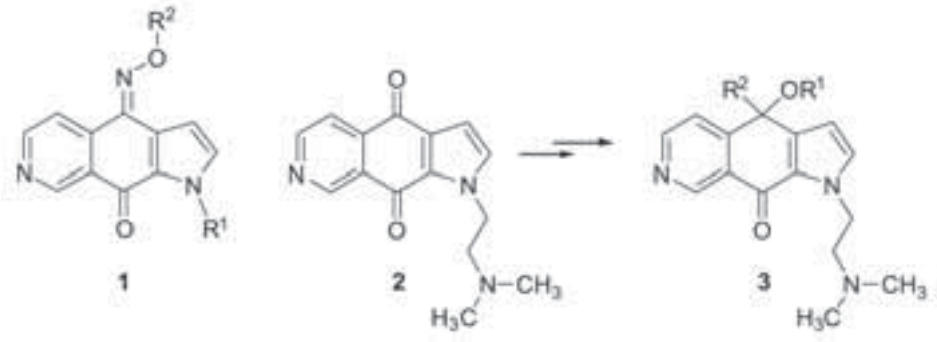

PO118

# Synthesis and antiviral activity of some benzisoselenazol-3(2*H*)-ones and their isostructural analogues

Magdalena Pietka-Ottlik, Jacek Mlochowski, Egbert Piasecki, **Piotr Potaczek**

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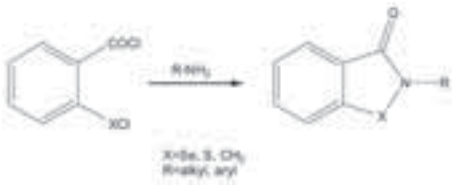
[b]*Laboratory of Virology, Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Weigla 12, 53-114 Wroclaw*

Recently, it has been reviewed that some of synthetically available organoselenium compounds, particularly benzisoselenazol-3(2*H*)-ones are strong inhibitors of viral cytopathogenicity [1-5]. Because the mechanism of their biological activity remains unknown we formulated the hypothesis that antiviral activity of these compounds strongly depends on the presence of selenium. For this purpose, we designed a group of isostructural analogues of benzisoselenazol-3(2*H*)-ones in which selenium has been replaced by sulphur or methylene group and tested them against selected viruses (HSV-1, EMCV and VSV), *in vitro,* in the same conditions as selenic compounds. For the synthesis, reaction of appropriate dichlorides with primary amines was applied.

Our study has demonstrated that antiviral activity of organoselenium compounds is much higher than their isostructural analogues which were almost completely inactive. These results suggest that selenium plays crucial role in this type of antiviral agents.



## References:

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This work was supported by Wroclaw University of Technology, grant 332093 and by Ministry of Science and Higher Education, grant N204 022 31/0458.

PO119

# Design and synthesis of novel antibiotics

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There is an urgent need to develop novel antibiotics to help overcome the increasing problem of bacterial resistance which is rapidly decreasing the therapeutic value of many, once potent, antibiotics.

The aminoacyl-tRNA synthetases (aaRS), in particular isoleucyl-tRNA synthetase (IleRS), are well-validated drug targets. Mupirocin is a commercially available inhibitor of IleRS which is marketed as Bactroban. Mupirocin can only be used as a topical antibiotic as it is metabolised *in vivo* to give inactive metabolites. Resistance to mupirocin is becoming increasingly widespread, thereby reinforcing the need for development of novel inhibitors of this family of enzymes.

Our approach involves using a combination of *in silico* methods, predominantly our in-house computer programme for constrained structure generation, SPROUT [1] and virtual high throughput screening (VHTS) carried out using the docking programme, eHiTS [2]. SPROUT design successfully resulted in the production of a range of potential targets for synthesis. These targets are currently being synthesised and each will to be sent for biological screening once synthesis is complete.

VHTS was carried out and 23 molecules were purchased for biological evaluation. Preliminary screening showed that approximately half of these ligands exhibited inhibition of IleRS at 500 μM. Further screening confirmed several of these molecules showed 90-100 % inhibition of IleRS at 5 μM. Hits resulting from VHTS are currently being resynthesised to verify activity.

Targets from *de novo* SPROUT design are synthesised and sent for biological testing; initial results showed that our first *de novo* designed compound exhibits weak activity against IleRS. A small, focused library based on this structure is being synthesised to probe biological activity.

A number of IleRS inhibitors have been identified, including several compounds showing 90-100% inhibition at 5 μM. Further biological evaluation of these ligands is ongoing.

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PO120

# Semisynthetic derivatives of vancomycin and eremomycin with improved chemiotherapeutetic properties.

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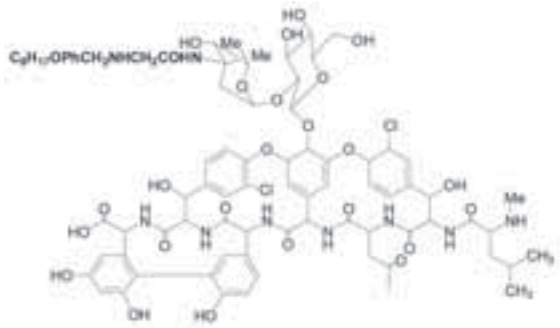
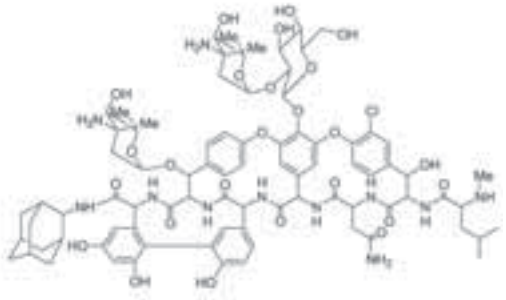


GINA-220



GINA-213

New semisynthetic derivatives of glycopeptides antibiotics vancomycin and eremomycin were obtained and their antibacterial and pharmacokinetic properties were investigated. N'-p-Octyloxybenzylglycyl-vancomycin (GINA-220) was obtained by the reaction of acylation of unprotected vancomycin with activated esters of α-N-alkyl-N-Fmoc-amino acid which is directed selectively to the amino group of disaccharide branch. GINA 220 was found to be highly active against glycopeptide-sensitive staphylococci and enterococci (MIC's are 0.25 – 1 μg/mL) and moderatly active against GISA (MIC's 1-2 μg/ml) and GRE (MIC's 4-8 μg/ml) at *in vitro* studies. At *in vivo* studies GINA-220 was active against *S. aureus* systemic infection at mice with $ED_{50}$ 1 mg/kg versus 2 mg/kg of vancomycin (*iv*) and substantially more effective than vancomycin in the mouse thigh infection model.

Adamantyl-2 amide (GINA-213) of eremomycin was synthesized by the reaction of eremomycin with amine in the presence of condensing reagent PyBOP and was found to be highly active against vancomycin-sensitive staphylococci and enterococci (MIC's 0.25-0.5 μg/ml) and moderately active against GISA (MIC's 1-2 μg/ml) and GRE (MIC's 8 μg/ml) at *in vitro* studies. GINA-213 is equally active *in vitro* against both ciprofloxacin-sensitive and -resistant *Bacillus anthracis* strains (MIC's 0.25-0.5 μg/ml) and 45 times more active than ciprofloxacin against resistant strains. I*n vivo* studies against *S. aureus* at mice (*iv*) showed that GINA-213 had long half-life (185 min), high tissue levels (Vss 26285 ml/kg) and deep tissue penetration (lung, spleen) in comparison with vancomycin.

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PO121

# The effects of proton pump inhibitors on the bacterial flora of gastroesophageal reflux disease (GERD) patients

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**Objectives:** Individuals diagnosed with gastroesophageal reflux disease (GERD) are thought to have a condition in which acid and pepsin from the stomach is being refluxed into the esophagus. As such, patients are often given proton pump inhibitors (PPIs), such as Protonix, in order to raise the pH of the stomach. However, recent studies have shown that acid-producing cells are also found in the oral cavity and upper gastrointestinal tract, suggesting that PPIs may have additional target cells outside the stomach. Bacterial and human DNA homology suggested that PPIs may affect the activity of both prokaryotic and eukaryotic proton pumps.

**Methods:** Nineteen different strains of *Lactobacilli* were grown in the presence of Protonix; the bacteria were inoculated in microtiter plates in media at a pH of 4.5 and exposed to two-fold dilutions of Protonix at a range of 2.5 mg/mL to 2.5 μg/mL. In cases where growth was not observed at pH 4.5, the strains were inoculated in media at higher pH values (5.0 and 5.5) to determine the level of sensitivity to the drug. For strains most sensitive to Protonix, the minimal inhibitory concentration (MIC) of the drug was determined, and growth curves were measured by exposing the bacteria to 5 μg/mL of Protonix (approximating the clinical dose). Gram-staining was carried out to visualize any potential morphological changes in the bacteria.

**Results:** Eight of the nineteen *Lactobacilli* strains tested were found to have an MIC below 313 μg/mL; *L. plantarum* 14917 was the most sensitive bacteria found, having an MIC of 20 μg/mL. Additionally, the growth curves measured for some strains, such as *L. gasseri* 9857, indicated noticeably slower growth rates upon treatment of Protonix, while the Gram-staining results revealed conformational changes in some of the bacteria, such as *L. s. salivarius* 11741.

**Conclusions:** A number of *Lactobacilli* strains were found to have increased sensitivity, slower growth, and/or conformational changes when grown in the presence of Protonix. These results suggest that the normal balance of flora found in the oral cavity and gastrointestinal tract of patients taking PPIs could be disrupted, possibly leading to the long-term complications observed in some patients. It also supports the idea that the proton pumps can be a target to suppress bacterial growth.

PO122

# HIV-RT inhibitors: developing of COMBINE, VALIDATE and GRID/GOLPE models from experimental available ligand-enzyme complexes and predictive ability.

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Human immunodeficiency virus (HIV) is the etiological agent of acquired immunodeficiency syndrome (AIDS), an infection characterized by loss of helper T lymphocytes and heavy damage of lymphatic tissues. The current therapy against AIDS is based on four classes of anti-HIV drugs: the nucleoside and nucleotide reverse transcriptase (RT) inhibitors (indicated as NRTIs and NtRTIs, respectively), the non-nucleoside reverse transcriptase inhibitors (NNRTIs), the protease inhibitors (PIs), and the fusion inhibitor (FI) enfuvirtide. Among the marketed anti-HIV drugs more of the half belongs to the NNRTI class. During the last two decades much effort has been done in developing new and more potent RT inhibitors. Many of the marketed NNRTI were disclosed with the aim of computational approaches using either LBDD or SBDD techniques. As a matter of fact, nowadays the NNRTIs identified so far include more than 50 structurally quite different classes of molecules. As a consequence, in the NNRTI field the efforts are now focused on the development of novel compounds endowed with higher anti-HIV-1 activity and a resistance profile different from that of known drugs. Continuous efforts in this field are documented by the wide number of NNRTIs described in the recent literature, some of which, such as capravirine and etravirine are under clinical trials.

Despite the massive effort in obtaining experimental information on ligand/enzyme interaction to the best of our knowledge only very few attempts have been done in obtaining 3-D QSAR models for the NNRTI of general applicability. Such a general model, in which several different chemical scaffolds are included, could be of help to overcome the RT mutagenic issue.

Here we present the developing of 3-D QSAR models using three different independent well known techniques: COMBINE, VALIDATE and GRID/GOLPE. This is the first time that a structure based 3-D QSAR (SB 3-D QSAR), a scoring function QSAR (SC QSAR) and a classical 3-D QSAR techniques are used to develop chemometric based models in the field of RT inhibitors.

Preliminary data are very encouraging and promising. Using 25 experimentally determined ligand/enzyme complexes high statistical coefficients were obtained for all the three different techniques. The COMBINE analysis was characterized by $r^2$, $q^2$ and SDEP values of 0.92, 0.74 and 0.56, respectively. The VALIDATE model showed lower statistical values ($r^2$ = 0.85, $q^2$ = 0.63, SDEP = 0.68) while the GRID/GOLPE procedure gave the highes values ($r^2$ = 0.99, $q^2$ = 0.90, SDEP = 0.35). Models details and applications will be reported

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PO123

# Ranking ligand docking poses by geometric scores: Application to the $GABA_A$ receptor

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Docking poses are routinely evaluated by energetic scoring functions. In case of low receptor resolution or protein homology models, docking results are unreliable due to insufficient knowledge on the binding site geometry. This lack of receptor resolution, however, is often compensated by plenty of biological data. In this study we present a method to include data from covalent labelling of $GABA_A$ receptors by ligands into the scoring of docking poses in order to refine rankings obtained by classical scoring functions.

The FlexX-docking tool is applied to dock the imidazobenzodiazepine flumazenil into homology models of the benzodiazepine binding site of an $\alpha1\beta2\gamma2$ $GABA_A$ receptor. Subsequently, a distance matrix based on covalent reactions between an NCS-analogue of flumazenil and selected Cys-mutants of the $GABA_A$ receptor is created for each pose. Adequate scoring points are assigned for each pose taking into account the difference between optimal distance for covalent reaction and actual distance found in the docking pose. Ranking using this distance-score differs from the FlexX-score ranking. Combination of both scores and subsequent ranking leads to feasible, energetically favourable poses that are in agreement with experimental data.

Due to poor homology with available templates, a large variety of structurally different but equally plausible $GABA_AR$ models do exist. The distance-score allowed to quickly evaluate different homology models of this receptor with respect to the topology of the benzodiazepine binding site. The best models feature both a satisfactory distance-score, and an energetically favourable FlexX-score.

Supported by grant P16397 of the Austrian Science Fund (to W. Sieghart)

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PO124

# Synthesis and biological evaluation of new thiazole derivatives

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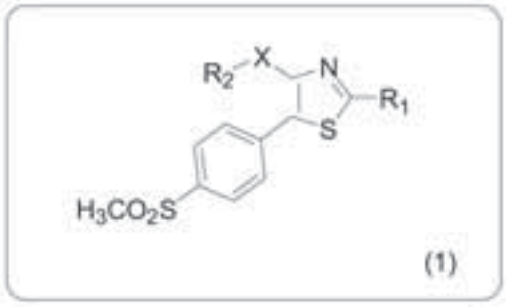
The discovery and characterization of the cyclooxygenase-2 (COX-2) enzyme early in the 1990s [1] led to new molecules which are selective inhibitors for this enzyme. The recent worldwide withdrawal of rofecoxib because of evidence of increased cardiovascular risk [2] has raised concern about the safety of COX-2 selective inhibitors. Research efforts [3] to clarify the reason for this undesired adverse effect are carried out at present. In connection with this, the research of new chemical structures of COX-2 inhibitors is very important. Moreover potential therapeutic applications of selective COX-2 inhibitors have been expanded beyond the areas of analgesia and inflammation, as shown by recent studies on COX-2 which have been focused on cancer [4] and neurodegenerative disorders [5].

As a part of a research project on this matter, new thiazole derivatives **(1)** have been synthesized:

The synthesis of **(1)** has been carried out from commercially available starting materials following known synthetic methods.




