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**BOOK OF ABSTRACTS**

## Plenary Lectures



## Key Lectures

**Oral Presentations**

## Poster Presentations

# PROTEIN STRUCTURE SIMILARITY CLUSTERING (PSSC) AS GUIDING PRINCIPLE FOR CHEMICAL GENOMICS

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In the everlasting effort to find small molecules which alter protein function and ultimately might lead to new drugs, combinatorial chemistry has emerged as a very powerful tool. Opposite to the original thought that large library sizes will result in the discovery of many hit and lead structures, it has been recognized that biological relevance, design, and diversity of the library are more important. As the universe of thinkable compounds is almost infinite, the question arises: Where is a biologically validated starting point to build a combinatorial library around? Nature itself might give an answer: Natural products have been evolved to bind to proteins. Recent results in structural biology and bioinformatics indicate that the number of distinct protein families and folds is fairly limited. Often the same structural domain is used by many proteins in a more or less modified form created by divergent evolution. This structural conservatism of Nature can be exploited for the design of biologically relevant molecules derived from natural products addressing the ligand-sensing cores of these domains. In the lecture arguments for a natural product guided library design will be discussed and highlighted by its recent application in the synthesis of combinatorial libraries of biologically active compounds.[1)]

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# STRATEGIES FOR LEAD FINDING AND LEAD OPTIMIZATION UTILIZING EXPERIMENTAL AND COMPUTATIONAL APPROACHES

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Lead Finding and Lead Optimization depend critically on our ability to integrate synthesis skills, automation technologies, design capabilities and medicinal chemistry knowledge into the discovery process. The discovery of novel Kv1.5 channel inhibitors, microtubule disassembly inhibitors, G6P-Translocase inhibitors, and factor Xa inhibitors will be discussed to describe various discovery strategies.

The voltage-gated potassium channel Kv1.5 is regarded as a promising target for the development of new atrial selective drugs with fewer side effects. Various approaches for lead finding including similarity search, rescaffolding, ligand- and structure-based design will be described. Emphasis will be given on synthetic strategies and the impact of automation technologies.

Besides synthetic small molecules natural products are a valuable source for novel lead structures. The impact of natural products on discovery will be briefly reviewed and illustrated based on selected project examples including microtubule disassembly inhibitors and G6P-Translocase inhibitors.

Factor Xa plays a critical role in the co-agulation pathways. Our efforts towards orally active agents will be described with an emphasis on aspects of multidimensional compound optimization.

# PURSUING LEADLIKENESS IN PHARMACEUTICAL RESEARCH

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Exhaustive enumeration of unique compounds with molecular weight (MW) <= 300 a.m.u., having only C, H and (in the future) O, N, S, P, and X is an on-going project between UNM and Daylight [1]. Based on Daylights GENSMI [1], all unique canonical non-isomeric SMILES are currently generated and stored in a database that can effectively become a resource for mining the entire virtual space of small molecules. The complexity of this problem at a computational and logistic level (over $10^{10}$ molecules are anticipated) is justified by recent trends in the pharmaceutical industry to move toward a fragment-based drug discovery approach [2], rooted in the concept of leadlikeness [3]. Existing chemicals significantly under-sample chemical space at MW > 300 [3]. The degree of overlap between the current enumeration effort and WDI, the World Drug Index is discussed based on descriptors related to branching, cyclization and molecular complexity [4]. Over 44 million unique SMILES meet the WDI criteria. We anticipate that these structures can become the basis for exploring novel chemistry spaces.

Our undestanding of the quality of leads rests on mining known biological actives. Such a source is the WOMBAT 2005.1 database [5], which contains over 104,000 unique chemicals and 230,000 biological activities. A derivative database related to clinical pharmacokinetics is the WOMBAT-PK (WB-PK) database [6]. WB-PK 2005.1 contains 656 drugs with multiple human ADME/Tox endpoints: > 600 oral bioavailability and half-life data, > 500 plasma protein binding and volume of distribution (steady state) values, > 400 total clearance, non-renal clearance and maximum recommended therapeutic daily dose values, etc. Matching clinical data with calculated properties, one can gain better insights for lead discovery. In particular, the relationship between the Maximum Recommended Therapeutic (daily) Dose, MRTD, and the partition coefficient (clogP and LogD74) will be discussed. Selection criteria that rely on ChemGPS [7], a principal components analysis-based model for, e.g., PK prediction [8], will also be highlighted.

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# FROM LEADS TO DRUGS

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The optimization of a lead structure is an evolutionary procedure. Medicinal chemists follow the similarity principle, based on the experimental experience that structural analogs most often have similar biological properties. Whereas there are many exceptions to this general observation, all rational lead optimizations are based on this concept. Every improvement of certain properties of a lead results in further structural modifications. This process ends when the final candidate has all desired properties to proceed to preclinical profiling and clinical investigation.

General procedures for lead structure modification are (bio)isosteric replacement of atoms and groups, formation of rings (rigidization), cleavage of rings, modification of side chains and linkers, use of „privileged“ structural elements, selective optimization of side activities (SOSA approach), virtual screening, structure-based ligand design, computer-aided ligand design, fragment-based ligand design, modification of ADME properties, prodrugs, soft drugs, and targeted drugs. Selected examples will illustrate most of these principles.

# IN SILICO DESIGN AND FOCUSED SOLID PHASE SYNTHESIS OF NEW, SELECTIVE ENZYME INHIBITORS WITH POTENTIAL IN CANCER AND NEUROLOGICAL THERAPIES

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Inhibitors of enzyme targets for cancer therapy. The successful clinical development of selective small-molecule protein kinase inhibitors, such as imatinib (Gleevec), in chronic myeloid leukaemia (CML) and gastrointestinal stromal tumors (GIST), and gefitinib (Iressa), in lung cancer, have established a new paradigm for the treatment of tumors, inaugurating the era of the so-called "molecular targeted cancer therapy". Imatinib, a specific inhibitor of the BCR-ABL tyrosine kinase, is an effective drug in CML, but in many patients with advanced disease a harmful drug resistance frequently develops after an initial positive response. Taking the numerous mutations observed in BCR-ABL into account, which mainly clustered within the ATP binding region, and on the basis of the information obtained from the 3D structures of diverse enzyme-inhibitor complexes, we designed two focused libraries *in silico* and synthesized them through an original and efficient solid phase synthesis (SPS) and a multiple parallel synthesis (MPS) in solution. The results allowed new structure-affinity (SAFIR) and structure-selectivity relationships (SSR) to be established.

Another important and widespread tumor, i.e. breast tumor, was targeted in a study aimed at identifying new aromatase inhibitors, antitumor agents expected to replace estrogen receptor antagonists, like *tamoxifene* and *raloxifene*. A combined application of 3D-QSAR and direct modelling approaches (based on a homology built 3D model of the enzyme) guided the design of new classes of highly potent aromatase inhibitors, prepared through MPS in solution, which showed outstanding selectivity over 17-alpha-hydroxylase/17-20 lyase, another important cytochrome P450 enzyme involved in prostate cancer.

Enzymatic inhibitors with potential for the treatment of neurodegenerative disease. Two large series of *safinamide* and coumarin derivatives were designed, prepared and tested as monoaminoxidase (MAO) inhibitors. The former were prepared through an efficient solid-phase synthesis allowing the introduction of several structural modifications at three different steps of the synthetic pathway. Molecular diversity was properly explored on both series of ligands following the suggestions from both 3D QSAR studies and flexible docking investigation on MAO-A and MAO-B binding sites, recently elucidated by high resolution x-ray crystallography . The comparative analyses of the main interactions governing the MAO inhibition, allowed a ligand-based design of novel, reversible, potent and selective MAO-B inhibitors, with favourable pharmacokinetic profiles for the therapy of Parkinson disease.

Finally, based on the assumption that a suitable modulation of multiple targets can provide improved therapeutic effects and safer toxicological profiles, a new class of molecules, acting as dual MAO-B and acetylcholinesterase enzyme inhibitors, was successfully designed for their potential in the therapy of Alzheimer's disease. Investigations aimed at obtaining inhibitors with differently balanced activities at each targeted enzyme will be discussed and preliminary pharmacological data shown.

# COMPUTATIONAL CHEMOGENOMICS: EXTRACTING KNOWLEDGE FROM BIOCHEMICAL DATA

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In recent years, the establishment of high-throughput screening technologies and recent advances in protein expression, production and X-ray crystallography have led to an explosion of both response data (e.g., potency, affinity, metabolism, toxicity) on the interaction between proteins and ligands, and structural data on proteins and protein-ligand complexes, respectively. The question now is, how should these data be organised and how could they be analysed so knowledge, in the form of some trends, rules, or models connecting both chemical and biological spaces, can be extracted from them? This talk will attempt addressing this question by presenting some of our recent efforts to, on one hand, identify and classify chemical and biological entities and, on the other hand, develop new biochemoinformatics tools to extract knowledge from chemical and biological data.

# FINDING PROTEIN TARGETS FOR DRUGS - THE CHEMOGENETIC APPROACH

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# MICROWAVE-ASSISTED HIGHTHROUGHPUT CHEMISTRY IN LEAD GENERATION AND OPTIMIZATION

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The increasing need for speeding up the entire drug discovery process is one of the reasons driving the recent success of novel technologies that can produce compounds at a very high rate. Microwave (MW)-assisted organic chemistry is a relatively new technology that has been shown to significantly improve productivity in the generation of combinatorial libraries and target molecules [1]. MW high-speed synthesis has attracted a considerable amount of attention in recent years; more than 2000 articles have been published in the area of MW-assisted organic synthesis since the first article on the use of MW heating was reported in literature in 1986. More recently, after the change of the millennium, pharmaceutical and biotechnology companies were increasingly taking on board this technology for highthroughput lead generation, hit validation and lead optimization, but only today we can say that this technique is in some laboratories the first choice of work.
The transition from the use of dangerous domestic MW ovens, that generated data difficult to reproduce due to cold and hot spot problems to very sophisticated professional equipments was very much facilitated by four main companies: Biotage, CEM, Milestone and Anton Paar; all them have large differences between their instruments (often being developed with continuous feedback from researchers in the big pharmaceutical companies), from reaction vessel size and vial option to temperature and reaction monitoring. Some of them allow the chemist to perform up to 50 reactions in parallel; alternatively, single-vessel instruments integrated with an automated arm are available to speed up the serial lead optimization process. Also the optimization of reaction conditions is easily performed since reagents, temperatures, solvents and reaction times can be programmed to run unattended.
All this has helped MW chemistry to gain greater acceptance in all fields of drug discovery and development, from process and analytical areas to organic synthesis and, more recently, scaling-up.
The clue concept is that MW heating dramatically accelerates reaction times when compared to conventional heating in an oil bath. In addition, side reactions are reduced and yields often increased, leading to rapid synthesis of novel and purer compounds. The spectrum of MW-assisted chemistry being explored is becoming larger, touching all fields of solution and solid-phase organic synthesis, including Suzuki, Sonogashira, Stille, Heck, Buchwald, transition-metal-catalyzed reactions and various type of heterocycle synthesis and solvent-free reactions.
The last challenge that producers of MW chemistry equipments are facing is the option to utilize them in scaling up and in the production of large amounts of intermediates or development candidates for preclinical and clinical trials.
Some examples of MW chemistry in the production of hit validation and lead optimization libraries will be discussed in the context of accelerating the drug discovery process.

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# LIPID RAFTS ANF THEIRBIOLOGICAL SIGNIFICANCE : ENGINEERING LIPID ORDER AND DENSITY

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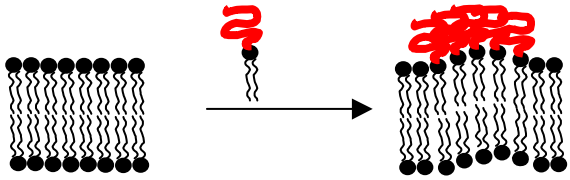
Biological membranes built from lipid bilayer membranes are highly valuable model systems for the description and investigation of membrane processes. Clustering effects, permeability studies as well as the influence of molecules onto the lipid-bilayer structure can be studied efficiently. The present talk will focus on two recent examples studying (a) the formation of raft-type lipid-conjugates on the surface of vesicles and planar bilayer membranes and (b) the action of small cationic polymers onto lipid bilayer membranes. In both cases vesicle systems serve as model membranes to study the distribution of lipid molecules by thermal, fluorescent and AFM-methods. A method controlling the density of surface receptors on vesicle surfaces relying on polymeric-lipid conjugates is presented.



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# DRUG TARGETING BY PEPTIDE CONJUGATES

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Antitumour effect of chemotherapy is frequently restricted by dose-limiting toxicities, including side effects (e.g. cardiotoxicity, multidrug resistance (MDR)). One of the novel approaches to destroy tumor cells is to deliver drug directly to the cancer cells by its covalent peptide conjugate [1,2]. We have developed two groups of conjugates in which drugs (e.g. acid labile *cis*-aconytil daunomycin (cAD), methotrexate, ferrocenecarboxylic acid) are coupled either to branched chain polymeric polypeptides, (poly[Lys-(DL-Ala$_m$-X$_i$)] (X = Glu, EAK, or X = Ser, SAK) with different charge characteristics [3] or to Arg-based oligopeptides. The synthesis, purification and structure determination as well as their biological properties (e.g. toxicity, fluorescence properties, *in vitro* cytotoxic effect) of these constructs will be outlined. We found that these peptide conjugates – depending on the structure of the peptide moiety - have enhanced antitumour effect *in vitro* even in MDR resistant cells. To understand the mechanism of action we have analysed the uptake of daunomycin and daunomycin-polypeptide conjugates by flow-cytometer on sensitive and multidrug resistant HL 60 cells and the localization of these compounds were compared by confocal laser microscopy. We observed that peptides studied have intracellular transporting ability to translocate attached entities across the cell membrane.

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# *IN SILICO* APPROACHES GENERATING NOVEL COMPOUND SERIES IN LEAD OPTIMIZATION

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Lead optimization (LO) is undoubtedly the major bottleneck in pre-clinical drug discovery. In LO utilizing typically 3-4 optimization cycles several goals should be achieved at the same time including: improved activity, selectivity, chemical and biological stability, enhanced bioavailability and safety. This process requires normally between 8 to 16 months depending on the target or the therapeutic area. In order to address the need to reduce the overall timeline, we developed an integrated *in silico* approach, which accelerates the re-design process after each iteration cycle. The key elements of this approach are library design and filtering/ focusing tools that define the LO library for the subsequent cycle.

In the library design we utilize several proprietary approaches for 'lead multiplying':

- 2D analog search based on structural similarity to the best compounds in the preceding iteration, selected from in-house or publicly available databases,
- A unique medicinal chemistry knowledge base (EMIL: Example Mediated Innovation for Lead evolution), which contains several thousands of structural "evolution" examples for bioanalogous sub-structural replacements
- Novel chemogenomics approach to increase the selectivity based on the genetic divergence of target family members ('selectivity jumping').

The subsequent filtering process comprises several *in silico* tools of various functions:

- 3D virtual screening if crystal structure or homology model is available
- *In silico* ADMETox filtering using the latest edition of Pallas™ software
- Synthetic Feasibility Analysis and Scoring
- Diversity selection ensures that all the relevant structural features are represented

In the present talk we describe the elements of this integrated *in silico* approach for LO together with case studies in the area of various target families (e.g. kinases, MMPs etc.).

# MODELING ROBUST QSAR

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It may look like a paradox but *the most fundamental and lasting objective of* (chemical) *synthesis is not a production of new compounds but the production of properties* [1]. Molecular design is a computational tool for screening virtual chemical compound space in a search for novel properties, and QSAR should work like a dictionary between molecular structures and properties. This clearly makes it an essential and irreplaceable method in molecular design. However, more and more sophisticated and robust tools are needed for the efficient transformation of the molecular structure space into the compound property space.

Molecular superimposition is a first problem during any QSAR procedure. Even if we use a method that does not require this operation, in fact, it is realized by default. This is convenient but we cannot control it. Although we usually do not realize this, in fact, such default superimposition is also performed by a variety of 2D QSARs. Recently, several improvements in the structure overlay have appeared that allow for more flexible or sophisticated superimposition.

Coding molecules in 3D or 4D QSAR is a next issue that does influence final model robustness. In the CoMFA-like fields a molecule is represented by a set of points determined in the space by a 3D grid. Different smooth and box fields have been thoroughly tested recently. A surface can be a base for the molecule description in several methods, e;g., Compass, CoRSA or CoMSA, that are based on sampling points or surface sectors. In Hopfinger's 4D QSAR a molecule is coded by the descriptors defining the pattern in which atoms occupy volume sectors. Alternatively, the self-organizing neural network can be used for the generation of molecular volumes.

Data handling is a next issue that can improve QSAR robustness. New computational methods including neural networks, data elimination, genetic algorithms, novel model validation schemes are some examples in this field. Generally, during QSAR modeling we operate on a strictly finite set of molecules for which activity is measured and described *a priori*. Eventually, before calculation we must have chosen the appropriate data for the compounds that are active. This decides that QSAR is more an *a posteriori* analysis of the SAR data structure than a strict method for the activity prediction in a sense of a novel compound design. Even a minute chemical structure modification can result in substantial activity changes. This similarity paradox decides that a virtual molecule, in reality, cannot only be a more or less substantial outlier in QSAR equation but can appear completely inactive. Thus, instead modeling by equations we can use more versatile techniques such as clustering or visualization. This allows us to avoid a paradox of producing an excellent model that completely fails when prediction is attempted. Eventually, it is better to have a vague idea on the trends than an illusion of a proper prediction. Finally, completely novel methods e.g., binary QSAR for HTS data appeared. This allows us to generate a model including both active and inactive compounds.

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# FUNCTIONAL OLIGOMERS OF RHODOPSIN - A G PROTEIN-COUPLED RECEPTOR TEMPLATE

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Recent biochemical and biophysical studies have challenged traditional view of GPCR as monomeric protein, and indicated that many of these receptors exist as homo- and hetero-dimers. Furthermore, their higher oligomeric assemblies may also have specific functional roles [reviews 1-3]. Cooperative interactions within such an oligomeric array may be critical for the propagation of an external signal across the cell membrane and to the G protein, and may therefore underlie the basis of signaling. Several studies have shown that dimerization occurs early after biosynthesis. It may help in receptor maturation. Other processes such as G-protein binding, downstream signaling and internalization have also been shown to be influenced by the dimeric/oligomeric nature of these receptors.

In addition to revealing key mechanisms of GPCR action, the concept of dimerization could be important in the development and screening of drugs that target GPCRs. The changes in ligand-binding, cross-talking and signaling properties that accompany oligomerization could potentially lead to new pharmacological classes of drugs.

Rhodopsin is still the only GPCR with three-dimensional structure known. To investigate mechanisms of activation, passing the signal and deactivation of rhodopsin, complexes of activated rhodopsin with its G-protein (transducin), rhodopsin kinase and arrestin were built. All complexes were generated based on oligomeric state of rhodopsin [4, 5]. Modeling revealed that both G-protein and arrestin bind to rhodopsin dimer, a basic unit of rhodopsin in paracrystal. In case of transducin adjacent dimer gives an additional surface to stabilize the complex. After unbinding of transducin beta-gamma subunit, remaining alpha part can bind to the second molecule of transducin and facilitate docking to rhodopsin [6]. The similar positive cooperativity effect was observed in arrestin-rhodopsin complex.

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# IN SILICO MEDICINAL CHEMISTRY AND PHARMACOLOGY OF NATURAL PRODUCTS: UNDERSTANDING BIOLOGICAL EFFECTS THROUGH MOLECULAR MODELLING AND MOLECULAR DYNAMICS SIMULATIONS

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Several important aspects of the chemistry and pharmacology of natural products are amenable to being studied by the same computational tools that are used in the case of synthetic compounds: from calculation of structures and chemical reactivity to ligand docking and structure-activity relationships. A variety of examples will illustrate the characterization of ligand-binding sites in proteins [1,2], the docking of small molecules into protein binding sites [1,2], the covalent and non-covalent binding of antitumor agents to DNA in a sequence-selective fashion [3-6], as well as the importance of molecular dynamics in the simulation of conformational changes [1,2] and in the determination of binding free energy differences [6,7]. The results obtained using these methodologies yield information that sometimes is beyond current experimental possibilities and can be used as a guide in the selection of experiments. On the basis of our improved level of understanding of molecular recognition, the widespread availability of target structures, and the ever-increasing power of computers, it is reasonable to assume that *in silico* methodologies will continue to aid in the interdisciplinary work aimed at the design and optimisation of new drugs.

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# DISCOVERY OF MULTIPOTENT DRUGS FOR THE TREATMENT OF ALZHEIMER'S DISEASE

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Alzheimer's disease (AD), the most common cause of dementia, is a complex neurological affection that is characterized by loss of memory and progressive deficits in different cognitive domains and by massive deposits of aggregated proteins to form the intracellular neurofibrillary tangles and the extracellular senile plaques. Even if the primary cause of AD is still speculative, early amyloid-β peptide (Aβ) aggregates are thought to be mainly responsible for the devastating clinical effects of the disease. Although, at present, the most followed approach to identify AD drugs is the amyloid hypothesis, significant research has been also devoted to the role of free radical formation, oxidative cell damage, and inflammation in the pathogenesis of AD, providing new promising targets and validated animal models. It is now clear that AD has a multifaceted etiology, hence, efforts to discover effective anti-Alzheimer drugs should be devoted to the design of new compounds that are able to hit different selected targets.

To this end, we applied a well-known design strategy in which distinct pharmacophores of different drugs were combined in the same structure leading to hybrid molecules. In principle, each pharmacophore of these new drugs should retain the ability to interact with its specific site(s) on the target and consequently to produce specific pharmacological responses that taken together should block or hopefully cure the neurodegenerative process leading to AD.

This research led to the discovery of *Lipocrine* that emerged in in vitro models as an effective candidate to be investigated in vivo for its multiple biological properties, namely, inhibition of acetylcholinesterase (AChE) and butyrylcholinesterase activities, inhibition of AChE-induced Aβ aggregation, and ability to protect cells against reactive oxygen species. Furthermore, among a series of polyamines, *Memoquin* displayed an even better pharmacological profile because, beside an in vitro profile similar to that of *Lipocrine*, in a transgenic mouse (AD11) model of AD, displaying a full complement of phenotypic hallmarks for the disease, it was able to decrease the cholinergic and cognitive impairment, Aβ deposition and tau hyperphosphorylation. Clearly, an approach based on a multiple intervention in the pathogenic pathway of the disease may have great potential to cure AD.

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# 2,3-BENZODIAZEPINES: MULTIPLE PHARMACOLOGICAL ACTIONS AND STRUCTURE-ACTIVITY RELATIONSHIP

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2,3-Benzodiazepines (BZDs) are members of a series of chemical entities that have been synthesized by changing the position of nitrogen atoms in the classical structure of 1,4-BZDs. Investigation of 2,3-BZDs emerged in EGIS Pharmaceuticals and Institute for Drug Research Hungary led to the conclusion that these molecules exhibit extremely rich pharmacology. The first remarkable molecule of this series was tofisopam, a drug acting as anxiolytic, vegetative modulator and antiparkinsonian agent. 2,3-BZDs with anxiolytic and antipsychotic characteristics were found and minor structural changes led to development of novel dopamine transporter inhibitors [1]. Compounds with AMPA receptor antagonistic properties were also identified in this series [2]. It was shown that methylene- or ethylenedioxy groups in 7,8-position or chlorine in C8 position and amino group in para position at the phenyl ring are requirement for blocking AMPA receptors [3]. Substitution of 2,3-BZD with cyclopropyl-carbamoyl group in C3 position further increases AMPA receptor antagonistic properties. Additional substitution of the phenyl ring with a methyl group in meta position enhanced the time-course of AMPA receptor blocking action. Although a series of modification in the molecular structures of AMPA receptor antagonist BZDs resulted in increased efficacy, lack of correlation was found between in vivo and in vitro pharmacological potencies. 2,3-BZDs with AMPA receptor-blocking activity may have therapeutic value in a wide range of CNS disorders such as Parkinson's disease, stroke, epilepsy, multiple sclerosis or motoneuron disease. Furthermore, lengthening the spacer between the phenyl and BZD rings led to the discovery of 2,3-BZDs containing stryryl double bound [1]. These changes resulted in anxiolytic compounds that devoid of binding to AMPA receptors. Some styryl-BZDs may exert anxiolytic effect acting on $GABA_A$ receptor subunits, their effect; however differ from that of 1,4-BZDs. Thus, the stryryl-2,3-BZD EGIS-8858, which exhibits anxiolytic effects in the elevated plus maze and the Vogel test, is not sedative, does not induce dependency, or amnesia and is not anxiogenic upon withdrawal.

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# USE OF SMALL INHIBITORY NUCLEIC ACIDS FOR DOWN-REGULATION OF GENES INVOLVED IN ALZHEIMER'S DISEASE

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According to the amyloid hypothesis, accumulation of Aβ is a primary factor driving Alzheimer's disease pathogenesis [1]. Lowering of Aβ secretion can be achieved by decreasing of β-secretase activity, rather than by down-regulation of the APP substrate protein. Therefore, BACE is a primary target for anti-amyloid therapeutic drug design [2]. Several approaches have been undertaken to find an effective inhibitor of human β-secretase, mostly in the field of peptidomimetic, non-cleavable substrate analogues [3].

Small inhibitory nucleic acids (siNAs) able to down-regulate gene expression include antisense oligodeoxy*ribo*nucleotides (antisense DNA), catalytic nucleic acids (ribozymes and deoxyribozymes) and short interfering RNAs (siRNAs).

While antisense oligonucleotides were first used to identify an aspartyl protease with β-secretase activity, all the strategies now demonstrate that siNAs are able to inhibit BACE biosynthesis in a sequence-specific manner, measured both at the level of its mRNA and the level of protein [3]. Moreover, knock-out of BACE reduces the intra- and extracellular population of Aβ40 and Aβ42 peptides. This anti-amyloid effect of siNAs was observed in a wide spectrum of cell lines as well as in primary cortical neurons. Thus targeting BACE with small inhibitory nucleic acids may be beneficial for the treatment of Alzheimer's disease and for future drug design.

**Acknowledgements**: Financial support from The State Committee for Scientific Research in Poland (project PBZ-KBN-059/T09/09) and from ICGEB grant CRP/POL04-01 is gratefully acknowledged.

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# THE ENDOGENOUS CANNABINOID SYSTEM: FROM MOLECULES TO THERAPEUTIC APPLICATION

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After decades of attempts to isolate the active principle of *Cannabis*, in 1964 Gaoni and Mechoulam described the major psychoactive component of the plant: (-)-$\Delta^9$-tetrahydrocannabinol (THC). Discovering the targets of THC took about 30 years more, since it was not until the 90s when the two cannabinoid receptors known to date, $CB_1$ and $CB_2$, were cloned. Then, a search began to identify their naturally occurring ligands. To date, four main endocannabinoids [1] have been isolated: anandamide (AEA), 2-arachidonoylglycerol (2-AG), 2-arachidonyl glyceryl ether and virodhamine. A major step was the discovery that the two best characterized endocannabinoids, AEA and 2-AG, were involved in key signaling pathways acting as endogenous immune, neuro and endocrine modulators [1]. This discovery was translated into an intense search for the elucidation of their synthesis and degradation pathways [2]. Regarding the biosynthesis, only very recently three enzymes have been characterized, a *N*-acylphosphatidylethanolamine-selective phospholipase D (NAPE-PLD) and two *sn*-1-selective-diacylglycerol lipases (DAGL-α and DAGL-β). The inactivation is a two-step procedure in which the endocannabinoid is first transported inside the cell by a facilitated mechanism, and subsequently degraded by fatty acid amidohydrolase (FAAH, for AEA) or by monoacylglycerol lipase (MAGL, for 2-AG). To date, it is widely accepted that the endogenous cannabinoid system (ECS) is constituted by two cannabinoid receptors, their endogenous ligands, as well as the enzymes responsible for their biosynthesis and degradation.

This lecture will attempt to review, from a medicinal chemistry perspective, both the classical and novel methodologies that target the different proteins of the ECS, as well as the actual and the potential therapeutic applications of these approaches. Agonists and antagonists of $CB_1$ and $CB_2$ receptors have been developed to establish the involvement of the ECS in many central and peripheral physiological processes. Examples of these agents in current clinical trials will be discussed. The process by which anandamide is transported has been a topic of much interest and advances have been made in the synthesis of compounds [2] that specifically inhibit anandamide transport [2,3]. The recent role [4,5] of these agents in excitotoxic processes and neurodegenerative diseases, such as Huntington´s chorea and multiple sclerosis, will be explored. Pharmacological studies, in combination with novel functional proteomic methods, have generated the first selective FAAH inhibitors inducing analgesic and anxiolytic effects [6]. With regard to the enzymes MAGL, NAPE-PLD, DAGL-α and DAGL-β, there are no available inhibitors and innovative approaches are required for the development of selective inhibitors in order to probe their potential pharmacological and therapeutic benefits.

Continued investigations of each of the targets regulating the ECS should greatly enrich our understanding of the physiological and pathological functions of this system and offer new therapeutic strategies for the treatment of diseases that still lack adequate medical therapies.

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# INHIBITION OF THE CELL CYCLE KINASE CDK1 BY 2,4-DIAMINO-PYRIMIDINES

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# NEW PARP INHIBITORS

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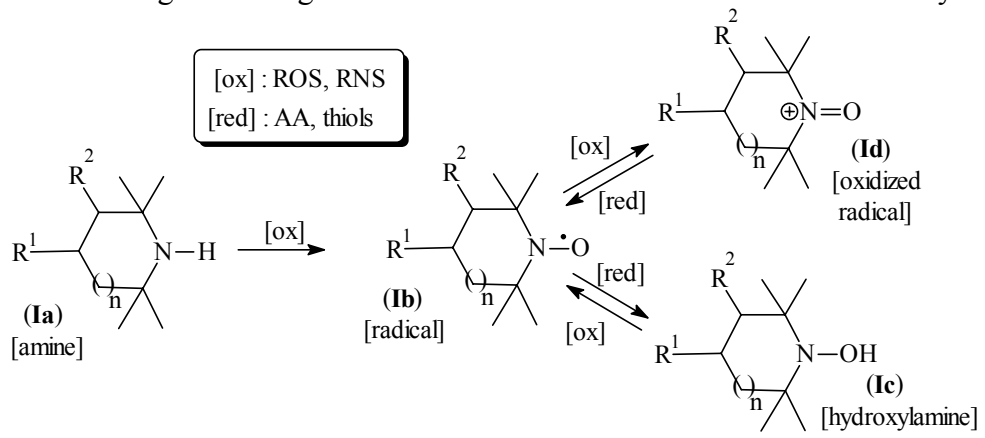
Reactive oxygen and nitrogen species (ROS, RNS) contribute to the ischemia and reperfusion induced tissue injury, and initiate lipid peroxidation, protein oxidation and the formation of single-strand DNA breaks. Single-strand DNA breaks can activate the nuclear poly-ADP-ribose polymerase (PARP) ultimately resulting in depletion of NAD. The inhibition of PARP can improve the recovery of different cells from oxidative damages. PARP inhibitors abrogated the ischemia-reperfusion induced lipid peroxidation and protein oxidation, and significantly decreased ssDNA break formation.

Several PARP inhibitors were synthesized and have shown efficacies in several animal disease models of cancer, ischemia and inflammation. Various 2-substituted-4-carboxamidobenzi-midazoles, mono- and bicyclic carboxamides, bi-, tri- and tetracyclic lactams and some other heterocyclic molecules were proposed as PARP-inhibitors [1]. The sterically hindered amines and non-toxic radicals may offer the exceptional advantage that they can fulfil the function of multi-step protectors in an antioxidant cascade system. The sterically hindered pyrroli(di)ne or piperidine-*N*-oxyl derivatives (Ib) and their amine precursors (Ia) exhibit a protective effect against damages caused by $H_2O_2$ and other ROS; they also exhibit a cardioprotective effect [2]. Molecules I having $R^1$ heterocyclic substituent (e.g. 4-carboxamidobenzimidazolyl-, quinazolyl-group) $R^2$ e.g. alkyl, aryl) with proper affinity to nicotinamide binding pocket of PARP 1 protein make these compounds capable of inhibiting the damages of DNA *via* the inhibition of the PARP activity.

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# 3-AMINO-1H-PYRAZOLE DERIVATIVES AS USEFUL SCAFFOLDS FOR THE GENERATION OF NEW KINASE INHIBITORS

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Sequencing of the human genome has revealed 518 protein kinases (PKs), which can be grouped into about 20 known families on the basis of their structural similarity. PKs are considered the second most important group of drug targets after G-protein-coupled receptors (GPCRs). Aberrant kinase activity is implicated in a variety of human diseases and members of the PK superfamily regulate key aspects of human neoplasia such as tumor cell proliferation, migration and survival .The ability to modulate kinase activity therefore represents an attractive therapeutic strategy for the treatment of human illness, such as cancer.

The vast majority of PKs are characterized by the presence of a highly homologous kinase catalytic domain (of about 250-300 amino acids residues), which folds into two lobes joined by a linker peptide coil of five to six residues, called the hinge region. The adenosine triphosphate (ATP) binding site, the common drugable feature of the kinase class, is situated at the interface of the two lobes.

As a part of our program towards the development of ATP-mimetic kinase inhibitors, we have designed new molecules based on the 3-aminopyrazole moiety, a well known adenino mimetic pharmacophore present in several classes of kinase inhibitors. The $NH_2$-C-N-NH pattern of the 3-aminopyrazole, which is stereochemically well suited to form hydrogen bonding interactions with the kinase hinge region of the ATP pocket, was embedded within the 1,4,5,6-tetrahydropyrrolo[3,4-c]pyrazole (A) and 1,4,5,7-6H-pyrazolo[3,4-c]pyridine (B) bicycles to give novel scaffolds endowed with additional diversity points.

An efficient solid-phase chemistry process, based on a novel linker to allow the attachment of pyrazoles to the resin, was developed for the combinatorial expansion of (A) and (B). Structural Chemistry information, including protein crystallography and computational modelling, was instrumental for a rapid and effective exploration of the high potential of (A) and (B) for the development of protein kinase inhibitors, as exemplified by the rapid identification of potent and selective Aurora and CDK2 inhibitors.

# PROGRESS IN THE TOTAL SYNTHESIS OF POLYCYCLIC NATURAL PRODUCTS

Johann Mulzer

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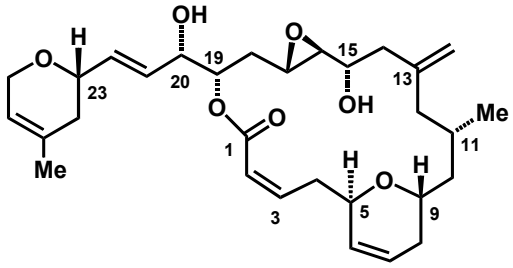
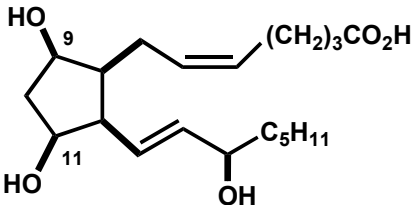
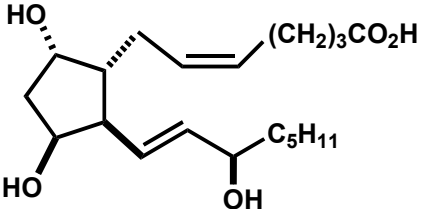
Progress and highlights in the total syntheses of the following natural products will be reported:

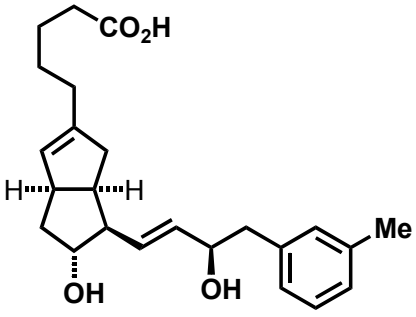



**Laulimalide**




**Isoprostanes**

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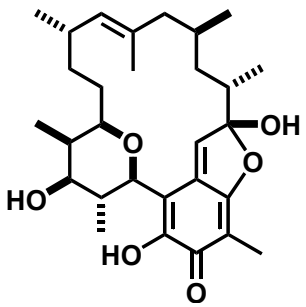


**Kendomycin**

# INHIBITION OF STEROID SULFATASE: A NEW APPROACH TO TREAT ESTROGEN- AND ANDROGEN-DEPENDENT DISEASES

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Steroid sulfatase (STS) catalyses the hydrolysis of the sulfate esters of 3-hydroxy steroids. Among the substrates are dehydroepiandrosterone sulfate and estrone sulfate, which are inactive transport or precursor forms of androgens and estrogens. STS regulates the local production of these hormones within target tissues. Thus, blockade of STS with pharmacological inhibitors could be a means to modulate local hormone levels only, without influencing systemic levels.