X= O, NH

$R_1$= $CH_3$, $CH_3CH_2$, $(CH_3)_2CH$, Ph,
4- chlorophenyl, 2-chlorophenyl
3-pyridinyl, 2-thienyl, Cyclohexyl etc

$R_2$= $CH_3$, n-$C_4H_9$, $PhCH_2$,
Cyclopentyl, Cyclohexyl etc

Analysis of the *in vitro* COX-2 inhibitory activity of these molecules have been determined by using purified enzyme and human whole blood (HWB) assays. In the first (purified enzyme) assay $IC_{50}$ values for the most active compounds were between 3nM and 100 nM (rofecoxib $IC_{50}$= 398nM). In the HWB assay the $IC_{50}$ values were higher than 1μM.

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This work was supported in part by the Ministry of Industry,Tourism and Commerce of Spain and the Department of Industry, Commerce and Tourism of the Basque Government

PO125

# Direct HPLC enantioseparation of new chiral sigma1 ligands on coated polysaccaride-based chiral stationary phases

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In the last years our research efforts have been focused on the synthesis and pharmacological evaluation of new potential sigma1 ligands. Preliminary binding assays of racemic N,N-disubstituted-3-aryl-butan-1-amines (Fig. 1) showed a good sigma1 affinity and interesting sigma2/sigma1 selectivity [1].

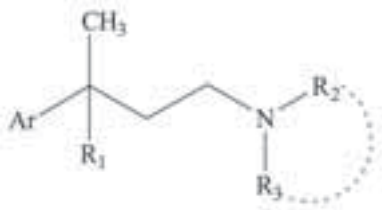


Fig. 1

To gain more insights into binding modes, we mapped conformers of all compounds onto a 5-point pharmacophore model developed by Laggner et al [2]. The role of stereochemistry in binding requirements was further investigated by extending fitting studies to the pure enantiomeric (R) and (S) forms. (S) isomers were predicted to be more active than the (R) isomers.

In order to obtain enantiopure compounds and to confirm the molecular modelling studies, here we report on direct HPLC enantioseparation by means of coated polysaccaride-based chiral stationary phases.

The chiral recognition was compared among four chiral columns: cellulose tris (3,5-dimethylphenyl carbamate) (Chiralcel OD-H); amylose tris (3,5-dimethylphenyl carbamate) (Chiralpak AD); amylose tris ((S)-1-phenyl- carbamate) (Chiralpak AS and AS-H). Different mobile phase compositions were investigated as well.

At the moment, the best resolutions were achieved by using Chiralpak AD column, providing a method that may be used for small scale preparation of pure enantiomers of the considered compounds.

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PO126

# Determination of trace amounts of endocrine disruptors by means of chemically bonded phases for pre-concentration and gas chromatography

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An excess of a female sexual hormone – estrogene – may show a negative influence on living organisms. The estrogene and its derivatives may disrupt natural hormonal balance, both for men, and women. This disruption is of particular importance in the case of young human bodies, as the triggered changes are irreversible. Recently, a large group of compounds has been enumerated having similar influence on humans as the estrogene, to mention: chloroorganic pesticides, alkylphenols, polichlorobiphenyles, organic compounds of the tin, and Bisphenol A and its derivatives. Substances of this type are known as endocrine-disrupting compounds, ECD [1, 2].

Recently, bisphenol A (BPA) has become a compound of particular interest of ecotoxicologists [3-6]. BPA is used for many industry activities, including the synthesis of polycarbonate plastics, epoxy resins, and polyacrylates. These plastic materials are massively used for the productions of (among others) baby bottles and internal coating layers for the packings for baby food industry, such as powdered milk and milk mixtures [6;7,8], as well as for lacquers for cans and vessels for storage of the food, drinking water, and medicines [2, 3]. Since second half of the 90-ties, numerous reports have arrived stating negative influence of BPA on human health. The newest research [4] classifies BPA as xenobiotic endocrine disruptor, disrupting the balance of the hormonal system of the humans and the animals. Since 1996, BPA has been classified by the European Commission as "external-derivative chemical influenced on human health and offspring".

Recently, many publications confirmed estrogenic activities of BPA *in vivo*, even taken in very little doses. These are foetus and little babies that are the most sensitive group taking into account hormonal aspects of BPA exposal. It seems that the main way of BPA penetration is related with milk, both breast milk and some diet supplements (e.g., powdered milk). The poster presents our research concerning determination of BPA in the milk and milk-derivative products. The main goal was to optimize the conditions for the isolation (by the use of SPE) and determination of BPA in the milk samples, by the use of gas chromatography coupled with Flame Ionization Detection (FID), and Low Resolution Mass Spectrometry (LRMS). In addition we prove that BPA is set free from the polycarbonate boxes and migrates to the food and water these boxes contain.

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PO127

# A new series of low-molecular-weight immunosuppressory isoxazoles

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In our earlier studies we showed that a new isoxazolotriazepine derivative, RM33, in the preliminary studies have immunosuppressive and antiinflammatory activities, in the mouse model. The compound inhibited both the humoral and cellular immune response and was effective not only upon *ip* administration but also when given orally, indicating good bioaccessibility and a potential therapeutic application. Other studies revealed the lack of toxicity of the compound even at very high doses.

We also showed that RM33 might effectively suppress the inflammatory reactions elicited by complete Freund's adiuvant (cFa). That effect was correlated with inhibition of pro- but not anti-inflammatory cytokines.

Although immunosuppressory isoxazoles, such as leflunomide, express anti-inflammatory properties and are sometimes used in combination with other immunosuppressors like Cyclosporine A (CsA)[1], their mechanism of action differs from that of CsA[2]. In particular, the mitogen-induced T cell proliferation is not affected[3].

That was also a case with regard to RM33 which stimulated rather than inhibited phytohemagglutinin-induced proliferation of human mononuclear blood cells, in addition, the compound did not affect IL-10 production, in contrast to CsA. Another isoxazole, RM11[4] also stimulated mitogen-induced T cell proliferation, and strongly stimulated both humoral and cellular immune response in mice.

Interesting, the structure of RM11 is very closely related to that of RM33, differing only in substitution of one group in the isoxazolo[5,4-e]triazepine ring. Therefore, within the family of isoxazoles, both immunosuppressive and immunostimulatory compounds may be found. It is also clear that immunostimulatory activity in some tests i.e. mitogen-induced cell proliferation does not exclude anti-inflammatory activity of a given compounds as in the case of RM33.

The inhibition of adjuvant-elicited foot pad endema by RM33 may, in part, be explained by its immunosuppressive action on the immune response since adiuvants provide c0-stimulatory signals in the initiation of the immune response[5]. The compound inhibited activity of TNF- which is important in the induction phase of the immune response and in mediation of inflammation[6].

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PO128

# Effects of donezepil on diisopropyl fluorophosphate toxicity

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Organophosphorus cholinesterase inhibitors, such as chemical warfare agents (i.e.sarin, soman,VX ) exert their effects by irreversibly inhibiting acetylcholinesterase (AchE), the major enzyme in the metabolism of acetylcholine (Ach), resulting in severe overstimulation of central and peripheral postsynaptic cholinergic activity.
This study evaluated the possible protective effects of reversible acetycholinesterase inhibitor donepezil on the toxic effects of acute intoxication with irreversible organophosphate cholinesterase inhibitor diisopropyl fluorophosphate ( DFP ), by animal model with rats.
We speculated, that donepezil could oppose the effects of DFP by pretreated either with physiologic saline or with donepezil and subsequently treaded with sublethal dose of DFP.
The effects of DFP toxicity were evaluated by behavioral observation and by measurements of body temperature and the degree of AchE inhibition and by the levels of c-fos mRNA induced in the brain.
We found,that the pretreatment by donepezil diminished the signs of behavioral toxicity and opposed the decrease of body temperature, AchE inhibition and the induction of c-fos mRNA by DFP.
We have also demonstrated that donepezil pretreatment by itself could provoke behavioral toxicity, decrease of body temperature and AchE inhibiton, although to a lower degree than the treatment by DFP. Donepezil by itself did not induce c-fos mRNA in the brain.
Our results demonstrate that donepezil pretretment could be an effective antidote against the intoxication by DFP.

PO129

# Development of a docking strategy for the generation of kinase-focused libraries

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Kinase enzymes play a crucial role in signal transduction and cellular proliferation and differentiation, and much effort is being done to generate chemical libraries of kinase ligands for high throughout screening, preferably on structure-based computational approaches[1].
We present here a docking and scoring strategy for the synthesis of kinase-focused libraries. To develop this approach, we have performed a systematic docking of two libraries in kinase crystal structures. The libraries tested are BioPrint (a “drug-like” database including more than 2500 drugs or drug related compounds) and a kinase-oriented library of 1440 compounds based around original pyrimidine scaffolds. We have also docked 123 well known kinase ligands described in publications and patents.
Kinase crystal 3D structures corresponding to ABL, EGFR and CDK2 were obtained from the Protein Data Bank. During docking, the protein remains rigid while the ligand is flexible allowing different conformations to be docked. Six scoring functions implemented in LigandFit have been calculated for each pose of each compound. The cut-offs were chosen according to the scores of the known inhibitors.
This strategy has been validated using the scoring of the known kinase ligands compared with BioPrint scoring, the kinase ligands being among the 5-7% best ranked compounds. When predicting the kinase activity of the compounds in the two libraries, approximately 86% of compounds from the kinase library passed the scoring filters for at least one kinase (for only 13% of compounds of BioPrint). Moreover, 8% of compounds from the kinase library passed the filters of the three kinases (for 0.6% of the BioPrint compounds), thus allowing to identify promiscuous scaffolds for kinase activity.

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PO130

# Constrained analogues of procainamide as novel small molecule inhibitors of DNMT1.

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Epigenetic alterations are increasingly recognized as valuable targets for the development of cancer therapies. They not only occur early in carcinogenesis but also are found in virtually all cases of cancer [1,2]. Importantly, epigenetic alterations do not involve changes in the DNA sequence and thus are potentially reversible. Of the epigenetic changes seen in cancer, the most extensively studied is the increase of CpG dinucleotide methylation at CpG islands in the proximal promoter regions of genes. This change in DNA methylation characteristically results in the transcriptional silencing of important cancer genes such as tumor suppressors and caretaker genes [3]. Nucleoside analogs like 5-azacytidine or 2-pyrimidone-1-ß-D-riboside (zebularine), though effective in inducing DNA demethylation and reactivation of hypermethylated genes, carry considerable concerns about toxicity [4,5]. These concerns have led to consideration of non-nucleoside inhibitors of DNA methyltransferases. Procainamide, originally approved by the U. S. Food and Drug Administration for the treatment of cardiac arrhythmias, specifically inhibits the maintenance methyltransferase activity of DNMT1 and reactivates genes silenced by promoter CpG island hypermethylation [6]. Herein we report the synthesis of *frozen analogues* of procainamide and their inhibitory activity towards DNA methyltransferases in vitro and in vivo.

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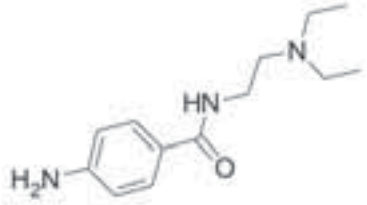
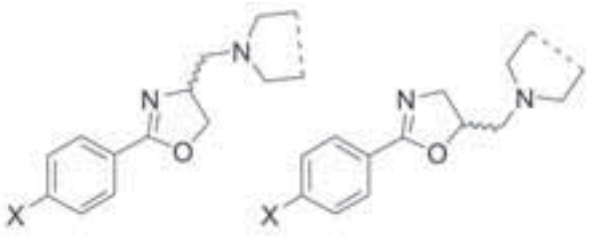



procainamide




frozen analogues
X= $NO_2$, $NH_2$

PO131

# The adenosine A3 receptor (A3AR) – a completely new target for PET-neuroimaging

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The adenosine A3 receptor subtype (A3AR) is involved in a variety of pathologies, especially neurological/psychiatric diseases (cerebral ischemia, glaucoma, stroke, epilepsy). The present study aims at the evaluation of [$^{18}$F]FE@SUPPY:1 **1** as PET tracer, on a new target receptor, the A3AR. FE@SUPPY:1 has been described to display high affinity and selectivity for the A3AR [1] and on the basis of the existing literature [2,3] it is conclusive, that the A3AR can serve as a new promising target for the PET-imaging of various pathologies.

The inactive precursors for [$^{18}$F]FE@SUPPY:1 have been synthesized by the Hantzsch´ pyridine condensation according to Li et al [1], for the hitherto unknown [$^{18}$F]FE@SUPPY:2 **2** a modified approach was developed.

The radiosynthesis of [$^{18}$F]FE@SUPPY:1 was successfully performed from the corresponding tosylate **3** by treatment with cyclotron produced [$^{18}$F]fluoride. [$^{18}$F]FE@SUPPY:2 **2** which was prepared by the same method is also submitted to biological evaluation. The biodistribution and Micro PET experiments are discussed.

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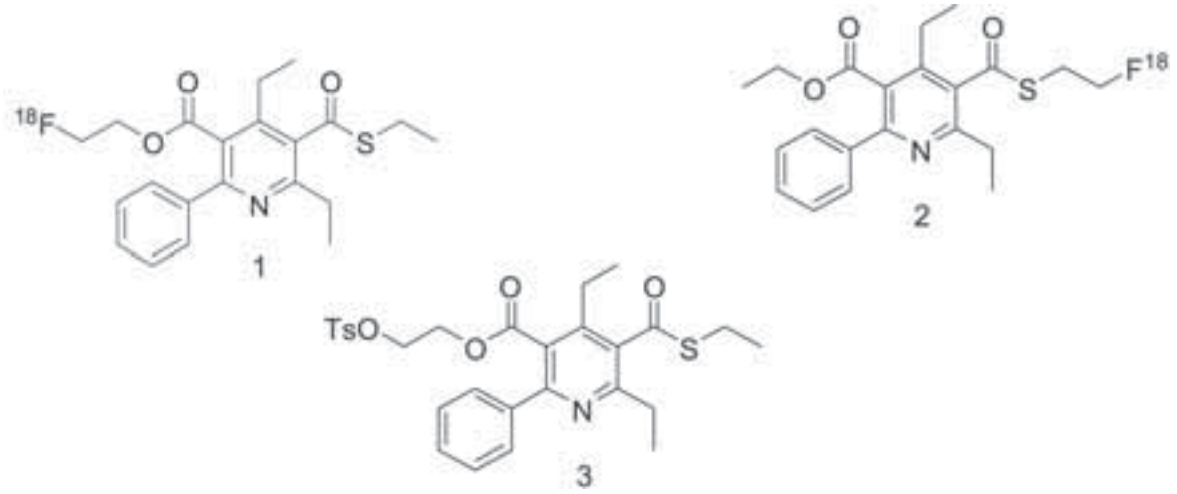

PO132

# Theoretical studies on the interaction of partial agonists with the 5-$HT_{2A}$ receptor

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SAR studies [1, 2] have recently identified chiral und achiral ligands of 5-$HT_{2A}$ receptors as partial agonists with much higher activity than common 5-$HT_{2A}$ agonistic primary amines like serotonin or mescaline. The data of about 50 derivatives, $pEC_{50}$ values and intrinsic activities ($E_{max}$) on rat arteries, allow a systematic exploration of the QSAR and the binding site, based on a hierarchical strategy using fragment regression analysis (FRA), receptor modeling, automatic docking and 3D-QSAR approaches. The compounds are primary and secondary arylethylamines ($R^N$ = H or substituted benzyl) from different structural classes (mainly indoles, methoxybenzenes and quinazolinediones), and show high variability of $pEC_{50}$ from 4 to 10 and of $E_{max}$ from 15 to 70%. Since $pEC_{50}$ values from *in vitro* organ assays are normally correlated with affinity, QSAR represent effects of structural variation on ligand-receptor interactions and even on receptor activation by application of an induced fit model (log K* based on $E_{max}$).