Preclinical development of STS inhibitors is most advanced for the indication of breast cancer, and there is increasing evidence that the steroid sulfatase pathway is the major source of estrogens in breast and endometrial tumours. Inhibitors of STS are thus considered potential new therapeutic agents for the treatment of estrogen-dependent cancers, but additional potential indications include androgen-dependent diseases such as prostate cancer, androgenetic alopecia, and acne [1].

In this review, we give a comprehensive summary of the current knowledge and problems in the field of medicinal chemistry of STS inhibitors. The various types of inhibitors are presented and structure-activity relationships are discussed. Until recently the field has been dominated by irreversible, arylsulfamate-based inhibitors, all derived from the lead estrone sulfamate (EMATE), the amido analogue of the natural substrate estrone sulfate. There are two major issues associated with STS inhibitors: potential estrogenicity and chemical stability. With the design of several potent, non-estrogenic inhibitors, the estrogenicity issue appears to be solved. The second issue is the inherent limited chemical stability of arylsulfamates in solution, even though they are stable in bulk. Stable, potent reversible inhibitors should be less problematic for development. However, the design was apparently hampered by the lack of the 3D structure of STS until recently, and the discovery of novel inhibitor types has been limited to (high-throughput) screening. We were successful with HTS to identify an interesting lead structure for further optimisation. Initial SAR of this novel STS inhibitor class will be presented. Besides increasing cellular and in vivo potency, one of the next challenges will be to identify the structural commonalities among the available, structurally diverse inhibitors. The recent publication of the 3D structure of STS is expected to help here and, in general, to further stimulate research in the area of STS inhibitors.

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# NEW DEVELOPMENT IN ALFA-1 ADRENERGIC RECEPTORS ANTAGONISTS

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$\alpha_1$-Adrenoceptors constitute a heterogeneous family of receptors belonging to the superfamily of G-protein coupled receptors. They own therapeutic interest because of their important role in control of blood pressure, contraction and growth of smooth and cardiac muscle.

$\alpha_1$-Adrenoceptors antagonists belong to a various chemical groups thus, quinazolines, phenylalkylamines, piperidines, dihydropirydines, and arylpiperazines. Currently, the arylpiperazines represent one of the most studied class of their antagonists. The typical $\alpha_1$-antagonist contains arylpiperazine moiety connects with one or two additional fragment of molecule. It was found that the protonatable nitrogen atom of piperazine ring play a crucial role in interaction with the receptor site. Moreover, an aryl moiety (preferentially substituted at the *ortho*- position with halogen or alkoxy group) connected to the second nitrogen atom of piperazine is the second structural feature which is needed for interacting with receptor. Finally, the basic nitrogen atom is bound to the spacer which is usually represented by polymetylene chains (substituted or not) with different number of methylene units. It is also important to note that the arylpiperazine and the terminal groups should be located at the proper distance to interact with the receptor counterparts.

In the course of our studies on $\alpha_1$-adrenoceptor antagonists, we designed and synthesized new arylpiperazine derivatives found to have affinity towards $\alpha_1$-adrenoceptors. We have chosen compounds whose common structural features are a substituted phenylpiperazine, the second terminal group is represented by pyrrolidin-2-one bounds to the second piperazine nitrogen atom with substituted or not substituted three methylene units chain. The compounds obtained displayed affinity to $\alpha_1$- and $\alpha_2$-adrenoreceptors, and possessed antiarrhythmic and hypotensive activity. Physicochemical properties such as lipophilicity determined using immobilized artificial membrane stationary phase, as well as calculated log D and pKa values have been evaluated for these new compounds. Preliminary molecular modeling study of new arylpiperazinepropylpyrrolidin-2-one derivatives enable to verify structural requirements of defined pharmacophore model of $\alpha_1$-adrenceptor antagonists.

# THE IDENTIFICATION OF HCV POLYMERASE INHIBITORS: A SHOW CASE OF MODERN DRUG DISCOVERY

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An estimated 3% of the world population (~170 million) suffers from chronic HCV infection that causes a progressive liver disease which can lead to fatal conditions such as cirrhosis or hepatocellular carcinomas. The leading cause for liver transplants in the US results from HCV infection, and some 10,000 deaths occur annually in that country that can be linked directly to the disease. Current therapies for HCV are based on combinations of pegylated interferon (IFN-α) and ribavarin, a broad spectrum antiviral agent. They are sub-optimal for a significant portion of the patient population, who fail to achieve a sustained response (50% in the case of the predominant genotype 1) or can not tolerate the adverse side effects associated with this treatment. Therefore, the discovery of specific antiviral agents would be of great benefit to HCV patients in general. In an effort to discover novel anti-HCV chemotherapeutics, two essential virally-encoded enzymes, NS3 serine protease and NS5B polymerase, were targeted in our drug discovery effort.

We recently reported the discovery of BILN 2061, a macrocyclic tripeptide inhibitor of NS3 protease that effectively reduced HCV RNA plasma levels in a short term study with HCV genotype 1 infected patients. NS5B polymerase inhibitors provide a complementary approach that likewise, offers opportunities for the development of novel HCV therapies. Screening of our corporate collection using a modified NS5B construct allowed the identification of specific benzimidazole 5-carboxamide derivatives originating from a large combinatorial screening library. These compounds were shown to specifically inhibit productive RNA binding to the polymerase through a novel and specific mechanism. NMR techniques were used to confirm interaction of compounds with the polymerase in the absence of RNA, and to determine the solution conformation of a bound inhibitor. Photo-affinity labeling experiments identified a putative binding region for these inhibitors which is located in the upper-thumb domain of the enzyme. The evolution of this class of compounds through a synergistic interaction between medicinal and combinatorial chemists allowed the rapid identification of derivatives which showed activity in a cell-based assay of *subgenomic* HCV RNA replication (replicons). Selection of resistant replicons using these analogs supported the existence of an allosteric binding site for this class of inhibitors. Further developments resulted from successful soaking of compounds into protein crystals and 3D-structure determination of inhibitors bound to NS5B using X-ray crystallography. These experiments confirmed the location of an allosteric binding pocket and provided insight into the mechanism by which this class of compounds inhibits polymerase activity. Using the cell-based replicon system, we also demonstrated that a combination of HCV protease and polymerase inhibitors are complementary in cell culture models of HCV RNA replication in suppressing the emergence of resistant variants, and expand the repository of potential treatments for chronic HCV infection.

# CHEMICAL MICROARRAYS,- TOOLS FOR HIGH THROUGHPUT FRAGMENT-BASED DRUG DISCOVERY

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Fragment based screening is a hot topic in lead discovery[1]. Fragments are compounds with lower molecular complexity and molecular weight (100-300 Da) than traditional screening library members. Fragments with low binding affinity to their drug target are still believed to be good starting points for drug discovery, due to the high 'ligand efficiency' of these compounds. A prerequisite to apply this approach is the availability of reliable and sensitive screening methods. Traditional biophysical methods (e.g. NMR, X-Ray) for the detection of low affinity interactions are not compatible with high throughput and often use artificial conditions. A powerful alternative is the simultaneous screening of ten thousands of compounds with microarray based SPR imaging in a very short time frame using small amounts of protein[2].

This function blind detection method[3] allows the direct analysis of binding events without the need of further reporter systems. Information of the fragment binding mode can be obtained by on array competition studies using compounds with known interaction mode. Key to the success in this area is generation of useful data by smart chemical library design using computational methods and medicinal chemistry knowledge.

This presentation focuses on several successful case studies in different therapeutic areas and shows how this technique in combination with rapid compound analoging as follow-up strategy efficiently accelerates early steps of the lead discovery process.

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# MICROWAVE ASSISTED AND CONVENTIONAL MONO ARYL SUBSTITUTION OF DIAZABICYCLOALKANES BY ENCAPSULATED PALLADIUM COUPLING

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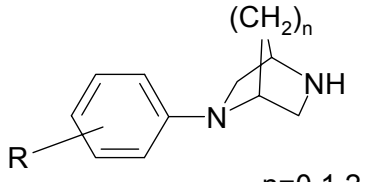
Aryl piperazines are recurring substructures in many pharmaceuticals, such as ligands of serotonin (5-HT) receptors, and key moieties of a variety of biologically active compounds, such as antifungals, antivirals, antibacterials or cholesterol ester transfer protein inhibitors.[1-5] The typical synthetic routes to the mono-aryl substituted piperazines are palladium catalyzed C-N coupling using an excess of piperazine or protected piperazines.




**1**

Our investigation was motivated by the possibility to substitute the piperazine part of several drugs with more rigid structures **1** to get more specific effects.

We will demonstrate the synthesis of substituted aryl diazabicycles of the type **1** utilizing encapsulated Pd catalyzed coupling reactions using protected and unprotected dibasic amines including the use of microwave reactions. The study of new catalysts and different ligand systems for the arylation of diazacycloalkanes still is continuing in our group.

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# APPLICATION OF SIMULTANEOUS pH AND ORGANIC SOLVENT GRADIENT RP HPLC IN DETERMINATION OF PHARMACOKINETICS-AFFECTING PARAMETERS OF DRUGS

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Pharmacokinetics and toxicity (ADMET – absorption, distribution, metabolism, excretion, toxicity) of xenobiotics depend on their $pK_a$ and lipophilicity. Therefore, there is a need for methods allowing fast testing of drug candidates for these properties.

We developed theoretical and experimental basis of determination of compound's $pK_a$ and lipophilicity ($log\ k_w$) employing reversed phase high-performance chromatography (RP HPLC) with simultaneous gradient of *pH* and organic solvent content in mobile phase. Advantages of our approach are that it can be applied to compound mixtures and it requires only minute amounts of substances.

A comprehensive theory of the combined pH/organic modifier gradient has recently been elaborated [1]. According to the theory, the determination of $log\ k_w$ and $pK_a$ consists in a series of gradient runs of programmed changes of pH and/or methanol content in the eluent and of different duration of the gradients. From a set of 18 retention data both the aqueous $pK_a$ value and the $log\ k_w$ value for both the ionized and the nonionized forms of the analyte are determined. A brief description of experimental procedure and computation steps will be given.

Verification of reliability of the acidity and liphophilicity parameters determined with the new method was done for a series of 100 acidic and basic analytes. A good agreement with the literature data is demonstrated.

[1] Wiczling, P., Markuszewski, M.J., Kaliszan, M., Kaliszan, R. Anal. Chem. 77, 449-458, 2005.

# MULTIDISCIPLINARY CHARACTERIZATION OF MOLECULAR INTERACTIONS BETWEEN NICOTINIC ACETYLCHOLINE RECEPTOR AND ITS LIGANDS

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Nicotinic Acetylcholine Receptor (nAChR) is a pentameric ligand gated ion channel found at the neuronal and nerve/muscle synapses. The broad range of various CNS drugs interact *directly* with the neurotransmitter binding sites or *allosterically* on several other binding domains of the receptor modulating its synaptic activity. Fast and reliable methods of measuring and characterization of this modulation is of great interest in CNS pharmacology and drug discovery.

New methods to characterize experimentally and theoretically the interactions between the receptor and various classes of drugs will be presented:

**Bio-affinity chromatography** technique, where the membranes containing nAChR were immobilized on the chromatographic stationary phase, can be used to describe the affinity of small ligands towards the receptor and the kinetics of this process. We used this method in fast screening of the series of constrained nicotine analogs and the method was able to successfully sort out the compounds with pronounced activity on nAChR. High agonistic activities were further confirmed in regular functional assays for these compounds.

Bioaffinity chromatography was also employed to determine the affinity of non-competitive (allosteric) inhibitors (NCIs) towards the receptor. This application is particularly important since other methods are hardly applicable to characterize the strength of binding for this class of ligands. The data collected for the series of non-competitive inhibitors was used to generate QSAR models describing the affinity of ligands towards the internal channel domain of nAChR.

**Computational modeling** techniques were used to elucidate the molecular mechanism of non-competitive inhibition. The inner surface of the nAChR channel is regarded as the most common active site for the binding and the inhibition of the ion flux by NCIs. The molecular model of the channel domain was generated by homology modeling. Molecular docking procedure was elaborated and the series of ligands-active site molecular complexes were developed. Free energies of binding ($\Delta G$) obtained in these simulations were found to be strongly correlated with ligands affinities measured in affinity chromatography experiments.

The results of the computational simulations and QSAR modeling suggested the alternative mechanism of blocking action for non-competitive inhibitors of ion channels. Both experimental procedures and theoretical modeling can be employed for fast screening of pharmacological modulation of nAChR by CNS drugs and new drug candidates. Various subtypes of the receptor are of increasing interest for medicinal chemists as potential drug targets and presented methods of ligand characterization can be used in drug discovery and design.

# 3D-QSAR AND VIRTUAL SCREENING OF PROTEIN-TYROSINE-PHOSPHATASE 1B INHIBITORS

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Insulin resistance in type 2 diabetes is due to a defect in the insulin receptor signalling pathway. The inhibition of protein tyrosine phosphatase (PTP) 1B, the key enzyme that disrupts this signal transduction system and abrogates the antihyperglycaemic effect of insulin, represents an effective target for the treatment of type 2 diabetes.
Beside the detailed investigation available X-ray crystal structures of PTP1B, we examined in our study a set of about 130 known inhibitors of PTP 1B [1]. Starting with the generation of traditional ligand based pharmacophore models with the use of the programs FlexS and GRID/GOLPE we obtained a 3D-QSAR model with significant correlation to the biological activities ($q^2_{L\text{-}20\%\text{-}O} = 0.71$). Subsequently a receptor based model was generated by an automatic docking procedure with an optimised version of the program Autodock. Although the ligand alignment obtained by means of this procedure shows marginal differences compared to the ligand based model, the 3D-QSAR analysis resulted in comparable statistical results ($q^2_{L\text{-}20\%\text{-}O} = 0.76$).
In order to identify new lead structures for the synthesis of novel ligands a virtual screening run of a database of 30,000 compounds that were already screened by *in vitro* methods for inhibitory activity on PTP1B was performed with the aim to compare the results of different *in silico* and in vitro screening methods. The first approach was a three step filtering with FeatureTrees, Autodock and GRID/GOLPE, a combination of ligand based and receptor based methods. Due to the differences in chemical space of the studied inhibitors and the compound database, the hits were filtered out with FeatureTrees, and a prediction with GOLPE was not expected to be successful. In a second, purely target based approach, a diverse subset of 1000 compounds from the original database was analysed by different docking and scoring combinations, using various methods (Autodock, FlexX, GOLD, C-Score and X-Score). Most of the results showed a significant enrichment of active compounds achieving the highest enrichment with the combination of Autodock and X-Score.

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# ADRENOMEDULLIN: A NEW TARGET FOR THE DESIGN AND SYNTHESIS OF DRUGS

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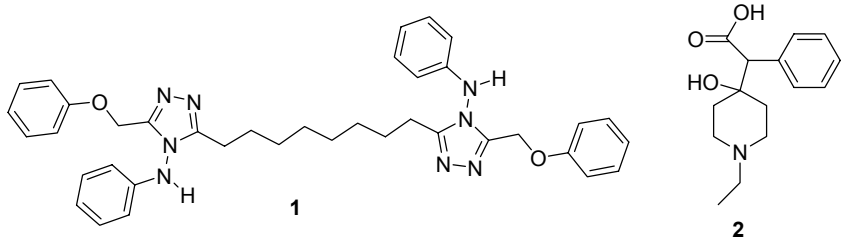
Adrenomedullin (AM) is a biologically active peptide isolated in 1993 from extracts of a human pheochromocytoma cell line, a cancer derived from the adrenal gland and whose function is related to several diseases, such as diabetes, hypertension and cancer.[1] AM levels are dysregulated in many human pathologies such as hypertension, heart failure, sepsis, cancer or diabetes. Several studies have demonstrated that changes in AM levels have opposite effects depending on the particular disease studied. Thus, while AM is a protective agent against cardiovascular disorders, it behaves as a stimulating factor in other pathologies such as cancer and diabetes.[2] Therefore, AM is a new and promising target in the development of molecules which, through their ability to positively or negatively regulate AM activity, could be used in the treatment of these pathologies.

In a recent study of High Throughput Screening for the search of positive and negative modulators of AM, several compounds have been detected, among them positive modulator **1** and negative modulator **2**.[3] Structurally related families of compounds, available from the small molecules NCI library, have been evaluated together with new series which have been synthesized in our laboratory, following a classical approach of molecular modulation. Three-dimensional structure-activity relationship 3D-QSAR analysis techniques, together with conformational and molecular dynamics studies have also been performed, in order to rationalize the chemical aspects required to bind AM, and to scrutinize the anchoring points that compose the binding site. This approach will help us in the further design of new analogues.



**1**



**2**

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# NOVEL HIGHLY POTENT ADENOSINE DEAMINASE INHIBITORS CONTAINING THE PYRAZOLO[3,4-*d*]PYRIMIDINE RING SYSTEM. SYNTHESIS, STRUCTURE-ACTIVITY RELATIONSHIPS AND MOLECULAR MODELING STUDIES

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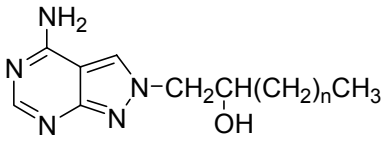
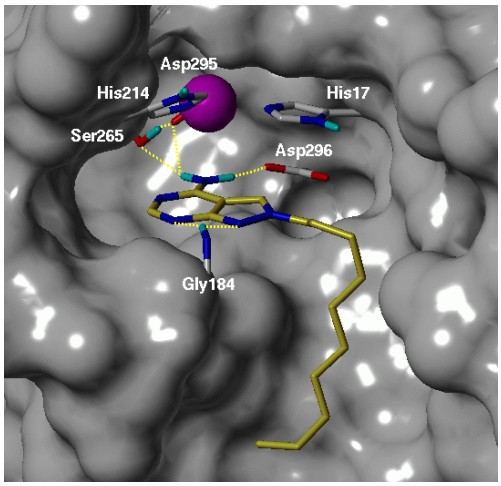
Adenosine deaminase (ADA) is a key enzyme in purine metabolism, playing a central role in controlling the effects of adenosine in a variety of system and a critical function in the development of the immune system. ADA inhibitors represent therefore an useful tool to potentiate the level of endogenous extracellular adenosine as well as an effective pharmacological approach towards the treatment of limphoproliferative disorders [1]. This study reports the synthesis of a number of 1- and 2-alkyl derivatives of the 4-aminopyrazolo[3,4-*d*]pyrimidine (APP) nucleus and their evaluation as inhibitors of ADA from bovine spleen. The 2-substituted APP compounds proved to be potent inhibitors, most of them exhibiting $K_i$ values in the nanomolar/subnanomolar range. In this series, the inhibitory activity enhances with the increasing of the length of the alkyl chain, reaching its maximum with the *n*-decyl substituent. Insertion of a 2'-hydroxy group in the *n*-decyl chain gave 4-amino-2-(β-hydroxydecyl)pyrazolo[3,4-*d*]pyrimidine **1**, whose (*R*)-isomer displayed the highest inhibitory potency of the series ($K_i$ = 0.053), showing an efficacy two order of magnitude higher than that of (+)-EHNA ($K_i$ 1.14) [2]. Thus, docking simulations of the most potent APP inhibitors into the ADA binding site were performed, in order to rationalize the SARs observed and to guide, perspectively, the design of new analogues.




**1** n = 7

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# SYNTHESIS OF PENTACYCLIC ALKALOID HYBRIDES

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Two groups of natural alkaloids – rutaecarpine and its derivates (*Evodia rutaecarpa*) and luotonin A and B (*Peganum nigellastrum*) – exhibit potent antitumor activity by activation of the caspase cascade and inhibition of the topoisomerase I. Both alkaloids are used as components of traditional Chinese folk medicines. *Evodia rutaecarpa* (Chinese name:Wu-Chu-Yu) has been used as a remedy for gastrointestinal disorders (abdominal pain, dysentery), headache, amenorrhea, and postpartum hemorrhage in Chinese medical practice for a long time. Rutaecarpine, a quinazolinocarboline alkaloid is the major component isolated from the fruit of *Evodia* species and has been shown to possess interesting pharmacological activity by having antiplatelet, vasodilator, diuretic, selective COX-2 inhibitor, cytochrome P450 activator properties. The plant *Peganum nigellastrum* is used for the treatment of rheumatism, inflammation and abscesses. Examination of the chemical components of this plant led to isolation of luotonine (A, B, E) alkaloids containing a pyrroloquinazolinoquinoline ring system.
The structural similarity and potent anticancer activity of these alkaloids promted us to synthesize hybrid compounds containing common structural features of rutaecarpine and luotonines.
Two alternative reaction routes have been developed for the synthesis of pentacyclic compounds, in which the key step is the Fischer indolization of the different phenylhydrazono-quinazolone derivatives. Starting from natural tricyclic alkaloid vasicinone the dimethylamino-methylene derivative was prepared by Vilsmeier-Haack reaction using DMF and $POCl_3$. Japp-Klingemann reaction of the product by coupling of different aryldiazonium salts led to the arylhydrazone derivatives of vasicinone. Fischer indolization of the phenylhydrazone has provided 8-nor-rutaecarpine (14-nor-luotonine A).
These bioisosteric analogues are the first representatives of a new heterocyclic ring system, indolopyrroloquinazoline.
In an alternative way indolyl-quinazolone was prepared starting from 2-alkyl-quinazolone via bromination, substitution with phenylhydrazine and indolization. Vilsmeier-Haack reaction of the indolyl-quinazolone and acid catalysed ring closure reaction of the formyl derivatives led to 14-nor-luotonine B(7-hydroxy-8-nor-rutaecarpine).
The 8-nor-rutaecarpines incorporate the structural elements of these two efficient herbal alkaloids, so they are promising lead molecules for our pharmacological researches.
The new compounds are characterized by UV, IR, $^{1}H$, $^{13}C$ NMR, MS spectroscopy.

# NOVEL QUINOLIZIDINYL DERIVATIVES AS ANTIARRHYTHMIC AGENTS

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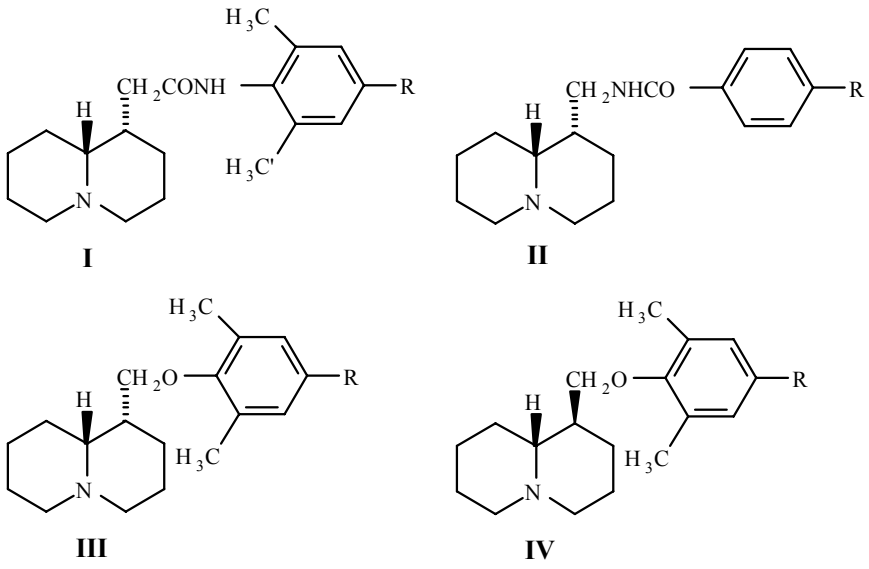
On the pattern of well known N-(dialkylaminoalkyl)anilides, particularly of lidocaine and pilsicainide, a set of N-homolupinanoyl anilides was prepared in the past [1] and found endowed with strong antiarrhythmic activity *in vitro* and *in vivo* tests. These compounds exhibited an unusual pharmacological profile, being devoid of local anaesthetic activity, $Ca^{2+}$ channel and β-adrenergic blocking activities. Since these peculiarities might be connected to the presence of the quinolizidine nucleus, new sets of compounds bearing this nucleus linked to different aromatic moieties (structures **I**-**IV**) have been prepared.




R= H; $NO_2$; $NH_2$; $NHCOCH_3$; $NHSO_2CH_3$; CO-Ar

Compounds of structure **III** and **IV** are somehow related to the 2,6-dimethyl-4-R-phenoxyalkylamines described by Mátyus et al [2], which exerted antiarrhythmic activity through the simultaneous block of $Na^+$ and $K^+$ channels.
The novel compounds are studied using isolated guinea pig dx atria spontaneously beating and Langendorff retrogradely perfused heart. Functional assays on adrenergic receptor subtypes are also performed.

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# *N*-(1,2,3,4-TETRAHYDRONAPHTHALEN-1-YL)-4-ARYL-1-PIPERAZINEALKYLAMIDES AS 5-HT$_7$ RECEPTOR AGENTS

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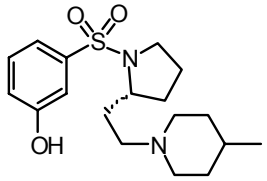
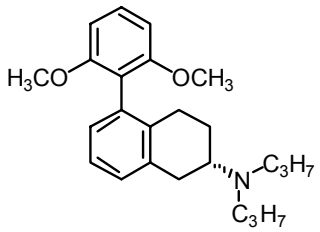
The 5-HT$_7$ receptor (5-HT$_7$R) has been found by the application of molecular cloning and it has been identified in rat, mouse, human, pig, and guinea pig. Although the biological functions of the 5-HT$_7$Rs are poorly understood, preliminary evidence suggests that it may be involved in depression, control of circadian rhythms, and relaxation of vascular smooth muscles. For these reasons the 5-HT$_7$R has become a target for the development of novel drugs. During the last decade considerable research efforts have been directed towards the identification of selective 5-HT$_7$R ligands, allowing the identification of several antagonists, such as SB-269970 (**1**) and a limited number of agonists, as compound **2**. [1]

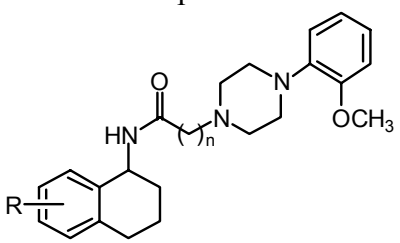



SB-269970 (**1**)




**2**

However, the reported 5-HT$_7$R agents display low potency, modest selectivity, and low metabolic stability. Therefore, the search for selectively-acting 5-HT$_7$R ligands as pharmacological tools or potential drugs is still open. In a recent paper [2], we have described the identification of a series of high affinity 5-HT$_7$R ligands based on the *N*-(1,2,3,4-tetrahydronaphthalen-1-yl)-4-aryl-1-piperazinealkylamide structure **3**. In particular, we observed that all structural modifications introduced on either the 1,2,3,4-tetrahydronaphthalenyl nucleus or on the linker between this particular group and the *N*-(2-methoxyphenyl)piperazine moiety influenced only the 5-HT$_7$R affinity and not the selectivity over 5-HT$_{1A}$ receptor. In contrast, modifications of the aryl group linked to the piperazine ring resulted in major changes in 5-HT$_7$R affinity. For this reason, we have further evaluated other substituents on the arylpiperazine moiety with the aim of modulating the activity on 5-HT$_7$ receptors of these ligands (structure **4**). The outcomes of this research will be presented.

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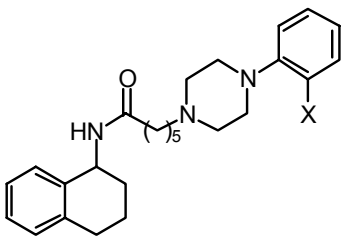


**4**

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# STRUCTURE BASED DESIGN OF CATHEPSIN S INHIBITORS

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Celera, South San Francisco, California, U.S.A.

Inhibitors of the cysteine protease cathepsin S, which is involved in antigen processing, have potential application for the treatment of autoimmune diseases. Additionally, the potent elastinolytic activity of cathepsin S may implicate this protease in atherosclerotic plaque destabilization and other tissue destructive diseases. Utilizing protein X-ray crystal structures and molecular modeling we have designed novel reversible inhibitors with picomolar potency and very high selectivity against the related cathepsins K, L, and B. Further optimization yielded orally bioavailable compounds with good pharmacokinetic profile.

# 8-ALKOXY-7-PHENYLPIPERAZINYLALKYL-PURINE-2,6-DIONES AS 5-$HT_{1A}$/5-$HT_{2A}$ RECEPTOR LIGANDS

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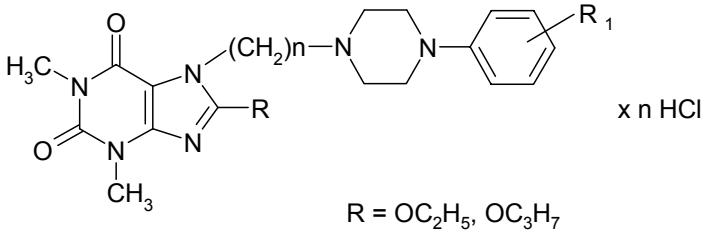
Our chemical and pharmacological studies on a group of the 1,3-dimethyl-8-[3-(4-phenyl-1-piperazinyl)-propylamino]-purine-2,6-dione derivatives with n-alkyl, arylalkyl or ester substituent in the 7-position showed that some compounds with arylalkyl group are selective 5-$HT_{1A}$ ligands ($K_i$ = 8 – 50 nM), with moderate affinity for 5-$HT_{2A}$ receptors ($K_i$ = 300-500 nM) [1, 2]. Several behavioral models demonstrated that these active compounds may be classified as 5-$HT_{1A}$ postsynaptic antagonists [2]. As a continuation of our research on the structure-activity relationship we designed and synthesized a set of new 8-alkoxy-1,3-dimethyl-7-(4-phenyl-1-piperazinyl)-alkyl-purine-2,6-dione derivatives by multi step procedure. The earlier obtained 1,3-dimethyl-7-(3-chloroalkyl)-8-alkoxy-purine-2,6-dione derivatives in the reaction with the appropriate piperazine derivatives yielded final products. The structures of the new compounds were confirmed by examination of their $^1$H-NMR, MS and UV spectra as well as by elemental analyses. For binding studies the free bases were converted into water soluble hydrochloride salts.




R = $OC_2H_5$, $OC_3H_7$
$R_1$ = H, 2-$OCH_3$, 3-Cl
n = 3, 4

The new series is under evaluation for their affinities for 5-$HT_{1A}$ and 5-$HT_{2A}$ receptors by determining their ability to displace [$^3$H]-8-OH-DPAT from the rat hippocampus or [$^3$H]-ketanserin from rat cortex membrane, respectively, according to previously described methods [1, 2].

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# NEW ARYLPIPERAZINYLALKYL DERIVATIVES OF 5,5-DISUBSTITUTED HYDANTOINS AS CNS RECEPTOR LIGANDS*

Anna Czopek[a], Hanna Byrtus[a], Maciej Pawłowski[a], Małgorzata Dybała[b], Gabriel Nowak[b]

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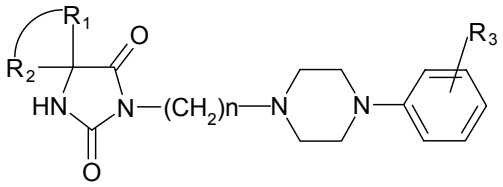
[b]Department of Cytobiology and Histochemistry,
Jagiellonian University Medical College

The important role in recognition of serotonin receptors 5-$HT_{1A}$ and 5-$HT_{2A}$, and stabilization of complex ligand – receptor acts the arylpiperazine ring system. Although the terminal amide moiety meaningfully increases affinity to these receptors, its role is still not fully explain [1].

In our earlier studies, we synthesized both 5,5 – dialkyl, 1',5-cyclohexanespiro- and 1',5-cyclopentanespiro hydantoins connected to phenylpiperazinealkyl moiety, which exhibited high 5-$HT_{1A}$ or 5-$HT_{2A}$ affinity (Ki = 17-33 or 34-37nM, respectively) [1]. In order to increase affinity to serotonin receptors the synthesis of new series of β-tetralonohydantoins were developed. Majority of obtained compounds have high affinity to 5-$HT_{1A}$ or 5-$HT_{2A}$ receptors (Ki < 50 nM) and represent various profile of pharmacological activity [2,3]. As a continuation of our research new derivatives of α-tetralonohydantoin, α-indanohydantoin, 5-cyclopropyl-5-phenylhydantoin and 5-methyl-5-phenylhydantoin were also synthesized. The structure of these compounds was confirmed by $^1$H-NMR spectral data as well as by C, H, N analysis. The purity of compounds was checked by TLC.




$R_3$ = H, $OCH_3$, Cl
n = 3, 4

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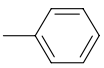
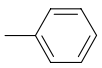
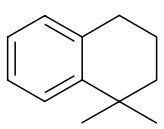
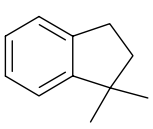
| $R_1$ |  |  |  |  |
| $R_2$ | $-CH_3$ |  | | |

The newly synthesized compounds as soluble in water hydrochlorides have been tested *in vitro* for their 5-$HT_{1A}$ and 5-$HT_{2A}$ receptor affinities. Pharmacological *in vivo* studies directing to 5-$HT_{1A}$ and 5-$HT_{2A}$ receptor activity profile are in progress.

*This study is supported by Polish Ministry of Scientific Research and Information Technology, grant No P22P05F04226.

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# MOLECULAR MODELLING OF SEROTONIN 5-$HT_{1A}$ RECEPTOR USING AUTOMATED DOCKING OF BIOACTIVE COMPOUNDS WITH DEFINED GEOMETRY. NOVEL APPROACH TO GPCR MODELLING

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Molecular model of serotonin 5-$HT_{1A}$ receptor was constructed by homology modelling (MODELLER 7v7) on the template of bovine rhodopsin X-ray structure. 400 conformations of the receptor were produced and evaluated by automated docking (FlexX) of bioactive, conformationally constrained arylpiperazine derivatives. Such inverse virtual screening was based primarly on the occurance of interactions between ligand and most important aminoacids (especially $Asp^{3.32}$) and the interaction energies scored by different scoring functions with concensus scoring (CScore) algorithm. The first step results showed the crucial role of $Asp^{3.32}$ conformation, the most probable binding site and ligand binding mode. The best models were used for docking of wide group of arylpiperazines with different structure and conformational flexibility. Ligand conformation, binding mode and possible interactions with binding site residues were observed and discussed with reference to previous studies in this field.

# ARYLPIPERAZINYLALKYL DERIVATIVES OF IMIDAZO[2,1-F]THEOPHYLLINE AS CNS RECEPTOR LIGANDS*

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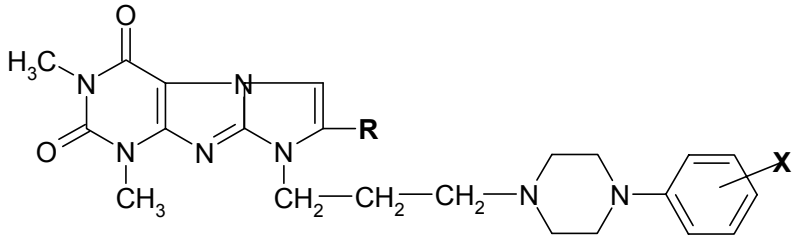
Arylpiperazines with an amide moiety are one of the most frequently investigated classes of 5-$HT_{1A}$/5-$HT_{2A}$ receptor ligands. Although the terminal amide fragment significantly affects binding of 1-arylpiperazine derivatives for serotonin receptors, its role is not clear yet [1]. In our earlier attempt to find new 5-$HT_{1A}$/5-$HT_{2A}$ receptor ligands, a series of arylpiperazinylalkyl derivatives with a complex terminal part based on the theophylline moiety had been synthesized. In the majority of the obtained compounds with pirymido[2,1-f]theophylline fragment, high ($K_i$<50nM) or very high ($K_i$<10nM) 5-$HT_{1A}$ receptor affinity and diversified pharmacological profile were observed[2,3]. The most potent for serotonin receptors were compounds with double bonds at annelated six member ring at 7,8 position of theophylline [4].