FRA indicates which substructures and substituents affect affinity or intrinsic activity. The large contribution of higher halogens in para position of phenethylamines to $pEC_{50}$ points to a specific hydrophobic binding site. Other results suggest the significance of hydrogen bonds of the aryl moiety for activation and the contrary effect of benzyl $R^N$ groups on affinity (increasing) and log K* (decreasing).

The results and suggestions from FRA and data on all available mutants have been considered for the derivation of putative binding sites at the human and the rat 5-$HT_{2A}$ receptor (h5$HT_{2A}$R, r5$HT_{2A}$R). The receptor models were generated from bovine rhodopsin by homology modeling using FUGUE. The compounds were placed into the r5$HT_{2A}$R binding site by automatic docking with SurflexDock. Analysis of the refined docking poses indicated common interaction patterns, e.g., with serine residues in TM3 and TM5 as well as with a cluster of aromatic amino acids in TM5 and TM6, and resulted in a binding-site based alignment of the whole series which is different to previous ligand based superpositions. This alignment was used for first 3D-QSAR analyses. CoMFA and CoMSiA of $pEC_{50}$ with steric, electrostatic, hydrophobic and H-bond fields in different combinations resulted in sufficient fit of the compounds ($q^2$ up to 0.82, $r^2$ up to 0.95). The important interaction regions largely reflect the patterns provided by the putative binding site.

With these results, a successive stepwise refinement of the binding site, of the alignment and of the 3D-QSAR should be possible, leading to more reliable receptor models, hypotheses on activation mechanisms and structure-based approaches like flexible docking, scoring and virtual screening.

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PO133

# De novo design and synthesis of Mur enzyme inhibitors

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*C. trachomatis* is the most common sexually transmitted pathogen in humans and can cause urethitis, cervicitis, pelvic inflammatory disease and infertility if left untreated. Increased emergence of pathogenic bacterial resistance to antibiotic therapy has created an urgent need for the development of new antibacterial agents directed towards novel targets, and in particular, the biosynthesis of the bacterial cell wall. Although much work has focussed on the later stages of this pathway, relatively little research has been done on the earlier steps, occurring in the cytoplasm. These steps could provide a wealth of potential drug targets, as this part of the biosynthetic route has no mammalian counterpart.

Biosynthesis of peptidoglycan is a two-stage process; the first of which involves the formation of the *N*-acetylglucosamine-*N*-acetylmuramyl pentapeptide. These steps are catalysed by a series of ATP-dependent amino acid synthetases, known as the Mur synthetases [1,2].

We have recently applied the *de novo* molecular design computer program SPROUT, developed at Leeds, to the rational design of inhibitors of *Chlamydia trachomatis* Mur ligases.

SPROUT *de novo* design requires the 3-D structure of the protein target. As the X-ray crystal structures for the chlamydial Mur synthetases are currently unavailable, predictive models of the structures of the chlamydial enzymes were produced from the known *Escherichia coli* crystal structures [3].

*De novo* design, using SPROUT has been accomplished for MurD and MurF. Synthesis of several classes of inhibitors has been achieved and biological evaluation of these is in progress.

In tandem to *de novo* design, VHTS for commercially available Mur ligase inhibitors has been completed using eHiTS [4]. This has identified a range of potential inhibitors for the Mur enzymes –B to –F. Biological evaluation of these compounds is underway.

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PO134

# New nonsteroidal inhibitors of fungal 17β-hydroxysteroid dehydrogenase based on 1,5-benzodiazepine scaffold

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17β-Hydroxysteroid dehydrogenases (17β-HSDs) play a key role in modulation of biological potencies of human sex hormones. Therefore, they represent emerging therapeutic targets for the control of estrogen- and androgen-dependent diseases, such as breast, endometrial and prostate cancer, disorders of reproduction and neuronal diseases. To date, 14 different mammalian 17β-HSDs have been described that belong to protein superfamilies of short-chain dehydrogenases/reductases (SDR) or aldo-keto reductases (AKR). In order to avoid residual steroidogenic activity, we became interested in developing inhibitors of 17β-HSDs consisting of a nonsteroid core. So far, flavonoids and some other nonsteroid compounds such as 1,4-benzodiazepines were demonstrated to inhibit the HSDs from AKR and SDR superfamilies. In our previous reports [1,2] cinnamic acids derivatives showed inhibitory activity on 17β-HSD from the fungus *Cochliobolus lunatus* (17β-HSDcl), a model enzyme of the SDR superfamily. Based on 1,4-benzodiazepines, which are potent inhibitors of the AKR1C1-AKR1C4 HSDs from the AKR superfamily [3], we designed and synthesized new nonsteroidal inhibitors with 1,5-benzodiazepine core. Inhibitory potential of all compounds was evaluated on 17β-HSDcl in oxidative and reductive direction. Many compounds were found to be potent inhibitors with $IC_{50}$ values in low micromolar range (Figure 1) [4].

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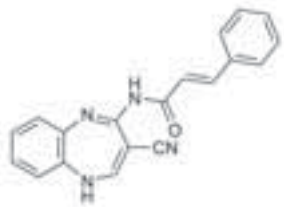
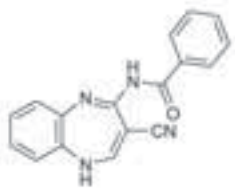


$IC_{50}$ (oxidation) = 3 μM
% inhibition @ 25 μM = 50%



$IC_{50}$ (oxidation) = 4 μM
$IC_{50}$ (reduction) = 10 μM

**Figure 1:** Structures and inhibitory activities of two best nonsteroidal inhibitors of 17β-HSDcl

PO135

# Isothiazolopyridines and their *in vitro* antibacterial evaluation

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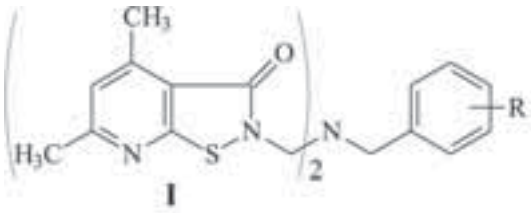
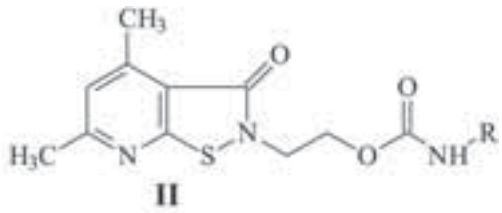



**I**

R = H, o-Cl, p-Cl,o-$OCH_3$, p-$OCH_3$, o,p-$CH_3$, 3,4-O-$CH_2$-O-




**II**

R = $C_2H_5$, *n*-$C_4H_9$, $C(CH_3)_3$, $C_6H_5$

A series of specific isothiazolopyridnies of Mannich base type possessing two isothiazolopyridine fragments at a single molecule (**I**) and carbamates of 2-hydroxyethylisothiazolopyridine (**II**) were prepared and tested *in vitro* in a microbiological evaluation at our laboratories.

The structures of the heterocycles **I, II** were assigned on the bases of IR and $^1$H NMR data. All new compounds **I**, **II** were tested against *Mycobacterium fortuitum* and *Propionibacterium acnes*. Activity against *M. fortuitum* correlated closely with that against *M. tuberculosis* and may be used as a measure of anti-*Mycobacterium tuberculosis* activity because of the potential hazards of using *M. tuberculosis* [1].

In microbiological evaluation against *M. fortuitum*, only Mannich base **II** (R=*o*-$OCH_3$) demonstrated activity at the $MIC_{50}$ and $MIC_{90}$ levels. Reduction of the microorganism was observed even at a submicrogram (μg/ml) concentration and this compound will be the object of further biochemical investigation. The activities of the remaining compounds against this strain were generally poor, with $MIC_{50}$≥125 μg/ml, or their limited solubility prohibited accurate determination.

Most of the compounds were found to be inactive against *Propionibacterium acnes*. Among eleven derivatives of isothiazolopyridine only two compounds [**II**: R=$C_2H_5$ ($MIC_{50}$ 6.9 μg/ml) and **I**: R= *o*-$OCH_3$ ($MIC_{50}$ 38 μg/ml)] possessed antibacterial action. However, to our complete surprise, five of the compounds tested helped stimulate the growth of *Propionibacterium acnes* at a concentration 1-15.6 μg/ml in the range of 10-50%. It is unclear if the observed stimulation was a consequence of enhanced bacterial replication by the preparations or by a lack of activity of the compounds, which allowed the natural growth of the microorganisms.

On the basis of these data it may be concluded that the Mannich bases **I**, except for R=*o*-$OCH_3$, and carbamates **II** do not seem to offer any specific antibacterial groups against *Mycobacterium fortuitum*(*tuberculosis*) and *Propionibacterium acnes*

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PO136

# Synthesis and biological investigation of new 1,2-diaryl heterocyclic compounds as anti-inflammatory agents

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Selective cyclooxygenase-2 (COX-2) enzyme inhibitors have a more advantageous gastrointestinal side effect profile than the traditional nonsteroidal anti-inflammatory drugs, however, extensive use of COX-2 inhibitors is likely to demonstrate gastrointestinal adverse effects similar to those caused by traditional NSAIDs and these drugs - except aspirin - are accompanied with cardiovascular concerns in case of a long-term use [1]. Celecoxib and diclofenac have similar COX-2 selectivity [2], and maybe therefore celecoxib is not associated with an increased incidence of cardiovascular events [3], but it is desirable to reduce the gastrointestinal side effects.

Our aim was to synthesize celecoxib analogues, which are more active and have a better side effect profile than the parent compound.

According to the above aspects 20 new celecoxib analogues have been synthesized. Among these compounds 2-[4-(5-*p*-tolyl-3-trifluoromethyl-pyrazole-1-yl)benzenesulfonylaminooxy]-propionic acid (1) displayed interesting pharmacological properties: it has no *in vitro* COX-1 and COX-2 inhibitory activity, it is more effective analgetic and anti-inflammatory agent than celecoxib, and its gastrointestinal side effect profile is essentially more favourable than that of celecoxib. The disodium salt of **1** is soluble in water, and this new chemical entity was identified as a potential candidate both for oral and intravenous administration [4].

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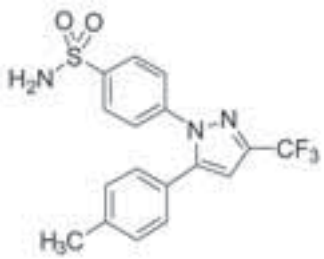
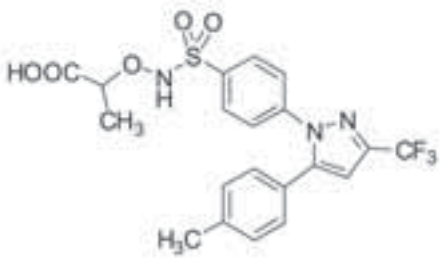


*celecoxib (Celebrex®)*



**1**

PO137

# Simple and efficient way of synthesis of sulfonylureas as potential antibacterial agents

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Bacterial resistance to antibiotic therapy has become a global health threat. To fight against this problem, new antibacterial compounds have to be discovered. Very well known and recognized as a potential targets for the antibacterial therapy are the enzymes that catalyze building of the peptidoglycan in bacteria. UDP-*N*-acetylmuramoyl: L-alanine ligase (MurC) and UDP-*N*-acetylmuramyl-L-alanine: D-glutamate ligase (MurD) catalyze two steps of the biosynthesis UDP-*N*- acetylmuramyl pentapeptide, which is an essential bacterial peptidoglycan precursor. Specially enzyme MurD is very appropriate as a target since it has no human counterpart. There are not many known good inhibitors of enzymes MurD and MurC, other than phosphinate inhibitors and none is used in antibacterial therapy. [1]

We synthesized series of sulphonylureas starting from various aminoacids. As aminoacid part we used glycine, L-alanine, D-glutamic acid, D-alanine and L-phenylalanine. Synthesis of compounds is shown in figure 1. [2, 3]

The compounds will be tested on MurC and MurD.

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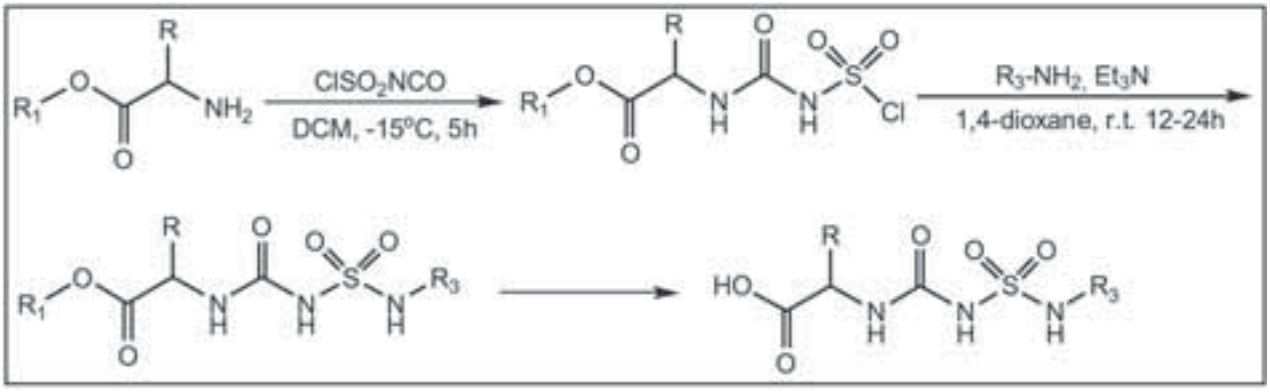


Figure 1: Synthesis of sulphonylureas

PO138

# Refinement of structure-activity relationships of the triazinobenzimidazole $A_1$AR antagonists.

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[a]*Dipartimento di Scienze Farmaceutiche,*
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Adenosine is probably the most important neuromodulator in the central and peripheral nervous systems, exerting many biological functions by activation of specific membrane G-protein coupled receptors (ARs), currently classified into $A_1$, $A_{2A}$, $A_{2B}$ and $A_3$ subtypes. Selective $A_1$AR antagonists have demonstrated promising therapeutic potential for the treatment of cognitive diseases, renal failure, Alzheimer's disease, and cardiac failure. We have disclosed a series of 3-aryltriazinobenzimidazoles (ATBIs) **I** as a novel class of $A_1$AR antagonists ($K_i$ values in the low nanomolar range), highly selective over $A_{2A}$ and $A_3$ARs.[1,2] Pharmacophore-based modelling studies suggested that three hydrogen bonding sites (HB1 acceptor, HB2 and HB3 donors) and three lipophilic pockets (L1, L2, and L3) might be available to antagonists within the $A_1$AR binding cleft. According to this pharmacophore scheme, our derivatives receive a hydrogen bond (via the N1 nitrogen) from the HB2 site and fill the L1, L2, and L3 liphophilic pockets with the 10-phenyl, 3-phenyl, and fused benzene rings, respectively. To probe the topology of the receptor lipophilic areas L1 and L3, in the present work we synthesized a new series of ATBIs **II**, featuring modified substituents at the 10-position, and a chlorine atom at the 7-position. Synthesis, biological evaluation and structure-activity relationships for these new derivatives **II** will be discussed.

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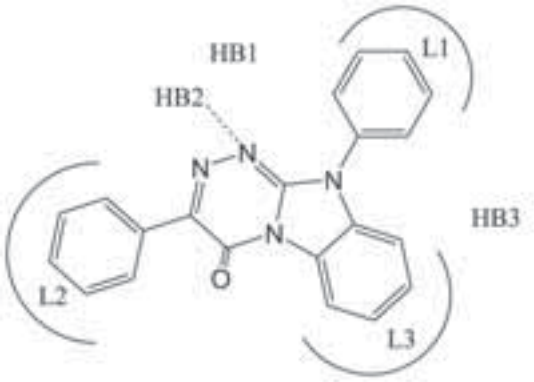
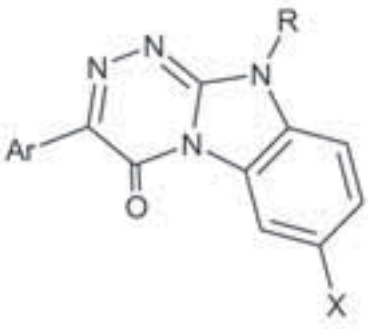



ATBIs **I**




ATBIs **II**

PO139

# Isomeric N,N-dicyclohexane-4-ol-amine aryl esters: The discovery of a new class of highly potent and efficaceous Pgp-dependant MDR inhibitors

**Elisabetta Teodori[a],** Cecilia Martelli[a], Milena Salerno[b], Nacira Darghal[b], Silvia Dei[a], Fulvio Gualtieri[a], Dina Manetti[a], Serena Scapecchi[a], Maria Novella Romanelli[a]

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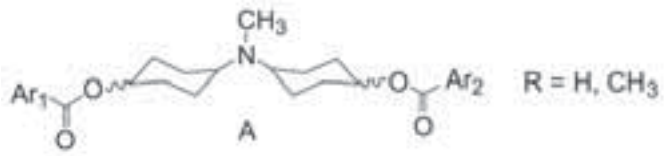
Multidrug resistance (MDR) is a kind of acquired drug resistance of cancer cells and microorganisms to a variety of chemotherapic drugs that usually are structurally and mechanistically unrelated [1]. Multidrug resistance may originate from several biochemical mechanisms; classical MDR is due to a lower cell concentration of cytotoxic drugs associated with accelerated efflux of the chemotherapic, as a consequence of the over expression of proteins such as Pgp and MRP1 [2] that act as extrusion pumps using ATP as energy source.

Direct information on the structure of Pgp and MRP1 are scarce [3] but all information collected so far point to the existence of a large, polymorphous drug recognition domain, where a variety of molecules can be accommodated in a plurality of binding modes [4].

Inhibition of the functions of Pgp and sister proteins, is considered a suitable approach to circumvent MDR and drugs possessing inhibitory properties have been and are actively sought [5,6], even if, so far, no drug has been approved for therapy.




Based on the present knowledge of the recognition site of Pgp [6], and as a follow up of our previous research [7], we have designed a new series of molecules with a N,N-dicyclohexylamine scaffold (general structure A) where asymmetrically substituted compounds give origin to four geometrical isomers of quite different shape [8]. Now we report an extension of structure-activity relationships and of pharmacological studies in this series of compounds.

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PO140

# New amphotericin B derivatives: Synthesis, antifungal and haemolitic activity.

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Despite the development of newer antifungal drugs, polyene antifungals continue to be the most potent broad-spectrum antifungals available for the clinical use. Amphotericin B (AmB, **1**) is the drug of choice for the treatment of mycotic infections caused by a wide range of fungi. Clinical use of this drug is continuously growing as a result of the increasing incidents of life-threatening fungal infections, particularly in cancer patients, patients who have undergo organ transplantation and patients with AIDS. However, AmB therapy is limited by considerable toxicity (nephrotoxicity, central nervous system and liver damage, side effects) and very poor water solubility. Earlier AmB modification was widely studied and several AmB derivatives with improved characteristics were described [1, 2], though no semi-synthetic polyene antibiotics were introduced to the clinic. We report now a new group of derivatives of polyene macrolides exhibiting improved water solubility and lower hematotoxicity comparing with AmB, and retaining the antifungal activity of the parent antibiotic (Fig. 1).

Reaction of AmB with the hydrochlorides of different amines in DMSO in the presence of condensing reagent led to corresponding amides of AmB (**2**), including amides with hydrophobic or hydrophilic moiety or aminoacid residue. Modification of the aminogroup of AmB was carried out by the reductive alkylation reaction (**3**, **4**), acylation (**5**) or Amadori rearrangement (**4**). Investigations of the antifungal and haemolytic activity of the new derivatives allowed concluding some structure-activity relationships for this class of antifungal antibiotics.

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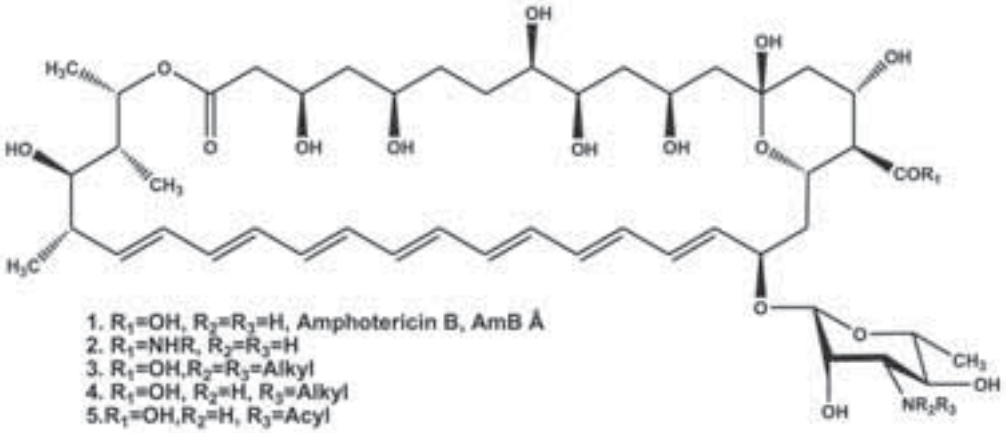

PO141

# Synthesis of achiral analogue of J-113397, a potent and selective NOP receptor antagonist

**Claudio Trapella**[a], Remo Guerrini[a], Laura Piccagli[a], Girolamo Calo'[b], Barbara Spagnolo[b], Giulia Fanton[a], Domenico Regoli[b], and Severo Salvadori[a]

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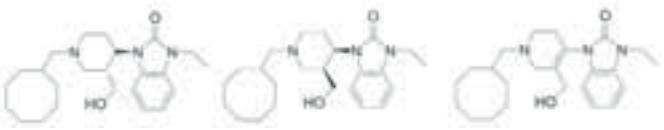
The N/OFQ-NOP receptor system has been shown to be involved in the regulation of a variety of central and peripheral functions [1] The first non-peptide NOP antagonist was J-113397 (chart 1), which was shown to bind with nanomolar affinity to NOP receptors and to display 100-300 fold selectivity over classical opioid receptors.
The present study was performed in an attempt to identify J-113397 derivatives with a similar pharmacological profile but obtainable with shorter/high-yelding synthetic procedures. We first considered the influence of the chirality at C3-C4 carbon atoms of the piperidine ring system (Chart 1), and proposed to simplify the molecule removing the two chiral centers through the introduction of a C3-C4 double bond. Interestingly, the derived compound, coded as Trap101 (Chart 1), displayed a pharmacological activity similar to that of (±)J-113397 and could be used as a novel template for a structure-activity study[2].
**Chart 1.** Structure of the compounds selected for molecular modeling studies.



J-113397 compound **1** Trap-101

Secondly we made an effort for exploring the conformational features of J-113397, compound 1 and Trap-101 (Chart 1). Since the absolute configuration of the active enantiomer (3R,4R)J-113397 was determined by X-ray crystallography [3] (CCDC 151492), we used this information as a starting point for further conformational analyses performed on the three compounds. (Figure 1)

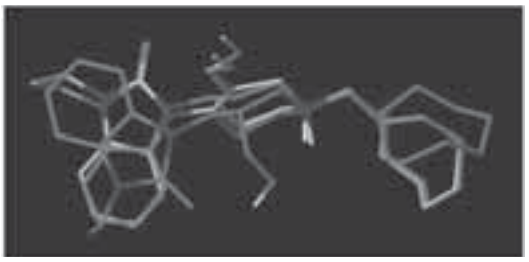


**Figure 1** Molecular modelling of J-113397, compound 1 and Trap-101

Trap-101 represents the first example of a NOP receptor ligand characterized by a 1,2,3,6-tetrahydro-pyridine nucleus, it shows a pharmacological profile very close to that of J-113397, however the preparation of Trap-101 is much easier than that of J-113397 and the yield much higher.

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PO142

# Selectively protected triaminobutane, synthesis and applications

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We present a method of preparation of $N^4$-acyl $N^4$-alkyl derivatives of (2*S*) and (2*R*)-1,2,4-triaminobutane [1,2] from (2*S*)-$N^4$-benzyloxycarbonyl-2,4-diaminobutanoic acid (1) as a result of multi step synthesis [3].

The substrate (1) was converted into selectively protected (2*S*)- or (2*R*)-$N^4$-benzyloxycarbonyl-1,2,4-triaminobutane (2). Then the free amino groups in the compound (2) were blocked with *tert*-butoxycarbonyl group followed by removal of benzyloxycarbonyl group to obtain compound (3). The product (3) was used as a building block for further acylation and alkylation reactions. The final cleavage of tert-butoxycarbonyl protecting groups furnished the target compounds (5a - 5c), that may be directly used for the preparation of heterocyclic compounds.

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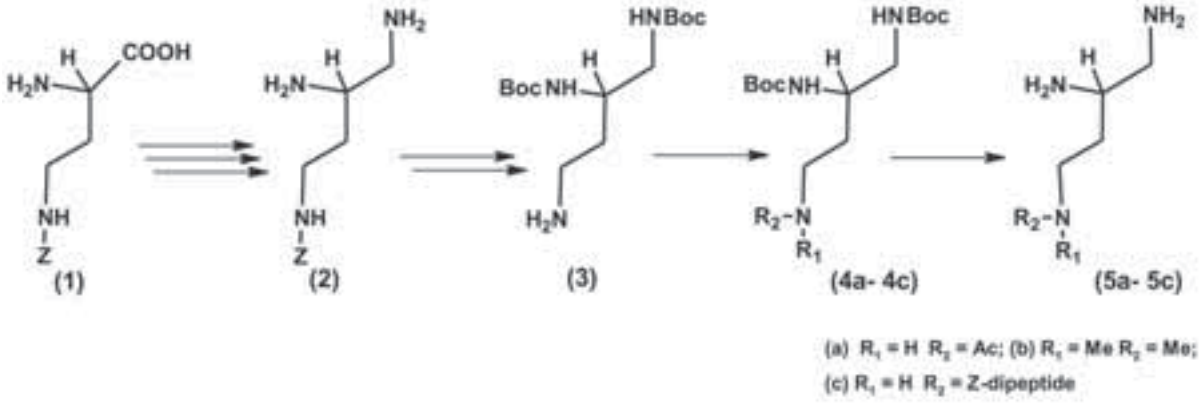

PO143

# A QSAR study on PPAR-γ agonists using multivariate data analysis

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Peroxisome proliferator-activated receptor gamma (PPAR- ) is a nuclear receptor and transcription factor that plays a crucial role in the regulation of lipid and glucose metabolism. Currently it offers a molecular target mainly for drugs developed for the treatment of type II Diabetes Mellitus. A large number of synthetic ligands with potency to bind to and activate the receptor have been reported. They belong to two major chemical classes, thiazolidinediones (TZDs) and tyrosine-based (TB) derivatives. Some QSAR studies have appeared in literature concerning mostly CoMFA and CoMSIA 3-D approaches or multiple linear regression analysis [1]. In the present study we applied Principal Component Analysis (PCA) and Projection to Latent Structures (PLS) in the aim to derive predictive models on the basis of 2-D and 3-D descriptors which refer to easily interpretable molecular properties and energy parameters. Binding data, expressed as pKi, and gene transactivation data, expressed as pEC50, were collected from literature for a large number of compounds containing mostly TB derivatives, classical TZDs, TZD-fatty acid hybrids and some natural fatty acids. The different compound classes and certain subclasses within them were distinctly discriminated in the score plot of the overall PCA model. An adequate 2 component PLS model could be derived for binding affinity ($r^2$=0.81, $q^2$=0.78) after exclusion of a limited number of compounds with special structural characteristics. The model was successfully validated by permutation test through randomly reordering response data and the use of a test set. The influence of individual molecular properties on activity was also evaluated. Characteristically, binding was found to increase with increase in volume parameters, while lipophilicity, polarity, flexibility and the degree of ionization expressed as fraction of negative charged species, although important for binding did not present a regular pattern. Energy parameters ($E_{HOMO}$, $E_{LUMO}$) were also found to exert considerable influence to binding. With gene transactivation data inferior models were derived for the whole data set. Adequate models were obtained for individual chemical classes or for the highly active compounds if considered separately. In those models structural specific descriptors were found to be more important than overall molecular parameters. These results may reflect the higher complexity incorporated in gene transactivation data.

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PO144

# Antioxidant and free radical scavenging activity of selenated extracts of lentinula edodes

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Many species of mushroom have been found to be highly potent immune system enhancers. *Lentinula edodes* ( Shii-take mushroom) is one of medicinal mushrooms from which extracted highly purified polysaccharide fraction (*Lentinan*) is an approved drug, used in cancer treatment as well as in AIDS research [1]. Hot water extracts from *L. edodes* mycelium and culture media (LEM, LAP) both demonstrate strong antitumor activity [2].