On the basis of the above data, we have synthesized the new tricyclic theophylline derivatives with five member ring, with double bond, annelated at 7,8 position and with arylpiperazinylpropyl substituent at N8 position, to study the influence of ring size and the presence of double bond, the kind of the substituent at N4 position of arylpiperazine moiety on serotonin receptors activity.



R: -H, -$CH_3$, -$C_6H_5$

X: -H, 2OMe, 3Cl

The newly synthesized compounds in a form of water-soluble hydrochlorides have been tested *in vitro* for their 5-$HT_{1A}$ and 5-$HT_{2A}$ receptor affinities. Pharmacological *in vivo* studies directing to CNS receptor profile of the synthesized compounds are in progress.

*This study is supported by Polish Ministry of Scientific Research and Information Technology, grant No P22P05F04226.

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# SYNTHESIS, ANTICONVULSANT ACTIVITY AND X-RAY ANALYSIS OF NEW N-(4-ARYLPIPERAZIN-1-YL)-ALKYL-3-SPIROSUCCINIMIDE DERIVATIVES

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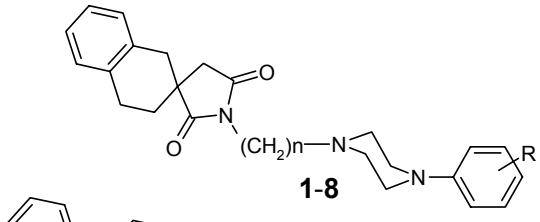
In the course of our earlier research on anticonvulsant activities of 1,3-substituted pyrrolidine-2,5-diones it has been found that introduction of the 4-substituted piperazine at the imide nitrogen atom caused considerable growth of the anti-seizure activity [1, 2]. The second factor essential for the activity is an aromatic ring at a 3-position of pyrrolidine-2,5-dione. On the other hand, it was proved that many of spirosuccinimides exhibited anticonvulsant activity [3]. Based on these findings, in effort to obtained compounds with enhanced anticonvulsant activity the following modifications in the 3-position of pyrrolidine-2,5-dione ring have been performed:

- introduction of aromatic area as a distal fragment with the conformational freedom,
- introduction of rigid tetrahydronaftalene skeleton.

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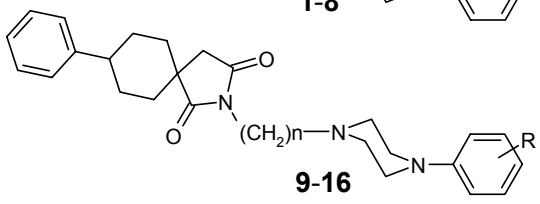


**9-16**

**n=2, 3**
**R = H, 2-$OCH_3$, 3-Cl, 3-$CF_3$**

The preliminary anticonvulsant assay for all the compounds were provided by the Antiepileptic Drug Development (ADD) program using the testing procedures described elsewhere [4]. The $ED_{50}$=26mg/kg for N-[{4-(3-trifluoromethylphenyl)-piperazin -1-yl}-propyl]-3-spiro-β-tetralone-pyrrolidine-2,5-dione was recorded. For all derivatives we determined the lipophilicity by use of the RP-TLC method. For selected compounds the structural characterization by crystal X-ray analysis has been done.

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# THE PACKING MOTIFS IN THE CRYSTALS OF N-SUBSTITUTED SUCCINIMIDES WITH CONFIRMED ANTICONVULSANT ACTIVITY

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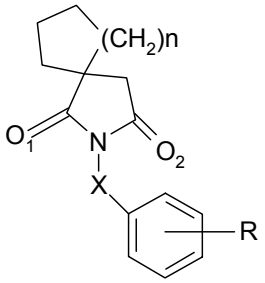


X = $CH_2$, NH
R = H, $CH_3$, Cl, $CF_3$, COOH

In our reaserch programe concerning compounds with confirmed biological activity, we have focused our attention on varius geomerical phenomena, important for ligand – receptor interactions [1]. In this aspect, beside molecule conformation, also intermolecular interactions of the molecules in the crystal should be taken into account. As epilepsy is a civilization disease, recently near 50 millions people in the world have been expecting help. For that reason, systematic search for new effective anticonvulsants has been still in focus of medicinal chemistry. All studied by us new succinimides with potential anticonvulsant activity are the subject of pharmacological and supplemental studies focused on drug design. Taking the above into consideration, we have oriented our recent structural study with new N-substituted-3-spiro-succinimides (see Schemat) to precise analysis of intermolecular interactions in the crystals. We have supposed that it would help us to recognize the connections pattern of succinimides to the respective receptor [2].

In view of one-atomic linker X (NH or $CH_2$), studied compounds have been divided into two groups of derivatives: N-amino and N-benzyl-derivatives respectively. The electronegativity of linking atoms is different. Therefore, the different opportunity of H-bond formation has been evident. Nevertheless, related main packing motif in both groups of derivatives has been identified. Thus, supramolecular synthons (dimmers or chains) are created with participation of linking atom (X = C or N) as the bonds of X - H···O = C. The weaker interactions [C(ph)- H(ph)···O = C] join the synthons into three-dimensional net within the crystals [3, 4].

Carboxyl oxygen atoms not identically participated as proton acceptor in the strongest H-bonds. This has been in agreement with different depths of MEP minimum in proximity of particular oxygens.

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

# 3D-QSAR COMFA STUDY ON ANNELATED XANTHINE DERIVATIVES - ADENOSINE $A_{2A}$ RECEPTOR ANTAGONISTS

Andrzej Fruziński[a], Janina Karolak-Wojciechowska[a], Anna Drabczyńska[b], Christa E. Müller[c] and Katarzyna Kieć-Kononowicz[b]

[a]Institute of General and Ecological Chemistry, Technical University, 90-924 Łódź, Poland;
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[c]Pharmaceutical Institute Poppelsdorf, University of Bonn, D53 115 Bonn, Germany

Adenosine is an endogenous nucleoside that modulates many physiological processes. Its actions are mediated by interaction with specific cell membrane receptors. Four subtypes of adenosine receptors (ARs) have been cloned: $A_1$, $A_{2A}$, $A_{2B}$, and $A_3$. In our research we are looking for ligands of $A_1/A_{2A}$ ARs among annelated oxygen or nitrogen containing xanthine derivatives. So oxazolo-, oxazino-, oxazepino- and imidazo-, pyrimidino- and diazepinopurindiones were synthesized and evaluated for their affinity to $A_1$, $A_{2A}$ ARs [1].
Conventional CoMFA [2] was applied to a series of 18 pharmacologically examined derivatives from different structural patterns in order to find activity trend of the molecules analyzed. SYBYL molecular modeling software [3] was used for structure generation. The molecular structures of the investigated compounds were optimized using the DFT (density functional theory) formalism [4]. The 3-21G basis set and the hybrid functionals B3LYP have been applied [5]. The final molecular electronic properties were calculated in a single point using the restricted Hartree-Fock (RHF) method and 6-31++G basis set [6]. Atomic charges were calculated to fit the electrostatic potential at points selected according to the CHelpG scheme [7].
The atom-based RMS alignment yielded good predictive CoMFA model (r2cv=0.752, r2cnv=0.994, F value=285.689) with six components. The 3D-contour maps generated from the above studies were assessed for the activity trend of the molecules analyzed. The interaction of ligand and receptor is under steric and electrostatic control. The steric (contribution of 79%) field is of greater importance for the predictivity than the electrostatic (21%) one.
CoMFA electrostatic contour map and molecular electrostatic potential map for the Connolly surface have been compared. The correlation found proved to be very interesting. The data generated from the present study can be useful in the design of novel compounds with higher predictive abilities.

Partly supported by Polish State Committee for Scientific Research (Grants No 2 P05F 014 28 and 2 P05F 022 26); Quantum chemical calculations were performed in the ACK CYFRONET Kraków, Poland; support through computational grants 030/1999 and 050/1999 is gratefully acknowledged.

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# TRICYCLIC CYCLOALKYLPURINEDIONES: POTENCY AS ADENOSINE RECEPTOR LIGANDS AND ANTICONVULSANTS

Anna Drabczyńska[a], Christa E. Müller[b], Elżbieta Pękala[a], Britta Schumacher[b], and Katarzyna Kieć-Kononowicz[a]

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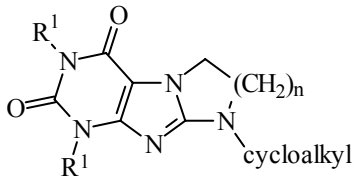
[b] Pharmaceutical Institute Poppelsdorf, University of Bonn, Bonn, Germany

The xanthine alkaloids caffeine and theophylline were the first antagonists for adenosine receptors (AR). During the past 20 years a large number of AR antagonists have been developed including xanthine bi- or tricyclic derivatives.

As a continuation of our studies [1, 2] tricyclic purinediones with cycloalkyl substituents were synthesized and their biological activity was evaluated *in vitro* and *in vivo*.

In order to investigate SARs the lead structure was modified by enlarging of the annelated ring (from 5 to 7 membered), by variation of the cycloalkyl moiety and by elongation of the substituents at the pyrimidinedione ring nitrogen atoms.




n = 1, 2, 3
$R^1$ = methyl, propyl

Compounds were tested *in vitro* for their affinity towards rat $A_1$ and $A_{2A}$ AR, with [$^3$H] CCPA and [$^3$H] MSX-2 as radioligands, respectively. Pyrimidine as the third annelated ring and propyl as substituent $R^1$ were beneficial for high potency at both receptor subtypes.

Cycloalkyl derivatives were also investigated *in vitro* as anticonvulsants. Some of them have shown anticonvulsive protection mainly in chemical seizures (ScMet).

Supported in part by Polish State Committee for Scientific Research (Grant No 2 P05F 022 26)

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# LISOFYLLINE AS A PRODUCT OF *IN VITRO* BIOTRANSFORMATION OF PENTOXIFYLLINE

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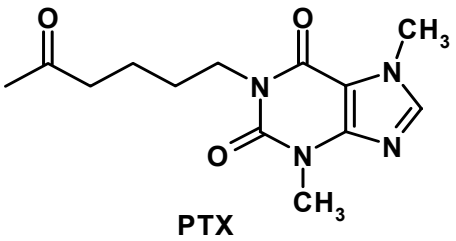
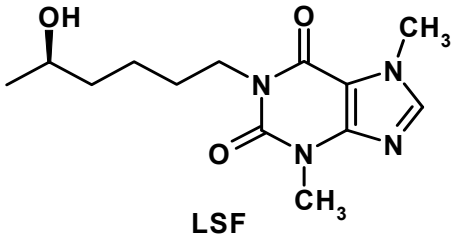
Lisofylline, 1-(5-R-hydroxyhexyl)-3,5-dimethylxanthine (LSF) is a novel methylxanthine with anti-inflammatory properties, that was originally developed to reduce cellular damage due to ischemic reperfusion, hypoxia, or autoimmune diseases. LSF is several hundred-fold more effective than its parent compound, pentoxifylline (PTX), at inhibiting responses to treatment with inflammatory cytokines. LSF inhibits stress-activated lipid metabolism, suppresses the production of inflammatory cytokines, such as interleukin (IL)-12,which exerts these effects through a common lipid intracellular signaling pathway, reduces toxicity and improves patient responses to cancer chemotherapy and radiation therapy. LSF can decrease dysfunction caused by IL-1ß in pancreatic islets, so may have therapeutic value in prevention of autoimmune disorders, including Type 1 diabetes, and autoimmune recurrence following islet transplantation, and in preservation of ß cell functional mass during isolation.




PTX




LSF

LSF can be synthesized in different ways (using chemical or microbial methods) [1-3]. As this agent is not commercially available in Poland we had to obtain it for the pharmacokinetic studies by new methods. One of them was biotransformation of PTX by enantioselective enzymatic reduction using alcohol dehydrogenase from *Lactobacillus kefi*r (LKADH-Fluka or Zyme) with ee = 100% [4].
In another type of experiments LSF was obtained under *Saccharomyces cerevisiae* – mediated reduction of PTX with different yield and enantiomeric excess. Five strains of yeast were used to the microbial conversion of PTX. Reductions were carried in water and in a few organic solvents. The yields of transformations and amounts of each enantiomer formation were examined by means of chiral HPLC.

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# (CYCLO)ALKYL 3-PIPERIDINOPROPYL ETHERS AS HISTAMINE $H_3$ RECEPTOR ANTAGONISTS

Dorota Łażewska[a], Kamil Kuder[a], Holger Stark[b], Walter Schunack[c], Xavier Ligneau[d], Jean-Charles Schwartz[d], and Katarzyna Kieć-Kononowicz[a]

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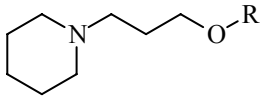
The histamine $H_3$ receptor belongs to the superfamily of G protein-coupled receptors. It has been described as a presynaptically located auto- and heteroreceptor in histaminergic and nonhistaminergic neurones mostly in the central, but also in the peripheral nervous system. Most of the histamine $H_3$ receptor antagonists consist of characteristic structural elements, such as a nitrogen-containig heterocyclic ring, connected with polar moiety by alkyl spacer, itself possibly connected by another spacer with hydrophobic residue [1]. This lipophilic part of compound is believed to be important for its biological activity as well as for pharmacokinetic and toxicological properties. Compounds are expected to act in CNS at logP ≈2 and cross the blood-brain barrier.

As a continuation of our previous works on piperidine derivatives [2, 3] possessing histamine $H_3$ receptor antagonist properties ethers were designed.




**1**

The aim of this work was to synthesize (cyclo)alkyl 3-piperidinopropyl ethers (**1**) in order to study the influence of the lipophilic residue on histamine $H_3$ receptor activity. Compounds were obtained using *N*-piperidinopropan-1-ol as starting material via classical Williamson's synthesis. Microwave oven method was used too with good results.

The novel compounds were evaluated for the histamine $H_3$ receptor activity *in vitro* in a binding assay for the human histamine $H_3$ receptor stably expressed in CHO-K1 cells (or HEK 293) and for *in vivo* activity in the brain after oral administration to mice. The tested compounds possess good antagonist activities at the histamine $H_3$ receptor, some compounds have shown *in vivo* activities in a low mg/kg dosage range as $ED_{50}$ value.

Lipophilicity of this series of compounds has been determined by means of logP values calculated using computer programs: Pallas 3.1, SciLogP, Alchemy, HyperChem. The influence of pH environment on lipophilicity was estimated by prediction of logD.

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# BIOTRANSFORMATION OF PRAZIQUANTEL BY CYTOCHROME C FROM *SACCHAROMYCES CEREVISIAE*

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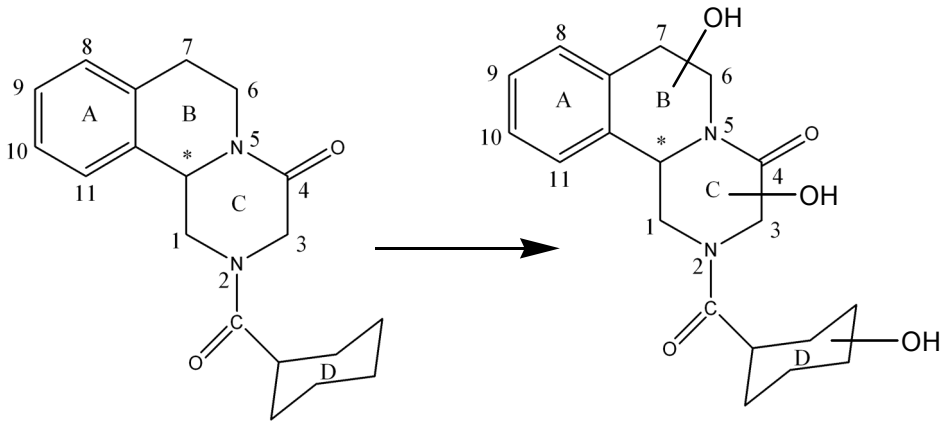
Praziquantel (PZQ) is a drug which is used by choice for schistosomiasis treatment. Currently it is used in malaria treatment. The usefulness of PZQ as antimalarial drug is important because of rapid development of resistance to usually applied drugs. PZQ undergoes extensive metabolism in human body, mainly in liver by two cytochrome P-450 isoenzymes 2B1 and 3A [1]. As the result of these biotransformation numerous mono- and dihydroxylated derivatives in B, C and D ring are formed. One metabolite has been fully identified and described, it is *cis*- and *trans*- 4-hydroxypraziquantel [2]. Up to now were created many different *in vitro* and *in vivo* models of PZQ biotransformations[3].




In our research we have created *in vitro* model of PZQ biotransformation by using of cytochrome c from *Saccharomyces cerevisiae*. This enzyme acts similarly to cytochrome P-450 [4]. We performed three types of experiments. In the first type of experiment we used only cytochrome c, in the second cytochrome c with $H_2O_2$ and in the third cytochrome c with NADP. All experiments were performed in two types of buffer: pH=7,0 and pH=6,0. The reactions were monitored by used of HPLC

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# SYNTHESIS OF 5-SUBSTITUTED HYDANTOINS IN THE MICROWAVE OVEN AND THEIR BIOTRANSFORMATION TO CORRESPONDING NONNATURAL AMINOACIDS USING HYDANTOINASE METHOD

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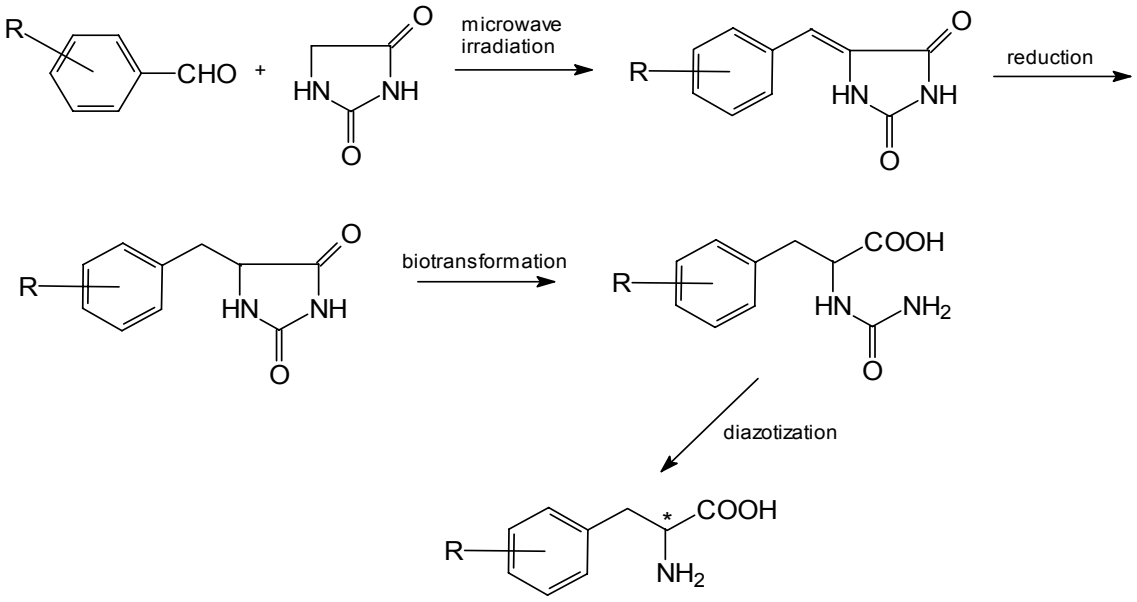
Biocatalyst is a very attractive and profitable alternative to classical organic methods of synthesis. Hydantoinases, commercially available enzymes, are of great value for biotechnological purposes. According to the EC nomenclature hydantoinases are classified as cyclic amidases (EC 3.5.2.). Hydantoinases catalyse the reversible hydrolityc ring cleavage of hydantoin and 5-monosubstituted hydantoin [1]. In the present work we described application of hydantoinase method to obtain enantiomerically pure nonnatural amino acids.




The synthetic approach to obtain substrates for hydantoinases involved condensation of aldehyde and hydantoin in microwave irradiation [2] and next the reduction of double bond in 5-position of the hydantoin-ring [3]. Synthesis under microwave irradiation offers several advantages: easy work-up after the reaction, reduction of the byproducts formed in the usual thermal degradation, better selectivity and short reaction time compared to conventional heating.

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# *IN SILICO* BIOTRANSFORMATION OF BUPRANOLOL

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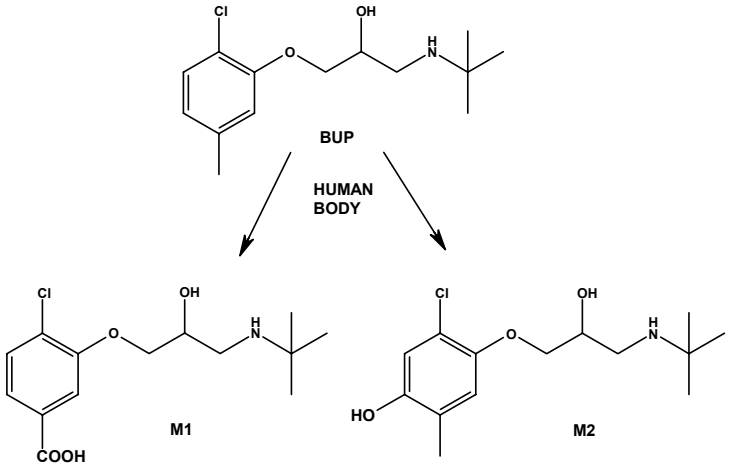
Bupranolol (BUP) is the only registered drug, which demonstrates antagonistic activity against all four known subtypes of β-adrenoceptors. It contains chiral center and its side chain is similar to that of most other β-blockers, except that tertiary butyl group replaces the more common isopropyl group. Because of its high lipophilicity it undergoes extensive metabolism in human liver to more hydrophilic compounds [1,2]. The major pathway of human metabolism is oxidation of the aromatic ring methyl group of bupranolol to carboxyl group (M1) [1]. Another metabolite is formed by hydroxylation of aromatic ring by polymorphic cytochrome P450 CYP2D6 (M2) [2]. Its high affinity to that monooxygenase and rapid metabolism are infrequent combinations in enzymology [2].




The aim of present study was to perform biotransformation of bupranolol *in silico* using available software: Metabol Expert [3], Metasite [4] and Meteor [5]. Additionally biocatalysis/biodegradation on-line database of the University of Minnesota was used to predict microbial biotransformation [6]. The obtained results were compared. Log P values of predicted metabolites were calculated since this factor plays important role as well in distribution and the fate of drugs in the body.

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Research supported by grant CMUJ WŁ 263/P/F

# APPLICATION OF MICELLAR LIQUID CHROMATOGRAPHY (MLC) TO DETERMINE LIPOPHILICITY OF NEW PURINDIONE DERIVATIVES OF POTENTIAL ANTICONVULSANT ACTIVITY

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Lipophilicity or lipid/water partition properties affect most of the processes at the basis of drug action. Therefore, determining lipophilicity parameters of drug candidates is necessary at the early stage of the drug development process. Modern, highly efficient synthesis procedures typically provide large numbers of target compounds. That requires procedures for determining lipophilicity parameters that are rapid and can be used with very small samples. For quantitative comparison of relative lipophilicities of drugs, the most suitable are the logarithms of retention factors corresponding to pure buffer as hypothetical mobile phase, $\log k_w$, obtained by extrapolation of the reversed-phase high-performance liquid chromatography (RP HPLC) retention coefficients, $\log k$, vs. volume fraction of organic modifier in the binary aqueous eluents. Micellar liquid chromatography (MLC) is a kind of RP HPLC which utilises a mobile phase that contains an amount of micelle-forming surfactant above its critical micellar concentration (CMC). The formed micelles have a structure that, contrary to the n-octanol-water or the classic RP HPLC system, contain both hydrophobic and electrostatic interaction sites, thus making them resemble biomembranes more than the classical RP HPLC stationary phase.

A series of 110 new aryl-, alkyl-, arylalkyl- and cykloalkyl-, substituted derivatives of pyrimido-, oxazolo- and diazepino-purindiones of potential anticonvulsant activity showing a large range of lipophilicity were subjected to micellar liquid chromatography (MLC). Three columns were used: a classic hydrocarbon silica *Symmetry* $C_{18}$ (Waters, Milford, MA, USA) column and two monolithic columns: *Chromolith SpeedROD RP-18e* and *Chromolith Performance RP-18e* (both from Merck, Darmstadt, FRG). Sodium dodecyl sulfate (SDS) was employed in concentrations of 0.075, 0.1125 and 0.15 M as a micelle forming agent. The organic modifier was n-propanol used in proportions of 5, 10 and 15 (% v/v) to phosphate buffer in the eluent. The obtained MLC lipophilicity parameters of the analytes were logarithms of retention factors, $\log k$, corresponding to a mobile phase containing a given molar concentration of SDS and volume fraction of n-propanol. For the columns studied relatively good correlations ($r > 0.8$) between $\log k$ and theoretically calculated n-octanol-water partition coefficients, *CLOGP*, of purindiones were found. The $\log k$ values of analytes were also related by the regression equations to their RP HPLC lipophilicity parameters, $\log k_w$. The highest correlation coefficients ($r \sim 0.9$) were observed in the case of *Symmetry* $C_{18}$ column. The results obtained confirm that MLC could be a convenient method of determination of lipophilicity of newly synthesized compounds of potential pharmacological activity.

# TRICYCLIC TRIAZINO- AND TRIAZEPINO[3,4-*f*] PURINEDIONES: NEW ADENOSINE $A_1$ AND $A_{2A}$ RECEPTOR LIGANDS

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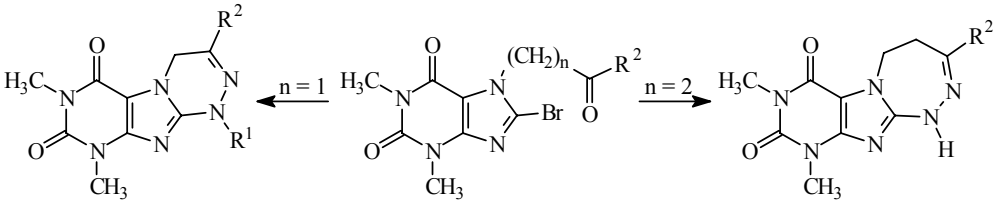
Extracellular adenosine regulates several physiological functions by activation of specific cell membrane receptors. There are adenosine receptor subclasses defined $A_1$, $A_{2A}$, $A_{2B}$ and $A_3$ARs. The most intensively studied subtypes are the high-affinity $A_1$AR and $A_{2A}$AR which adenosine activates in nano- to sub-micromolar concentrations. Since the first reports on the adenosine $A_1$ and $A_{2A}$ receptors appeared, efforts have been made to identify ligands for these receptors and xanthine derivatives have been described to posses high antagonistic activity [1]. As a continuation of our research [2, 3] a series of [1,2,4]triazino- (**I–VIII**) and [1,2,4]triazepino[3,4-*f*]purinediones (**IX, X**) has been synthesised.

Tricyclic theophylline derivatives (**I–X**) were obtained as a result of cyclocondensation of 8-bromotheopylline derivatives (**A**) bearing a keto group at the 7-alkyl substituent and hydrazine, or phenylhydrazine, respectively.




**I - VIII** **A** **IX , X**

| № | $R^1$ | $R^2$ | № | $R^1$ | $R^2$ |
|---|---|---|---|---|---|
| **I** | H | $C_6H_5$ | **VI** | $C_6H_5$ | p-Cl–$C_6H_4$ |
| **II** | H | p-Cl–$C_6H_4$ | **VII** | $C(O)CH_3$ | $C_6H_5$ |
| **III** | H | m-$OCH_3$–$C_6H_4$ | **VIII** | H | p-F–$C_6H_4$ |
| **IV** | H | $CH_3$ | **IX** | | $CH_3$ |
| **V** | $C_6H_5$ | m-$OCH_3$–$C_6H_4$ | **X** | | $C_2H_5$ |

The prepared compounds (**I-X**) were evaluated *in vitro* for adenosine $A_1$ and $A_{2A}$ receptor binding affinities. Adenosine $A_1$ and $A_{2A}$ receptors binding was measured in rat cortical membranes using [$^3$H]CCPA and in rat striatal membranes using [$^3$H]MSX-2 respectively. Amongst them, compounds **I, III, VII** were found to be adenosine $A_1$ receptor ligands; **I, IV, X** showed adenosine $A_{2A}$ receptor affinity.

Molecular modelling and SAR studies of **I–X** were performed to investigate structure–activity relationships using programs CAChe 6.1, HyperChem 7.5 and Alchemy2000.

Supported in part by Polish State Committee for Scientific Research (Grant No 2 P05F 014 28)

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# AFFINITIES FOR $\alpha_1$-AR/5-HT$_{1A}$ RECEPTORS AND WATER SOLUBILITY EVALUATION OF PHENYLPIPERAZINE PHENYTOIN DERIVATIVES

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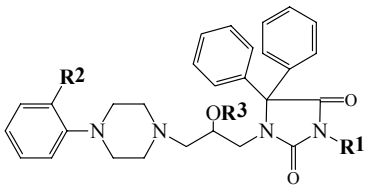
During our previous investigation to search for new antiarrhythmic agents, compound **AZ-99** was obtained (**Fig.1**, **R**$^1$=$C_2H_5$, **R**$^2$=H, **R**$^3$=H) as amine derivative of phenytoin [1].This compound possesses structural similarities to known $\alpha_1$-adrenoceptor antagonists [2] and has shown hypotensive as well as antiarrhythmic activity in rats and significant affinities for $\alpha_1$- and $\alpha_2$-adrenoceptor. Bad water solubility of compound **AZ-99** restricted its pharmacological properties. As chemical modification of this, a series of new compounds were synthesized (**Fig.1**)




**R1= CH 3, CH 2COOCH 3**
**R2=H, OC 2H5, F**
**R3=H, COCH 3**

**Fig.1**

The obtained compounds were evaluated on their affinities for $\alpha_1$- and $\alpha_2$-adrenergic and 5-HT$_{1A}$ serotoninergic receptors in radioligand binding assays. Selected compounds were assessed on their affinity and selectivity for $\alpha_1$-adrenoceptor subtypes in functional bioassays. Water solubility of the compounds was tested experimentally using UV-spectroscopy for evaluation of compounds concentration. Furthermore, theoretical prediction of the water solubility was carried out by the use of different computational methods. Results of theoretical and experimental determination of compounds solubility were compared and an influence of solubility on pharmacological properties was circumscribed and graphically displayed. The most promising compound (**Fig.1**, **R**$^1$=$CH_3$, **R**$^2$=$OC_2H_5$, **R**$^3$=H) with highest affinities for 5-HT$_{1A}$ and $\alpha_1$-adrenergic receptors showed the highest water solubility, too.

Partly supported by grant no. 3P05F 03125.

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# MOLECULAR MODEL OF 5-HT$_7$ RECEPTOR: INTERACTION WITH ARYLPIPERAZINE LIGANDS

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Molecular model of serotonin 5-HT$_7$ receptor was constructed, based on the methodology used earlier for the 5-HT$_{1A}$ modeling [1]. The final model was selected from initial 400 models via inverse virtual screening with the use of high and low affinity compounds. The group of arylpiperazine ligands having different spacer structure was used for further model validation. It was found that conformational flexibility influences the affinity for 5-HT$_7$ and selectivity towards 5-HT$_{1A}$ [2]. The binding mode of arylpiperazine derivatives within the 5-HT$_7$ receptor is proposed. The correlation between experimental binding affinities of these compounds and FlexX docking energies is discussed. In addition, the impact of Asp$^{3.32}$ conformation on the quality of the model is investigated in comparison with analogous residue in 5-HT$_{1A}$.

This study was supported by the research Grant no. 012/2002 from the Polish Pharmacy and Medicine Development Foundation, given by the POLPHARMA Pharmaceutical Works.

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# SYNTHESIS AND SAR STUDIES OF 1,2,3,4-TETRAHYDRO-β-CARBOLINE DERIVATIVES AS NEW 5-$HT_7$/5-$HT_{1A}$ RECEPTOR LIGANDS

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Since its discovery in 1993, the 5-$HT_7$ receptor is gaining increasing interest as a potential drug target. Studies utilizing recently developed selective antagonists revealed that 5-$HT_7$ receptors play a role in thermoregulation, learning and memory, hippocampal activity, sleep, circadian rhythms and mood. Due to a relatively limited number of papers describing structure-activity relationship (SAR) studies of 5-$HT_7$ receptor ligands further research in this field is of particular interest.

It is known that pharmacophoric arylpiperazine fragment is well recognized by 5-$HT_{1A}$, 5-$HT_{2A}$ as well as 5-$HT_7$ receptors. Indeed, 1-(2-methoxyphenyl)piperazine (oMPP) derivatives were among the most active 5-$HT_7$ receptor ligands identified by the screening of our compounds library. In the structure of a few selected compounds oMPP fragment was replaced with a 1,2,3,4-tetrahydroisoquinoline, 1,2,3,4-tetrahydro-β-carboline (THBC) or 9-methylcarbamoylmethyl-THBC moiety. The impact of the applied structural modifications on the 5-$HT_7$ affinity and selectivity for 5-$HT_{1A}$ receptor is discussed. For three selected compounds their functional profile, at both receptors, was determined in electrophysiological experiments. The extracelelural recording of epileptiform activity of hippcocampal CA3 neurons was used. To induce that activity the brain slices were perfused with physiological salt devoid of magesium ions, and the frequency of bursting events was the measured parameter.

This study was supported by the research grant no. 012/2002 from the Polish Pharmacy and Medicine Development Foundation, given by the POLPHARMA Pharmaceutical Works.

# SYNTHESIS AND BIOLOGICAL EVALUATION OF 4-[2-ALKYLTHIO-4(5)-(4-SUBSTITUTEDPHENYL) IMIDAZOLE-5(4)-YL] BENZENESUFONAMIDES AS SELECTIVE COX-2 INHIBITORS

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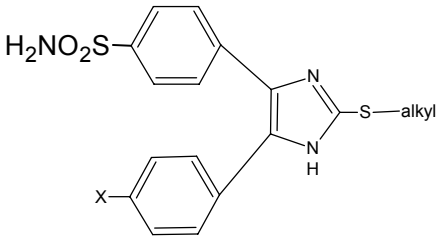
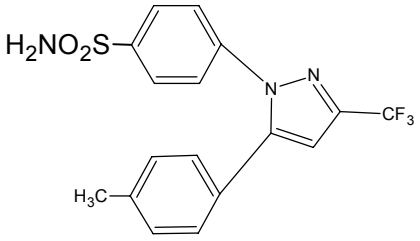
Selective COX-2 inhibitors currently provide effective treatment of inflammatory disease states such as rheumatoid arthritis and osteoarthritis[1,2].Diarylheterocycles constitute a major class of selective COX-2 inhibitors. In this regard celecoxib possesses a central five-membered pyrazole ring [3,4]. Structural-activity relationship (SAR) studies for the diarylheterocycles class have shown that $SO_2NH_2$ pharmacophore and SEt substituents provides optimum COX-2 selectivity and potency [5]. We designed, synthesized and evaluated biologically 4-[2-alkylthio-4 (5)-(4-substitutedphenyl) imidazole-5 (4)-yl] benzenesufonamides as selective COX-2 inhibitors. Conformational analysis and superimposition of energy minima conformers on celecoxib along with biological evaluation provided a good explanation that compounds with high COX-2 inhibitory potency and selectivity can obtain by placement of $SO_2NH_2$ at the *para*-position of one of the phenyl ring, thioalkyl group on the imidazole ring and various substituents at the *para*-position of the other phenyl ring. COX-2 inhibitory potency and selectivity of the synthesized compounds are equal or better than celecoxib as the reference drug.