Our previous works show, that submerged cultivated mycelium of *Lentinula edodes* very effective accumulates selenium from cultivation medium in form of the high bioavailable selenoamino acids [3]. Selenium is a constituent of certain selenoproteins of important enzymatic function: selenium-dependent enzymes act as antioxidants. The other important selenium health effect, not exclusively linked to enzymatic functions is connected with functioning of the immune system. It appears to be a key nutrient in cancer prevention and inhibiting HIV progression to AIDS. We hope, that high concentration of organic forms of selenium in submerged cultivated mycelial biomass would enhance anticancer and inhibiting HIV progression activity of mushroom extracts. Our objective of present research was to evaluate the antioxidant properties( DETBA and methods) [4, 5], reducing power (modified Oyaizu method) [6] and scavenging effects on free DPPH radicals [7] of water and methanolic extracts of selenated and non-selenated *L. edodes* mycelium. The content of total selenium and selenated polysaccharides and amino acids in tested extracts was also determined. In all tests non-selenated extracts in concentration of 5-20 mg/ml show moderate to high antioxidant activity, good reducing power and very good scavenging effects on DPPH radicals. Whereas water and methanol extracts of the mycelium cultivated in medium containing 10 μg/ml of selenium expressed excellent scavenging effects ( over 98%) and – in concentration of 10 mg/ml - antioxidant activity and reducing power comparable with that of BHT and BHA. The selenium content in tested extracts, determined by atomic absorbtion spectrometry, was low and equaled for water extract … and for methanolic extract …. . Nevertheless selenium-containing compounds present in extracts enhanced antioxidant and reducing power and free radical scavenging effect for almost100%. Extracts of the selenated mycelium of Lentinula edodes are actually prepared for tests for antitumor activity.

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PO145

# The use of novel continuous-flow reactors to facilitate faster and safer medicinal chemistry

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Organic chemistry and especially combinatorial and high throughput chemistry in the general laboratories are performed in a narrow parameter space, because the "Synthesis Feasibility Space" – the vectorially arranged set of theoretically synthesizable organic compounds-together with the reactions are limited when applying conventional synthetic methods. Eg. reactions that have safety limitations, or require high pressure, temperature were not suitable for combinatorial synthesis earlier. By applying enabling microfludics based techniques, and utilizing members of the Cube™ reactor family such as H-Cube™, X-Cube™ and O-Cube™, we have made difficult chemistries accessible to medicinal chemists.

The Cube reactors mentioned above are bench-top continuous-flow reactors which employ a continuous stream of substrate passed through a catalyst cartridge system (CatCart™),a stainless steel tube, filled with heterogeneous or immobilized homogeneous reagents/catalyst, and reacted, in the case of the X-Cube, at temperatures and pressures of up to 200°C and 150 bar respectively. The H-Cube[1,2] and O-Cube employ electrolysis of water to generate hydrogen and ozone gas respectively in a safe and controlled manner without the need for gas cylinders.

The poster presentation will provide a general overview of utilizing the micro-flow reactors in overcoming difficult processes involved in the synthesis of drug targets in medicinal chemistry. Focus will be on enantio- and chemoselective synthesis, catalytic hydrogenation, other triphasic reactions, such as carbonylation, reactions under supercritical conditions and the notoriously dangerous ozonolysis.

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PO146

# New imidazolidin-4-ones based on dipeptide derivatives of primaquine as potential prodrugs for antimalarial therapy

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In the last few years our research group has been working on imidazolidin-4-ones based on amino acid derivatives of the antimalarial drug primaquine (PQ,I). These compounds were active against *Plasmodium berghei* activity *in vivo* and thus may constitute a new sub-class of 8-aminoquinolines useful for malaria therapy. [1-3]

We have recently introduced the imidazolidin-4-one scaffold on dipeptide derivatives of PQ (II), at either the *N*-terminal primary amino group (III) or embedded between both the amidic nitrogens (IV) [4]. The reactivity of the target compounds was evaluated in isotonic buffer at physiological pH and temperature, and compounds III were seen to rapidly release the parent drug whereas compounds IV were highly stable. Biological assays on the activity of these compounds against *P. berghei* are now under course and their prospective application as prodrugs (III) or drugs (IV) for antimalarial therapy will be discussed.

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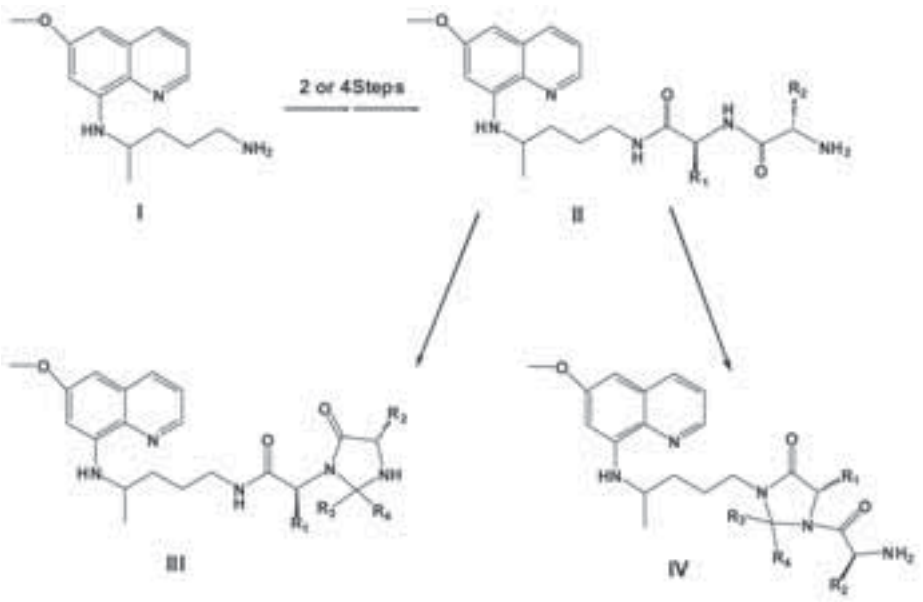

PO147

# Synthesis and biological properties of novel arylacetamido-arylacrilate as histone deacetylase inhibitors (HDACi)

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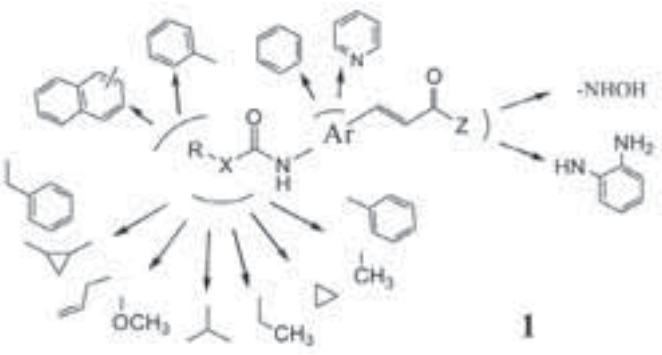
Chromatin remodelling is one of the most important mechanism in the epigenetic regulation of gene expression [1]. Histone deacetylases (HDACs) are highly involved in chromatin remodelling, thus being an important target for cancer terapy. The use of HDAC inhibitors (HDACi) activate the transcription of a small set of genes regulating cell proliferation and cell cycle progression, bearing growth arrest, apoptosis and/or differentiation in tumor cells.

Starting from the pharmacophore model for HDACi design, we have recently reported a novel class of hydroxamates as HDACi active at nanomolar range [2]. Although hydroxamates, such as the recently FDA-approved suberoylanilide hydroxamic acid (SAHA) [3], are well known to provide the most effective HDAC inhibition, and furnished the best values of apoptosis in tumor cells, the limited *in vivo* efficacy due to their metabolically labile nature prompted us to look for alternative zinc binding groups. In particular, the 2'-aminoanilides such as MS-275 [4] show cytodifferentiating effect on tumor cells. So, we prepared a new series of HDACi based on the (4-(2-arylacetamido)arylacrylate scaffold, belonging to both the hydroxamate and 2'-aminoanilide family (compounds **1**).




Compounds 1 exhibited high efficacy in enzymatic assays and displayed very promising cytodifferentiating (2'-aminoanilides) and pro-apoptotic (hydroxamates) properties in human leukemia U937 cell line.

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PO148

# Novel synthetic histone/protein methyltransferase inhibitors

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[b]*University of Texas M.D. Anderson Cancer Center, Science Park-Research Division, Smithville, Texas 78957, USA;*
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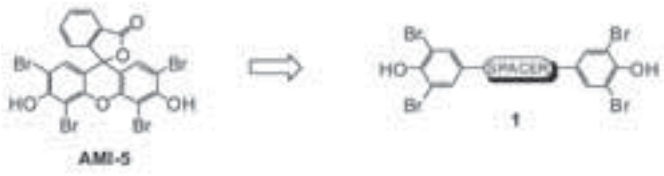
Histones can be modified in many ways (acetylation, phosphorylation, ubiquitination, methylation) affecting gene expression [1]. Evidence accumulated over the past few years suggests that such modifications constitute a "histone code" that directs a variety of chromatin-involving processes. There are currently many known sites of lysine and arginine methylation on histones. Depending on the context, lysine methylation provides either activating or repressing modification. Most lysine methyltransferases (HKMTs) are characterized by a conserved SET domain and certain SET domain proteins are under abnormal control in tumors [2]. In addition to their role in histone modification, protein arginine methyltransferases (PRMTs), such as PRMT1 and CARM1, target several proteins involved in signal transduction, mRNA splicing, RNA transport, and protein-protein interactions [3].
In 2004, a series of dyes and dye-like compounds were evaluated as small molecule modulators of PRMT and HKMT activity. In this screen, AMI-5 was one of the most potent, though less selective, compound [4]. Following our medicinal chemistry effort aimed to discover new small molecule epigenetic modulators [5], we chose the AMI-5 chemical structure as a template and designed a new series of simplified analogues starting from a pharmacophore hypothesis. We identified the presence of two *o*-bromo- or *o,o*-dibromophenol moieties as crucial for having anti-methyltransferase activity, and inserted a number of different hydrophobic spacers between the above fragments.




Such compounds were tested against fungal and human PRMT1, against human CARM1 and against the HKMT SET7. Selected compounds were tested on the human leukemia U937 cell line showing high cytodifferentiation effects.

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PO149

# Structural investigation, in vitro antitumor profile and aromatase inhibition effects of new triorganotin analogues of 4-OH-androstenedione (formestane©)

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**Introduction:** The interaction of tin with currently used drugs or steroids has brought up new data on the anticancer profile of organotins given that many of them have been found to act as endocrine disruptors while others as sex-steroid enzymes inhibitors, allowing for a wide discussion on their ability to act as aromatase inhibitors and therefore as their potential to be applied for treatment in endocrine cancers.
**Aim:** We present herein, preliminary results on the structure, antitumor profile and aromatase inhibition effects of the trimethyl and triphenyltin analogues of 4-hydroxy-androst-4-ene-3,17-dione (Formestane©), which is a synthetic steroidal aromatase inhibitor.
**Experimental:** The complexes are prepared by reacting the ligand with the corresponding triorganotin hydroxide. The antitumor profile is determined by measuring the % cytotoxicity against human tumor and normal cell lines, using the SRB assay (NIH screening protocol). The aromatase inhibition effect is studied via ELISA techniques.
**Results-Discussion:** The spectroscopic analysis provides evidence on the formation of a chelate ring, responsible for the stabilization of the triorgano-tin cation with the Sn central atom in a five-coordinated environment exhibiting distorted trigonal bipyramidal geometry. The new complexes exhibit remarkable cytotoxicity against all cell lines tested. The aromatase inhibition is comparable to that of free 4-OH-A. The structure-activity relationships will be discussed.

PO150

# Stable helical b3-peptides in water via side-chain cyclization

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β-Peptides have been regarded as suitable foldamers for modulating protein-protein interactions. They fold in a varied set of secondary structures in a range of solvents (helices, sheets and turns) and, additionally, they are resistant to degradation by peptidases, in contrast to natural α-peptides [1-3].

Due to its similarities in side chain positioning with certain biologically active helical α-peptides, the 14-helix of $\beta^3$-peptides has been successfully explored as a modulator of protein-protein interactions [4-6].


Overlay of a α-helix (blue) and a 14-helix (cyan) from a $\beta^3$-peptide. (A) side view, (B) top view.

In order to stabilize the 14-helical secondary structure of β-peptides in water we have introduced a rigidification element on the backbone via cyclization through side chain to side chain lactam bridges. An on-bead cyclization protocol of $\beta^3$-peptides was developed, providing easy access to cyclic $\beta^3$-peptides. The optimal positioning and length of the constraining lactam bridge were studied using a library of foldamers synthesized on the solid-phase.

The effect on the stabilisation of the 14-helix was investigated with CD spectroscopy, showing that covalent bridging of two side chains in $\beta^3$-peptides significantly stabilized their helical conformation in aqueous solutions; and turned out to be superior to the previously described electrostatic interactions [7].

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PO151

# Multiple ligands applied to dopamine and adenosine receptors

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The application of a multiple ligand approach is a new trend in medicinal chemistry and in some cases has superseded the traditional "one target-one disease" philosophy [1]. This is especially true for disorders in which the alteration of a single receptor is therapeutically insufficient. A relevant example is Parkinson's disease (PD), where patients receiving traditional treatment based on single dopamine interactions, obtain only partial or transient benefits at best [2]. However, balanced modulation of dopamine and adenosine receptors showed promising efficacy and fewer side effects than single-target treatments [3]. In the present work, we report the synthesis and biological evaluation of novel compounds with the capacity to interact at both adenosine and dopamine receptors. The combination of different privileged heterocycles, such as ergolenes [4] and indolo[2,3-a]quinolizidines, with several tripeptide libraries has resulted in heterocycle-peptide hybrids (Figure 1) with dual activity at both receptors. Structure-activity relationships, binding mode characteristics and pharmacological applications for these new molecules will be discussed in depth.

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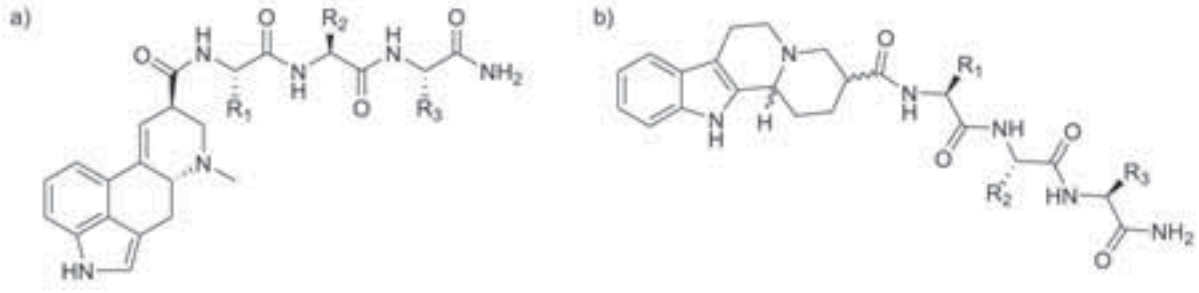


Figure 1. Heterocycle-peptide hybrids based on: a) ergolenes, b) indolo[2,3-a]quinolizidines.

PO152

# Synthesis and biological activity of new antiviral compounds

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HIV (Human Immunodeficiency Virus) is the virus that causes AIDS (Acquired Immune Deficiency Syndrome). Approximately 39.5 million individuals worldwide were living in 2006 with HIV, 2.6 million more than in 2004 [1]. The advent of Highly Active Anti-Retroviral Therapy (HAART), a combination cocktail of inhibitors of HIV reverse transcriptase and protease, has provided an effective means of controlling viral load and disease progression in HIV-infected individuals [2]. However, the global epidemic of infection by HIV-1, the emergence of drug-resistant HIV strains, a significant pill burden and adverse side effects are problems that require the development of new classes of anti-retroviral agents that interfere with different targets in the HIV life cycle. The viral entry process can provides new anti-HIV-1 targets for a novel class of drugs called "*entry inhibitors*" [3, 4].

BMS-378806 (**1**) [5, 6] developed by Bristol-Myers Squibb is a promising entry inhibitors compound in clinical trials [4]. In this context we have synthesized analogs of compound **1** introducing different changes in the chemical structure instead of the methyl-piperazine group.
The new compounds of general formula **2** were tested at the Department of Biochemistry, School of Medicine, of the University of Utah, Salt Lake City (USA).

The chemistry and the biological data will be discussed in the poster.

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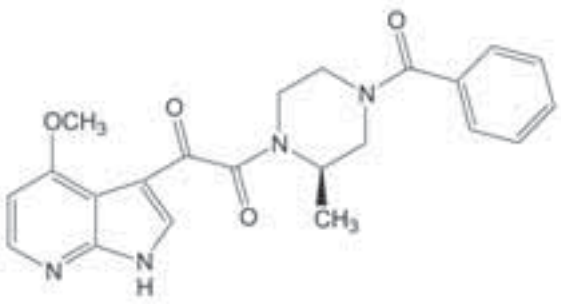


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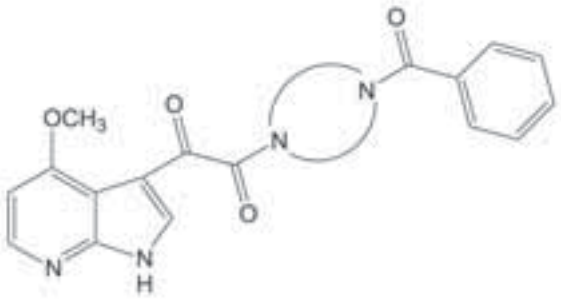


**2**

PO153

# Preparation of new hydrazonoformamide derivatives of isoniazid

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Tuberculosis remains in the beginning of 21st century the worlds leading infectious diseases caused by a single infectious *Mycobacterium tuberculosis* bacteria. According to alarming data from World Health Organisation, one-third of the world's population is currently infected; more than 5000 people die of this infection every day. Problems in the chemotherapy rise with amplificatory bacterial resistance.

In the quest for biologically more potent anti-tuberculosis compounds we have synthesised several derivatives where either isoniazid (INH) or pyrazinamide (PZA) were linked with another conventional drug by the CH fragment [1]. Some of them had exhibited higher activity against non-tuberculous strains than INH or PZA. These results stimulated us for other modifications of the structure where INH is similarly linked with various aniline derivatives as demonstrated by compounds of type **1** (Scheme). The aniline partner was selected having in mind the most active INH-hydrazine and hydrazone derivatives [2]. Reactions of *N´*-isonicotinoylhydrazonoformamide with substituted anilines didn't lead to the desired outcome. As the formylation of anilines was also unsuccessful we activated anilines using diethoxymethyl acetate and then treated their products with INH.

The synthesis, general characteristics, physical and antimycobacterial activity evaluation will be presented.

The study was supported by MSM 0021620822, GAUK and Slovenian/Czech bilateral program Contact 06/2006-7 (BI-CZ/06-07-006).

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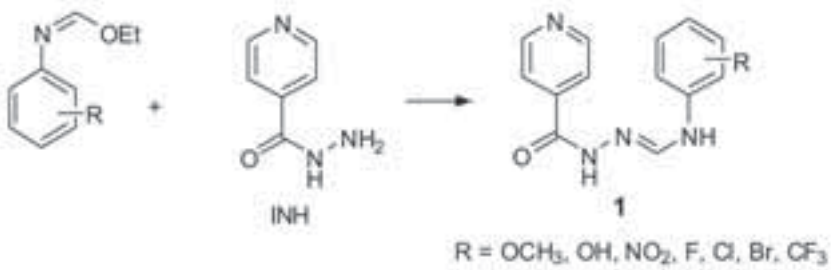
Scheme



PO154

# Synthesis and antifungal activity of new N-[1-aryl-2-(1H-imidazol-1-yl and 1H-1,2,4-triazol-1-yl)-1-ethylidene]-N'-2,4-dichloro-phenylhydrazine derivatives.

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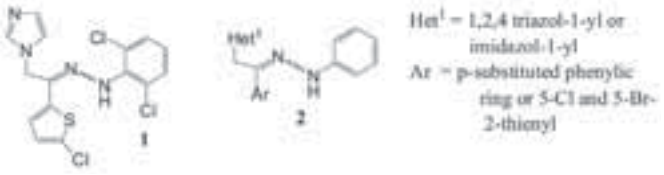
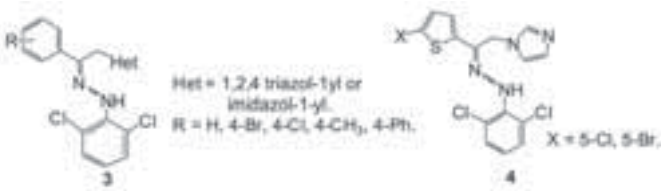
Considering the increased incidence of severe opportunistic fungal infections in immunocompromised patients together with the development of resistance among pathogenic *Candida spp.*, there is a great need for new antifungal compounds. On the other hand, the increase of tuberculosis due to emergence of multidrug-resistant strains (MDR) of *Mycobacterium tuberculosis*, together with the increased incidence of severe disseminated infections produced by mycobacteria other than tuberculosis (MOTT) in immunocompromised patients, have prompted the search for new antimycobacterial drugs.
Our search consists on the synthesis and *in vitro* evaluation of antifungal activity of new zinoconazole **1**[1] analogues. Therefore we synthesized N-[1-aryl-2-(1*H*-imidazol-1-yl and 1*H*-1,2,4-triazol-1-yl)-1-ethylidene]-N -phenylhydrazine derivatives **2** that showed a significant antifungal activity.




Trying to enhance the antifungal activity of the compounds **2**, we synthesized N-[1-aryl-2-(1*H*-imidazol-1-yl and 1*H*-1,2,4-triazol-1-yl)-1-ethylidene]-N -2,4-dichloro-phe-nylhydrazine derivatives **3, 4.**




All compounds will be tested to evaluate their *in vitro* antifungal and antimycobacterial activity.

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PO155

# 3-Piperidin-4-ylmethyl-3H-benzoxazol-2-one derivatives as σ ligands.

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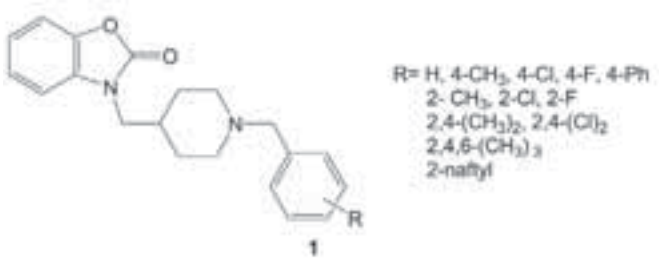
Sigma receptors are involved in several functions such as modulation and biosynthesis of several neurotransmitters, motor control, cell growth and proliferation [1]. The lack of any endogenous ligand and the existence of at least two sigma receptors subtypes $\sigma_1$ and $\sigma_2$ make it difficult to characterize their biological role. Several classes of structurally unrelated compounds interact with σ receptors, but only few σ ligands are gifted with affinity and selectivity against a specific receptor subtypes. The interest in σ ligands stems from the possibility to develop clinical agents for the treatment of several CNS diseases (affective and motor disorders, cocaine abuse, cognitive impairment), for neuroprotection, tumor treatment and diagnosis[2]. Moreover $\sigma_1$ receptor ligands could be involved in treatment of schizophrenia, depression, lack of memorization skill, difficulty of learning and for increase the action of analgesic drugs.
With the aim to obtain new σ selective ligands, we synthesized some 3-piperidin-4-ylmethyl-3*H*-benzoxazol-2-one derivatives **1**.




**1**

All synthesized compounds have been tested for their σ receptors affinity and demonstrated a good affinity and selectivity against $\sigma_1$ receptors. Specifically, the p-substituted derivatives resulted as the compounds with the greatest affinity and selectivity against $\sigma_1$ subtype receptor (R=4-Cl: $\sigma_1K_1$=0.098nM; $\sigma_2K_i/\sigma_1K_i$= 4357).

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PO156

# Aroylhydrazones of 2-phenylindole-3-carbaldehydes as novel antimitotic agents

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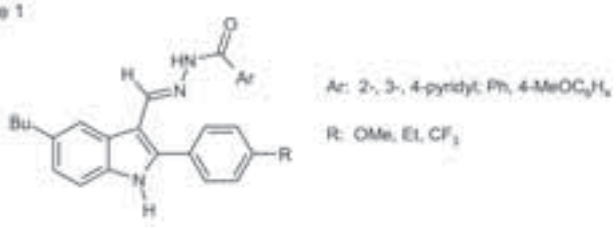
In a previous study [1] we showed that 2-phenylindole-3-carbaldehydes with lipophilic substituents in 5- and 4'-position strongly inhibit the growth of breast cancer cells *in vitro*. The lack of *in vivo* activity of these derivatives prompted us to modify the aldehyde function. The reaction with various hydrazides of (substituted) benzoic acid and pyridine carboxylic acids afforded the corresponding hydrazones (Fig. 1) which were shown to be stable to hydrolysis. They strongly inhibit the growth of human MDA-MB 231 and MCF-7 breast cancer cells with $IC_{50}$ values between 30 and 100 nM. Contrary to the starting aldehydes they do not interfere with tubulin polymerization. However, they block the cell cycle in $G_2$/M phase similar as vincristine does as demonstrated by flowcytometry. In these experiments, a substantial amount of debris (sub-$G_1$ peak) was detected probably due to apoptotic processes. This observation is in accord with preliminary data showing that caspases 3 and 9 are up-regulated by the hydrazones. Apoptosis can be considered the result of the cell cycle arrest in $G_2$/M phase and may be responsible for the antimitotic effect exerted by the 2-phenylindole-based hydrazones. However, the primary target of these agents remains to be elucidated.

Figure 1

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PO157

# (S)-1-benzyl-6-methylpiperazine-2,5-dione: a simple cyclodipeptide based chiral solvating agent

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Determination of enantiomeric excess (e.e.) of chiral compounds is a very important subject, especially in the area of medicinal chemistry. There are several different methods for e.e. determination, of which NMR method is one of the most common.

In our work, we wish to present a novel chiral solvating agent (CSA) for NMR use that we discovered in the synthesis of simple indole alkaloid analogues of *Dipodazine* [1, 2]. Our CSA **1** is structurally a cyclodipeptide of *N*-benzylated (*S*)-alanine and glycine. As such it possesses an H-bond donor group (position 4) and an H-bond acceptor group (position 5). Through these groups the CSA is able to form homointermolecular H-bonds and, what is of importance to us, heterointermolecular H-bonds with various chiral amides **2**. Through formation of these H-bonded associates between **1** and **2**, we were able to distinguish between both enantiomers of **2** in NMR spectra and also to determine the e.e. of **2** [3]. Use of **1** was also successfully implied in determining of enantiomeric purity of intermediate **3** in our synthesis of *Tryprostatin B* analogues.

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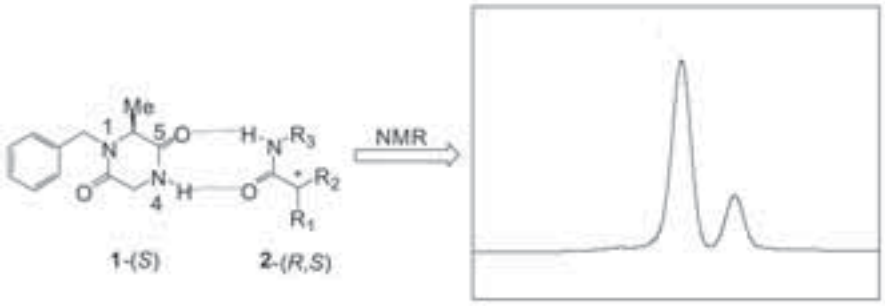


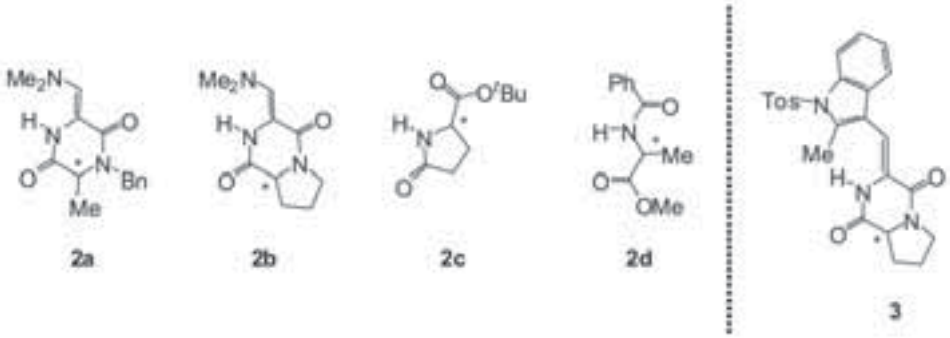

PO158

# Determination of butanetriols in glycerine by gas chromatography

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Glycerine added to diet supplements must satisfy certain rigorous requirements including that on the content of butanetriols. A method for determination of their concentration in glycerine based on gas chromatography was published in 1976 by JECFA. Butanetriols are separated on a packed column filled with Chromosorb W using the liquid stationary phase (polyoxyethylene-(8)-ethylenediamine) currently commercially unavailable. Moreover, the stationary phase proposed to be used is characterised by very low stability.

In view of the above a new method for butanetriols in glycerine determination has been proposed based on a commonly commercially available column. The column was HP-INOWAX (30m x 0.32mm x 0.5 m) characterised by a polarity close to that of the liquid stationary phase of polyoxyethylene-(8)-ethylenediamine.

Chromatographic analysis was made on a gas chromatograph made by Hewlett-Packard HP 5890 series II, equipped with a split-splitless injector and flame-ionisation detector (FID). The temperatures of the injector and detector were 320°C and 250°C, respectively, while the temperature programme of the column was 80°C 5°C/min 240°C. The carrier gas (helium) flow rate was 3.20cm$^3$/min. The split was fixed at 1/15. The internal standard used was 1,4 – butanediol. The method proposed was characterised by repeatability, detectability and determinability.