**Celecoxib**

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# SYNTHESIS OF NOVEL GLYCYRRHIZIC ACID CONJUGATES WITH AMINOACIDS POSSESSING IMMUNOMODULATE AND ANTIVIRAL PROPERTIES

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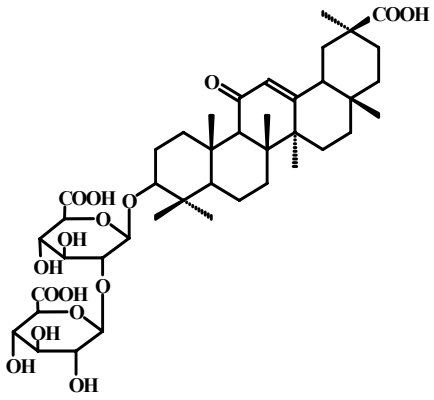
Triterpene glycosides are natural compounds widely distributed in high plants. 18β-Glycyrrhizic Acid (GL) (1), the major component of licorice roots (Gl. uralensis Fisher), is known as an anti-inflammatory, anti-ulcerogenic, anti-allergic, antiviral and γ-interferon stimulating agent [1]. To continue our studies of the structure-activity relationships among GL derivatives and related compounds we synthesized novel compounds to be conjugates of GL with aminoacids (Leu, Val, Pro, Met, Glu, Lys etc.) or their dipeptides. The selective incorporation of aminoacids residues into GL carbohydrate part was carried out by the activated ester method using N-hydroxysuccinimide-N,N'-dicyclohexylcarbodiimide. 18α-stereoisomeric derivatives of some aminoacids and 18α-GL were prepared by using the same method. Target compounds were isolated by column chromatography on silicagel and their structures were established by NMR $^{1}H$ and $^{13}C$ experiments.

Among GL conjugates synthesized stimulators of humoral immune response in mice were found. Some GL derivatives were active against human immunodeficiency virus type 1 and SARS-associated corona virus in vitro.




This work was supported by RFBS and Austria grant 03-03-20004 BNTS_a and grant 1488.2003.03 for scientific schools.

1. Baltina L.A. Curr Med Chem, 2003, 10, 155-171

# DESIGN AND SYNTHESIS OF NOVEL ANTIVIRAL TRITERPENE DERIVATIVES FOR MEDICINE

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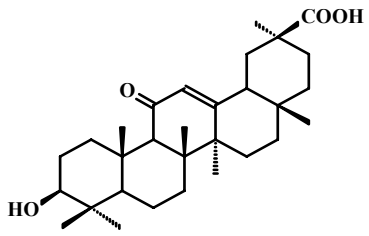
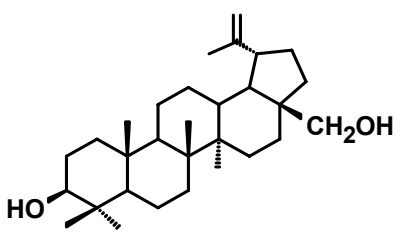
Triterpene compounds of natural origin, Glycyrrhetinic Acid (GLA) (1) to be the major triterpenoid of oleanene type isolated from licorice roots (Gl. uralensis Fisher) and Betulin (BL) (2), the main lupane group triterpenoid of the birch bark of Betula pendula, are of great interest for medicine due to wide range of biological and pharmacological activities (anti-inflammatory, anti-ulcerogenic, antibacterial, hypolipidemic, antitumor, antiviral, etc.) [1]. Using software PASS prediction of biological activity spectra on the basis of structure of compound quantity relations of "structure-antiviral activity" for BL, and related compounds were obtained and used for the chemical design of new bioactive derivatives for structure-antiviral activity relationships studies.

Selective chemical transformations of GLA, BL, allobetulin, betulinic, and betulonic acids were carried out by using simple protocols. Novel groups of nitrogen containing derivatives (amides, ureids, peptides, hydrazides etc.) of GLA and its related compounds (11-desoxo-GLA, 18,19-dehydro-GLA), betulonic and betulinic acids were synthesized. A-nor- and seco-derivatives of allobetulin and 11-deoxo-GLA were synthesized by using dehydrating agents and ozonolysis. Selective oxidative transformations of BL and its derivatives, GLA and related compounds have been studied by using ozone, peracids, dimethyldioxirane, and NaOCl. Lactones and lactames were synthesized on the basis of 3-oxo-derivatives. Structures of novel compounds were confirmed by high resolution NMR spectroscopy.

Among BL, betulonic acid and GLA derivatives produced potent inhibitors of influenza A, herpes simplex type 1, ECHO 6 enterovirus, HIV-1 inhibitors and anti-SARS corona virus active compounds were found.




This work was supported by RFBS and Austria grant 03-03-20004 BNTS_a and grant 1488.2003.03 for leading scientific schools.

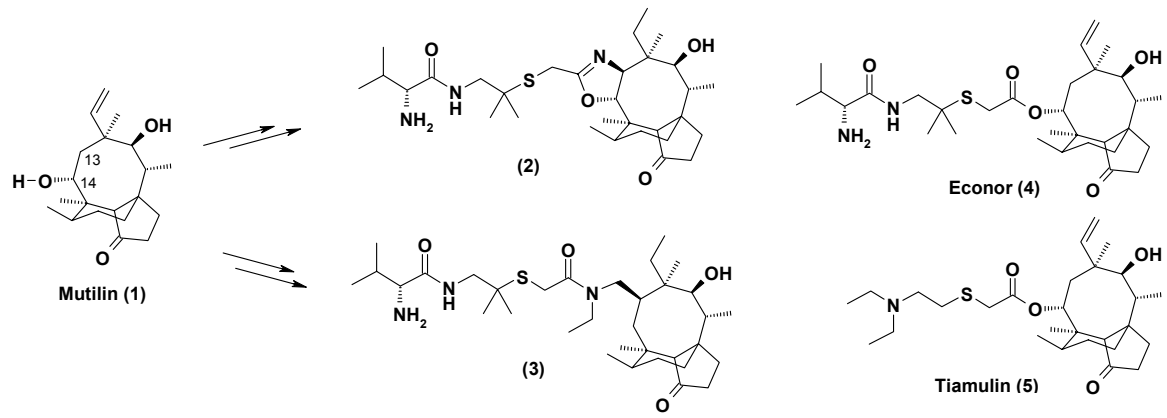
# VIRTUAL DOCKING EXPERIMENTS ON PLEUROMUTILIN DERIVATIVES FUNCTIONALIZED IN POSITION 13

Heinz Berner[a], Rosemarie Mang[a], and Sonja Schindler[a]

[a]Antibiotic Research Institute, Sandoz GmbH, Vienna, Austria

The emergence of multiple resistance in bacteria demands for new antibiotic classes for human application. Pleuromutilins are a class of antibiotics solely used in veterinary medicine so far, containing a tricyclic scaffold with a unique anellation of 5, 6 and 8 membered rings.

Here we present synthesis and docking experiments of two example compounds (2,3) of our research program concerning new substitution patterns at the tricyclic skeleton of pleuromutilin derivatives. These substances represent different orientation or transposition of the C14-side chain of Econor (4) and are realized via a multiple-step synthesis starting from mutilin (1) using different hydride shifts [1].




We performed our virtual docking experiments [2] on the basis of a recently published X-ray structure of the 50S ribosomal subunit of *Deidococcus radiodurans* co-crystallized with Tiamulin (5) [3]. The docking studies elucidate the weak antimicrobial activities of (2) and (3). Alterations in substrate orientation and hydrogen bonding of (2) and (3) at the binding site will be discussed in comparison to their parent compound Econor.

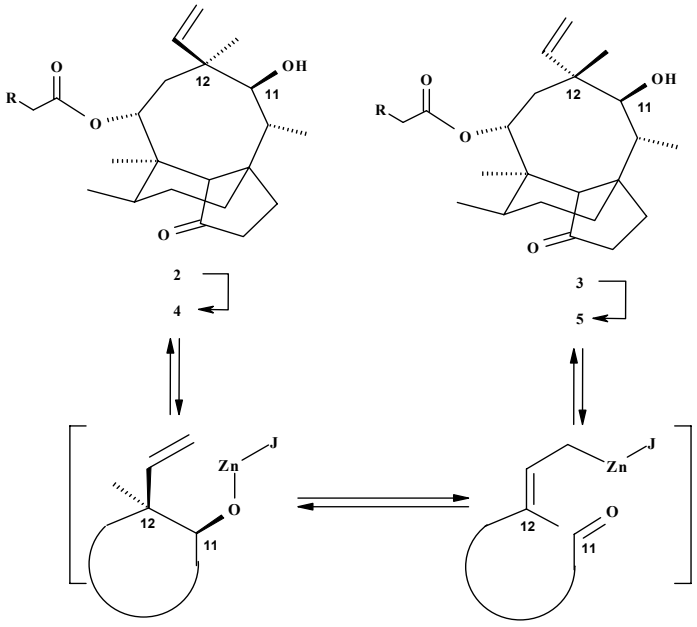
[1] Berner H.; Vyplel, H.; Schulz, G. *Monatshefte für Chemie* **1983**, 114, 501.
[2] MOE Software, Computational Chemistry Group.
[3] Schlünzen, F.; Pyetan, E.; Fucini, P.; Yonath, A.; Harms, J.M. *Molecular Microbiology* **2004**, 54, 1287.

# SELECTIVE C-12 EPIMERIZATION OF THE TRICYCLIC SCAFFOLD OF PLEUROMUTILIN (1)

Rosemarie Mang[a], Sonja Schindler[a] und Heinz Berner[a]

[a] Antibiotic Research Institute, Sandoz GmbH, Vienna, Austria

Investigating the specificity of action within the tricyclic skeleton of pleuromutilin based antibiotics the C-12 Epimer (**5**) of Econor (**4**) [1] was synthesized by a reversible metal-retro-ene-reaction of the homoallylic moiety[2].




**1** R=OH
**2** R=OTos, 12*S*
***3*** R=OTos, 12*R*
**4** R=SC(CH$_3$)$_2$CH$_2$NHCOCH(CH(CH$_3$)$_2$)NH$_2$ (12*S*, ECONOR)
**5** R=SC(CH$_3$)$_2$CH$_2$NHCOCH(CH(CH$_3$)$_2$)NH$_2$ (12*R*, epi-ECONOR)

Treatment of the tosylate **2** with EtZnJ ends up in a equilibrium mixture of compounds **2** and **3** which after separation are transformed to the epimers of Econor (**4** and **5**). Besides of a suggested mechanism for this tranformation the antibiotic activity of **4** and **5** will be discussed based on comperative modelling considerations at the recently published ribosomal active site [3] of this type of substances.

[1] Berner,H.; Vyplel,H. Eur. Pat. EP 153277 A2 19850828
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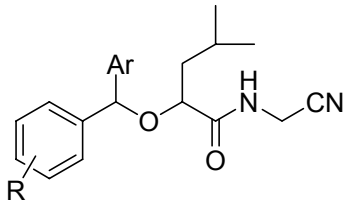
[3] Schluenzen,F.; Pyetan,E.; Fucini,P.; Yonath,A.; Harms,J.M. Molecular Microbiology **2004,** 54, 1287**.**

# DEVELOPMENT OF CATHEPSIN K INHIBITORS FOR THE TREATMENT OF BONE DISORDERS

Cheuk K. Lau[a], Christophe Mellon[a], and Elise Isabel[a], Sylvie Desmarais[b], Jean-Pierre Falgueyret[b], Frederic Massé[b], M. David Percival[b], Gregg Wesolowski[c]

[a]Department of Medicinal Chemistry, [b]Department of Biochemistry, Merck Frosst Centre for Therapeutic Research, Kirkland, Quebec, Canada. [c]Department of Molecular Endocrinology, Merck Research Laboratories, West Point, PA, USA.

A series of potent diarylmethylether cathepsin K inhibitors was developed. Replacement of the P2-P3 amide bond of dipeptide based nitrile inhibitors with a diarylmethylether gave potent and selective cathepsin K inhibitors.



# SUBSTITUTED DIARYLSULFIDES AND THEIR ANTIPLATELET ACTIVITY

Josef Jampílek[a], Martin Doležal[b], Eliška Brojerová[c], Lubomír Opletal[c], and Daniel Jun[d]

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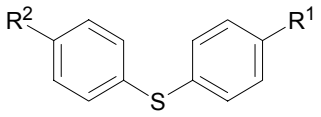
[b]Department of Pharmaceutical Chemistry and Drug Control,
[c]Department of Pharmaceutical Botany and Ecology, Faculty of Pharmacy in Hradec Králové, Charles University in Prague, 500 05 Hradec Králové, Czech Republic
[d]Department of Toxicology, Military Medical Academy, PO BOX 35/T, 500 01 Hradec Králové, Czech Republic

A number of intermediates based on substituted phenylsulfanylphenyl derivatives were obtained when preparing potential antileukotrienic agents [1-3]. These intermediates were tested for their antiplatelet activity as potential cyclooxygenase-I (COX-I) inhibitors.




$R^1$: -CHO, -$COCH_3$, -COOH, -$CH_2COOH$, -$CH(CH_3)COOH$, -$C(CH_3)_2COOH$, -$C(CH_3)(O)CHCOOC_2H_5$, -$C(CH_3)(O)CHCOOH$, -$CH=C(CH_3)COOH$, -$CH_2CH(CH_3)COOH$
$R^2$: -$OCH_3$, -OH

The above-mentioned compounds were evaluated using an *in vitro* antiplatelet assay in human platelet-rich plasma [4]. Arachidonic acid was used as an inductor of the aggregation process. Acetylsalicylic acid was used as a positive control. The results were expressed as $EC_{50}$ values. Some substances showed very interesting activity.

*The project was supported by the Ministry of Education of the Czech Republic (No. LN00B125) and by the League Against Cancer (funded from Terry Fox Run).*

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[2] Jampílek, J.; Doležal, M.; Kuneš, J.; Víchová, P.; Jun, D.; Raich, I.; O´Connor, R.; Clynes, M.: *Curr. Org. Chem.* **2004**, *8*, 1235.
[3] Jampílek, J.; Doležal, M.; Kuneš, J.; Raich, I.; Liška, F.: *Chem. Pap.* **2005**, *59*, in press.
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# DISCOVERY OF NOVEL TETRACYCLIC TETRAHYDROFURAN DERIVATIVES AS POTENT, ORALLY ACTIVE, BROAD SPECTRUM PSYCHOTROPIC AGENTS

José M. Alonso[a], José I. Andrés[a], José M. Cid[a], Adolfo Díaz[a], Laura Iturrino[a], Pilar Gil[a], Anton Megens[b], Victor K. Sipido[b], and Andrés A. Trabanco[a]

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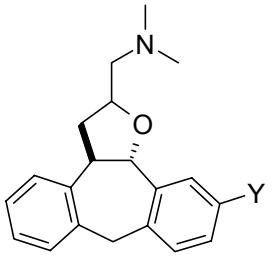
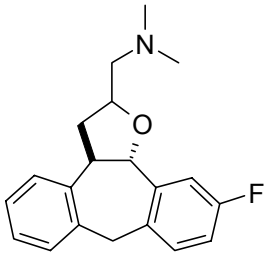
We have recently described a series of tetracyclic tetrahydrofuran derivatives represented by structure **I** [1,2]. These tetracycles proved to have a rich pharmacological profile as it was shown through their interaction with multiple dopaminergic, serotonergic, $\alpha$-adrenergic, and histamine receptors and for the norepinephrine transporter. These binding properties translated into several interesting activities in some vivo assays for antipsychotic, anxiolytic, and antidepressant potential [3].

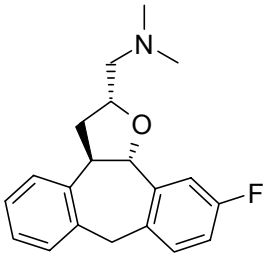



I

[X = O, S; Y = H, halo]




II




III

Herein we will report on the synthesis and pharmacological characterization of new "carbon bridged" analogues represented by formula **II**, which have led to the identification of **III** as a potent orally active broad spectrum psychotropic agent.

[1] Trabanco A. A.; Alonso, J. M.; Andrés, J. I.; Cid, J. M:; Fernández, J.; Iturrino, L.; Megens, A. *Chem. Pharm. Bull.* **2004**, *52*, 262.
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[3] Fernández, J.; Alonso, J. M.; Andrés, J. I., Cid, J. M.; Díaz, A.; Iturrino, L.; Gil, P.; Megens, A.; Sipido, V. K.; Trabanco, A. A. *J. Med. Chem.*, **2005** in press.

# STEROID SULPHATASE INHIBITION: FROM CONCEPT TO CLINICAL TRIAL

L. W. Lawrence Woo,[a] Atul Purohit,[b] Susannah Stanway,[b] R. Charles Coombes,[c] Michael J. Reed,[b] Barry V. L. Potter[a]

[a]Medicinal Chemistry, Department of Pharmacy and Pharmacology & Sterix Ltd, University of Bath, Bath BA2 7AY, UK; [b]Endocrinology and Metabolic Medicine & Sterix Ltd, Imperial College, Faculty of Medicine, St. Mary's Hospital, London W2 1NY, UK; [c]Cancer Research UK Laboratory, Hammersmith Hospital, London, W12 0NN, UK

Breast carcinoma is the most common form of female cancer. Many tumours which are hormone-dependent are treated with anti-oestrogens or aromatase inhibitors. There is strong evidence to suggest that an inhibition of steroid sulphatase (STS), which converts oestrone (E1) sulphate to E1 and also dehydroepiandrostenedione (DHEA) sulphate to DHEA, will further attenuate oestrogenic stimulation in HDBC. E1-3-*O*-sulphamate (EMATE) was the first potent, orally active, irreversible STS inhibitor developed by us a decade ago. However, the unexpected high oestrogenicity of EMATE in rodents prompted us to develop non-oestrogenic agents. A series of bicyclic coumarin sulphamates was produced whose SARs led to the discovery of a series of tricyclic coumarin sulphamates of which STX64 (667COUMATE) was highly promising [1]. In a placental microsomes preparation, STX64 was found to be about 3-fold more potent than EMATE as an active site-directed inhibitor [1]. *In vivo*, STX64 inhibited rat liver STS activity by 93% (single dose, 10mg/kg, p.o.), inhibited E1S-stimulated uterine growth in ovariectomised (ovx) rats and caused regression of E1S-stimulated tumour growth in a NMU-induced mammary tumour model in a dose-dependent manner [2]. More importantly, in contrast to EMATE, STX64 is non-oestrogenic, as shown by its failure to stimulate uterine growth in ovx rats [2]. Toxicological studies in rats performed on STX64 showed no significant or irreversible toxicity. STX64 has an oral bioavailability of 95% in rats [3] which is attributed to its protection from metabolic degradation via sequestration into red blood cells as a result of binding to carbonic anhydrase II [3]. The crystal structure of hCAII bound with STX64 has recently been elucidated [4]. With these favourable toxico-pharmacological and pharmacokinetic parameters, STX64 became the first STS inhibitor to enter clinical trial for treating postmenopausal women with HDBC. Treatment consisted of a test dose of the drug followed by 3 two weekly cycles. Each cycle consisted of daily dosing for 5 days followed by 9 days off treatment. Patients were then followed up for 1 month. To date, 8 patients have been treated at the 5mg dose and 3 at the 20mg dose. For all subjects >90% of STS activity in PBLs was achieved 24h after the single dose was given or on Day 5 of cycle 1. In 3 tumor samples inhibition of STS activity ranged from 78-100%. Reductions in serum androstenediol concentrations of 44-90% were detected in 3/4 subjects studied. So far 4 subjects have shown clinical evidence of stable disease. No serious drug-related adverse effects have been detected to date. These preliminary Phase I trial results demonstrate that STX64 inhibits STS potently in humans. Thus, laboratory- and clinical results are encouraging, validating the concept that inhibiting STS constitutes a novel form of anti-endocrine therapy for the treatment of HDBC.

*This work is supported by Sterix Ltd as a member of the Ipsen Group and clinical studies are coordinated by CRUK.*

[1] Woo et.al. (2000) Chem. Biol., 7: 773; [2] Purohit et.al. (2000) Cancer Res., 60: 3394. [3] Ireson et. al. (2004) Br. J. Cancer 91: 1399. [4] Lloyd et. al. (2005) Biochem. J., 385: 715.

# NEW 5β,6β-EPOXYSTEROIDS WITH CYTOTOXIC ACTIVITY

Vânia M. Moreira[a], Jorge A. R. Salvador[a], M. L. Sá e Melo[b], James R Hanson[c] Andreia Marques[d] and Sérgio Simões[d]

[a]Laboratório de Química Farmacêutica, Faculdade de Farmácia, Universidade de Coimbra, Rua do Norte, 3000-295 Coimbra, Portugal
[b]Centro de Estudos Farmacêuticos, Laboratório de Química Farmacêutica, Faculdade de Farmácia, Universidade de Coimbra, Rua do Norte, 3000-295 Coimbra, Portugal
[c]The School of Chemistry, Physics and Environmental Science, University of Sussex, Brighton, Sussex, BN1 9QJ, UK
[d]Laboratório de Tecnologia Farmacêutica, Faculdade de Farmácia, Universidade de Coimbra, Rua do Norte, 3000-295 Coimbra, Portugal and Centro de Neurociências e Biologia Celular de Coimbra, Departamento de Zoologia, Universidade de Coimbra, 3004-517 Coimbra, Portugal

The synthesis of 5β,6β-epoxides from $\Delta^5$-steroids is a very important reaction since the former functionality is present in a number of biologically active steroids. Thus, natural occurring compounds such as Withaferin A [1], Withanolide D [2], Jaborosalactone A [3] or marine steroids isolated from *C. viridis* [4], all possess 5β,6β-epoxide functions and have demonstrated antitumor activity.
Steroidal 5β,6β-epoxides can easily be obtained from $\Delta^5$-steroids by use of numerous potassium permanganate-metal sulfate and nitrate systems for the oxidation of C-C double bonds [5]. Starting with 16-dehydropregnenolone acetate, new compounds were synthesized as promising antitumoral agents.
Compounds 1 and 2 (Fig. 1) showed good cytotoxic activity against HT-29 colon cancer cells, with an IC50 of 181 and 20 μM respectively, in preliminary studies. Further studies of cytotoxic activity against lung cancer cell lines, such as A549, are currently in progress.

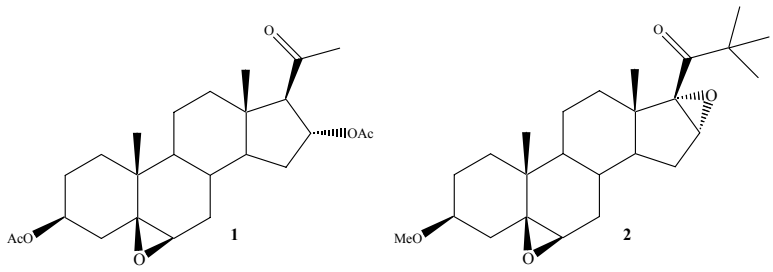


**Fig. 1**

[1] S. M. Kupchan, W. K. Anderson, P- Bollinger, R. W. Doskotch, R. M. Smith, J. A. S. Renauld, H. K. Schnoes, A. L. Burlingame, D. H. Smith, J. Org. Chem., 1969, 34, 3858.

[2] K. Gamoh, M. Hirayama, N. Ikekawa, J. Chem. Soc. Perkin Trans. 1, 1984, 449.

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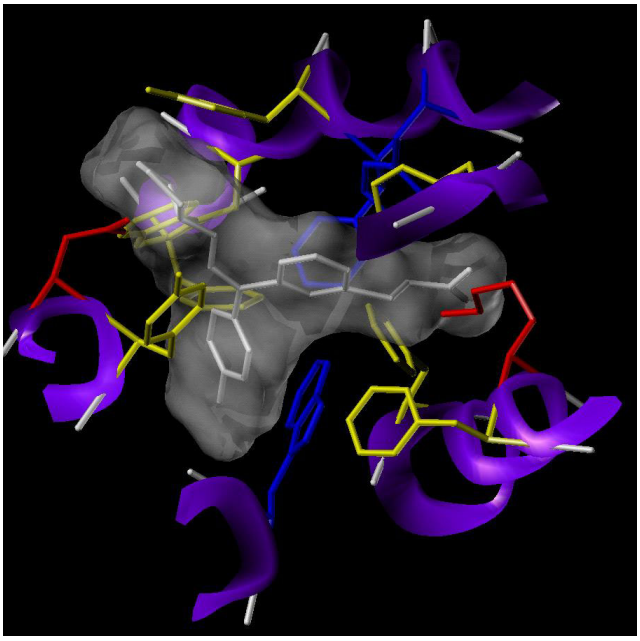
# HOMOLOGY MODELLING AND BINDING SITE MAPPING OF THE HUMAN HISTAMINE H1 RECEPTOR

Róbert Kiss [a], Zoltán Kovári [a,b], and György M. Keserű [a,b]

[a] Department of Computer Assisted Drug Discovery, Gedeon Richter Ltd., H-1475, Budapest 10, P.O. Box 27, Hungary
[b] Department of Chemical Information Technologies, Budapest University of Technology and Economics, H-1111, Budapest, Szt. Gellért tér 4.

Three dimensional model of the human histamine H1 receptor was developed by homology modelling using the high resolution structure of bovine rhodopsin as template. Genetic algorithm based docking calculations were used to identify the role of several amino acids having an effect on agonist or antagonist binding. A reasonable theory for receptor activation mechanism of histamine, based on mutational data is presented. Binding mode analysis of four H1 antagonists (mepyramine, desloratadine, loratadine and acrivastine) allowed us to rationalise their binding affinity. Binding site mapping resulted in seven new potential aromatic interaction points (Tyr 108, Phe 184, Phe 190, Phe 199, Phe 424, Trp 428, Tyr 431), that took part in forming the lipophilic pocket of the antagonist binding cavity.

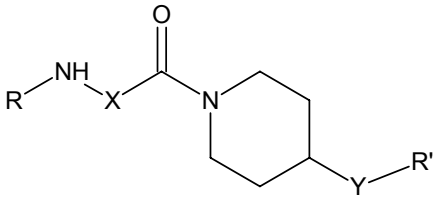


# COMSIA STUDY OF OXAMIDE- AND GLYCINEAMIDE-TYPE NR2B SELECTIVE NMDA RECEPTOR ANTAGONISTS

Ildikó Magdó, István Borza, Gizella Barta-Szalai, Éva Bozó, Anikó Gere, Csilla Horváth, Sándor Farkas, György Domány and György M. Keserű

Gedeon Richter Ltd., Budapest 10. POB 27, H-1475, Hungary

N-methyl-D-aspartate (NMDA) receptor has attracted recently significant interest as a target for the development of central nervous system (CNS) therapeutics. NMDA receptor antagonists have therapeutic potential as neuroprotectants, anticonvulsants and analgesics. The efficacy of NR2B selective NMDA antagonists has been proven in animal models as well as clinical trials. The CNS side-effect profile of NR2B selective compounds appears to be improved compared with non-subtype-selective NMDA antagonists.
A novel series of oxamides and glycineamides of the general formula **1** ( X = C=O, $CH_2$; Y = $CH_2$, O, $CH_2O$; R, R' = substituted aryl) was synthesized and tested in a binding assay using [H3]-Ro 25-6981 as radioligand.




**1**

3D-QSAR Comparative Molecular Similarity Indices Analysis (CoMSIA) was performed during the lead optimization process. In a total 27 oxamide and glycineamide derivatives were used for this CoMSIA study. 20 molecules were used as training set for the 3D-QSAR analysis, 7 randomly selected molecules were kept as test set for external validation of the CoMSIA model.
Regression analysis was performed by partial least square (PLS) method. The optimal number of components was chosen by leave one out (LOO) crossvalidation method on the basis of the highest $q^2$ value. Statistically significant CoMSIA model has been derived with a $q^2$ =0.544 and $r^2$ =0.938. The robustness of this final model was validated with the external test set The predicted $pIC_{50}$ values differ less than 0.7 from the measured ones. This model was then used to predict the activities of further designed compounds.

# CHARACTERISATION OF $Na^+$ CHANNEL BLOCKERS BY A SIMPLE, SPECTROPHOTOMETRIC ASSAY FOR THE DETERMINATION OF GLUTAMATE RELEASE FROM RAT CORTICAL SYNAPTOSOMES

A. Gere, B. Kiss and Zs. Szombathelyi

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A correlation exists between the modulation of $Na^+$-channel activity and endogenous glutamate (GLU) release evoked by depolarization. Until recently, for the in vitro determination of GLU release a very low throughput enzyme-linked fluorometric assay based on the measurement of formation of NADPH in the presence of NADP and glutamate dehydrogenase (GDH) has been used. Here, we describe and validate a simple spectrophotometric method for GLU release from rat cortex synaptosomes suitable for medium throughput purposes.

Synaptosomal GLU release was measured from rat cortical synaptosomal preparation (P2 fraction, 1 mg protein/well) by continuous spectrophotometric monitoring of β-$NADP^+$ (1.2 mM) reduction by GDH (5 Units/well) in 96-well plates incubated at 37°C (incubation volume 200 µl) using plate reader (Labsystems iEMS Reader MF) at 340nm. As depolarizing agents ($Na^+$ channel site 2 agonists) veratridine and aconitine were used. Tetrodotoxin (TTX), crobenetine, lifarizine, riluzole, eperisone, tolperisone, inaperisone, lamotrigine and phenytoin as $Na^+$ blockers were tested.

Increase in absorbance (i.e. GLU release) strongly depended on the synaptosomal protein content in the presence of depolarizing agents: > 0.8 mg protein/well was needed to detect the release of GLU. Basal GLU release was 0.75±1.4 nmol GLU/mg protein/10 min. Veratridine and aconitine caused a concentration-dependent release of endogenous GLU from synaptosomes. Maximal induction was evoked by 20 µM veratridine. Submaximal concentration of veratridine (3 µM) elicited GLU release of 13.5±1.2 nmol GLU/mg protein/10 min. All sodium channel blockers inhibited veratridine (3 µM) evoked GLU release in a concentration-dependent manner. TTX was the most potent ($IC_{50}$=0.022±0.001µM) followed by crobenetine ($IC_{50}$=0.29±0.15 µM). Lifarizine ($IC_{50}$=4.7±0.9 µM), riluzole ($IC_{50}$=8.4±0.9 µM) were active at low micromolar concentration while eperisone ($IC_{50}$=39.3±4.1 µM), tolperisone ($IC_{50}$=41.9±7.7 µM), inaperisone ($IC_{50}$=75.2 µM), lamotrigine ($IC_{50}$=95.5 µM) and phenytoin ($IC_{50}$=178.6 µM) were active in the high micromolar concentrations. A competitive interaction between veratridine and inhibitors such as tolperisone, crobenetine on GLU release was found. A good correlation between the potency of the drugs to inhibit the increase of GLU release induced by veratridine, their binding affinity for the [$^3$H]Batrachotoxin ([$^3$H]BTX) binding in rat cortical synaptosomes and inhibition of veratridine induced intracellular $Ca^{2+}$ elevation measured by $Ca^{2+}$ sensitive fluorescent dye, fluo-4 was observed.

This simple, spectrophotometric 96-well plate technique allowed medium throughput and still accurate determination of the effects of various drugs on depolarization evoked GLU release. There is a close correlation between the inhibitory activity of drugs on veratridine evoked glutamate release and displacement of [$^3$H]BTX binding and inhibition of veratridine evoked increase of intracellular [$Ca^{2+}$] level.

# ACTIVITY *IN VITRO* AND *IN VIVO* OF NEW DERIVATIVES OF ANTHRACYCLINE ANTIBIOTICS

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Anthracycline antibiotics belong to a broad spectrum of antitumor drugs which for a period of almost three decades have been extensively applied for the treatment of patients with different tumor deseases, particularly with acute leukemia or solid tumors. The basic clinical problems connected with using of these antibiotics in therapy appeared to be their toxicity, mainly cardiotoxicity and the drug resistance of tumor cells to these drugs.

In the search for new analogs of anthracycline antibiotics with lower toxicity or/and higher cytotoxic activity, a series of new derivatives with modified amino group in daunosamine moiety have been synthesized. These derivatives revealed similar or higher antiproliferative activity than the parent drugs. Toxicity of these analogs were significantly lower, as shown by the values of $LD_{50}$ being from 2 to 20 fold higher in comparison to the parent antibiotics. The results of determinations of cardiotoxicity, using male mice indicated that cardiotoxicity of the new analogs is also significantly lower than that of the referential antibiotics. Besides, the majority of the new analogs apppeared to be able to overcome, completely or partially, drug resistance of cancer cells.

On the base of these results the analogs of daunorubicin, doxorubicin, epidaunorubicin and epidoxorubicin, containing morpholine ring at the 3' position, were selected to a preliminary *in vivo* screening. The transplantable mouse tumor models such as P388 leukemia, L1210 leukemia, colon carcinoma C38 and breast carcinoma 16/C growing in (BALB/c x DBA/2)F1 or C57B1/6 x DBA/2(BDF1) mice were applied as the models in these studies. The new derivatives and referential antibiotics were administered intraperitoneally. The obtained results have shown that cytotoxic activity *in vivo* of the new analogs of daunorubicin and epidaunorubicin against P388 leukemia were higher then that of the parent drugs *e.g.* the value of ILS (increase in life span) for these derivatives were 49.6% and 33.6% and for the referential antibiotics 36% and 28% respectively. The activity of the new analogs against rectal carcinoma C38 and breast carcinoma 16/C were lower than that of the referential drugs.

It should be pointed out that the new derivatives of anthracycline antibiotics only in some cases revealed the increase of activity *in vivo* but because of lower toxicity and possibility to overcome the drug resistance to cancer cells may appear as valuable drugs in antitumor therapy.

# *IN VITRO* CYTOTOXICITY OF DEXTRAN-METHOTREXATE CONJUGATES IS DEPENDENT ON THE MOLECULAR WEIGHT OF THE CARRIER USED

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**Introduction:** Methotrexate (MTX) is one of the drugs widely utilized in the treatment of oncological and hematological diseases. There were numerous attempts to amend this agent by chemical modifications or conjugations with different carriers. The conjugations of the MTX could prolong the time of the drug activity, therefore improving its antitumor effect. Our aim was to investigate *in vitro* cytotoxicity of MTX coupled with different dextran macromolecules in comparison with free drug. We were also interested in establishing the possible dependency of cytotoxicity on the molecular weight of the carrier.

**Materials and methods:** Five dextran-MTX conjugates were synthesized in our laboratory using dextrans with different molecular weights as the carriers ($T_{10}$, $T_{40}$, $T_{70}$, $T_{110}$ and $T_{500}$ respectively). Conjugation was performed by adding active form of MTX to dextran-containing solution. The stability of prepared conjugates was assessed at different conditions and compounds were stored at -20ºC after lyophilization. All conjugates had approximately the same level of substitution. Free MTX was used as the reference compound. A549 (human non-small cell lung carcinoma), SW707 (human rectal adenocarcinoma) and P388 (murine leukemia) cell lines were used for *in vitro* cytotoxicity assay. Compounds were tested at four consequent concentrations and all tests were repeated three times. The *in vitro* cytotoxic effect of all agents was examined after 72-hour exposure of the cultured cells to varying concentrations of the tested compounds, using the SRB assay and computing average 50% inhibitory concentration ($IC_{50}$) dose as an endpoint.

**Results:** Cytotoxicity studies *in vitro* revealed that all dextran-MTX conjugates had approximately 10-fold higher $IC_{50}$ values in comparison with free drug, therefore had lower cytotoxicity, and this difference was statistically significant. There was also difference in the cell line sensitivity to the free MTX and all conjugates. The P388 cell line was the most sensitive and had approximately 10-fold lower $IC_{50}$ in comparison with respective $IC_{50}$ in A549 and SW707 cell lines. The sensitivity of SW707 was just a slight higher than of A549, though still statistically significant. Further analysis established that there was negative correlation between molecular mass of the dextran used as a carrier and the cytotoxicity of dextran-MTX conjugates *in vitro*.

**Conclusions:** Data of *in vitro* experiments revealed that dextran-MTX conjugates have lower cytotoxicity in comparison with free MTX. The results also showed that there is dependency of the *in vitro* cytotoxicity of dextran-MTX conjugates on the molecular weight of the dextran macromolecule. This fact probably could be explained by different *in vitro* bioavailability of the compounds tested and deserve further investigation. We also consider that *in vivo* tumor models have to be applied to determine whether or not this decreased cytotoxicity *in vitro* would be accompanied by a decrease of an overall toxicity and antitumor activity *in vivo*. These studies are actually undertaken.

# ANTIMICROBIAL ACTIVITY OF *CHELIDONIUM ALKALOIDS* INVESTIGATED BY BIOARENA SYSTEM

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BioArena system, which integrates the up-to-date methodology and biological results of bioautography with TLC, is especially suitable for investigating biochemical interactions in a sorbent bed after chromathographic separation. The antimicrobial effect of *Chelidonium alkaloids* has been investigated in this system.

Greater celandine (*Chelidonium majus* L.), a member of Papaveraceae family, is widely distributed in Europe and Western Asia. This plant is a well known source of benzophenanthridine (chelidonine, sanguinarine and chelerythrine), protoberberine (berberine) and protopine (coptisine) alkaloids. These alkaloids exhibit choleretic, colagogue, spasmolytic, antitumor, antiinflammatory and antimicrobial actions.

Although the antimicrobial activity of *Chelidonium alkaloids* against human bacteria has been reported the mechanism of this action is almost unknown. In this case for bacterial biotest, the phytopathogen *Pseudomonas savastanoi pv. phaseolicola* race 6, causing halo blight on bean, was used. The antibacterial activity of *Chelidonium alkaloids* was visualised by staining of bioautograms with aqueous solution of MTT (3-[4,5-dimethiltiazol-2-yl]-2,5-diphenyltetrazonium bromide). To study the mechanism of action of *Chelidonium alkaloids* co-factor substances, as L-arginine, glutathione and copper(II)-sulphate were added separately to the cell suspension in different concentration before inoculation [1].

An important fact is that there is a relationship between the amount of HCHO-capturing agent and antimicrobial activity of *Chelidonium alkaloids*, that is, larger quantities of L-arginine and glutathione always resulted in a greater decrease in antimicrobial activity. These alkaloids exhibite an antibacterial activity, while their methylated derivates promote cell proliferations under the same conditions. The Cu(II), the oxidized form of copper, an essential trace metal found in all biological systems which enhances the antibacterial activity of HCHO.

It is obvious that BioArena provides more information than conventional bioautography. We can change the incubation time, and may observe the changes on the sorbent layer during 5-6 or more days, using endogenous and/or exogenous substances in culture medium on the antimicrobial action of the separated components.

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# HIT OPTIMISATION AND SAR OF 3-PHENOXYPROPYL PIPERIDINE ANALOGUES AS ORL1 (NOP) RECEPTOR AGONISTS

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For several decades the focus of the field of pain has been to find an analgesic with the efficacy of morphine (μ opioid) but without the adverse side effect profile (e.g. respiratory depression, nausea, constipation, dysphoric effects and abuse potential). The ORL-1 receptor is the most recently discovered member of the opioid receptor family. It purportedly plays an important role in pain transmission, cognition and anxiety and carries a high degree of sequence homology with the other opioid receptors μ, κ and δ, but does not bind typical opioid ligands with high affinity.

Organon therefore initiated a program to develop potent ORL-1 receptor agonists as potential sedative analgesics for perioperative pain following surgery, which could replace the currently used combination of opioids (analgesic) with midazolam (sedative).

High throughput screening followed by hit optimisation led to a lead candidate that bound to the ORL1 receptor. This demonstrated full agonist activity and reversed hyperalgesia in a variety of animal models.

A successful lead optimisation program was initiated focusing on this compound to improve bio-activity (Ki <10 nM), selectivity (>20-fold selectivity over other opioid receptors) and solubility (~2mg/ml).

This poster will cover the hit optimisation phase of the project from screening hit to lead candidate, and will include details of the SAR developed within the 3-phenoxypropyl piperidine series during lead optimsation.

# SYNTHESIS OF NEW AMIODARONE DERIVATIVES

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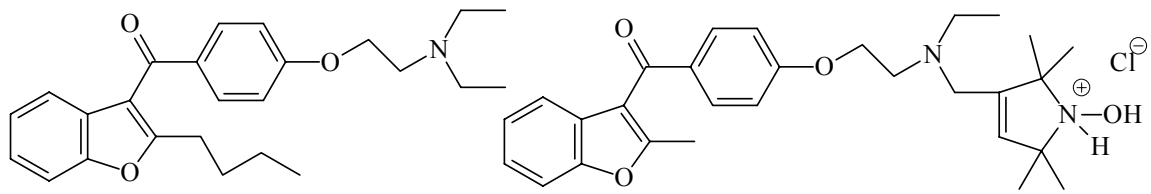
Amiodarone **1**, a widely used antiarrhythmic agent, has a mixed effect on the mitochondrial permeability transition (MPT) in low concentration, however induces mitochondrial swelling when administered in higher doses [1]. To eliminate the side-effects of amiodarone, several structural changes have been attempted [2]. In this work we report the butyl side-chain alteration for methyl and diethylamino side-chain modification with 2,2,5,5-tetramethylpyrroline nitroxide or its hydroxylamine or secondary amine precursor. The latter type of modification was proved to be advantageous previously in the case of class Ib antiarrhythmic agents: H-2545 [3], mexiletine [4]. This idea was confirmed again by the biological effect of compound **2** which inhibited MPT in isolated mitochondria in a concentration-dependent manner with an IC50 value of 4.9 ± 0.5 μM [5]. It also inhibited the release of mitochondrial pro-apoptotic proteins cyt c, AIF and endoG and exhibited antioxidant effect in cultured cell lines.



**1** **2**

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# THE EFFECT OF RADIATION STERILISATION ON DISULFIRAM IN SOLID STATE

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Disulfiram (Antabuse, Esperal, bisdiethylthiocarbamoyl disulphide) is a well-known drug administered in treatment of chronic alcohol disease as a substance producing many unpleasant side effects such as headaches, memory lapses, cardiac and circular system problems, alimentary system problems, allergies and others in response to alcohol consumption. These symptoms appear because disulfiram inhibits alcohol oxidation at the stage of acetate aldehyde, binds alcohol forming strongly toxic ammonium complexes and blocks synthesis of norepinefrine.

Disulfiram is applied in the form of tablets or hypodermal implants. According to the pharmacopoeia recommendations the latter as well as many other forms of drugs (injections, infusions or eye drops) should be sterile. In the 1980s and 1990s the first attempts were made at application of radiation sterilisation. According to literature [1] small doses of radiation < 1 kGy can be used without any risk of degradation of disulfiram.

Results of our recent study performed for the standard doses (10-25 kGy) recommended for radiation sterilisation by European Union Standards (EN 552) do not confirm the earlier reports. As a result of radiation sterilisation disulfiram emits specific unpleasant smell and changes its colour from white to grey-green. To recognise fully the character of these changes a thorough study of disulfiram sterilised *in substantia* has been performed in an extended range of radiation doses from 10 to 100kGy. Sterilisation was carried out in solid state, at room temperature, and at normal air humidity with the electron beam of 9.96 MeV from an accelerator. The irradiation caused changes in the substance were analysed 2 days after the irradiation. All measurements were made simultaneous for the irradiated and non-irradiated substance.

The analyses were performed by a number of instrumental methods including: spectrophotometric (UV, IR, NMR, MS), chromatographic (TLC, HPLC), thermal (TG, DSC), X-ray diffraction (XRD) and other (SEM, EPR).

The results obtained were discussed against a background of literature data and the final conclusion was that disulfiram in solid state is characterised by a too low radiochemical stability to ensure no risk of its degradation on its radiation sterilisation with an electron beam.

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# PREPARATION OF ISOINDOLE-CONDENSED HETEROCYCLES VIA RETRO DIELS-ALDER REACTIONS

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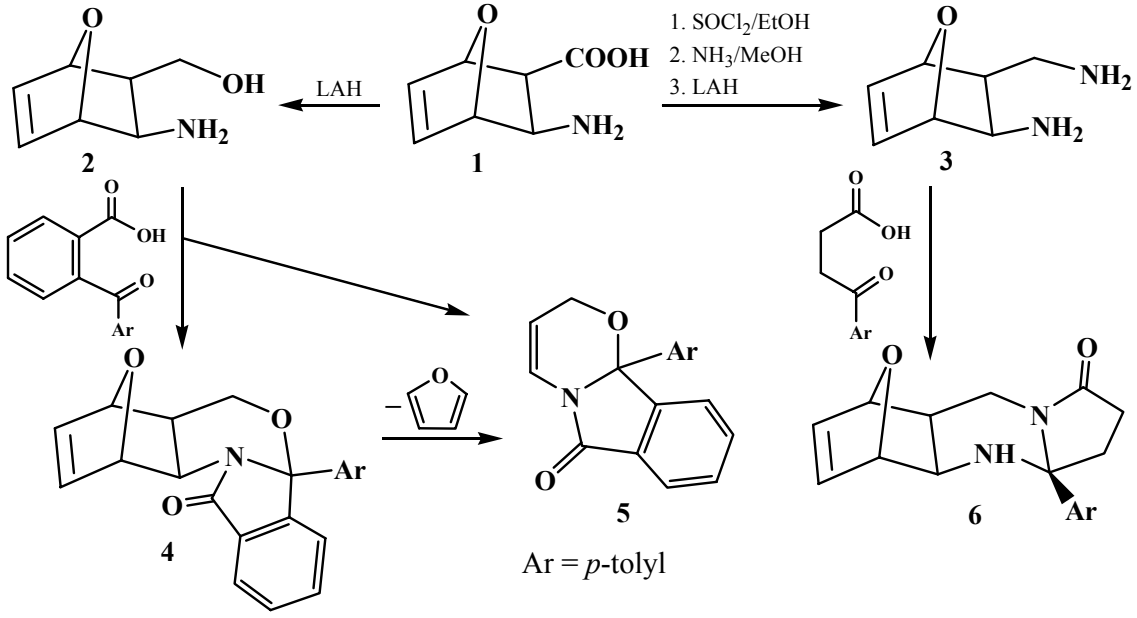
In our earlier work, the cyclization of *diexo*- and *diendo*-3-aminobicyclo[2.2.1]heptane- and -hept-5-ene-2-carboxylic acids with oxocarboxylic acids resulted in pyrimidinediones, thioxopyrimidinones and 1,3-oxazines. On heating, cyclopentadiene (CP) was cleaved off and mono-, bi- and tricyclic heterocompounds were obtained [1].
As a continuation, furane has now been used as a diene which was expected to be removed more easily than CP. Its Diels-Alder adduct with maleic anhydride was transformed to aminoalcohol **2** by ammonolysis, which was followed Hofmann degradation and reduction of β-aminoacid **1**. Diamine **3** was synthesized by esterification of **1,** then ammonolysis and finally LAH-reduction of the carboxamide. Derivatives **2** and **3** were reacted with the oxocarboxylic acids **A** or **B** to obtain tetra-, penta- and heptacyclic compounds, *e.g.* **4-6**. On the basis of known compounds with similar structures, anorectic and HIV-1 reverse transcriptase inhibitor effects are expected [2].




Scheme

From **4**, furane was removed by heating, which resulted in 1,3-oxazinoisoindole **5** in good yield, in a basically new preparation. **6** did not undergo the retro Diels-Alder reaction.
The structures of the new compounds were established by NMR spectroscopy.

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# THE MOST LIKELY BINDING CONFORMATION AND 3D STRUCTURE OF THE BINDING POCKET FOR BENZOXAZINE OXYTOCIN ANTAGONISTS

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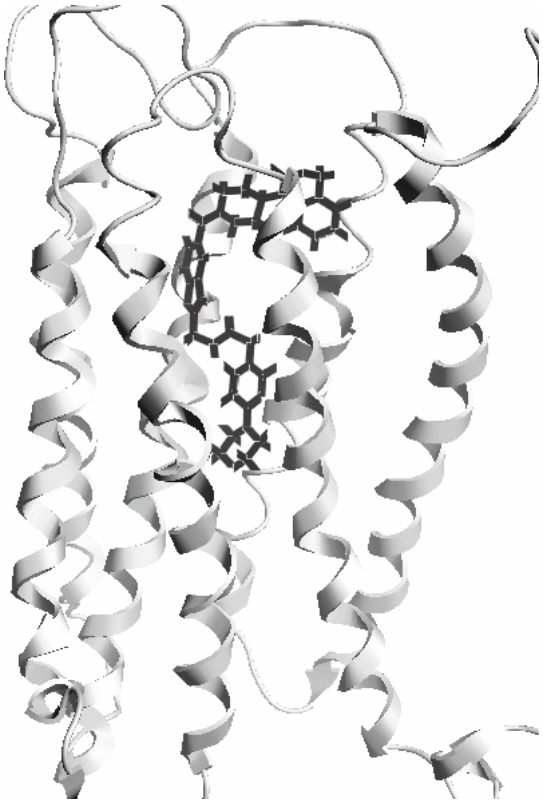
Molecular docking and 3D-QSAR studies were performed to determine the binding mode for a series of benzoxazine oxytocin antagonists taken from the literature [1,2]. Structural hypotheses were generated by docking the most active compound to the rigid receptor by means of AutoDock 3.05. The cluster analysis yielded 7 possible binding conformations.

These structures were refined by using constrained simulated annealing, and the further ligands were aligned in the refined receptor by molecular docking. A good correlation was found between the estimated $\Delta G_{bind}$ and the $pK_i$ values for complex **F**. The Connolly-surface analysis, CoMFA and CoMSIA models ($q^2_{CoMFA} = 0.653$, $q^2_{CoMSIA} = 0.630$ and $r^2_{pred,CoMFA} = 0.852$, $r^2_{pred,CoMSIA} = 0.815$) confirmed the scoring function results. The structural features of the receptor-ligand complex and the CoMFA and CoMSIA fields are in closely connected. These results suggest that receptor-ligand complex **F** is the most likely binding hypothesis for the studied benzoxazine analogs.



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# INVESTIGATION OF CYTOTOXIC ACTIVITY OF NATURALLY OCCURING ACRIDONE ALKALOIDS

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Acridone alkaloids constitute a small group of natural products found exclusively in the family Rutaceae. They are known to exhibit a wide range of pharmacological activities including cytotoxic effect, which are presumed to be exerted by the inhibition of an intracellular enzyme, topoisomerase-II [1].

The aim of the present study was the investigation of cytotoxic effect of 7 furanoacridones (rutacridone, isogravacridone chlorine, gravacridondiol, gravacridontriol, gravacridondiol monomethyl ether and the mixture of gravacridontriol monoglycoside and gravakridondiol monoglycoside) and 2 further akridones (arborinine and evoxanthine) isolated from *Ruta graveolens* L.

Cytotoxic effects were measured *in vitro* on three human cell lines: MCF-7 (breast adenocarcinoma), HeLa (cervix adenocarcinoma), and A431 (skin epidermoid carcinoma).The cytotoxicity of the compounds was measured by the MTT ([3-(4,5-dimethylthiazol-2-yl)2,5-diphenyltetrazolium bromide]) assay [2]. Doxorubicin and cisplatin were used as positive controls. Oil/water partition constants (expressed as log P) were calculated for the furanoacridones with the computer program MOE 2004.03.

Arborinine proved to be an outstandingly potent cytotoxic agent, especially on HeLa cells. Its calculated $IC_{50}$ values are comparable with those of cisplatin. The tested furanoacridones exhibited a wide range of activity ($IC_{50}$ values ranging from 3.02 to over 90 μM). The antiproliferative potency of these compounds and lipid solubility displayed a clear parallelism. Evoxanthine was not effective on any cell line.

In summary, our results indicate that naturally occurring acridone alkaloids, including furanoacridones, may be used as starting structures for the development of novel anticancer agents.

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# PHARMACOLOGICAL EVALUATION OF ORIGINALLY SYNTHESIZED ESTRONE AND ESTRADIOL STEREOISOMERS

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As the structure of the estrogen receptor (ER) ligand binding domain (LBD) determines a well defined cavity for binding each ligand, at the synthesis of new ligands it is necessary to investigate the influence of the functional groups position on the *in vitro* receptor binding ability and *in vivo* effectiveness.

Our present aim was to characterize the *in vitro* ER binding affinity and selectivity, and *in vivo* effectiveness of four estrone and twelve estradiol stereoisomers determined by radioligand binding assay and uterus weight gain measurement. Our molecules were modified estrone and estradiol analogs in the position of C3-, C13-, C16- and C17.

We proved that the ER recognized our molecules, although the differences among the inhibition constant ($K_i$) values reached the three orders of magnitude. The 16α-bromomethyl-estra-1,3,5(10)-triene-3,17β-diol exhibited the highest affinity to ER ($K_i = 2.55 \pm 0.64$ nM), so this compound bound to ER as strong as the endogenous estradiol does. The selectivity ratio (SR) of the compounds showed high divergence depending on the position of the different substituents. Moreover some isomers possessed higher affinity to progesterone or androgen receptor than to ER, although these $K_i$ values (196.1 – 2963 nM) were not comparable to $K_i$ values of the reference molecules (7.24 – 9.15 nM).

Only two compounds evoked significant *in vivo* uterotrophic effect. 16α-Bromomethylestra-1,3,5(10)-triene-3,17β-diol displayed strong, dose-dependent estrogenic effect on the uterine tissue. Surprisingly, the lowest dose (10 μg) of 16β-hydroxymethyl-estradiol induced significant increase in the uterus weight that could not be observed after the administration of the higher doses (30 or 50 μg).

Our results reflect that the stereoisomery possesses great influence on the ligand-receptor interaction, although a comparison can not made routinely between the *in vitro* and *in vivo* results. 16α-Bromomethylestra-1,3,5(10)-triene-3,17β-diol is considered a good ER-ligand with excellent specificity and its *in vivo* efficacy was also acceptable.

# SYNTHESIS OF STEROIDAL HETEROCYCLES BY 1,3-DIPOLAR CYCLOADDITION

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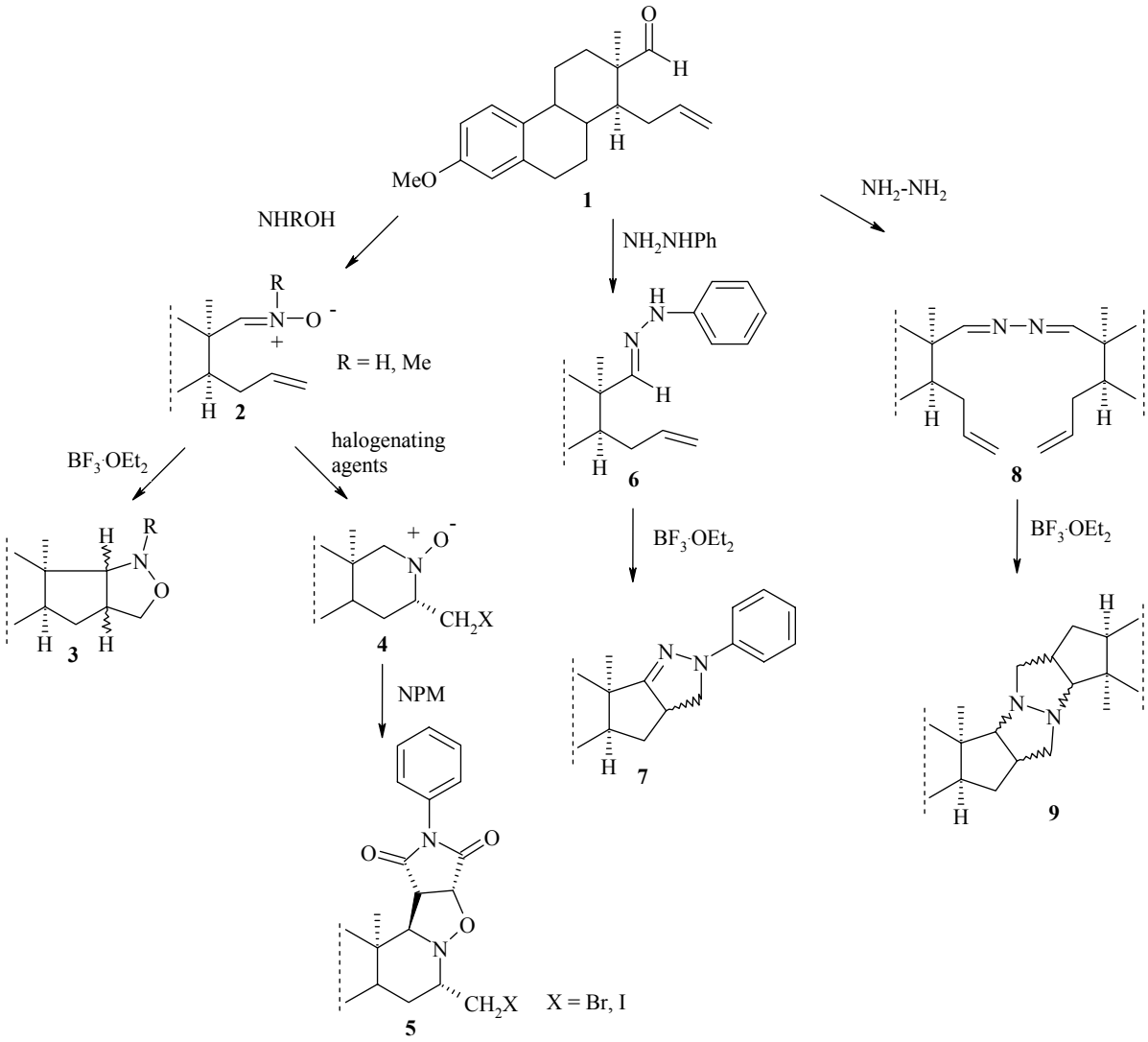
The D-secoaldehyde (**1**) of the 13α-estrone-3-methylether was transformed into dipoles, of which intra- and intermolecular dipolar cycloaddition reactions were investigated. Cyclization of oximes (**2**) with $BF_3.OEt_2$ led to isoxazolidines (**3**) with *cis* ring anellations. The halogen-induced formation of cyclic nitrones (**4**) and their subsequent intermolecular 1,3-dipolar cycloaddition with *N*-phenylmaleimide (NPM) yielded the cycloadducts (**5**) with high chemo- and stereoselectivity. Reactions of the hydrazone (**6**) and the aldazine (**8**) with Lewis acid yielded nitrogen-containing 13α-estrone derivatives (**7**, **9**).



# SYNTHESIS OF 13$\alpha$-DEHIDRO-EPIANDROSTERONE DERIVATIVES BY DOMINO KNOEVENAGEL HETERO DIELS-ALDER REACTION

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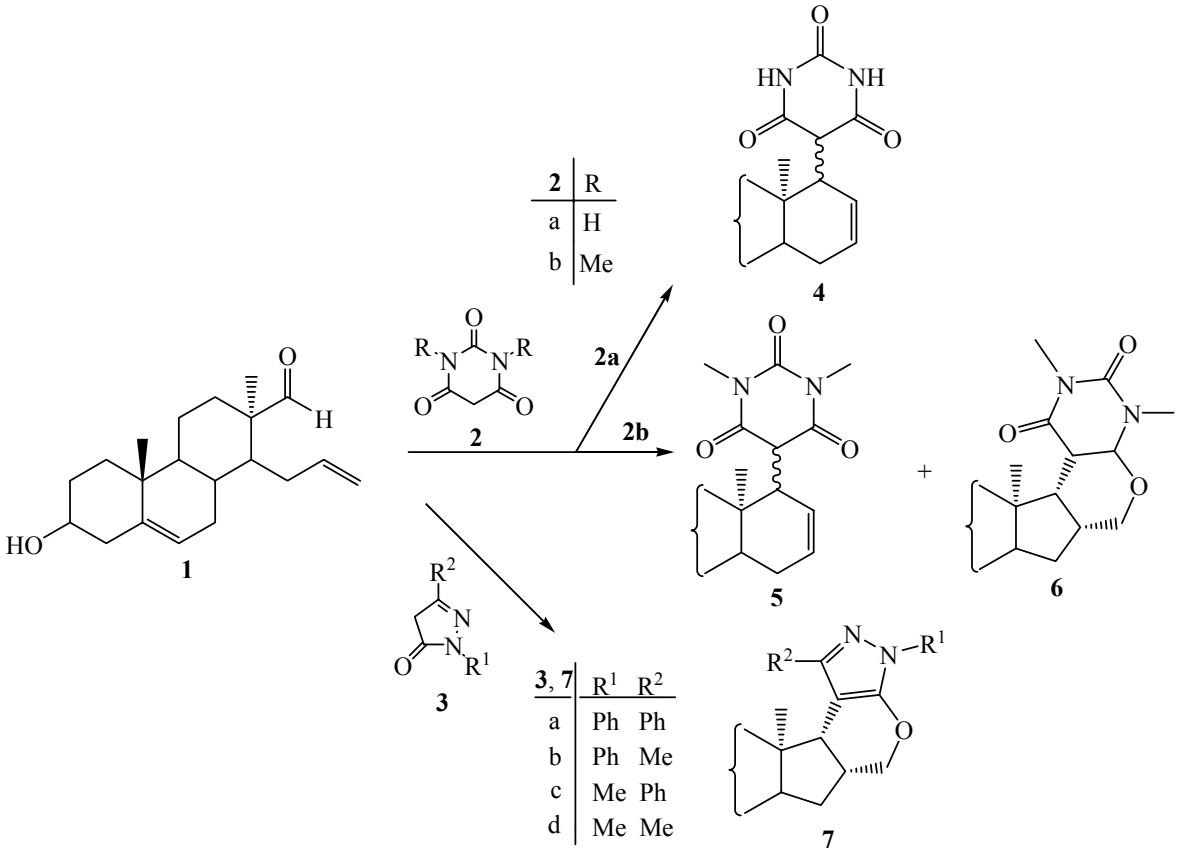
Based on our earlier results [1, 2] we studied the chemo-, regio- and stereoselectivity of the domino Knoevenagel hetero Diels-Alder reaction of a 13α-seco-dehidro-epiandrosterone derivative with different 1,3-dicarbonyl compounds. The reaction of **1** with barbituric acid (**2a**) produced unsaturated 13α-D-homo-dehidro-epiandrosterone derivative (**4**). **1** with 1,3-dimethylbarbituric acid (**2b**) gave unsaturated D-homo (**5**) and 16$\alpha$,17$\alpha$-condensed compound (**6**). In the case of substituted pyrazolone reagents (**3a-d**) we obtained 16$\alpha$,17$\alpha$-condensed compounds (**7a-d**) as main products.

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[2] L. F. Tietze, J. Wölfling, Gy. Schneider, *Chem. Ber*. **124** (1991) 591.

# STEREOSELECTIVE APPROACH TO SOME NOVEL D-DIHOMO-ESTRONE DERIVATIVES IN THE NORMAL SERIES

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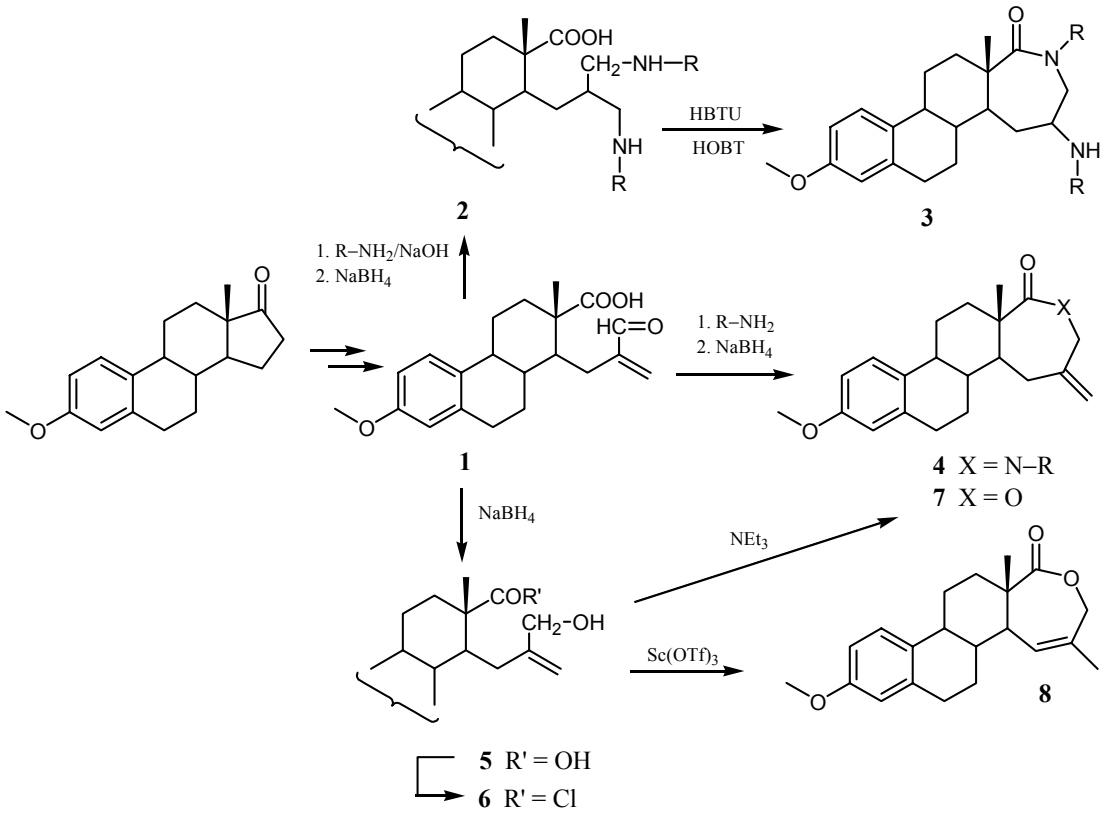
New D-dihomo-heteroestrone derivatives were synthesized from an unsaturated carboxylic estrone secoaldehyde **1**, easily accessible from estrone-3-methylether. Michael-addition and reductive amination of **1** in alkaline solution led to carboxylic diamines **2**, which were cyclized to seven-membered amino-lactams **3**. Only reductive amination and subsequent cyclization could occur to obtain methylidene-lactams **4** when the reaction was carried out without applying sodium hydroxide in the first step. The analogous D-dihomo-lactone **7** was also prepared by the intramolecular substitution of the carboxylic chloride **6** of the corresponding secoalcohol **5** derived from **1** by reduction. At the same time, the direct ring-closure of **5** with scandium(III)-triflate resulted in the unsaturated 16-methyl derivative **8**.



# NEW DERIVATIVES OF ARYLSULFONYLIMIDAZOLIDINE WITH POTENTIAL PHARMACOLOGICAL ACTIVITY

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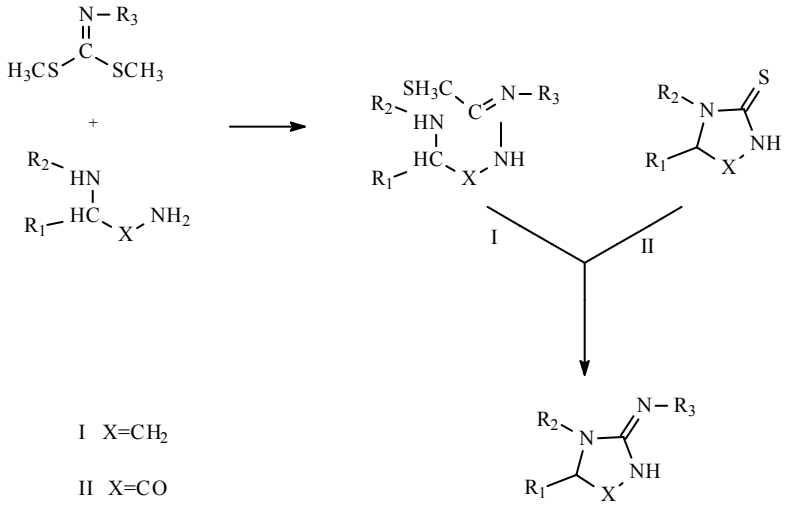
Synthetic derivatives of imidazolidine are an important group of medicines, charcterized by varied pharmacological activity. Some derivatives of arylsulfonylimidazolidne show hypoglycaemic activities [1-3 ].

New cyclic derivatives of 1-arylguanidine were tested for their activity as potential hypoglyceamic agents in type II diabetes. Among them TES 6, TES7 revealed stronger hypoglyceamic activity them Tolbutamide in mice with diabetes induced by single injection of Streptozocin (2000mg/kg; i.p) [ ].

In the synthesis of the required compounds we applied two methods according to the scheme 1.1-arylsulfonyl-2-hydrazonoimidazolidine-4-one [4,5] were obtained in the reaction of 1-arylsulfonyl-2-tioxoimidazolidine-4-ones with 50% solution of hydrazine hydrate. 1-alkyl-2-arylsulfonylimino-5-arylimidazolidines were synthesized by condensation of dimethyl N-arylsulfonyliminodithioic acid esters with respective 1-alkyl-2-arylethylene-diamines.

Scheme 1:

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# OPIOID ACTIVITY OF NEW CARBONYL DERIVATIVES OF 1-ARYL-2-AMINOIMIDAZOLINE-2

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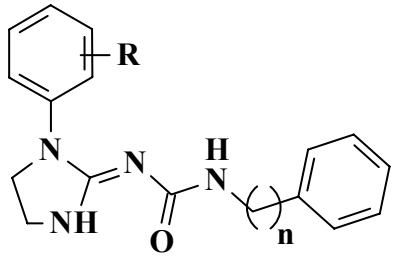
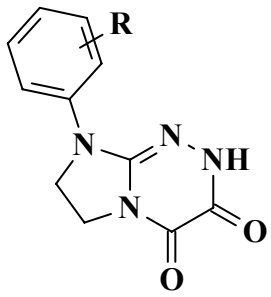
The carbonyl derivatives of 1-aryl-2-aminoimidazoline-2 both chain and fused structure were found to have significant antinociceptive activity connected with activation of the MOP (mu opioid protein) receptor [1-3]. The analyze of their structures allowed formation of non-classical MOP agonist pharmacophor [1,3]. Recent results on the chain (imidazole-2-yl)urea (**A**) and imidazo[2,1-c][1,2,4]triazine (**B**) [4] derivatives are implementing the model with new features, which have to be taken under consideration in planning further synthesis.




**n = 1,2**

**A**




**B**

Series **A** compounds exhibited significant antinociceptive activity in the "writhing test", reversed by small dose (5 mg $kg^{-1}$ i.p.) of naloxon. Antinociceptive activity of series **B** compounds depended on the substituent in the N8-phenyl ring location and the tautomeric keto-enol equilibrium shift toward the 3-oxo form. Compounds existing predominantly in 3-hydroxy form do not show any antinociceptive activity and their serotonergic activity is strongly increased.

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[3] D. Matosiuk, S. Fidecka, L. Antkiewicz-Michaluk, I. Dybała, A. E. Kozioł, *Eur. J. Med. Chem.* **2002**, 37 845.
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# COMPARISON OF E099-25011 AND ITS RIGID OR STERICLY CROWDED ANALOGUES STRUCTURES IN VIEW OF THEIR PHARMACOLOGICAL ACTIVITY

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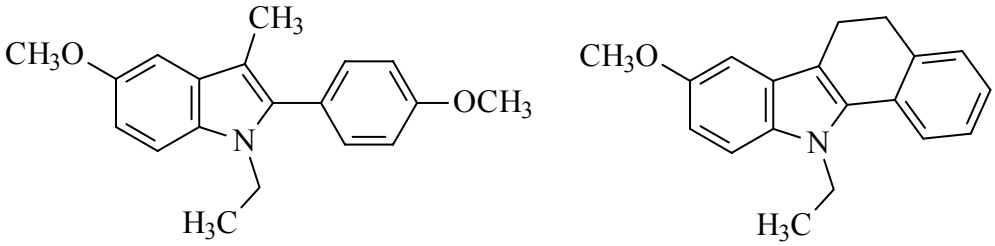
Glutamate is one of the most important excitatory neurotransmitters in the central nervous systems and plays a significant role in the pathophysiology of different neurological and psychiatric diseases [1,2]. This presentation deals with the structural studies (X-ray analysis and theoretical calculation) of the non-competitive GluR5/GluR6 antagonist E099-25011 [1-ethyl-2-(methoxyphenyl)-3-methyl-5-methoxy-indole] found in a HTS and its analogues having rigidly bonded or stericly hindered C-2 pharmacophoric aromatic substituent.




E099-25011

Basic structural and conformational information obtained from X-ray investigations were used to explain the dramatic changes in the glutamate receptor GluR5/6 affinity with the changes of the spatial location of the C-2 substituent. The molecular modeling studies using molecular mechanic method and MNDO-AM1 approximation were undertaken to investigate the conformational preferences of searched derivatives.