PO159

# Fluorescent studies on adenosine $A_{2A}$ receptors homodimerisation

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In recent times it has become obvious that adenosine $A_{2A}$ receptors exist as dimers or higher-order oligomers in living cells. Association of these receptors can involve homodimerization, in which multiple copies of the same receptor are shown to coexist [1], as well as heterodimerization with different members of the G protein-coupled receptors superfamily [2]. It seems that dimers are formed constitutively on the level of the early protein biosynthesis and delivered to the plasma membrane in this form [3]. Since dimers are pharmacologicaly working units, important implications for the way in which ligands are rationally designed come from the existance of these structures.

In our studies, we used Förster resonance energy transfer (FRET) technique, based on the non-radiative transfer of energy between adenosine $A_{2A}$ receptor proteins, tagged with donor and acceptor fluorophores. Human adenosine $A_{2A}$ receptor cDNA was inserted into pECFP-N1 and pYFP-N1 vectors, then transiently transfected into HEK293 cells. The functionality of expressed receptors was evaluated by radioligand binding assays with [$^3$H]CGS216880 to compare binding activities of wild-type and fluorescent-tagged receptors. Various independent fluorescent methods, like flow cytometry, fluorescence spectroscopy, laser confocal microscopy and fluorescence lifetime microscopy, were used in order to confirm constitutive homodimers formation.

Afterwards, the influence of ligands derivatives on adenosine $A_{2A}$ receptors complexes was measured by time-resolved FRET, which allows discrimination of the emitted fluorescence not only by wavelength and fluorescence intensity but also according to the lifetimes of the excited states of the fluorophores. Since fluorescence lifetime measurements are independent of any change in fluorophore concentration or excitation intensity, this kind of measurements provide quantitative information about interaction between labeled receptors of interest. Various antagonists, with predefined binding activities to adenosine $A_{2A}$ receptors were used in this research, in order to check influence of selective ligands on receptors dimerization state. Moreover, we developed dimeric compounds with antagonistic properties for adenosine $A_{2A}$ receptors, according to the concept of “bridging” of dimeric receptors by ligands containing two pharamcophores linked through a spacer of appropriate length [4].

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PO160

# Structural characterization of 3,3'-dithiobisindoles potential antitumoral agents

**Irena Wolska**[a], Maria Niemyjska[b], Dorota Maciejewska[b]

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The antiproliterative activity of bisindoles against human tumor cell lines is well known. Their mode of action is dependent on the substituents present at indole parts of molecules and kind of linker between these rings. It has been suggested that some of them induced apoptosis by changing the Bax/Bcl-2 ratio and can induce a G1 cell-cycle arrest [1], but another ones inhibit the tyrosine-kinase activity [2]. On the other hand, bisindoles could interact with DNA by classic minor groove binding *via* interactions within the AT-rich region [3]. Moreover, X-ray studies of bisindoles revealed its 'butterfly' conformation [4], which is analogous to proposed for inhibitors of HIV-1 reverse transcriptase, sharing mode of action of nevirapine [5]. On the basis of this knowledge, we have decided to obtain the compounds structurally related to the 3,3'-diindolylmethane, namely 3,3'-dithiobisindoles. In the poster we report the synthesis, cytotoxicity, $^{13}C$ CP/MAS NMR spectral data, as well as the results of X-ray analysis some of these dithiobisindoles. We compare their molecular organization in solid state with 3,3'-diindolylmethane taking into account that small variations of the chemical structure may modify the transmission of antiproliferative signals.

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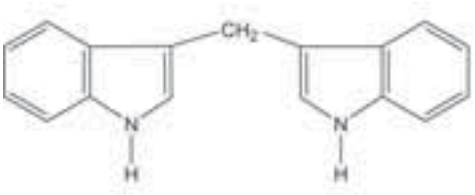
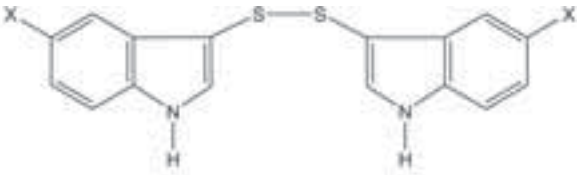



3,3'-diindolylmethane




X=H, $OCH_3$, F, J, Br
3,3'-dithiobisindoles

PO161

# Synthesis and biological activity of 3-[(tetrazol-5-yl)methyl]-4-substituted-1,2,4-triazoline-5-thione

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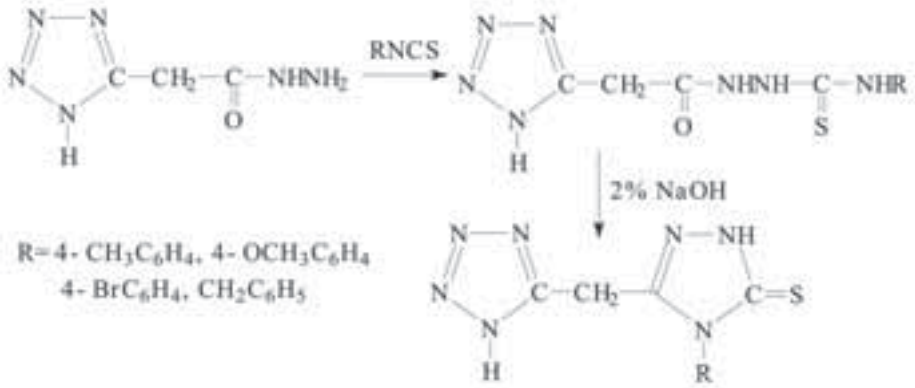
1,2,4-Triazole derivatives have been reported to associate with antimicrobial, fungicidal, anti-inflammatory, antiparastatic, insecticidal, herbicidal, antiviral, antitumor, anticonvulsant, antidepressant and hypotensive effects. The development of novel synthesis strategies for achieving compounds containing the 1,2,4-triazole system has been the subject of our research for the past few years.

In this work we have synthesized new derivatives of 1,2,4-triazole which have tetrazole system in position 3. Five compounds were screened for their in vitro activity against 22 of clinical or reference species of aerobic bacteria. Using the agar diffusion method, microbial suspensions (0.5 McFarland standard) were put onto Mueller-Hinton agar containing several concentrations of the tested compounds (31.25 - 500 mg $L^{-1}$). Estimation of MIC (Minimal Inhibitory Concentration) was performed by the Mueller-Hinton broth-dilution method only for the compound 1-[(tetrazol-5-yl)acetyl-4-bromophenyl-thiosemicarbazide showing promising antibacterial activity assessed by the agar dilution method. The optical density ($OD_{600}$) measurements were determined for bacterial culture in Mueller-Hinton broth containing from 0.49 to 500 mg $L^{-1}$ of the compound 1-[(tetrazol-5-yl)acetyl-4-bromophenyl-thiosemicarbazide.

It was found by the agar diffusion method that all tested compounds were not active against all Gram-negative and the majority of Gram-positive bacteria. However, the compound 1-[(tetrazol-5-yl)acetyl-4-bromophenyl-thiosemicarbazide was active against *Micrococcus luteus* ATCC 10240 with MIC value of 125 mg $L^{-1}$ and strongly inhibited the growth of this species at values ranging from 3.91 - 62.5 mg $L^{-1}$ (by about 80-90%).



PO162

# Detection of single-mismatch oligonucleotides on functionalized diamond surface by optical and electrochemical methods

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Diamond has a number of advantages for biomaterial detection, such as its wide potential window, simple functionalization, physiochemical stability, and biocompatibility. In addition, the diamond Solution-Gate Field-Effect Transistors (SGFETs) on the hydrogen-terminated (H-terminated) diamond surface, which includes a conductive layer of p-type without doping, is suitable due to the lack of passivation layers and membranes on the channel surface. Here, we investigate simple and direct immobilization methods of DNAs for applying diamond electrolyte solution-gate FET.

The functionalized diamond surface for direct immobilization and detection of DNAs was fabricated on H-terminated diamond. For decreasing physical adsorption of DNAs by neutralized surface, the H-terminated diamond surface was treated partial oxidation(0.5ML). And then, amino group was functionalized on the partial oxidized surface(0.1ML) by UV irradiation for immobilization with carboxylated probe DNAs. The specific hybridization with 100nM concentrations of 21 base pair DNA can be clearly detected by two methods, fluorescence microscopy and diamond Solution-Gate Field-Effect Transistors(SGFETs). The DNA hybridization was confirmed by Cy-5 labeled target DNA on micropatterned diamond surface.

The change of gate potential by negative charge of immobilized or hybridized DNA was measured on SGFET in static methods. Considering the 0.1 ratio of N/C on partial functionalized surface, the increased charge by immobilized probe DNA is calculated to be $2.1\times10^{12}/cm^2$ and the shift of the threshold by hybridization is calculated to be $8.1\times10^{11}/cm^2$ and hybridization efficiency on functionalized diamond surface was shown about 40%. In addition, a single-mismatch oligonucleotide was successfully distinguished on diamond by controlling various factors, such as intrinsic hybridization affinity of target oligonucleotides including a single mismatch and negative surface charge of oxidized diamond surface in real-time detection. A deep understanding of the hybridization phenomenon on the diamond surface, and detection of single-mismatch target oligonucleotides are of great interest not only for application to DNA sensor fabrication, but also to achieve a fundamental understanding of many key physiological processes.

PO163

# Synthesis and antimicrobial activity of some 2-benzenesulfonamidothiazoles

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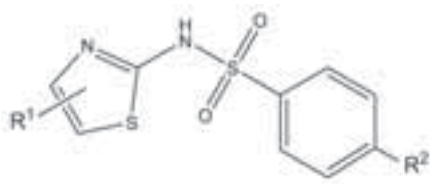
The emergence of microbial resistance to existing antibiotics generated an urgent need for novel antimicrobial agents. In our recent studies we found out several thiazole derivatives of particular interest in the inhibition of bacteria [1] and benzenesulfonamido derivatives of heterocyclic systems possessing significant antibacterial activity [2-3]. Thus, with the aim to develop new and more potent sulfonamides and to evaluate the influence of the chemical modifications on the antimicrobial properties of these compounds, some novel 2-sulfonamidothiazoles variously substituted in the heterocyclic portion and carrying an amino, nitro or methyl group in the *para* position of the benzene ring were prepared.



$R^1$ = H, 4-$CH_3$, 5-$CH_3$, 4-$CH_2COOC_2H_5$ $R^2$ = $NH_2$, $NO_2$, $CH_3$

The sulfonamidothiazoles were screened *in vitro* for their antimicrobial properties against Gram positive (bacilli, staphylococci, streptococci, *Sarcina lutea*) and Gram negative (*Escherichia coli*) bacteria, yeasts (*Candida tropicalis*, *Saccharomyces cerevisiae*) and moulds (*Aspergillus niger*) up to the maximum concentration of 100 g/ml. Sulfamethoxazole was used as reference drug. Whilst the number of the studied compounds does not permit a detailed structure-activity relationship, it is possible to identify those structural features that are conducive to antimicrobial activity. The presence of the amino group promotes the antibacterial activity. In fact, the sulfanilamidothiazole shows the best inhibition of all the Gram positive bacteria, including methicillin-resistant *Staphylococcus aureus*, at concentrations comparable with or slightly higher than those of sulfamethoxazole. The activity detected in the *p*-amino derivative decreases in the *p*-nitro substituted compounds, being limited to bacilli, whereas disappears in the *p*-methyl derivative. The antibacterial properties of the active substances against *Bacillus subtilis* increase when tested in combination with trimethoprim. This synergistic effect suggests that these compounds share the same antibacterial mode of action as sulfamethoxazole. Moreover, all sulfonamidothiazoles are inactive against Gram negative bacteria, yeasts and mould.

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PO164

# Antimicrobial activity of the natural lactobacillus paracasei isolate

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Apart from other products of the lactic acid bacterial (LAB) metabolism, such as $H_2O_2$, $CO_2$ and bacteriocins, lactic acid is known of posessing significant antimicrobial activity[1-3]. The objective of our study was to investigate into antimicrobial activity and identify extracellular metabolites of the natural *Lactobacillus paracasei* LAB-1R (isolated from the generic Serbian cheese from the locality of Rajac), as well as to examine possibilities for the utilisation of the supernatant obtained in the role of natural antibiotic. Following the *Lactobacillus paracasei* cultivation in MRS broth the resulting supernatant exerted various degrees of inhibition of indicator Gram (+) (*S. aureus* ATCC 6538, *B. subtilis* ATCC 6633, *C. sporogenes* ATCC19404), and Gram (–) bacterial strains (*E. coli* ATCC 8739 and *P. aeruginosa* ATCC 9027*),* as well as fungistatic effect against *C. albicans* ATCC 10231. Our results have demonstrated rather significant inhibitory activity against the Gram (+) strains (*S. aureus, B. subtilis)*, while the effect on the Gram (–) strains (*E. coli, P. aeruginosa*), proved to be less pronounced. Fungistatic effect on *C. albicans* has only been detected in concentrated supernatant. In order to determine bacteriocin antimicrobial activity, the lactic acid effect has been neutralized by an appropriate modification of the supernatant pH. The HPLC methods (RI and Diaodaray) have been employed for the lactic acid identification and for the quantification of its content in an overnight La*ctobacillus paracase*i LAB-1R culture supernatant (18-20g/L).The supernatant of the standard *Lactobacillus rhamnosus* ATCC 7469 has been subject to concurrent testing for the comparison and the lactic acid content amounted 15-17g/L.

Investigation into antimicrobial activity of extracellular metabolites of the natural LAB-1R isolate identified as *Lactobacillus paracasei* by means of diffusion and dilution methods (with MICs determined), gave evidence that such metabolites can be utilized as natural antibiotic and preserving agents.

Key words: LAB, *Lactobacillus paracasei*, lactic acid, antimicrobial activity

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PO165

# Asymmetric synthesis of bupranolol and its analogues.

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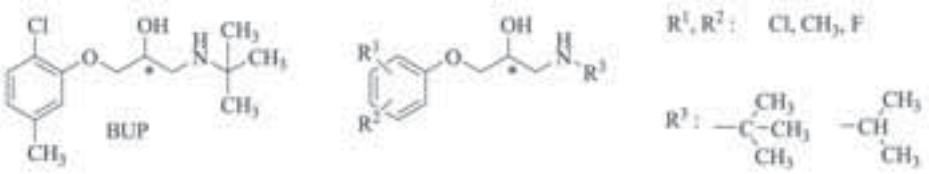


Bupranolol (BUP) is a lipophilic $\beta_1/\beta_2/\beta_3$–adrenoceptors ($\beta$-ARs) antagonist. It contains a chiral center and its side chain is similar to that of the most other β-blockers, except that a tertiary butyl group replaces the more common isopropyl one.