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# THE ANTITUMOR EFFECTS OF 3'-*C*-METHYLADENOSINE MEDIATED BY INHIBITION OF RIBONUCLEOTIDE REDUCTASE

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Several *C*-branched nucleoside analogues emerged as promising therapeutical agents. As a part of our continued effort to identify nucleoside inhibitors of target enzymes in DNA/RNA biosynthesis as potential chemotherapeutic compounds, we have examined the antitumor activity of a series of adenosine derivatives substituted at the 1'-, 2'-, and 3'-*C*-position of the ribose ring with a methyl group. From this study 3'-*C*-methyladenosine (3'-Me-Ado) emerged as the most active compound showing activity against human leukemia and carcinoma cell lines. Structure-activity relationships studies showed that the structure of 3'-Me-Ado is crucial for the activity. The replacement of a hydrogen atom of $N^6$-amino group with small alkyl or cycloalkyl groups, the introduction of a chlorine in the 2-position of purine ring, or moving the methyl group from the ribose 3'-position to 1'- or 2'-positions brought about a decrease or loss of activity. The pronounced antiproliferative activity of 3'-Me-Ado, which makes this compound particularly interesting, appears to be related to its ability to deplete both intracellular purine and pyrimidine deoxynucleotides through ribonucleotide reductase (RR) inhibition.

# STRUCTURE-AFFINITY RELATIONSHIPS OF 5'-CARBAMOYL- AND 5'-THIOCARBAMOYL DERIVATIVES OF THE $A_1$ SELECTIVE ADENOSINE RECEPTOR AGONIST 2'-ME-CCPA AS PARTIAL $A_1$ AGONISTS

Loredana Cappellacci[a], Palmarisa Franchetti[a], Patrizia Vita[a], Riccardo Petrelli[a], Michela Pasqualini[a], Barbara Costa[b], Claudia Martini[b], Antonio Lavecchia[c], Karl-Norbert Klotz[d], Mario Grifantini[a]

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The $A_1$ adenosine receptor ($A_1$AR) agonists have many potential therapeutic applications. The receptor subtype is widely distributed in the body and in the heart is found in higher density in supraventricular tissues than in the ventricles. Many agonists with high affinity and efficacy for the $A_1$ receptor subtype have been investigated and selective agonists for the $A_1$AR have been developed for their potential use for the treatment of cardiovascular diseases. However, the ubiquitous distribution and wide range of physiological actions mediated by $A_1$AR are obstacles to development of $A_1$ agonists as therapeutic agents. In this respect, partial agonists that may produce less receptor desensitisation and exhibit fewer side effects may be advantageous for certain indications.

It is known that the substitution of the 5'-hydroxyl group of adenosine with different groups, such as ethoxy, phenyloxy, alkylthio, phenylthio, alkylamino, carbamate or thionocarbamate, induces partial agonism at $A_1$AR. In this communication we describe a series of 5'-carbamates and 5'-thionocarbamates of 2-chloro-2'-*C*-methyl-$N^6$-cyclopentyl-adenosine (2'-Me-CCPA), a potent and highly selective $A_1$ agonist at the bovine and human receptors [1]. The new compounds were tested in radioligand binding assays at bovine and pig receptors and compared with similar derivatives of the CVT-510 ($N^6$-[(*R*)-3-tetrahydrofuranyl]adenosine, Tecadenoson) an $A_1$ agonist that is currently being developed for the potential control of rapid heart rate during atrial arrhythmias. Some compounds proved to be partial agonists with good affinity and selectivity for pig $A_1$AR. Interestingly, $N^6$-[(*R*)-3-tetrahydrofuranyl]-derivatives of adenosine such as Tecadenoson and its 2-chloro- and 2'-*C*-methyl-analogues showed species selectivity having high affinity for pig $A_1$AR and low affinity for bovine receptor. This selectivity is the opposite of the selectivity displayed by 2'-Me-CCPA. Preliminary molecular modeling studies to explain the species selectivity will be reported.

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# 2-PHENYL[1,2,3]TRIAZOLO[1,2-*a*][1,2,4]BENZOTRIAZIN-1-ONE DERIVATIVES AS A NEW CLASS OF ADENOSINE RECEPTOR ANTAGONISTS.

Primofiore G.[a], Da Settimo F.[a], Taliani S. [a], Sergianni V. [a], Simorini F. [a], La Motta C.[a], Marini A.M. [a], Tuscano D.[b], Martini C.[b], Greco G.[c], Cosimelli B.[c], Novellino E.[c]

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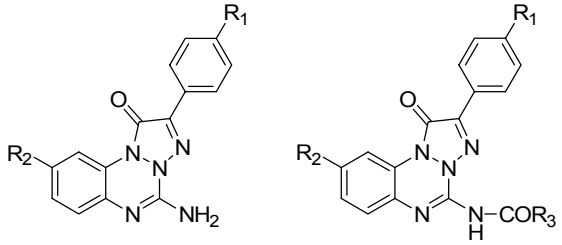
A wide variety of physiological functions on central nervous, cardiovascular, immune, and hormonal systems are modulated by adenosine. This ubiquitary molecule also inhibits lipolysis, platelet aggregation, and neurotransmitter release from nerve endings and potentiates histamine release from mast cells. Adenosine modulates these physiological functions acting via at least four specific cell surface receptors (ARs), classified as $A_1$, $A_{2A}$, $A_{2B}$, and $A_3$.

Selective antagonists at $A_1$ receptors have demonstrated promising therapeutic potential for the treatment of cognitive disease, renal failure, Alzheimer's disease, and cardiac arrhythmias; $A_{2A}$ antagonists could find applications in Parkinson's disease, Huntington's chorea, and myasthenic syndromes. $A_{2B}$ selective antagonists may prove useful in the treatment of asthma, and $A_3$ selective antagonists could act as antiasthmatic, cerebroprotective, and antiinflammatory agents [1-4].

In the present study a number of 2-(4-substituted-phenyl)-[1,2,3]triazolo[1,2-*a*][1,2,4]benzotriazin-1-one derivatives **1** and **2** were synthesized and tested for their affinity at $A_1$, $A_{2A}$ and $A_3$ adenosine receptors.




**1** **2**

The preparation of compounds **1** was accomplished by cyclization with cianogen bromide in absolute methanol of the appropriate 1-(2-amino-5-substituted-phenyl)-4-(4-substituted-phenyl)-[1,2,3]triazol-5-ones [5]. The reaction of the 5-amino group of compounds **1** with the appropriately substituted phenylisocyanates or benzoyl chlorides gave the uretane or the amide derivatives **2**, respectively. Careful examination of the binding data for the triazole derivatives **1** and **2** revealed that the effects of the $R_1$, $R_2$, and $R_3$ substituents on affinity and selectivity for the adenosine receptor subtypes were not constant but interdependent.

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# $F_2$-DABOs ANTAGONIZE CELL PROLIFERATION AND INDUCE CELL DIFFERENTIATION BY INHIBITING A NON-TELOMERIC ENDOGENOUS REVERSE TRANSCRIPTASE

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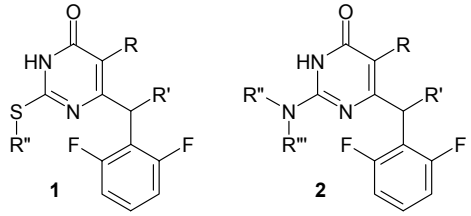
Endogenous, non-telomeric reverse transcriptase (RT) is encoded by two classes of repeated genomic elements, retrotransposons and endogenous retroviruses [1], and is an essential component of the retrotransposition machinery of both types of elements. Expression of RT-coding genes is generally repressed in non-pathological, terminally differentiated cells, but is active in early embryos, germ cells, embryo and tumour tissues, all of which have a high proliferative potential. To clarify whether reverse transcription is functionally implicated in control of cell growth, differentiation and in embryogenesis, recent experiments [2] have been undertaken to inactivate the endogenous RT activity. Indeed, we found that RT inhibition by nevirapine and efavirenz, non-nucleoside RT inhibitors (NNRTIs) widely used in the treatment of AIDS, has a great impact on a variety of cell lines, of both murine and human origin, and can cause a significant decrease of cell growth concomitant with the stimulation of differentiation [2].
2,6-Difluorobenzyl-*S*-DABOs and -*N*-DABOs ($F_2$-DABOs, **1** and **2**) are the latest generations of a class of NNRTIs developed by our group in the past decade [3]. They are active at low nanomolar concentrations in cell-based and enzymatic assays. With the aim to further investigate the antiproliferative and cytodifferentiating activity of NNRTIs we chose MC1047 and MC1220, respectively, as representatives of the $F_2$-DABO classes and tested them against endoRT. In experiments with human differentiating cell systems, the two compounds significantly reduced cell proliferation and facilitated the morphological differentiation of cells. These results propose $F_2$-DABOs as useful tools in preventive and/or curative therapy to counteract the loss of differentiation in de-differentiating pathologies and as antiproliferative drugs in tumour therapy.




R,R'= H, Me, Et, *i*-Pr
R",R'"= alkyl, cycloalkyl

References:
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# URACIL-BASED HYDROXY-AMIDES (UBHAs) AS A NEW CLASS OF PICOMOLAR HDAC INHIBITORS

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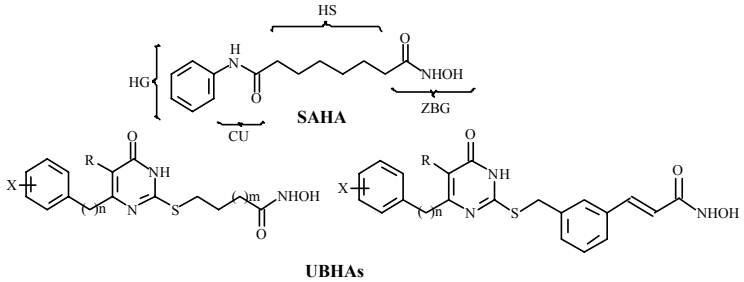
Histone deacetylase (HDAC) enzymes play an important role in the epigenetic regulation of gene expression. Since HDAC inhibitors have been reported to induce cell cycle arrest, differentiation and/or apoptosis, they are considered a promising class of new generation anticancer agents. Most of HDAC inhibitors generally consist of a zinc-binding group (ZBG) and a five- or six-carbon hydrocarbon spacer (HS) attached to a hydrophobic group (HG) via a connection unit (CU). Suberoylanilide hydroxamic acid (SAHA), a well-known HDAC inhibitor, shows a hydroxamate moiety (ZBG), a six-carbon linear aliphatic chain (HS), and an amide function (CU) connected to a benzene ring (HG), so perfectly exemplifying this general model for HDAC inhibition [1].

A comparison of amino acid sequences of the HDAC active site showed that its structural features are well conserved across all the HDACs, except for the rim of the catalytic pocket. Therefore, it has been reasoned that changes of the CU and/or HG, assumed to interact with the entry area of the catalytic pocket, could provide potent and possibly selective HDAC inhibitors. However, only a few connection units, such as amide, sulphonamide, ketone, ether, oxazole and thiazole have been reported [2]. Prompted by these evidences, we planned the synthesis and anti-HDAC evaluation of a new series of hydroxamates carrying an uracil moiety as CU, differently sized thioalkyl aliphatic and cinnamyl chains as HS, and several differently hindered (un)substituted aryl and arylalkyl moieties at the C6-position of the uracil ring as HG. Preliminary results showed that some of these derivatives are very potent HDAC inhibitors, with $IC_{50}$ values in the subnanomolar range (600-800 pM), and are also endowed with promising class II HDAC selectivity and very interesting antiproliferative and cytodifferentiating activities.




R = H, alkyl, aryl, halogen, NHCOR, etc. X = H, eldonor and el-withdrawing groups n = 0-3; m = 0-5.

[1] Mai A, Massa S, Rotili D, Cerbara I, Valente S, Pezzi R, Simeoni S, Ragno R. Histone deacetylation in epigenetics: an attractive target for anticancer therapy. *Med. Res. Rev*. **2005**, in press.
[2] Dai Y, Guo Y, Curtin ML, Li J, Pease LJ, Guo J, et al. A novel series of histone deacetylase inhibitors incorporating hetero aromatic ring systems as connection units. *Bioorg. Med. Chem. Lett*. **2003**, *13*, 3817-3820.

# (ARYLOXOPROPENYL)PYRROLYL HYDROXYAMIDES AS NOVEL HUMAN CLASS II HISTONE DEACETYLASE INHIBITORS

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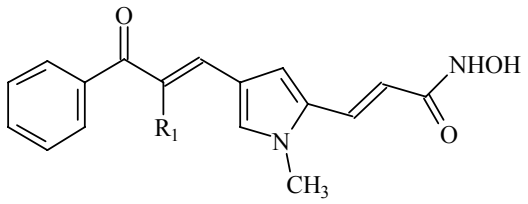
Histone deacetylase (HDAC) is a family of enzymes playing an important role in the control of gene expression. Because it has been reported that its inhibition brought about cell-cycle arrest and induced apoptosis and/or differentiation, HDAC is considered a promising target for new types of pharmaceuticals.

Recently, we reported a new series of hydroxamic acid-containing compounds, *ie* aroyl-pyrrolyl-hydroxyamides (APHAs), as HDAC inhibitors [1]. Various chemical modifications on different portions of APHA lead compound have been performed, with the aim to define structure-activity relationships and to improve its HDAC inhibitory activity. Among them, the insertion of an alkyl/alkenyl chain between the benzoyl portion and the pyrrole C4-position of the lead compound has been produced. All the newly synthesized derivatives were tested against maize HD2 and maize HD1-B and HD1-A, two deacetylase enzymes homologues of mammalian class I and class II HDACs, respectively. Interestingly, properly substituted (aryloxopropenyl)pyrrolyl hydroxyamides **1** were not very potent against HD2, slightly active against HD1-B, and endowed with high inhibitory activity against HD1-A enzyme [2]. $IC_{50}$ values of such compounds against HD1-A are in the range 12-200 nM, the class II selectivity ratio is 30-180 depending on the type and the position of substituents on the benzene ring. Tested on human HDACs to confirm their class II HDAC selectivity in mammals, our selective pyrrole derivatives showed no activity against human HDAC1 (0% of inhibition at 5 μM) whilst were able to inhibit human HDAC4 (55% of inhibition at 5 μM). In functional assays, such compounds failed in p21 induction, whereas produced hyperacetylation of α-tubulin in ZRT5.1 breast cancer cells.




**1**

[1] Mai A, Massa S, Rotili D, Cerbara I, Valente S, Pezzi R, Simeoni S, Ragno R. *Med. Res. Rev.* **2005**, in press.
[2] Mai, A.; Massa, S.; Pezzi, R.; Rotili, D.; Loidl, P.; Brosch, G. *J. Med. Chem*. **2003**, *46*, 4826-4829.

# SYNTHESIS AND BIOLOGICAL EVALUATION OF CURCUMINE ANALOGUES AS APOPTOSIS-INDUCING AGENTS

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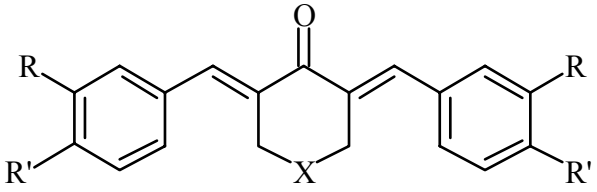
Apoptosis, also known as programmed cell death, is a physiological cell suicide mechanism, whose morphological appearance relies on the activation of caspase-family cisteine proteases and on the alterations in mitochondrial membrane structure. Recent studies focused the attention on the potential anticancer properties of hydroxylated compounds such as curcumin **1** and resveratrol [1,2].
In particular curcumin (**1**), a natural product endowed with a wide range of pharmacological properties, displayed good antiproliferative activity due to its ability to induce apoptosis in tumor cells. On the bases of these premises, we designed various derivatives in order to explore new areas of structural alteration of curcumin. In particular, we replaced the α,γ-diketo moiety of **1,** with a cyclohexanone, *N*-methylpiperidone, *N*-ethylpiperidone, and *N*-benzylpiperidone rings, obtaining derivatives **2** as conformationally restrained analogues of curcumin.




**2**

X= $NCH_2CH_3$, $NCH_3$ , NBz, $CH_2$
R = OH, $OCH_3$, $NO_2$ , Cl

The antiproliferative ($IC_{50}$, inhibition concentration 50%) and apoptotic ($AC_{50}$, concentration able to induce apoptosis in 50% of cells) activities of these compounds were evaluated *in vitro* on a myeloblastic leukemia cell line (HL60).
Interestingly, a number of derivatives **2** were endowed with antiproliferatie and apoptotic activities at concentrations ranging from 0.5 to 3 μM.

[1] H. Ligeret, S. Bathelemy, R. Zini, JP. Tillement, S. Labidalle, D Morin, et al. *Free Radical Biology and Medicine*, *Vol 36*, No. 7, pp 919-929, **2004** .
[2] M. Roberti, D. Pizzani, D. Simoni, R. Rondanin, R. Baruchello, C. Bonora, F. Buscemi, S. Grimaudo, M. Tolomeo, et al. *J. Med. Chem*. **2003**, *46*, 3546.

# CONFORMATIONALLY CONSTRAINED ANALOGUES OF THE hFcεRIα STALK PEPTIDE KAPREKY

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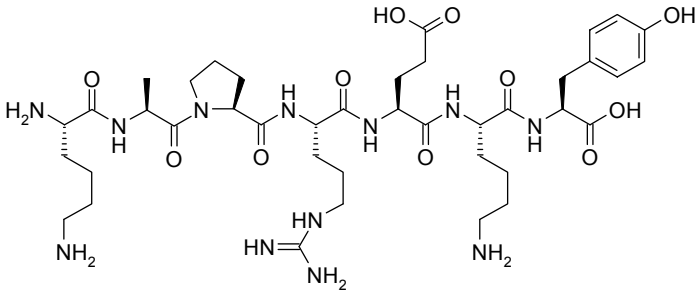
Brunner Strasse 59 A-1235 Vienna, Austria

The signaling of mast cells and basophils is triggered by the binding of IgE to the α-chain (FcεRIα) of the high-affinity IgE-receptor followed by cross-linking with polyvalent antigen.[1] It was shown previously that the anti-human FcεRIα-monoclonal antibody 5H5F8 which recognizes the membrane-proximal epitope $^{171}$KAPREKY$^{177}$ inhibits the signaling of mast cells without affecting the binding of IgE to the same receptor.[2] This epitope thus represents a novel molecular target for the modulation of FcεRIα-mediated cell activation.
Goal of the project described here is the generation and characterization of conformationally constrained KAPREKY analogues which mimic the secondary structure given in the corresponding part of the FcεRIα. These conformationally constrained KAPREKY-analogues are intended to serve as tools for studying the interaction of potential LMW binders to the KAPREKY sequence.



The poster presentation describes the design, modeling studies and the synthesis of constrained analogues of the KAPREKY peptide. Basis for the design of these mimics is an X-ray structure of the 5H5F8-Fab fragment in complex with the KAPREKY peptide. Our approach covers the introduction of modified peptide-backbones as well as the replacement of stabilizing hydrogen bonds between side chains by covalent bonds.

[1] Nadler, M.J.; Metthews, S.A.; Turner, H.; Kinet, J.-P. Signal Transduction by the High-Affinity Immunglobulin E Receptor FcεRI: Coupling Form to Function. *Adv. Immunol*. **76**, 325 – 355, (2000).
[2] Nechansky, A.; Robertson, M. W.; Albrecht, B. A.; Apgar, J. R.; Kricek, F. Inhibition of antigen-induced mediator release from IgE-sensitized cells by a monoclonal anti-FcεRI α-chain receptor antibody: implications for the involvement of the membrane-proximal α-chain region in FcεRI-mediated cell activation. *J. Immunol*. **166**, 5979-5990, (2001).

# DESIGN, SYNTHESIS AND BIOLOGICAL ACTIVITIES OF PYRROLYLETHANONEAMINE DERIVATIVES, A NOVEL CLASS OF MAO INHIBITORS

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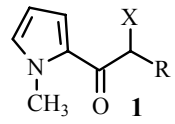
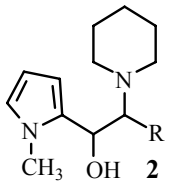
Mitochondrial monoamine oxidases (MAOs) are flavin-containing enzymes that catalyze the oxidative deamination of neurotransmitters and exogenous aryl-alkylamines. In mammals, two different types of MAOs are present in most tissues, namely MAO-A and MAO-B. MAO-A preferentially deaminates aromatic monoamines, such as the neurotransmitters serotonin, noradrenaline and adrenaline, while MAO-B oxidizes β-phenylethylamines (PEA) and benzylamines. Selective MAO-A inhibitors are currently used for treating neurological disorders, such as anxiety or depression, while selective anti-MAO-B agents (e.g., selegiline) are administered alone or together with *L*-DOPA for the treatment of Parkinson's syndrome or Alzheimer's disease. Actually, the studies on new MAO inhibitors have been focused on reversible and selective agents. In fact, irreversible and/or non-selective inhibitors showed shortcomings including cumulative effects, loss of selectivity after chronic treatment, and interaction with tyramine-containing foods (cheese effect) [1].




**1**

R = H, $CH_3$, Ph;
X = aliphatic and alicyclic amines




**2**

R = H, Ph

Pursuing our studies on pyrrole analogs of cathinone [2-4], we synthesized and tested against MAO-A and MAO-B isoenzymes a series of pyrrolylethanoneamine derivatives **1** and **2** which share chemical features similar to that of moclobemide, brofaromine, toloxatone and other reversible MAO inhibitors used in clinical practice.
In general, aminoketones **1** were found to be potent and selective A-inhibitors. In particular, derivative (-)-(*R*)-**1a** (X = 1-pyrrolidinyl, R = Ph) was more potent ($K_i$(MAO-A) = 0.0035 μM) and selective (A-selectivity 200000) against A isoenzyme than toloxatone and moclobemide used as reference drugs. Interestingly, aminoalcohol (+)-**2a** selectively inhibited MAO-B enzyme ($K_i$(MAO-B) = 1.24 μM; $K_i$(MAO-A) = 7 μM).

[1] Wouters, J. *Current Med. Chem.* **1998**, *5*, 137.
[2] R. Di Santo, et al. *Arch. Phar.* (Weinheim) **1992**, *325*, 403-409.
[3] F. La Torre, R. Costi, R. Di Santo, et al. *Chromatographia*, **2004**, *60*, 171-178.
[4] R. Di Santo, R. Costi, A. Roux, et al. *J. Med. Chem.* submitted.

# DOCKING STUDIES ON BIFUNCTIONAL QUINOLINYL DIKETO ACIDS AS HIV-1 INTEGRASE IHNHIBITORS

Roberto Di Santo[a], Roberta Costi[a], Alessandra Roux[a], Michela Forte[a], Marino Artico[a], Antonio Lavecchia[b], Ettore Novellino[b], Lucia Palmisano[c], Clementina Galluzzo[c], Mauro Andreotti[c], Stefano Vella[c], Lucia Nencioni[d], Annateresa Palamara[d], Christophe Marchand[b], Yves Pommier[e]

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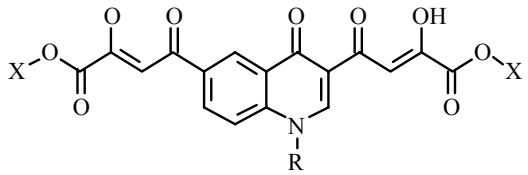
[c]ISS, Istituto di Virologia, V.le Regina Elena 299, I-00161 Roma, Italy,
[d]Istituto di Microbiologia, Università di Roma, P.le Aldo Moro 5, I-00185 Roma, Italy,
[e]Laboratory of Molecular Pharmacology Division of Basic Sciences, National Cancer Institute Building 37, National Institute of Health, Bethesda, MD 20892-4255, USA.

Combination therapy using RT and PR inhibitors is nowadays the best clinical approach in the acquired immunodeficiency syndrome (AIDS), caused by infection with the human immunodeficiency virus type-1 (HIV-1). However, the emergence of resistant strains calls urgently for researches on inhibitors of further viral targets such as integrase (IN), the enzyme that catalyzes the integration of the proviral DNA into the host chromosomes. In the past several years, numerous compounds with diverse structural features have been reported as IN inhibitors, of which the most promising are compounds characterized by $\beta$-diketo acid moiety (DKAs). Several reported DKAs selectively inhibit the strand transfer reaction of IN and exhibit potent antiviral effects against HIV-infected cells [1]. It is believed that DKAs function by competing with substrate DNA in binding to the in active site.

Recently, we were engaged in studies on quinolinyl-2,4-dioxobutanoic acids as potent IN inhibitors [2], endowed with selective activity against strand transfer step. Surprisingly, bifunctional compounds such as derivatives **1** showed lower selective activity if compared with the monofunctional counterparts. Docking studies are in progress to elucidate the binding mode of compounds **1** to IN in the presence of DNA substrate.



R = H, $CH_2C_6H_4F$
X = H, $CH_2CH_3$

[1] R. Costi, R. Di Santo, M. Artico, A. Roux, et al. *Bioorg. Med. Chem. Lett.* **2004,** *14*, 1745.
[2] R. Di Santo, R. Costi, M. Artico, Y. Pommier, C. Marchand, US Patent 2004, 60,552,423.

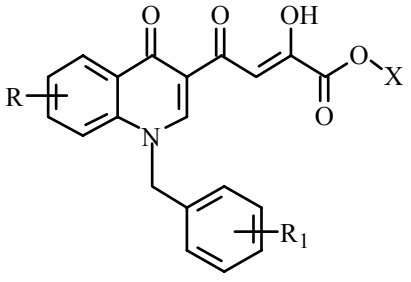
# SAR STUDIES ON QUINOLINYL-2,4-DIOXOBUTANOIC ACIDS, HIV-1 INTEGRASE INHIBITORS THAT BLOCK HIV-1 REPLICATION IN INFECTED CELLS

Roberto Di Santo[a], Roberta Costi[a], Alessandra Roux[a], Michela Forte[a], Marino Artico[a], Antonio Lavecchia[b], Ettore Novellino[b], Lucia Palmisano[c], Clementina Galluzzo[c], Mauro Andreotti[c], Stefano Vella[c], Lucia Nencioni[d], Annateresa Palamara[d], Christophe Marchand[e], Yves Pommier[e]

[a]Istituto Pasteur - Fondazione Cenci Bolognetti, Dipartimento di Studi Farmaceutici, Università degli Studi di Roma "La Sapienza", P.le Aldo Moro 5, I-00185 Roma, Italy,
[b]Dipartimento Chimica Farmaceutica e Tossicologica, Università degli Studi di Napoli "Federico II", via D. Montesano 49, I-80131 Napoli, Italy,
[c]ISS, Istituto di Virologia, V.le Regina Elena 299, I-00161 Roma, Italy,
[d]Istituto di Microbiologia, Università di Roma, P.le Aldo Moro 5, I-00185 Roma, Italy,
[e]Laboratory of Molecular Pharmacology Division of Basic Sciences, National Cancer Institute Building 37, National Institute of Health, Bethesda, MD 20892-4255, USA.

Acquired immunodeficiency syndrome (AIDS), caused by infection with the human immunodeficiency virus type-1 (HIV-1), remains a serious global health problem. Drugs approved so far include nucleoside (NRTIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs) and protease inhibitors (PRIs). Due to failure of monotherapy with the above inhibitors, combination protocols were designed with both RTIs and PRIs in order to increase the clinical efficacy and reduce the emergence of resistant variant. However, the emergence of resistant strains calls urgently for researches on inhibitors of further viral targets such as integrase (IN), the enzyme that catalyzes the integration of the proviral DNA into the host chromosomes.

Aryl diketo acids (DKAs) containing a distinct dioxobutanoic acid moiety (i.e. L-731,988) have been identified as potent and specific inhibitors of HIV-1 IN, which block HIV-1 replication in infected cells. Pursuing our studies on HIV-1 IN inhibitors [1,2] we recently reported the potent anti-IN activity of pyrrolyl diketo hexenoic acids (PDKHAs) characterized by elongated diketo acid chain [3]. More recently, we designed and synthesized quinolinyl diketo acids **1** as conformation ally restrained analogues of PDKHAs [4]. SAR and MM studies on these novel HIV-1 IN inhibitors will be shown.




**1**
R = H, F, Cl
$R_1$ = H, F, Cl, OH, $OCH_3$, $NO_2$
X = H, $CH_2CH_3$

[1] M. Artico, R. Di Santo, R. Costi, et al. *J. Med. Chem.* **1998**, *41*, 3948.
[2] R. Costi, R. Di Santo, M. Artico, et al. *Bioorg. Med. Chem.* **2004**, *12*, 199.
[3] R. Costi, R. Di Santo, M. Artico, A. Roux, *Bioorg. Med. Chem. Lett.* **2004,** *14*, 1745.
[4] R. Di Santo, R. Costi, M. Artico, Y. Pommier, US Patent 2004, 60,552,423.

# DISCOVERY OF NOVEL ANTI-MALARIAL AGENTS: 1-[(ARYL)(4-ARYL-1*H*-PYRROL-3-YL)METHYL]-1*H*-IMIDAZOLES TARGETED TO FARNESYL TRANSFERASE

Roberto Di Santo[a], Roberta Costi[a], Michela Forte[a], Carlo Galeffi[a], Marino Artico[a], Carsten Peters[b], Herbert Waldmann[b], Reto Brun[c]

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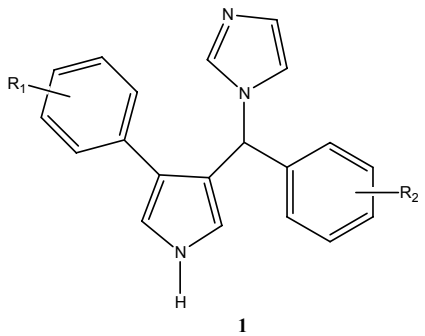
Malaria is one of the most important infectious diseases that causes about 500 million clinical cases resulting in over one million deaths annually, especially small children in disease endemic countries. The fatal cases are generally caused by the most virulent human malaria parasite *Plasmodium falciparum*. Current clinical treatment involves the use of antifolates and the quinoline-containing drugs. The antifolates include diaminopyrimidines (such as pyrimethamine, trimethoprim), the biguanides of which proguanil is a representative, and sulfa drugs, namely sulfonamides and sulfones. The quinoline-containing drugs are represented by quinine, aryl aminoalcohols related to quinine such as mefloquine, 4-aminoquinolines (such as chloroquine), and 8-aminoquinolines like primaquine. These drugs are currently failing at an accelerating rate in most malaria-endemic regions, with consequent increases in malaria-related morbidity and mortality. Therefore, to combat malaria new drugs are needed. Other malaria control efforts have focused on new drug targets such as isoprenoid biosynthetic pathway inhibitors. Pursuing our decennial studies on azole derivatives as chemotherapeutic agents [1-3], we discovered the inhibitory activity against the enzyme farnesyl transferase (FT) of a series of 1-[(aryl)(4-aryl-1*H*-pyrrol-3-yl)methyl]-1*H*-imidazoles **1**. Derivatives **1** showed anti-*Plasmodium falciparum* activity at micromolar concentrations. SAR studies of these new antimalarials targeted to FT will be reported.




**1**

$R_1$ = H, alkyl, aryl
$R_2$ = H, F, Cl,

[1] Artico, R. Di Santo, R. Costi, et al. *J. Med. Chem.* **1995**, *38*, 4223.
[2] A. Tafi, M. Artico, R. Costi, R. Di Santo, et al *J. Med. Chem.* **1996**, *39*, 1227.
[3] A. Tafi, R. Costi, M. Botta, R. Di Santo, et al. *J. Med. Chem.* **2002,** *45*, 2720.

# 2,4-DIOXO-5-HEXENOIC ACID DERIVATIVES ARE NOVEL SELECTIVE NON-NUCLEOSIDE INHIBITORS OF MAMMALIAN TERMINAL DEOXYNUCLEOTIDYL TRANSFERASES, WITH POTENT CYTOTOXIC EFFECT AGAINST LEUKEMIC CELLS

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Mammalian terminal deoxynucleotidyl transferase (TDT) catalyzes the non-template-directed polymerization of deoxynucleoside triphosphates *in vitro*. It was initially isolated from thymus, but it is also expressed in bone marrow. Evidences continue to accumulate that TDT is a key player in influencing the outcome of V(D)J recombination during lymphocyte and repertoire development. Over 90% of leukemic cells in acute lymphocytic leukemia and approximately 30% of leukemic cells in the chronic myelogenous leukemia crisis exhibit elevated TDT activity, and the TDT activity of such leukemic cells is associated with a poor prognosis on chemotherapy and survival time.

TDT belongs to the family X of DNA polymerases (pols), whose other members are pol β, pol λ and pol μ in mammalian cells and pol IV in the yeast *S. cerevisiae*. Pol β and *Sc*pol IV do not display terminal transferase (tdt) activity, whereas pol λ has been shown to be endowed with strong *bona fid*e tdt activity. Biochemical studies suggested that pol λ and pol μ might be involved in the nonhomologous end joning (NHEJ) recombinational repair pathway of DNA double strand breaks (DSBs). The availability of specific inhibitors for these enzymes might help the investigation of their cellular functions. Moreover, the correlation between high TDT activity and malignancy of acute lymphocytic leukemia, further increases the interest in developing TDT-specific inhibitors.

In an effort to identify potent and selective inhibitors of the tdt activity of pol λ and TDT, we undertook a random screening of synthetic non-nucleoside analogues. Here, we report the characterization of the mechanism of action of three diketo hexenoic acid (DKHA) derivatives [1], which proved to be extremely selective for the tdt activity of pol λ and TDT. To the best of our knowledge, these are the first non-nucleoside specific inhibitors of mammalian terminal transferases reported so far. Moreover, these DOHA analogs were not toxic towards HeLa cells ($CC_{50}$ > 100 μM), whereas they showed potent cytotoxicity against the $TDT^+$ acute leukemia cell line MOLT-4 ($CC_{50}$ = 14 μM).

[1] R. Costi, R. Di Santo, M. Artico, A. Roux, *Bioorg. Med. Chem. Lett.* **2004,** *14*, 1745.

# SAR STUDIES ON INDOLYL DIKETO ACID DERIVATIVES AS HCV RNA-DEPENDENT RNA POLYMERASE INHIBITORS

Roberto Di Santo[a], Roberta Costi[a], Marino Artico[a], Alessandra Roux[a], Michela Forte[a], Luigi Maria Longo[a], Giovanni Maga[b], Ketith Tardif[c], Aleem Siddiqui[c], Maurizio Fermeglia[d], Marco Ferrone[d], Maria Silvia Paneni[d], Sabrina Pricl[d]

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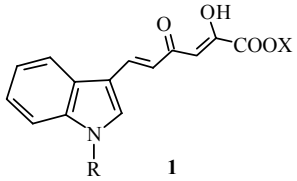
*Hepatitis C virus* (HCV) is a RNA virus of the *Flaviviridae* family. Since the advent of serological assays for HCV in 1990, it has been shown to be the major etiological agent for post-transfusion and sporadic non-A, non-B hepatitis worldwide. Further, it is estimated that the 3% of the world's population, or about 170 million people, are seropositive for HCV. Unlike hepatitis B, which is associated with chronicity in approximately 5% of adult infections, more than 80% of HCV-infected individuals develop chronic hepatitis. Chronic hepatitis C can lead to cirrhosis and end-stage liver disease in 20-30% of the patients and, among these, 1-4 % may develop hepatocellular carcinoma. The currently approved therapeutic protocol includes a combination of pegylated interferon and ribavirin. However, these regimens have limited efficacy (10-40% of the patients) and significant side effects, causing up to 20% of the patients to discontinue the therapy. As a result, there is an urgent need for developing safe and effective antiviral agents. In the last decade the development of inhibitors targeting the HCV NS5B polymerase (RdRp) has attracted the attention of investigators worldwide. Specific inhibitors for HCV RdRp were however not identified until recently. These include nucleoside analogues and various non-nucleosides from different chemical classes. Belonging to the latter group, over 200 compounds including alkyl-, phenyl-, pyrrole- and thiophene-substituted diketo acids (DKAs) were evaluated by Merck Company in the HCV NS5B polymerase assay: of these, several compounds demonstrated low micromolar $IC_{50}$ values. Pursuing our studies on DKAs as inhibitors of viral and human polymerases, we recently designed and synthesized indolyl-2,4-dioxo-5-hexenoic acids **1** as HCV RdRp inhibitors [1]. A number of derivatives **1** showed interesting activity against recombinant HCV RdRp at micromolar concentrations. QSAR studies are ongoing to elucidate the binding mode of these inhibitors to the biological target.




**1**

R = $CH_2CH_3$, $CH_2CH_2CH_3$, $C_6H_4CN$, $C_6H_4OH$
X = H, $CH_2CH_3$

[1] R. Di Santo, R. Costi, A. Roux, S. Pricl et al. *J. Med Chem.* submitted.

# 3D-QSAR MODELS FOR SELECTIVE CLASS I (HD1-B) AND CLASS II (HD1-A) HDAC INHIBITORS

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Histone deacetylases (HDACs) are known to play an important role in the regulation of gene expression by catalyzing the deacetylation of the acetylated ε-amino groups of specific histone lysine residues. In eucaryotes, three deacetylase families have been identified so far (class I, class II and class III HDACs) with different sensitivity to different inhibitors, but no strongly selective compounds. Because of class I HDACs selective inhibitors may have great therapeutic potential as anticancer agents, and class II selective inhibitors can represent useful tools to explore or dissect the role of a given HDACs in different protein complexes, 3D-QSAR analyses have been conducted on a series of 25 (aryloxopropenyl)pyrrolyl hydroxyamides [1] active against both maize HD1-B (homologous of mammalian class I HDACs) and HD1-A (homologous of class II HDACs) enzymes, with the end of interpreting their HD1-B and/or HD1-A selectivity. The starting compound conformations have been performed using a molecular dynamic run with simulated annealing procedure as implemented in Macromodel 7.1 and the 3D-QSAR models have been built using the Grid Independent Descriptors (GRIND) calculated by Almond 3.2.0a software.
Satisfactory results have been obtained: the $r^2$ and $q^2$ have been 0.96 and 0.81 for the HD1-B model, and 0.98 and 0.85 for the HD1-A model.
The data analysis has revealed that the main characteristic for the HD1-A selectivity is the *shape* of compounds. In fact, the importance of shape description have been recognized by many authors and it is considered crucial for the ligand ability to bind to a receptor. In this case, the exactly shape has been calculated using one of the GRIND descriptors (TIP-descriptor), which selects the most descriptive molecular surface regions according a criterion based on the local curvature.
Molecules with a bowed shape and consequently with an exact distance (around 8.5 Å) between the H-bond acceptor region of the hydroxamic acid and the hydrophobic region of the cap group of the pyrrole compounds have high HD1-A activity and low HD1-B activity. It seems that when the molecules are bended, their caps can interact with an external hydrophobic region of the enzymes and that is relevant for the HD1-A activity, but not for the HD1-B one. Infact, all the most HD1-B-active pyrroles have a straight shape.

[1] Mai, A.; Massa, S.; Pezzi, R.; Rotili, D.; Loidl, P.; Brosch, G. *J. Med. Chem*. **2003**, *46*, 4826-4829.

# REVERSED AMIDE DERIVATIVES OF ARACHIDONOYL ETHANOL AMIDE -SYNTHESIS, CB1-RECEPTOR ACTIVITY AND ENZYMATIC STABILITY

Teija Parkkari,[a] Juha R. Savinainen,[b] Tapio Nevalainen,[a] Laura Mäkipaja,[a] Tuomas Sirviö,[a] Susanna M. Saario,[a] Jarmo T. Laitinen,[b] Riku Niemi,[a] and Tomi Järvinen[a]

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The growing understanding of the endogenous cannabinoid system (ECS) as a new neuronal signaling system has offered fascinating opportunities for the drug discovery. Drugs effecting through the ECS have been suggested for the treatment of several disorders; pain, anxiety, glaucoma, nausea, obesity, head injuries and Parkinson's disease as a few examples. The ECS consists of cannabinoid receptors (CB1 and CB2) and their ligands (endocannabinoids), membrane bound carrier proteins and metabolizing enzymes (FAAH, MGL). The cannabinoid receptor ligands and enzyme inhibitors are believed to provide new therapeutical insights for the future. Two major endocannabinoids, *N*-arachidonoyl ethanol amide (AEA) and 2-arachidonoyl glycerol (2-AG), are excellent ligands for the cannabinoid receptors, and they have been shown to act as therapeutic agents in several *in vitro* and *in vivo* models. However they degrade enzymatically very fast *in vivo*. In order to provide good probes for the cannabinoid drug research and design based on endogenous cannabinoids, their structures must be modified. In the present study, seven reversed amide derivatives **(**Fig. 1.; **1a-d, 2a-c**) of AEA were synthesized and evaluated for their CB1 receptor activation by a [$^{35}$S]GTP$_\gamma$S binding assay using rat cerebellar membranes. The primary goal of the study was to develop CB1 receptor agonists having improved enzymatic stability compared to endogenous AEA. Furthermore, by reversing the amide bond of AEA, the formation of arachidonic acid would be prevented. Finally, an effect of the carbonyl carbon position of the CB1 receptor activity was explored by synthesizing analogues having different chain lengths (**1a-d**, $C_{20}$; **2a-c**, $C_{19}$). All the synthesized compounds, except **1d**, showed dose-dependent CB1 activity. For example, the potency values for the compounds **1b** and **2b** were $E_{max}$ = 305 ± 10 % basal, $p$EC$_{50}$ = 5.7 ± 0.1 and $E_{max}$ = 222 ± 9 % basal, $p$EC$_{50}$ = 5.4 ± 0.2, respectively, and for the reference compound AEA $E_{max}$ = 415 ± 3 % basal, $p$EC$_{50}$ = 5.3 ± 0.1. In rat brain homogenate, the reversed amides possessed significantly higher stability against FAAH induced degradation than AEA. Therefore, the reversed amide analogues of AEA may serve as enzymatically stable structural basis for the drug design based on the endogenous cannabinoids.

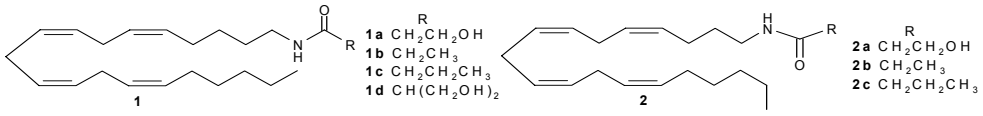


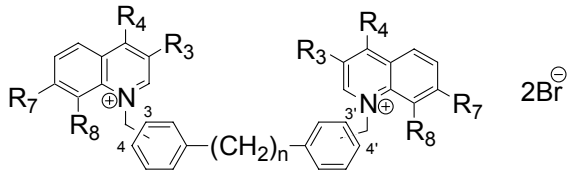

**Figure 1.** Chemical structures of reversed amides **1a-d, 2a-c**.

# NEW CHOLINE KINASE INHIBITORS WITH ANTIPROLIFERATIVE ACTIVITY AGAINST *ras*-TRANSFORMED CELLS

Rosario Sánchez-Martín, Ana Conejo-García, Olga Cruz-López, Joaquín M. Campos, Miguel A. Gallo, Antonio Espinosa,

Departamento de Química Farmacéutica y Orgánica, Facultad de Farmacia, c/ Campus de Cartuja s/n, 18071 Granada (Spain)

We have recently carried out studies aimed to establish the SARs that define ChoK inhibitory potency and antiproliferative activity of a set of bispyridinium compounds [1]. The aim of this communication specifically focusses on studying the effect to be expected on the biological activities by a variation in the linker that connects the quinolinium cations having electron-releasing groups at their position 4, with other different groups at positions 3, 7 and 8 of the heterocycle (compounds **1)**.




**1**

On one hand, according to their inhibitory activities against human ChoK, it is found that the enzymatic inhibitory potency is closely related to the size of the linker, the 3,3'-biphenyl moiety being the most suitable one, followed by the 4,4'-bibenzyl fragment and finally, the 4,4'-biphenyl one. On the other, the antiproliferative activity against the HT-29 human colon cancer cell line is less influenced by the linker type and by the substituent $R_4$. The corresponding QSAR equation was obtained for the whole set of compounds for the antiproliferative activity, the electronic parameter $\sigma_R$ of $R_4$, the molar refractivity of $R_8$ ($MR_8$) and the lipophilic parameters clog *P* and $\pi_{linker}$:

$p(IC_{50})_{HT\text{-}29} = -2.66 - 0.03\ (\pm 0.00)\ MR_8^2 + 0.10\ (\pm 0.02)$ clog $P + 1.05\ (\pm 0.31)\ \pi_{linker} - 3.73\ (\pm 0.71)\ \sigma_R$

$n = 40$, $r = 0.920$, $s = 0.223$, $F_{4,35} = 47.856$, $\alpha < 0.001$

Toxicity assays were performed for the most active compounds *in vitro*, the most promising compounds being **1a** ($R_3 = R_7 = R_8 = H$; $R_4$ = 4-chloro-*N*-methylanilino; n = 2; 4,4'), and **43** ($R_3 = R_8 = H$; $R_4$ = 4-chloro-*N*-methylanilino; $R_7$ = Cl; n = 2; 4,4') as a consequence of their interesting antiproliferative activities [$IC_{50\ HT\text{-}29}$ = 0.70 and 0.80 μM] and low toxicity [$LD_{50}$ = 16.7 and 12.5 mg/Kg of mouse]. These biological activities justify further analysis for antitumoural assays under *in vivo* conditions.

[1] Conejo-García, A.; Báñez-Coronel, M.; Sánchez-Martín, R. M., Rodríguez-González, A.; Ramos, A.; Ramírez de Molina, A.; Espinosa, A.; Gallo, M. Á.; Campos, J. M.; Lacal, J. C. Influence of the linker in bispyridinium compounds on the inhibition of human choline kinase. *J. Med. Chem.*, **2004**, *47*, 5433-5440.

# SYNTHESIS OF *N*-ALKYL-1-PHENYL-5-METHYLSULFONYLINDOL-2-CARBOXAMIDE AS NEW COX-2 SELECTIVE INHIBITORS

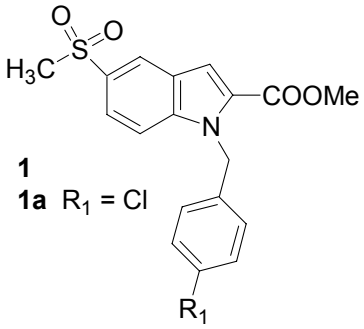
Olga Cruz-López, Juan José Díaz-Mochón, Joaquín M. Campos, Miguel Á. Gallo, Antonio Espinosa

Departamento de Química Farmacéutica y Orgánica, Facultad de Farmacia, c/ Campus de Cartuja s/n, 18071 Granada (Spain)

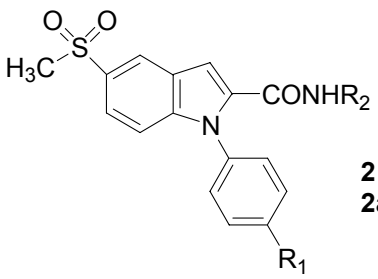
We have identified novel COX-2 selective inhibitors using pharmacophore models. This approach was used to design indomethacin analogues **1** that exhibited consistent structure-activity relationships leading to the potent and selective COX-2 inhibitor **1a** [1]. In this communication, a series of molecules with an *N*-alkyl-1-phenyl-5-methylsulfonyl indol-2-carboxamide structure is presented as new ciclooxygenase-2 selective inhibitors.

This study was undertaken according to the following steps:

1. A butyl group as an *N*-alkyl group was introduced. Five compounds with this group were synthesized, in which the *para* substituent of the phenyl group was modified. Of all the studied substituents the compound with the methylthio group showed a better potency and an inhibitory selectivity**.**
2. We studied how the length of the *N*-alkyl moiety affects the biological activity while maintaining the methylthio substituent.
3. With the most active compound so far obtained (**2a**) a series of modifications that affect the CO-NH fragment were carried out.




**1**
**1a** $R_1$ = Cl




**2**
**2a** $R_1$ = SMe; $R_2$ = *n*-Bu

From the analysis of the biological results obtained for these compounds it can be stated that the presence of an electron-releasing group at the *para* position of the phenyl group, specifically the methylthio group, and the lengthening of the *N*-alkyl chain with a butyl or a pentyl group, leads to an increase in both potency and selectivity in the COX-2 inhibition.

[1] Palomer, A.; Cabré, F.; Pascual, J.; Campos, J.; Trujillo, M. A.; Entrena, A.; Gallo, M. A.; García, L.; Mauleón, D.; Espinosa. A. Identification of novel cyclooxygenase-2 selective inhibitors using pharmacophore models. *J. Med. Chem.*, **2002**, *45*, 1402-1411.

# DOCKING STUDIES ON THE INHIBITION OF CHOLINE KINASE

Antonio Entrena,[a] Lara Milanese,[b] Joaquín Campos,[a] Miguel A. Gallo[a] and Antonio Espinosa[a]

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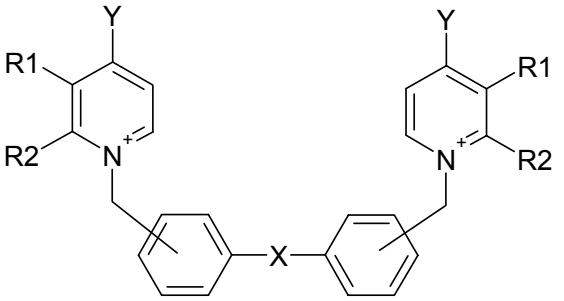
Our research group has described several human choline kinase (ChoK) inhibitors that show antiproliferative activity [1]. Recently, a crystal structure of *C. elegans* Choline Kinase (CKA2) has been resolved and important residues for the catalytic activity has been identified by site-directed mutagenesis [2,3].

In this communication, we present our findings on a possible mechanism for the inhibition of choline kinase by a series of bispyridinium and bisquinoliniun compounds of general formula **1.**




X= $CH_2$, $(CH_2)_2$, CH=CH
Y= $NH_2$, $NR_2$, etc
$R_1$=$R_2$= H, -$(CH)_4$-

**1**

A homology model of ChoK has been constructed based on the crystal structure of CKA2, and putative binding-sites for both choline and ATP have been identified according to the known site-directed mutagenesis data.

Docking studies reveal that most of these inhibitors occupy simultaneously both choline and ATP binding-sites. A qualitative structure-activity relationship between docking geometries and inhibition activity has been found, that supports our ChoK model.

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[2] Daniel Peisach, Patricia Gee, Claudia Kent and Zhaohui Xu. The Crystal Structure of Choline Kinase Reveals a Eukaryotic Protein Kinase Fold. *Structure*, **2003**, *11*, 703–713.
[3] Chong Yuan and Claudia Kent. Identification of critical residues of choline kinase A2 from Caenorhabditis elegans. *J. Biol. Chem*., **2004**,. *279 (17),* 17801-17809.

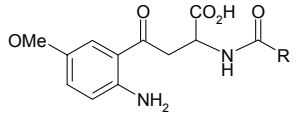
# KINURENAMINES AS A NEW TYPE OF POTENT nNOS INHIBITORS.

Antonio Entrena,[a] M. Encarnación Camacho,[a] M. Dora Carrión, [a] Germaine Escames,[b] Miguel A. Gallo[a], Dario Acuña-Castroviejo[b] and Antonio Espinosa[a]

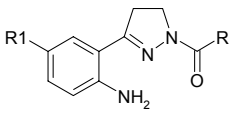
[a] Dpto. de Química Farmacéutica y Orgánica, Universidad de Granada, 18071 Granada, Spain.
[b] Dpto. de Fisiología, Instituto de Biotecnología, Universidad de Granada, 18071 Granada, Spain.

We have recently synthesized and evaluated a series of kynurenine and 4,5-dihydro-1H-pyrazole derivatives of general formulae **1** and **2,** respectively, as neuroprotective agents**.** Such compounds show a significant nNOS inhibitory activity [1,2,3], are inactive againts KYN3OH and consequently, their neuroprotective properties are due to the inhibition of the first enzyme.

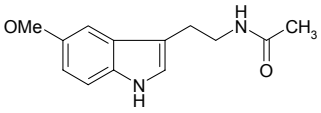
The side chain conformational mobility in kynurenine compounds is restricted by the formation of an intramolecular hydrogen bond between both the 2-$NH_2$ and the carbonyl groups, and as a consequence of this restriction, the kynurenine derivative can mimic the active conformation of melatonin **3** when it interacts with its biological target [2]. 4,5-dihydro-1H-pyrazole derivatives **2** are more rigid compounds that interact with nNOs in a similar manner to compounds **1** and **3.** A model for the interaction can be drawn on comparing both types of compounds[3].



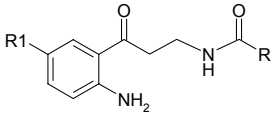

**1**, R = Me, Pr




**2**, $R_1$ = H, Cl, OMe
R = Me, Et, Pr, etc.




**3**




**4**, $R_1$ = H, Cl, OMe
R = Me, Et, Pr, etc.

We present in this communication our previous results on the inhibition of nNOS by a new type of kynurenamines derivatives **4**, that are well suited to our interaction model described for the nNOS inhibition [3].

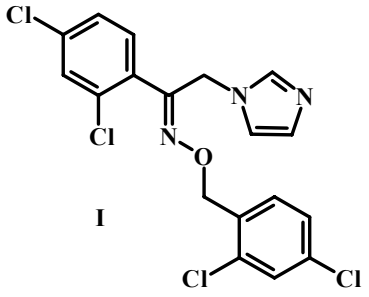
[1] J. León, F. Vives, E. Crespo, M.E. Camacho, A. Espinosa, M.A. Gallo, G. Escames, D. Acuña-Castroviejo. Modification of Nitric Oxide Synthase Activity and Neuronal Response in Rat Striatum by Melatonin and Kynurenine Derivates. *J. Neuroendocrinol*. **1998**, *10*, 297-302.
[2] M.E. Camacho, J. León, M.D. Carrión, A. Entrena, G. Escames, H. Khaldy, D. Acuña-Castroviejo, M. A. Gallo, A. Espinosa. Inhibition of nNOS Activity in Rat Brain by Synthetic Kynurenines: Structure-Activity Dependence. J. Med. Chem. **2002**, *45*, 263-274.
[3] M.E. Camacho, J. León, A. Entrena, G. Velasco, M.D. Carrión, G. Escames, A. Vivó, D. Acuña-Castroviejo, M.A. Gallo, A. Espinosa. 4,5-Dihydro-1H-pyrazole Derivatives with Inhibitory nNOS Activity in Rat Brain: Synthesis and Structure-Activity Relationships. *J. Med. Chem.* **2004**, *47*, 5641-5650

# NEW OXICONAZOLE ANALOGUES AS ANTIFUNGAL AGENTS

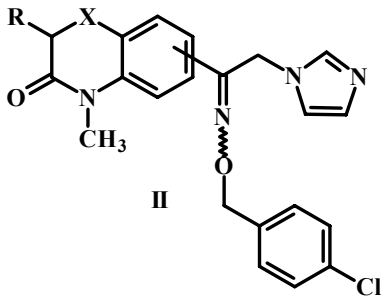
Renata Fringuelli[a], Lara Milanese[a], Antonio Macchiarulo[a],
Anna Vecchiarelli[b], Gabriele Costantino and Fausto Schiaffella[a]

[a]Department of Drug Chemistry and Technology. [b]Department of Experimental Medicine and Biochemical Sciences, Microbiology Section. University of Perugia, 06100 Perugia, Italy

The incidence of systemic fungal infections in immunocompromised patients has increased greatly during the last 20 years. Despite the growing list of antifungal agents, their clinical value has been limited by toxicity, pharmacokinetic deficiency or insufficiency in antifungal activity, partly imputable to the emergence of drug resistance.
Among antifungal agents, azole derivatives are still a viable lead structure in pursuit of a more efficacious, broad spectrum, systemic antifungal drug.
They act by inhibiting the cytochrome P450-dependent lanosterol 14α-demethylase ($P450_{14DM}$, CYP51), a key enzyme in the fungal ergosterol-biosynthesis pathway [1].
Among the azole antifungals, oxiconazole (I) is a well-known agent with a broad spectrum of activity [2], structurally characterized by an oxime ether group.
Previously we reported 1,4-benzothiazine and 1,4-benzoxazine azole compounds that are active against an experimental model of systemic candidiasis [3].
As part of this research project, this work focuses on the synthesis and evaluation of new oxiconazole analogues, structurally characterized by a 1,4-benzothiazine or 1,4-benzoxazine moiety (II).




I




II

Synthesis, docking studies and microbiological evaluation will be discussed.

[1] Lamb D.; Kelly D.; Kelly S. *Drug Res Updates* **1999**, *2*, 390.
[2] Mixich, G.;Thiele, K. *Arzneim.-Forsch.* **1979**, *29*, 1510.
[3] (*a*) Fringuelli, R.; Schiaffella, F.; Bistoni, F.; Pitzurra, L.; Vecchiarelli, A. *Bioorg. Med. Chem.* **1998**, *6*, 103. (*b*) Pitzurra, L.; Fringuelli, R.; Perito, S.; Schiaffella, F.; Barluzzi, R.; Bistoni, F.; Vecchiarelli, A. *Antimicrob. Agents Chemother.* **1999**, *43*, 2170. (*c*) Schiaffella, F.; Guarraci, A.; Fringuelli, R.; Pitzurra, L.; Bistoni, F.; Vecchiarelli, A. *Med. Chem. Res.* **1999**, *9*, 291. (*d*) Fringuelli, R.; Pietrella, D.; Schiaffella, F.; Guarraci, A.; Perito, S.; Bistoni, F.; Vecchiarelli, A. *Bioorg. Med. Chem.* **2002**, *10,*1681. (*e*) Macchiarulo, A.; Costantino, G.; Fringuelli, D.; Vecchiarelli, A.; Schiaffella, F.; Fringuelli, R. *Bioorg. Med. Chem.* **2002**, *10*, 3415.

# ASSESSMENT OF THE CONFORMATIONAL DEPENDENCE OF THE 'MIPHAK' DESCRIPTORS FOR IN-SILICO ADME APPROACHES

Mirco Meniconi, [a], Gabriele Costantino[a], Vladimir Palyulin[b] and Roberto Pellicciari[a]

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[b]Department of Chemistry, Moscow State University, Moscow 119992 Russia

The MiPhaK program generates a number of molecular descriptors based on evaluation of molecular properties on the molecular or solvent accessible surface. The interaction energy between the molecule and a probe atom is calculated all around the molecular or the solvent accessible surface. Subsequently, this three-dimensional map is transformed in a molecular spectrum and then in a set of molecular descriptors. Five probe atoms are available within the MiPhaK program, related to (i) the molecular electrostatic potential, (ii) the H-bond donor/acceptor capabilities, and (iii) the hydrophobicity of the molecule. When used in conjunction with a PLS analysis, the MiPhaK descriptors yielded successfully QSPR models for the prediction of ADME-related properties.

In this work, we engaged ourselves in the assessment of the conformational dependence of our MiPhaK descriptors. Indeed, the impact of the molecular conformation on the predictive ability of any QSPR model should be carefully evaluated for every method based on 3D-derived properties. Quite surprisingly, despite the variety of methods currently available, such an evaluation is only seldom presented.

The MiPhaK methodology, based on the evaluation of an interaction energy spectrum, is particularly suited to this kind of evaluation given the ease of comparison between spectra obtained for different conformers of a same molecule.

The obtained results outline a sensible dependence on molecular conformation for the MiPhaK descriptors related to the molecular electrostatic potential. On the contrary, descriptors related to the H-bond and to the hydrophobic character of the molecule do not show dependence on the molecular conformation, even if the involved molecule has an high conformational freedom. As a further investigation, we carried out a PCA study aimed at evaluating the ability of the MiPhaK descriptors to discriminate between different chemotypes when represented by large conformational ensembles. Also in this case we obtained a different result for MiPhaK descriptors related to the molecular electrostatic potential, which do not cluster together conformations of the same molecule, and those related to the H-bond and Hydrophobic probes, which show a clear conformation-independent behaviour.

In conclusion, our results indicate that the conformational dependency of 3D-derived descriptors should carefully evaluated and that the choice of the appropriate probe atom can be crucial for achieving conformational independency.

# UNDERSTANDING PPARα RECEPTOR ACTIVATION BY TARGETED MOLECULAR DYNAMICS

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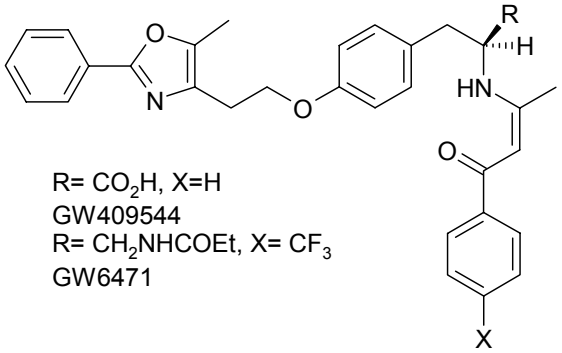
The peroxisome proliferator-activated receptor α (PPARα) regulates hepatic fatty acid metabolism and mediates the effects of fibrate lipid-lowering drugs [1]. Recently, crystal structures of the agonist- (GW409544) [2] and the antagonist- (GW6471) [3] bound PPARα Ligand Binding Domain (LBD) have been determined.




R= $CO_2H$, X=H
GW409544
R= $CH_2NHCOEt$, X= $CF_3$
GW6471

The agonist stabilizes the active conformation of LBD by direct interaction with Y464 situated on the C-terminal AF-2 helix, thus allowing the coactivator binding and gene transcription. Conversely, the antagonist disrupts the interaction between PPARa receptor and coactivator and promotes the binding of the co-repressor peptide. This indicates that antagonist induce an LBD conformation that interacts efficiently with co-repressors. The main difference between both molecules is the volume and nature of R group that prevents the proper disposition of AF-2 in the active conformation

We present in this communication a targeted molecular dynamics study of the transition between the active and inactive PPARα-LBD conformations in the presence of both the agonist and antagonist ligands. This study aims to get insights into the specific and coordinated ligand-receptor interactions that stabilize one or another conformation. The results thus obtained can be paradigmatic for the molecular mechanism of activation of other members of the nuclear receptor superfamily.

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# QSPR MODELS FOR CATIONIC NEUROTRANSMITTER RECEPTORS: 5-$HT_{1A}$ RECPETOR LIGANDS

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Serotonin 1A (5-$HT_{1A}$) receptor is among the most studied types of G-protein coupled receptors, and the role of 5-$HT_{1A}$ receptors in various central nervous system diseases have been well documented. The recently growing interest for 5-$HT_{1A}$ receptor agonists has been due to their neuroprotective action in different models of CNS injury. Activation of neuronal $5HT_{1A}$ receptors appears to be involved in neuroprotective effects.
Most of the previous QSAR models of 5-$HT_{1A}$ ligands have been developed by using small number of ligands some of them possessing similar chemical structures. The quantitative models, moreover, have not been validated by strict statistical procedures.
The aim of our study was to develop a more general model for ligands of $5HT_{1A}$ receptors using the 3DNET method. In the study a database was built, which contained structurally diverse ligands with their affinities. We selected 167 compounds with pKi values (measured in rat cortex); the affinities spanned a difference of 5 orders of magnitude (the highest and the lowest pKi values were 5.00 and 9.82, respectively). For model validation, leave-one-out, leave-n-out cross-validations, and an external validation were used. As the statistical test, a new method (shuffle) was also applied.
The 3D structures were obtained by (PM3 method, Hyperchem Release 7). The descriptors were calculated with Dragon program (Version 3.0, Milan Chemometrics, Milan). The 3DNET program (Version Beta 1.1.50) was used for selection of descriptors and for MLR, PLS and neural network computations. The predictivity of the artificial neural network was compared to other model building methods, like multiple linear regressions and partial least square projection to latent variables.
In the best model we have developed, values of $Q^2$ obtained by the bootstrap and external validation were higher than 0.4 and 0.3, respectively. The shuffle test did not draw any overlap, re-affirming the validity of the model.

# QSPR MODELS FOR CATIONIC NEUROTRANSMITTER RECEPTORS: $\alpha_1$-ADRENOCEPTOR LIGANDS

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The $\alpha_1$-adrenoceptors are involved in the pathomechanisms of various diseases. In particular, $\alpha_1$-adrenoceptor antagonists have attracted much attention over the past decade, due to their potential utility for the treatment of benign prostatic hiperplasia. In parallel, structure - affinity relationship studies on various classes of ligands of $\alpha_1$-adrenoceptors have been carried out to develop three dimensional models for qualitative or even quantitative prediction of the binding affinity. However, most of the models reported so far have been elaborated using ligands with a common structural skeleton. Consequently, their predictivity with acceptable accuracy has generally been limited to structurally relating compounds. In an effort to develop a model with wider scope, which can even be useful for screening of libraries with high diversity, we now describe our QSPR approach using DRAGON (Version 3.0, Milan Chemometrics, Milan) and, for the descriptor selection, 3DNET (Version 1.1.50 beta, Compelit, Budapest) programs.

In the database we included 230 compounds with published $\alpha_1$-adrenoceptor affinities (measured uniformly in rat brain using radioligand [$^3$H]-prazosin). For the model building, two third of the data was used, whereas one third of the full data set was used for external validation.

UFS (Unseen Forward Selection) method was applied for descriptor selection.

Several MLR (multilinear regression), PLS (partial least square) and ANN (artificial neural network) models were developed using scout scan, sequential trial & error and genetic algorithms. In generation of the model, model banks were built, and the best models were selected from the banks using bootstrap test.

All models we developed were carefully validated using bootstrap test, shuffle test and external validation. The best model we have reached is characterized by a value of 0.4 for $Q^2$ in external validation. Taking into consideration the high number and diversity of compounds included, the model seems to be acceptable for first prioritizations in virtual screening.

# NEW ANTI-VIRAL DRUGS FOR THE TREATMENT OF COMMON COLD

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Human Rhinovirus (HRV) is the most important etiologic agent of common cold in adults and children. At the present, no antirhinovirus agent exists in the market and the large number of different serotypes (~105) makes unlikely the development of vaccines. HRV is a single-stranded, positive sense RNA virus. Protease 3C is a cysteinyl protease which hydrolises Gln-Gly, Gln-Ser and Gln-Ala pairs and it is an essential protein for viral replication. Also, despite the high level of conservation among different serotypes, sequence allignment of viral protease 3C with mammalian protease reveals no homology. Thus, protease 3C is an optimal target for the development of anti-HRV agents. In the present work we investigated the design, the synthesis and the development of new potential reversible inhibitors against HRV protease 3C. In the past, several peptidic and non-peptidic structures have been formulated in order to act as analogues of the substrate. They all contain groups which can be attacked by the SH of the cysteine present in the active site of the protease, thus generating a reversible analogue of the transition state. Aldehydes, fluoromethylketones, isatines are just examples with excellent in vitro activity. Peptidic lactam AG7088 is now in Phase II clinical trial. Docking studies on the crystallized structure of HRV2 protease 3C, led us to the design and the synthesis of a series of 3,5 disubstituted benzamides, carrying electrophile moieties able to act as analogues of the substrate. Electrophile moieties include amides, esters and aloketones. Different substitutions on the aryl ring led us to investigate the importance of $\pi$-$\pi$ interaction on the stabilization of protease 3C-inhibitor complex. All structures were tested for enzimatic inhibition on HRV14 protease 3C at 10μM and, for the more active compounds, at 100 μM, 10 μM 1 μM and 0.1 μM. Results showed good improvement compared to the reference compound and need to be further investigated.

# SYNTHESIS AND EVALUATION OF TRIAZENE PRODRUGS AS CANDIDATES FOR MELANOCYTE-DIRECTED ENZYME PRODRUG THERAPY (MDEPT)

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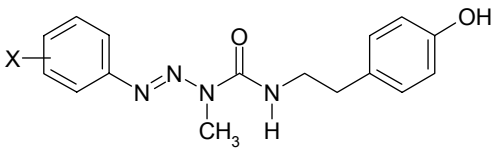
The approach which has been developed for triazene prodrugs is based on melanocyte-directed enzyme prodrug therapy (MDEPT). Triazenes have significant activity against malignant melanoma but show intolerable side effects. The mechanism sustaining their ability to act in cancer chemotherapy is the alkylation of DNA, through *in vivo* generation of methyldiazonium cations. Based on the unique occurrence of tyrosinase expression in melanocytes, the attachment of a drug to tyrosine would delivery the free drug only at the tumour site. To avoid systemic toxicity, the drug linker must be stable until drug release is required, and enzyme activity in blood and normal tissues must be very low.
To evaluate the feasibility of this approach, we have synthetised a range of triazene derivatives **1**. The stability of compounds **1** in phosphate buffer pH 7.4 and human plasma at 37ºC has been determinated. Compounds **1** are stable in pH 7.4 buffer for prolonged times (10 days) and in human plasma half-lives are 〉 70 h. The ability of prodrugs to liberate toxic agents upon exposure to tyrosinase is under study.

The authors thank Fundação para a Ciência e Tecnologia (Portugal) and FEDER for financial support.




**1**

# SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF DIPEPTIDE ESTERS OF THE ANTI-RETROVIRAL DRUG ACYCLOVIR

Cláudia S. G. P. Pereira[a,b], Cledir Santos[a *], João C. M. Barreira[a,b], Rui Moreira[c], Isabel C.F.R. Ferreira[b], Letícia Estevinho[b] and Paula Gomes[a]

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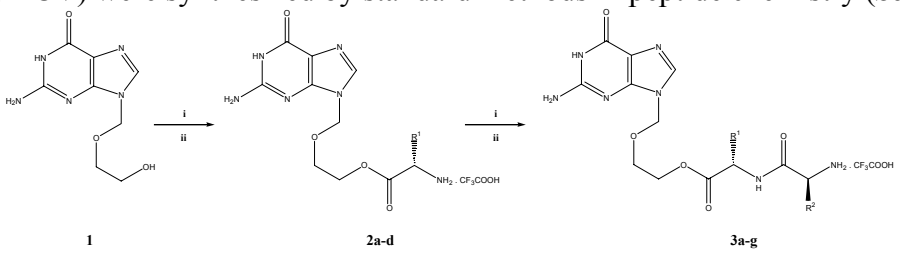
Seven dipeptide esters of the anti-retroviral drug 9-[(2-hydroxyethoxy)methyl]guanine (acyclovir, ACV) were synthesized by standard methods in peptide chemistry (*Scheme 1*).




**Scheme 1.** (i) $N^{\alpha}$-Boc-protected amino acid (BocAAOH), N,N'-dicyclohexylcarbodiimide (DCCI), N,N-dimethylaminopyridine (DMAP), dichloromethane (DCM); (ii) trifluoroacetic acid (TFA).

The derivatization of ACV with amino acids has been used for the development of ACV prodrugs. In fact, the valine derivative of ACV – valacyclovir – is a pro-drug of widespread use in herpes treatment. Mitra and co-workers have recently reported the synthesis and properties of novel dipeptide prodrugs of ACV, two of which are included in the present work (**3c** and **3d**) [1,2]. The compounds **3a-f** prepared were screened for their *in vitro* antimicrobial activity against Gram positive (*Bacillus cereus*, *Bacillus subtilis*) and Gram negative (*Pseudomonas aeruginosa*, *Escherichia coli*) bacteria, and also for their fungicidal activity using *Candida albicans*. The antimicrobial activities were evaluated by diameter measurement of haloes formed by growth inhibition caused by compounds **3** at different concentrations in DMSO [3]. Compounds **3** exhibited antimicrobial activity preferentially against Gram-positive bacteria and were all inactive against *Pseudomonas aeruginosa*. The minimal inhibitory concentrations (MIC) could be determined for some of the compounds in the concentration range assayed. Further dilutions are being done for MIC determination of most compounds against *B. cereus* and *B. subtilis*. The determined MIC were significantly lower than those of typical standards such as ampicilline, cloramphenicol or cyclohexamide. The parent drug, ACV, was also assayed and found to be less active than the corresponding dipeptide ester derivatives. These results suggest that compounds **3** might be activated by bacterial peptidases or actively transported through bacterial cell membrane.

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Thanks are due to FCT (Portugal) for financial support to CIQUP (Oporto), CECF (Lisbon) and CIMO (Bragança), and for doctoral research grant SFRH/BD/9277/2002 (C. Santos). We also thank MEDINFAR (Portugal) for their kind gift of acyclovir.