The current concept assumes that there are two states of the cardiac $\beta_1$-AR [for literature, see 1-3] classic or high-affinity state and a low-affinity state of $\beta_1$-AR (atypical $\beta$-adrenoceptor). The stimulation of the latter by non-conventional partial $\beta$-ARs agonists (e.g. CGP 12177) causes positive chronotropic, inotropic and lusitropic effects [2]. These effects, although not affected by many classical $\beta$-adrenoceptor antagonists (e.g. propranolol), are antagonized by BUP at high doses. We have previously shown the stereoselective effect of BUP against cardiostimulation of low-affinity state of $\beta_1$-AR in pithed rats [3]. Studies *in vitro* proved that among 12 $\beta$-blocking agents - levorotatory enantiomer of (S)-BUP is the most potent [1]. With this respect, and due to the fact that (-)-BUP is not commercially available, we aimed to synthesize (-)-bupranolol hydrochloride. Additionally, for further investigation of structural properties responsible for the antagonistic effects of BUP at the cardiac low-affinity state of $\beta_1$-ARs, slightly modified bupranolol analogues were synthesized as pairs of enantiomers. The asymmetric synthesis origined from the optically active epichlorohydrin. The purity and enantiomeric excess of the products was determined using a chiral amylose column (ChiralPak AD) which allowed direct separation of the enantiomers. We found in functional studies performed in pithed rats that (1) the antagonistic effect of BUP at the low-affinity state of $\beta_1$-ARs was retained when Cl at $R^1$ was replaced by F or methyl or $R^3$ was replaced by an isopropyl group; (2) the stereoselectivity of two new analogues of BUP proved again that the low affinity state of$\beta_1$-ARs is indeed a receptor.

## Acknowledgment

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PO166

# Alpha 2B adrenoceptor agonist ST-91 antagonizes beta 2 adrenoceptor-mediated relaxation in rat mesenteric artery rings

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We sought an isolated vascular preparation and experimental setting where the function of alpha 2B adrenoceptors could be demonstrated by non-recombinant technique. ST-91 (2-[2,6-diethylphenylamino]-2-imidazoline), an alpha 2B adrenoceptor agonist with a mixed alpha adrenergic receptor type/subtype selection profile antagonized the relaxant effect of isoproterenol in endothel-denuded rat mesenteric artery rings precontracted with phenylephrine. At $10^{-7}$ M of ST-91, the antagonism was characterized by a rightward shift of isoproterenol dose-response curve ($A_{50}$= 6.81±1.40 e-7 (n=4) vs the control 1.29±0.25 e-7 M (n=4)) with no $E_{max}$ depression. At $10^{-6}$ M the $E_{max}$ depression was prevalent (36.1±7.0 % (n=4) vs the control 79.9±5.1 % (n=4)); both actions could be antagonized by the alpha 2 adrenoceptor antagonist yohimbine. The not subtype-selective alpha 2 adrenoceptor agonist xylazine ($10^{-7}$ M) did not affect the relaxant action of isoproterenol. Present findings are discussed in the light of previously reported hemodynamic effects attributed to alpha 2B adrenoceptors in receptor subtype-knockout animals.

PO167

# Induction of synaptotagmin4 protein in hippocampus of rats after systemic kainic acid treatment

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Synaptotagmin IV (Syt IV) is a member of synaptotagmin family and is thought to be a secretory vesicle protein that is expressed in the brain. However, the involvement of Syt IV in vesicular transport and its localization in secretory vesicles is still a matter of debate as some authors claim that there is evidence that Syt IV is not a synaptic vesicle protein. The distinctive characteristic of Syt IV is that it is inducible by massive membrane depolarization during seizures. Seizures are characterized by activity-dependent changes in synaptic plasticity, including alterations in synaptic efficacy and synaptic rearrangement. Here we used kainic acid (KA) model of seizures in rats to investigate the time course of hippocampal induction of Syt IV. Rats received a subcutaneous injection of KA (13 mg/kg) and immunohistochemical analysis was done on brain sections of rats that were transcardially perfused 90 minutes, 4, 8, 12 and 24 hours after the injection. We found that the induction of Syt IV in hippocampus follows a distinct time course: its levels were increased in the granular and hillar cell layer of dentate gyrus 30 minutes following seizure activity (approximately 90 minutes after the injection of KA). 8 hours after injection of KA the signal appeared in the pyramidal cell layer of CA3, CA2 and CA1. There was still strong immunoreactivity in the neurons of CA1 region and in the hilus 12 and 24 hours after the injection of KA, while in other hippocampal regions the intensity of the signal reverted back to the basal level of expression. The downregulation in dentate gyrus granule neurons could be attributed to the downregulation of the protein while the downregulation in pyramidal layer of CA3 could be in correlation with cell damage and cell death. We propose that Syt IV may be used as an excellent marker for the activation of hippocampus during seizure activity.

PO168

# NSAID hydroxamic acids: Synthesis, cytostatic and antiviral activity evaluations

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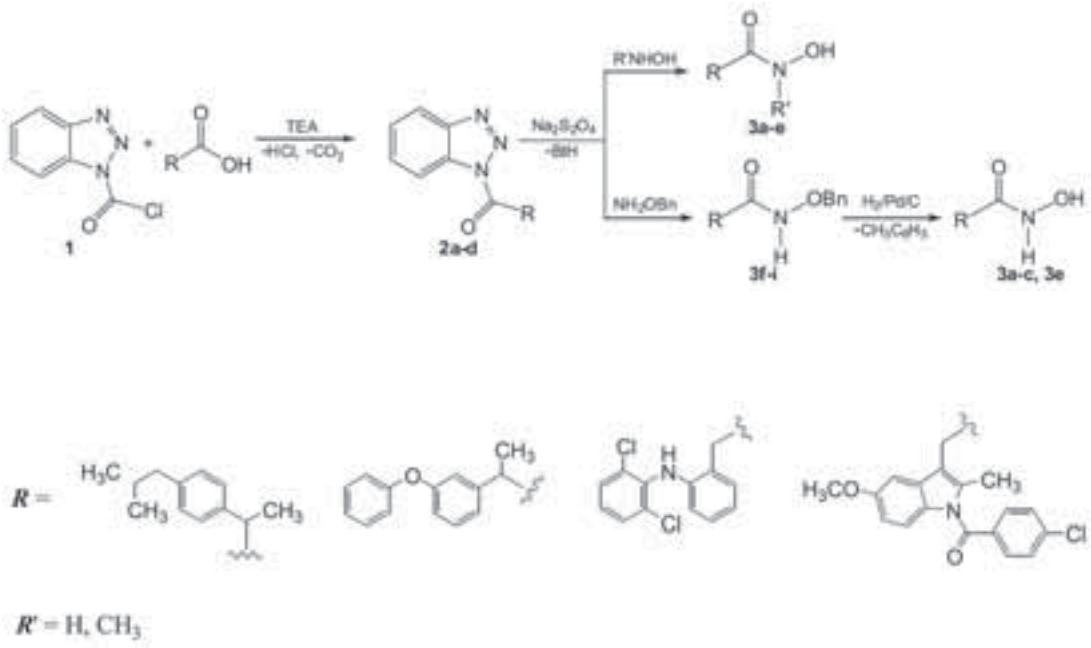
A new synthetic method for preparation of NSAID (non-steroidal anti-inflammatory drug) hydroxamic acids was developed. NSAIDs (ibuprofen, fenoprofen, diclofenac or indomethacin) were first converted to benzotriazolides **2a-d** which readily reacted with hydroxylamine, *N*-methyl-hydroxylamine or *O*-benzylhydroxylamine to afford the corresponding NSAID hydroxamic acids or *O*-protected derivatives **3a-i**. The products **3a-i** were evaluated for their antiviral and cytostatic activity against malignant tumor cell lines and human normal fibroblasts (WI38). Among the new NSAID derivatives of hydroxamic acid, **3e**, **3f**, **3g** and **3i** specifically inhibited the proliferation of non-small cell lung cancer cell (mean $IC_{50}$ = 0.21 – 0.55 μM) over that of normal human fibroblasts (selectivity index 55 – 140). Compounds **3b**, **3e** and **3i** showed differential inhibitory activity against the growth of HeLa cells ($p < 0.05$) at $10^{-5}$ M. Antiviral activity evaluations results indicated that **3f** had a minor activity against the influenza virus A/H1N1 subtype with a selectivity index of 7 – 10.



PO169

# Spinal cord protein hydrolisates in oral tolerance development

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Recently, it has been proposed to apply method of oral tolerance to the treatment of autoimmune diseases. The aim of our study was to use hydrolisate of pig spinal cord proteins (mixture of neuroantigens) to induction oral tolerance in animal model of sclerosis multiplex (SM) - experimental allergic encephalomyelitis /EAE/. Hydrolisates has been generated by digestion of pig spinal cord homogenates with pepsin. The female Lewis rats were fed with pig spinal cord hydrolizate in high dose for one week before immunization, which was induced by injection of guinea pig spinal cord homogenate. As a control were used animals with EAE only, hydrolizate only, albumine fed and intact ones. Clinical course was observed and graded in five steps scale. On the top of clinical symtoms (13-th day post immunization) the rats were sacrificed and the spleens were removed. Spleen calls were used for culture. Proliferation of them was measured by [$^3$H]thymidyne incorporation and expressed in cpm (average of triplicate samples). After 7 days of cells culture the inhibition of proliferation was observed in hydrolizate fed animals in comparison to control ones. The results shows that hydrolizate of pig spinal cord proteins has a modulatory effect on the immune reaction particularly on the orally induced antigen specific modulation of autoimmune response.

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P170

# QSAR study of antiproliferative activity of α-tocopherol and cholesterol derivatives against human breast cancer cells

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α-Tocopheryl-lysine derivatives, α-tocopheryl-succinate, α-tocopheryloxy butyric acid, cholesteryl-succinate and cholesteryloxy butyric acid are inducing human MCF-7 breast cancer cells to undergo apoptosis via destabilization of lysosomal and mitochondrial membranes [1, 2]. Here the QSAR models of the anticancer agents were performed to correlate their physicochemical properties with the antiproliferative $IC_{50}$ activities against the MCF-7 cell line [1, 2]. Geometry optimizations of the compounds were obtained by using the MOPAC/$PM_3$ method [3]. The CS Gaussian 98 program [4] by density functional theory (DFT) using the B3LYP hybrid functional including 6-31G (d, p) basis [5], was applied for partial atomic charge and dipole moment computations of the optimized models. The partition coefficient octanol/water (ClogP), pKa, and distribution coefficient (logD) were determined by using the ChemPro, Marvin 4.0.5 ChemAxon programs.
Liposolubility at pH 5.0 (logD), number of methyl groups in aliphatic side chain, partial charge of oxygen in the α-tocopheryl/cholesteryl moiety and dipole moment, account for the antiproliferative activity of the compounds. Multiple linear regression models with two, three and four variables, $pIC_{50}$ = f (N-$CH_3$, $logD_{pH\ 5.0}$), $pIC_{50}$ = f (N-$CH_3$, $logD_{pH5.0}$, O-atomic charge) and $pIC_{50}$ = f (N-$CH_3$, $logD_{pH5.0}$, O-atomic charge, dipole moment), were obtained with $R^2 > 0.83$ and cross-validation parameter, $q^2_{pre} > 0.66$. According to the regression and validation parameters calculated, the four-variable model was selected as the QSAR model with the biggest prognostic capacity. The QSAR approach can help in understanding the structural features that contributes to the action of the molecules. Therefore the theoretical method presented could be used as a fast, easy, and reliable tool for design of novel drugs with enhanced anticancer activities.

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P171

# Design of novel organoselenium and α-tocopherol compounds with enhanced chemotherapeutic activity

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Se-Allylselenocysteine, Se-propylselenocysteine, Se-methylselenocysteine, Se-cystamine, Se-methionine, S-methylcysteine are active in suppressing chemically induced mammary carcinogenesis in rats [1-3]. Here the QSAR study of the anticancer agents was performed to correlate their structural properties with the measured chemotherapeutic activities ($ED_{50}$) [1-3].

The CS Gaussian 98 program [4] with *Hartree-Fock* functional including 3-21G (d) basis [5] was applied for molecular properties computations of the optimized models. Energy of the Lowest Unoccupied Molecular Orbital (LUMO) and Electron Density on chalcogen (ED(X)) expressed the strongest influence on anticancer activity of the examined molecules. Multiple linear regression model of the alkyl-chalcogens, $pED_{50}$ = f (LUMO, ED(X)), was obtained with $R^2$=0.979 and cross-validation parameter $q^2_{pre}$=0.966. Relatively high statistical and validation parameters values pointed on good prognostic capacity of the created QSAR model.

Synergy between α-tocopheryl-succinate and methylseleninic acid in induction of cancer cells apoptosis [6] indicated on novel organoselenium-α-tocopheryl compounds as potent anticancer agents. Thus was decided to use the most active alkyl-chalcogens, Se-allylselenocysteine, Se-propylselenocysteine, and Se-methylselenocysteine, for design of novel compounds with α-tocopherol.

Anticancer activities ($ED_{50}$) of the designed molecules were evaluated using the created $pED_{50}$ = f(LUMO, ED(X)) regression model and the most potent chemotherapeutic agents were selected.

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P172

# Pharmacological inhibitors of protein kinases: screening and development

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During the past decade various drug discovery programs have led to the identification of many potent and selective ATP antagonist inhibitors of protein kinases. Generally, such inhibitors consist of structurally distinct, flat heterocyclic molecules, which enter the active site and compete with ATP, a feature usually demonstrated by enzyme kinetics and co-crystal analyses. Anti-kinase drugs possess prominent inhibitory properties against cancer cells both *in vitro* and *in vivo*, and several are currently being evaluated in clinical trials as a new generation of chemotherapeutics directed towards cancer and other proliferative diseases.

We routinely screen small-molecule compound collections for novel hits in a panel of biochemical (inhibition of cyclin-dependent kinases and BCR/Abl) and cellular assays (antiproliferative activity, cell cycle inhibition, activation of p53, induction of apoptosis) and we have identified several selective inhibitors of cyclin-dependent kinases (CDKs), *e.g.* purine derivatives roscovitine [1] and olomoucine II [2] or 4-arylazo-3,5-diamino-1*H*-pyrazole CAN508 [3]. A chirally and chemically defined and pure form of R-roscovitine (seliciclib, CYC202, Cyclacel) is currently in phase II clinical trials in patients with cancer.

The novel compound CAN508 displays surprising selectivity towards cyclin-dependent kinase CDK9, a positive regulator of transcription [3]. All biochemical and cellular effects of the inhibitor are fully consistent with direct inhibition of CDK9, i.e. decreased phosphorylations of the retinoblastoma protein and the C-terminal domain of RNA polymerase II, inhibition of mRNA synthesis, and induction of the tumor suppressor protein p53. A comparison of the kinase selectivity profiles of our inhibitors suggests that inhibition of CDK2, CDK7, and CDK9 may be responsible for the antiproliferative and pro-apoptotic potency of CDK inhibitors. In conclusions, the inhibitors represent good points for further structural optimization of specific CDK9 inhibitors, with potential pharmacological applications in oncology and virology.

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