# SYNTHESIS OF DIPEPTIDE ESTERS OF AZT AND THEIR INTERACTION WITH THE hPEPT1 PEPTIDE TRANSPORTER[1]

Cledir R. Santos[a], Rui Moreira[b], Bente Steffansen[c], Carsten U. Nielsen[c] and Paula Gomes[a]

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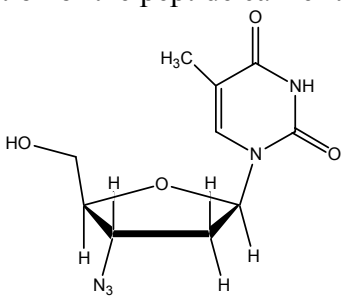
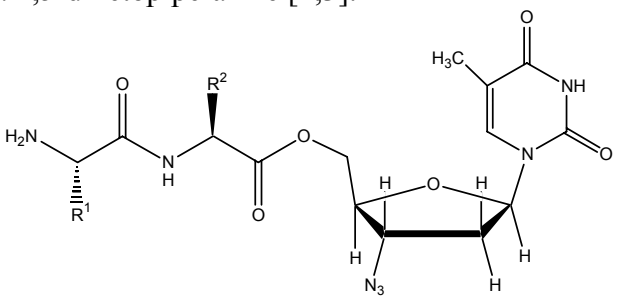
In the last decades, the acquired immunodeficiency syndrome – AIDS – has been a major health problem worldwide. The disease is caused by a retrovirus, the human immunodeficiency virus – HIV – and much research has been devoted to the development of efficient anti-retroviral agents, lethal to HIV while innocuous for the patient. Zidovudine, best known as AZT (**1**), has become the most widely employed drug in AIDS chemotherapy. However, AZT is associated with many adverse effects such as anaemia and leucopoenia [1]. Further, AZT is insoluble in the cerebrospinal fluid and does not penetrate into the brain tissue, and therefore may not prevent viral replication in the brain [1]. Thus, pro-drugs of AZT appear as a possible means to obviate the problems posed by the employment of AZT to treat AIDS. We have been working on drug derivatization with dipeptides, in order to obtain potential pro-drugs that could be activated by intramolecular cyclization of the peptide carrier to a 2,5-diketopiperazine [2,3].



**1**



**2**

We have recently published quite promising results regarding the application of this strategy to the phenolic analgesic paracetamol [3], which makes this approach potentially applicable to other hydroxyl-containing drugs such as AZT. Thus, we now wish to present the synthesis of dipeptide esters of AZT (**2**) as well as preliminary results on their interactions with the intestinal oligopeptide transporter hPEPT1. Out of eight different prodrugs tested, the Val-Ala and Val-Gly prodrugs were seen to have highest affinity (IC50 < 0.5 mM) for hPEPT1.

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[1] *Thanks are due to Fundação para a Ciência e Tecnologia (FCT, Portugal) for financial support to CIQUP and CECF research units, and also for the doctoral research grant SFRH/BD/9277/2002 (C. R. Santos). We also thank LAFEPE (PE, Brazil) and FARMANGUINHOS (RJ, Brazil) for their kind gift of AZT.*

# SYNTHESIS AND BIOLOGICAL EVALUATION OF NEW TERPENYLFURANES

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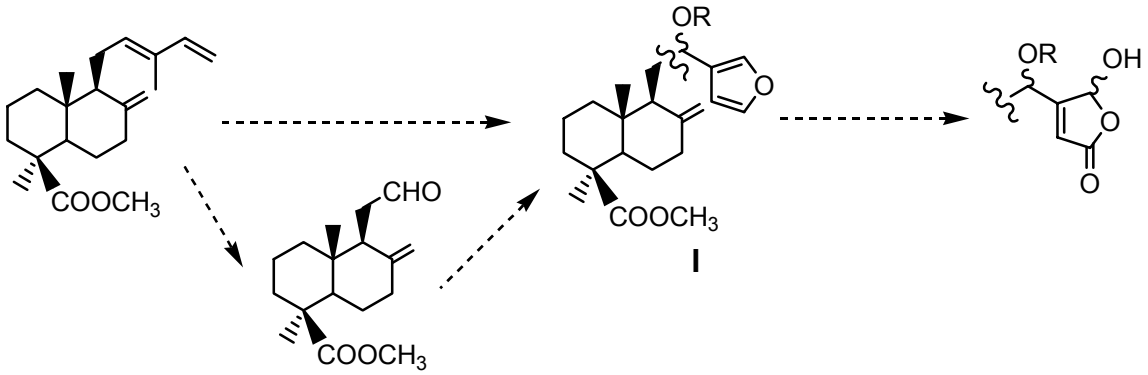
There is a large number of secondary metabolites isolated from marine sources that present very interesting cytotoxic properties. They have been isolated from sponges, tunicates, algae, etc and show great structural diversity [1]. Among these cytotoxic natural products, we have focused our attention on terpenoid derivatives bearing a furane ring such as thorectandrols or dysidiolide, sesterterpenoids with cytotoxicity values in the μM level [2, 3]. Thus, we have designed the estructures of new terpenylfuranes and planned their synthesis starting from labdane diterpenoids which are readily available in large quantities from *Cupressaceae* species.

Our group has wide experience on the isolation and characterization of this kind of natural products [4] and we have chosen *trans*-communic acid as starting material for the synthesis of the derivatives included in this communication.

*Trans*-communic acid was isolated from the berries of *Cupressus sempervirens* [5] and its methyl derivative was transformed into the furylderivate **I** by two procedures. The first one was based on the fotooxidation of the conjugate diene and the second involved the degradation of the side chain to the *tetranor*-aldehyde and the further condensation with furyllithium. The furylderivatives were also transformed into the corresponding lactols, fragments also present in many cytotoxic marine natural products.




The prepared compounds are being tested as cytotoxics against several neoplastic cell lines and the results will be presented in this communication.

**Acknowledgements:** Financial support came from came from Junta de Castilla y León (SA 068/04).

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# SYNTHESIS AND CYTOTOXICITY OF NEW 1,4-ANTHRAQUINONES

Ana Mª Gamito[a], Simone A. Gualberto[a], Mª Ángeles Castro[a], J. Mª Miguel del Corral[a], Marina Gordaliza[a], Carmen Cuevas[b], Arturo San Feliciano[a]

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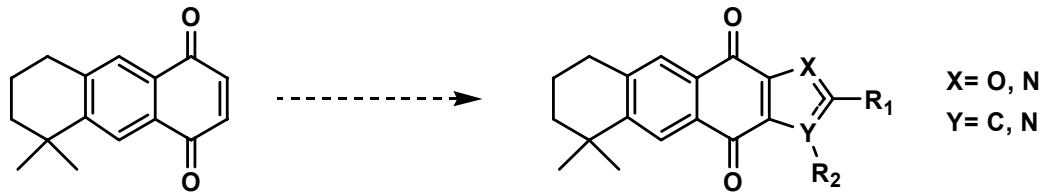
Many planar tri- and tetracyclic quinones have been found to display interesting antineoplastic properties and although the exact contribution of the quinone ring to their bioactivity remains uncertain, several studies have shown that the quinone moiety is essential for their cytotoxicity [1].

Among the different families of quinones [2], 1,4-naphthoquinones (1,4-NQs) and 9,10-anthraquinones (9,10-AQs) have been the subject of a great deal of biological studies. However the antitumor potential of 1,4-AQs has been little explored and only recently the antineoplastic activity of simple 1,4-AQs has been reported [3].

In the past years, our research group has been involved in the preparation of a large number of terpenyl-NQs that have shown high citotoxicity with $IC_{50}$ values under the μM level [4].

In this sense, we present now our studies on a straightforward synthesis of new 1,4-AQs through Diels-Alder addition of myrcene and *p*-benzoquinones and their further transformations into new polycyclic systems, in which the 1,4-AQ moiety is fused to five-membered heterocyclic rings such as furane, pirrole or imidazole.




The prepared compounds are being evaluated against different neoplastic cell lines and the results will be presented in this communication.

**Acknowledgements:** Financial support came from Junta de Castilla y Leon (SA 068/04)

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# NEW CYTOTOXIC TERPENYLNAPHTHOHYDROQUINONES FROM MYRCEOCOMMUNIC ACID

Mª Lucena Rodríguez[a], Mª Angeles Castro[a], José Mª Miguel del Corral[a], Marina Gordaliza[a], Carmen Cuevas[b] and Arturo San Feliciano[a].

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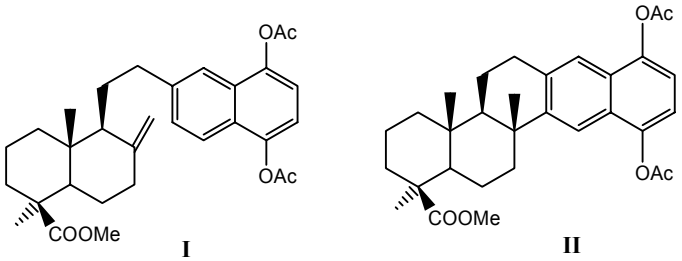
Every year many compounds showing interesting cytotoxic properties are isolated from marine sources. Many of these cytotoxic natural products are constituted by a quinonic or hydroquinonic moiety attached to a terpenoid skeleton, as it is the case of Avarol and Avarone [1].

Our research group has used these compounds as models for the design of new cytotoxic terpenylquinones and has chosen myrceocommunic acid (easily available from berries of *Juniperus oxycedrus*) as starting material for the synthesis of numerous analogues. We have studied the influence of both the terpenic and the quinonic residues on the cytotoxicity of these derivatives. Some of them have shown a very interesting potency and selectivity [2].

Among them, the terpenylnaphthohydroquinones **I** and **II**, obtained by Diels-Alder cycloaddition between methyl myrceocommunate and *p*-benzoquinone followed by oxidation, reduction and acetylation, were selected for introducing further modifications on the terpenic and quinonic moieties. Such transformations included oxidation to corresponding quinones, isomerizations, oxygenated functionalities, etc. accompanied by differents rearrangements in the decaline core.




**I** **II**

The synthesised analogues have been evaluated against several neoplastic cell lines and the evaluation results, mostly in the micromolar range, will be presented.

**Acknowledgements:** Financial support came from Junta de Castilla y León (SA 068/04).

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# VASORELAXANT ACTIVITY OF A NEW PHTHALAZINONE. SYNTHESIS AND STUDIES ON THE MECHANISM OF ACTION

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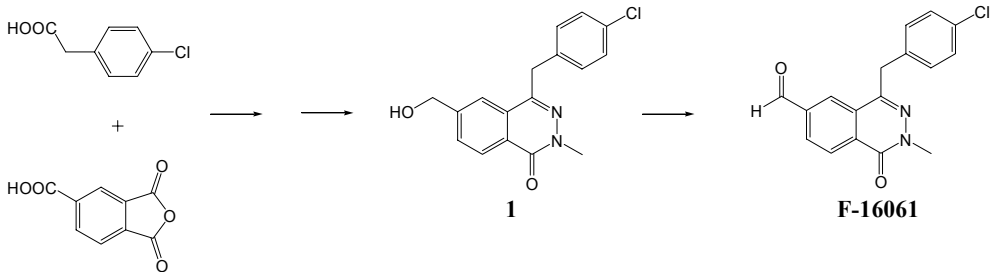
Hypertension is one of the most common cardiovascular diseases, leading to the development of stroke, coronary heart disease, cardiac failure or renal insufficiency [1]. Consequently, much work is ongoing with the aim of developing novel and more efficacious antihypertensive drugs. Drugs which increase vascular relaxation through different mechanisms are a major focus of such work.

Many studies have shown that phthalazinone derivatives can display important antihypertensive and anti-asthmatic effects, and such drugs have also been demonstrated to exert inhibitory activity on phosphodiesterases and on platelet aggregation [2].

We have synthesized a new family of phthalazinones displaying vasorelaxant activity. Among them, compound **F-16061** exerted the greatest vasorelaxant effect on rat aortic rings previously stimulated with phenylephrine (PE). Additionally, when pre-incubated with rat aorta, this compound reduced the subsequent vasoconstrictive effect of PE. The phthalazinone was prepared, as shown in the scheme below, through condensation of trimellitic anhydride with *p*-chlorophenylacetic acid to give an intermediate benzalphthalide, which was properly reduced to the corresponding alcohol, further treated with methylhydrazine to provide phthalazinone **1**, whose Swern oxidation led to the corresponding aldehyde, **F-16061**.




We have performed further experiments in order to establish the mechanism of action of **F-16061**. We examined its inhibitory effects on aggregation of human platelets, as well as intracellular calcium changes in platelets loaded with the calcium-sensitive dye Fura-2-AM, and $^{45}Ca^{2+}$ uptake / release in saponin-permeabilized human platelets. Results suggest that **F-16061** inhibits platelet aggregation, and that its effects are mediated through inhibition of calcium release from intracellular stores by blocking $IP_3$ receptors. In conclusion, this compound has important vasorelaxant and platelet inhibitory actions, which appear to be mediated through $IP_3$ receptor blockade resulting in a decrease in intracellular calcium release. Whether this compound will have therapeutic usefulness remains to be determined.

**Acknowledgements:** Financial support came from "Junta de Castilla y León" (JCyL Grant: SA 25/00B). AEGC also thanks to JCyL fellowship for supporting her placement in London.

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# ANXIOLYTIC EFFECTS OF PHTHALAZINONES AND HETEROCYCLIC RELATED COMPOUNDS

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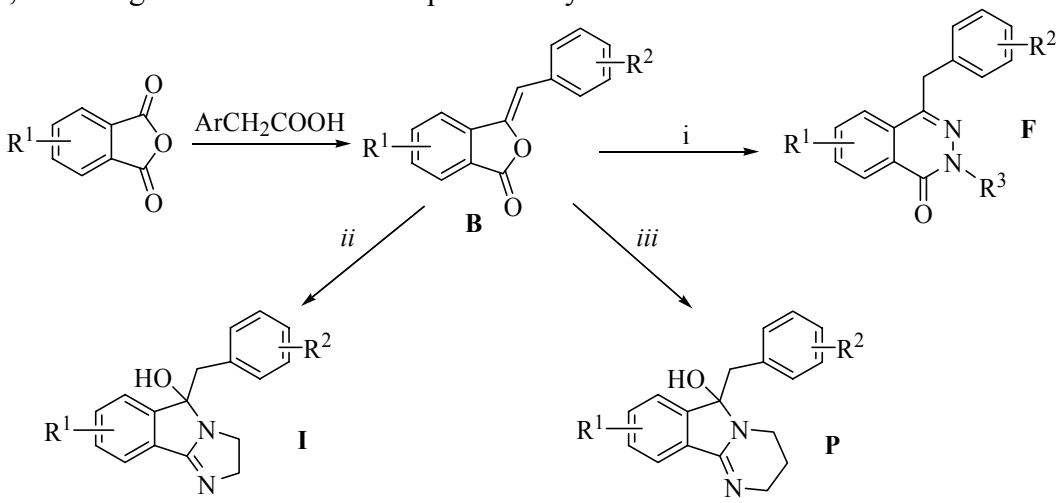
Anxiety disorders are common with high prevalence indexes in both developed and developing countries [1]. Pathological manifestations of anxiety are often chronic and include generalised anxiety disorder, obsessive–compulsive disorder, panic disorder, post-traumatic stress disorder and social and specific phobias [2].

We have reported previously the synthesis and evaluation of the title compounds in other bioactivity areas [3]. We describe here the anxiolytic effects observed through the elevated plus maze test in mice of some heterocyclic compounds belonging to the families of benzalphthalides (**B**), phthalazinones (**F**), imidazo[2,1-*a*]isoindoles (**I**) and pyrimido[2,1-*a*]isoindoles (**P**). Anxiolytic effects were estimated on the basis of the spent time and the number of entries into the open arms. Among the compounds evaluated two benzalphthalides, five phthalazinones, one imidazoisoindole and one pyrimidoisoindole, induced significant increments in the spent time and the number of entries into the open arms, after single administration intraperitoneally.




*i*) $H_2N\text{-}NHR_3$.; *ii*) $H_2N\text{-}(CH_2)_2\text{-}NH_2$.; *iii*)$H_2N\text{-}(CH_2)_3\text{-}NH_2$

**Acknowledgements**: Financial support came from Junta de Castilla y León. (Grant: SA25-00B). AZ thanks to the CIS-IMSS (México) for a post-doctoral fellowship. Collaborative research performed under the auspices of the "Programa Iberoamericano de Ciencia y Tecnología para el Desarrollo (CYTED), Sub-Programa X".

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# CALPAIN INHIBITORS AS POTENTIAL DRUG CANDIDATES FOR THE TREATMENT OF DUCHENNE MUSCULAR DYSTROPHY

Cyrille Lescop[a], Hervé Siendt[a], Reto Bolliger[a], Marco Henneböhle[a], Holger Herzner[a], Philipp Weyermann[a], Andreas von Sprecher[a], Mark Foster[b], Isabelle Courdier-Fruh[b], Michael Erb[b], Alexandre Briguet[b] and Josef P. Magyar[b]

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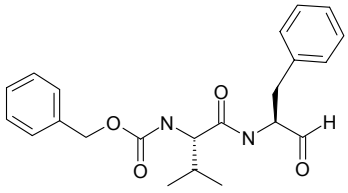
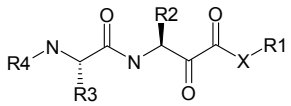
Calpain I and II are calcium-dependent cytosolic cystein proteases which are widely distributed in mammalian cells. They hydrolyze physiologically important proteins when activated by elevated intracellular concentration of calcium cation and are therefore implicated in a variety of disorders such as muscular dystrophy [1,2].
Duchenne Muscular Dystrophy (DMD) is a fatal inherited neuromuscular disease which is caused by the mutation on the gene encoding for dystrophin. DMD is associated with progressive deterioration of muscle function and no effective treatment is currently available [3]. Calpains are over-activated in dystrophin-deficient muscles and contribute to muscle wasting through increased proteolysis. Inhibition of calpain is therefore regarded as a possible therapeutic strategy for the treatment of DMD.
Starting from known calpain inhibitor lead compounds such as MDL28170 **1**, we initiated a lead optimization program to develop calpain inhibitors **2** with improved uptake into muscle cells. The presentation will describe the synthesis and evaluation of peptide keto-carbonyl compounds. These inhibitors exhibited activity with IC50 value at nanomolar range and they showed greatly improved potency over reference compound MDL28170 in the cellular assay. Several drug candidates were tested *in vivo* in *mdx* mice [4], a well established mouse model for DMD. They showed positive effects on two histological parameters demonstrating their potential as a treatment option.




**1**, MDL28170




**2**

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# NEW BUILDING BLOCKS FOR DRUG SYNTHESIS: 4-ACYL-5-HYDROXY-1-PHENYL-3-TRIFLUOROMETHYLPYRAZOLES

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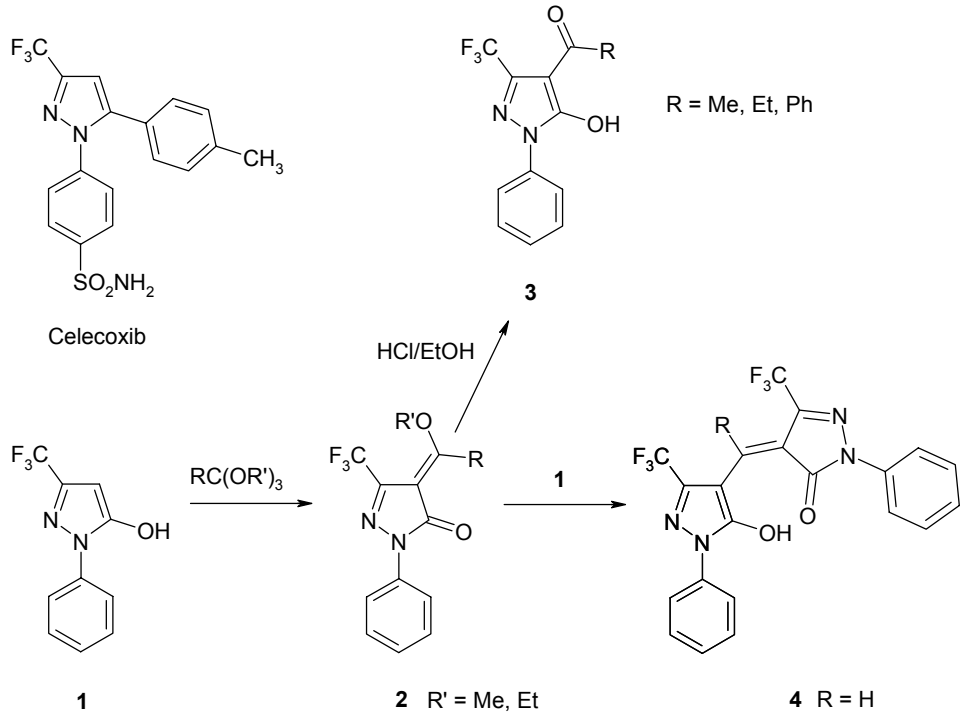
The 1-aryl-3-trifluoromethyl-1*H*-pyrazole unit is a substructure of many biologically active compounds including, for instance, the well-known cyclooxygenase-2 (COX-2) inhibitor Celecoxib (Celebrex™). Here we describe a simple synthesis of somewhat related building blocks **3** characterized by a 4-acyl-5-hydroxy-1-phenyl-3-trifluoromethyl-1*H*-pyrazole structure. Thus, condensation of pyrazolone **1** with various ortho esters leads to enol ethers **2** which can be easily cleaved by treatment with ethanolic hydrochloric acid to afford the target ketones **3**. In contrast, reaction of **1** with ethyl orthoformiate gives the dimeric product **4**. Detailed NMR spectroscopic investigations with the title compounds, utilizing also through-space $^{19}F,^{13}C$ couplings, provided far-reaching hints regarding their structure in solution.

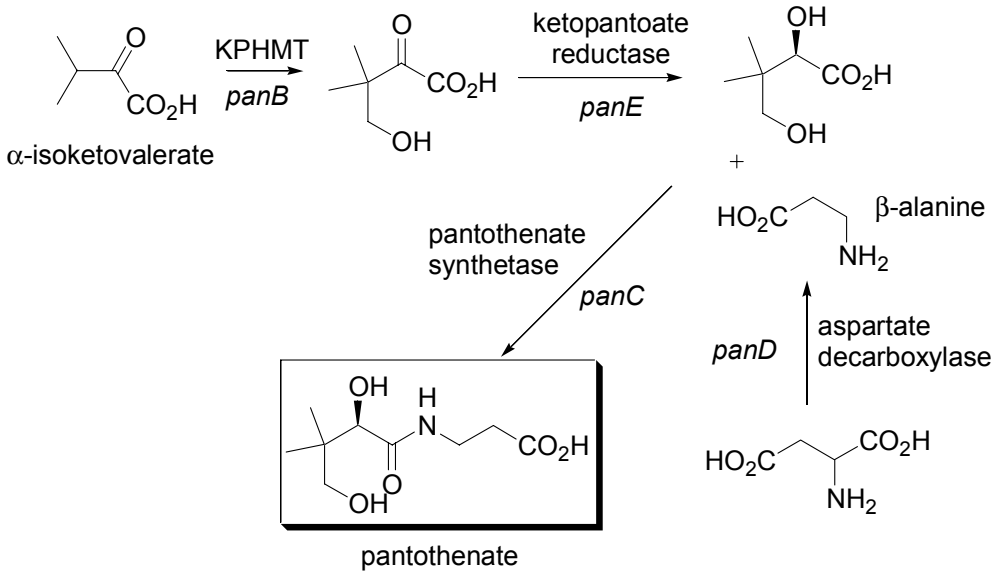


# INHIBITION OF PANTOTHENATE SYNTHETASE, AN ENZYME ON THE PANTOTHENATE PATHWAY

Chris Abell[b] and Kellie L. Tuck[a,b]

[a]School of Chemistry, Monash University, Clayton, Victoria, Australia 3800
[b]University Chemical Laboratories, University of Cambridge, Cambridge, UK CB2 1EW.

Pantothenate (otherwise known as Vitamin B5) is a key precursor for the biosynthesis of coenzyme A (CoA) and acyl carrier protein (ACP). Both of these are necessary cofactors for cell growth and are involved in essential biosynthetic pathways. The pantothenate pathway is not present in mammals and consequently represents an exciting target for the development of novel antibiotics and herbicides. The pathway is best understood in *E. coli*, where it comprises four enzymatic reactions.




***The biosynthesis of pantothenate (Vitamin B5) in bacteria, yeast and plants.***

Pantothenate synthetase is the last enzyme in the pathway, it catalyses the condensation of pantoate and β-alanine in the presence of ATP to give pantothenate. The overall reaction consists of two sequential steps, initial formation of a cofactor-substrate intermediate, followed by subsequent nucleophilic attack on the activated carbonyl by β-alanine
Inhibition of pantothenate synthetase by compounds that mimic the cofactor-substrate intermediate adduct will be discussed.

# THE BASAL ACTIVITY OF CONSTITUTIVE ANDROSTANE RECEPTOR (CAR) - HOMOLOGY MODELLING VERSUS X-RAY CRYSTALLOGRAPHY

Björn Windshügel[a], Johanna Jyrkkärinne[b], Antti Poso[c], Paavo Honkakoski[b] and Wolfgang Sippl[a]

[a]Institute for Pharmaceutical Chemistry, Martin-Luther-University Halle-Wittenberg, 06120 Halle, Germany
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The constitutive androstane receptor (CAR, NR1I3) belongs to the superfamily of nuclear hormone receptors that function as ligand-activated transcription factors. CAR plays an essential role in the metabolism of hormones and xenobiotics. In contrast to related nuclear hormone receptors CAR exhibits a constitutive activity for which the structural basis remains unclear.

To investigate the basal activity of CAR and the effect of co-activator binding homology models of the ligand binding domain (LBD) were generated [1]. Available x-ray structures of the related pregnane X (PXR) and the vitamin D receptor (VDR) were used as templates. Molecular dynamics (MD) simulations of CAR alone and in complex with a co-activator peptide (SRC-1) revealed a hypothesis for the activation mechanism. The basal activity of CAR can be explained by specific interactions between amino acids on the LBD and its C-terminal activation domain (AF-2).

To support the derived activation hypothesis, site directed mutagenesis studies were carried out on amino acids of the ligand binding pocket (LBP) [2]. In addition virtual receptor mutants were created and examined by MD simulations. The results not only support the proposed mechanism of constitutive activity but also give insights into the structural changes in the ligand binding pocket (LBP) upon mutation.

Docking studies carried out with program GOLD yielded the interaction modes of structurally diverse agonists unravelling the mechanisms by which ligands enhance CAR activity.

Compared to recently published x-ray structures of human CAR (1XV9, 1XVP) our homology model shows only minor deviations. However, the crystal structures - complexed with agonists - contain an unique additional helix that is supposed to keep the receptor in an activated state. It remains unclear whether this newly found helix is involved in maintaining constitutive activity or rather an effect of agonist binding, indeed.

[1] Windshügel et al. (2005) *J Mol Mod*, published online
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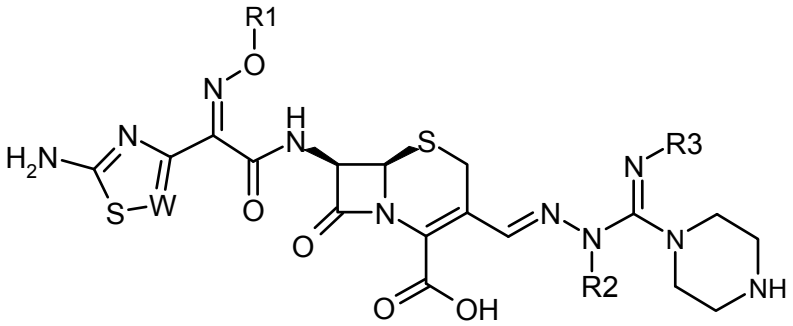
# SYNTHESIS AND SAR (STRUCTURE ACTIVITY RELATIONSHIP) OF NOVEL BROAD SPECTRUM CEPHALOSPORINS INCLUDING MRSA-ACTIVITY

Werner Heilmayer, Gerd Ascher, Rodger Novak, Lars Hesse, Susanne Paukner, Hildegard Sieberth, Josef Wieser, Klaus Thirring

Antibiotic Research Institute, Sandoz GmbH, Vienna, Austria

We describe the synthesis and discuss the SAR of new parenteral cephalosporins (cephem-azomethines), which display highly improved activity against clinically relevant pathogens, i.e. MRSA (*methicillin resistant Staphylococcus aureus*), *Enterococcus faecalis* and *Enterobacter cloacae*. Until now, such activity was unknown for this class of compounds.

In general, the residue in position 7 was introduced by acylation of 7-amino-3-cephem-3-aldehyde with the heterocyclic acid chloride [1]. Condensation of the resulting hydroxylactone with aminoguanidines gives the desired cephalosporines. The most interesting compound found in this series, BC-1175 [2], was selected for further investigation.




**BC-1175: W = N; $R^1$ = $CH_2F$; $R^2$ = Methyl; $R^3$ = H**

On a molecular basis, the corresponding $IC_{50}$-values obtained from measurements of the binding of these compounds to PBP2a (Penicillin Binding Protein 2a) will be given. A correlation to the *in vitro* MRSA activities is discussed.

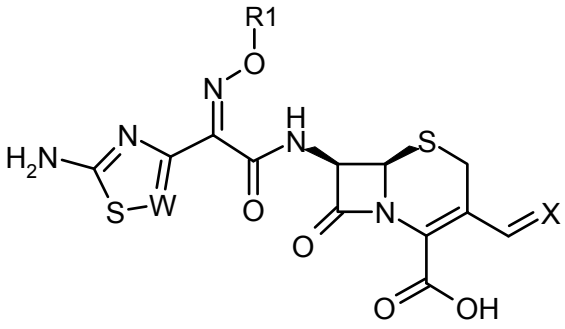
[1] Gerd Ascher, Johannes Ludescher, Hubert Sturm, Josef Wieser; WO 9529182
[2] Gerd Ascher, Josef Wieser, Michael Schranz, Johannes Ludescher, Johannes Hildebrandt; WO 9843981

# PREPARATION OF NEW AMINOGUANIDINES AS BUILDING BLOCKS FOR THE SYNTHESIS OF NOVEL HIGHLY ACTIVE CEPHALOSPORINS

Klaus Thirring, Gerd Ascher, Hildegard Sieberth, Rodger Novak, Josef Wieser, Werner Heilmayer

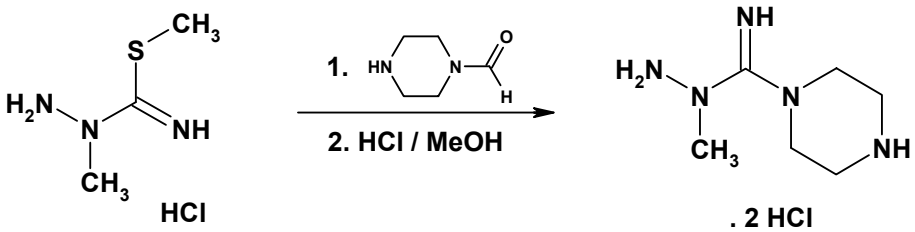
Antibiotic Research Institute, Sandoz GmbH, Vienna, Austria

Cephalosporins are widely used for the treatment and prophylaxis of bacterial infections. Emergence of resistant bacterial strains (e.g. *methicillin resistant Staphylococcus aureus* MRSA) against available antibiotics creates the need for new agents such as Cephalosporins with increased activity against these pathogens.
An approach to target resistant bacterial strains results from the discovery of Cephem-azomethines with broad antibacterial spectrum (gram-positive and gram-negative). Additionally, they also demonstrate activity against MRSA which is until now an unknown effect in the Cephalosporin class.
The synthesis of new aminoguanidine moieties used as substituents for cephalosporins in position 3 is described. Novel synthetic paths are discussed along with the description of unexpected differences in physicochemical parameters.




**X = aminoguanidine-moiety**

For example, S-methyl-2-methyl-isothiosemicarbazide is reacted with N-formylpiperazine followed by removal of the formyl group under acidic conditions to yield the corresponding aminoguanidine [1].



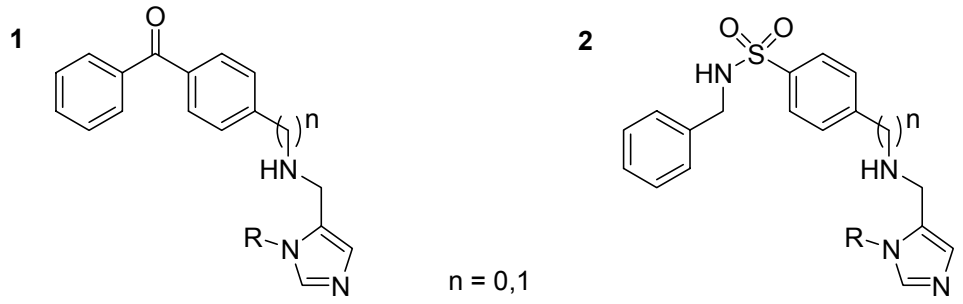

[1] Gerd Ascher, Josef Wieser, Michael Schranz, Johannes Ludescher, Johannes Hildebrandt; WO 9843981

# NOVEL LEAD STRUCTURES FOR ANTIMALARIAL FARNESYLTRANSFERASE INHIBITORS

Katrin Kloth[a], Katja Kettler[a], Jochen Wiesner[b], Hassan Jomaa[b] and Martin Schlitzer* [a]

[a]Department Pharmazie, Ludwig-Maximilians-Universität München, Germany
[b]Biochemisches Institut der Justus-Liebig-Universität Gießen, Germany

Farnesyltransferases (FTases) were identified in pathogenic protozoa including *Plasmodium falciparum*, the causative agent of malaria tropica [1]. Malaria tropica is the most important protozoa caused disease accounting for 2 to 3 million death cases each year. There is an urgent need for new anti-malarial therapeutics because of the accelerated occurrence of malaria parasites resistant to chloroquine and other commonly used drugs. For this reason we are searching for novel lead structures for antimalarial farnesyltransferase inhibitors. Previously, we have described the development of p-aminobenzophenone- and sulfonamide-based farnesyltransferase inhibitors with a nitrophenylfurylacryloyl moiety as a substructure which show significant activity ($IC_{50}$-values in the nanomolar range) as antimalarial agents [2].
FTase is a heterodimeric zinc metalloenzyme. It is already known that benzylimidazole-derivates are excellent cysteine surrogates of CAAX-peptidomimetic farnesyltransferase inhibitors by serving as an alternative ligand for the FTase's active site zinc ion [3].
Therefore, we combined 4-benzophenone- (**1**) and *N*-benzylsulfonamide-based (**2**) analogues with different imidazoles to improve the antimalarial activity.

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# STRUCTURE-BASED DRUG DESIGN OF NEW DXR-INHIBITORS AS ANTI-INFECTIVE AGENTS

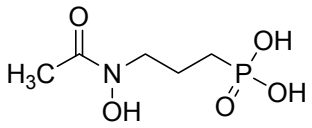
Johann Perruchon,[a] Regina Ortmann,[a] Jochen Wiesner,[c] Katrin Silber,[b] Hassan Jomaa,[c] Gerhard Klebe,[b] and Martin Schlitzer* [a]

[a] Department Pharmacy, Ludwig-Maximilians University, D-81377 Munich, Germany
[b] Institute for Pharmaceutical Chemistry, Philipps University, D-35032 Marburg, Germany
[c] Institute for Biochemistry, Justus-Liebig University, D-35392 Gießen, Germany

In spite of half a century development of antibiotics infectious diseases still pose one of the most serious health problems due to the unavoidable development of resistant germs. Therefore, increased efforts in anti-infective drug discovery are urgently needed. Enzymes involved in the nonmevalonate (DOXP/MEP) pathway of isoprenoid biosynthesis pathway are promising targets for the development of anti-infective agents [1], since this pathway is present in many pathogenic micro-organism while absent in humans.

Fosmidomycin and FR900098 are well-known as potent inhibitors of 1-desoxy-D-xylulose-5-phosphonate (DOXP) reductoisomerase (DXR), one enzyme of the DOXP/MEP pathway. However, their effectiveness against certain pathogens is hampered by their high polarity.

Based on crystal structures and flexible docking, we designed FR900098 derivatives with reduced polarity.




***FR900098***

[1] Jomaa, H. *et al.*, *Science*, **1999**, *285*, 1573-1576.

# BENZOPHENONE-BASED FARNESYLTRANSFERASE INHIBITORS AS NOVEL ANTI-MALARIALS

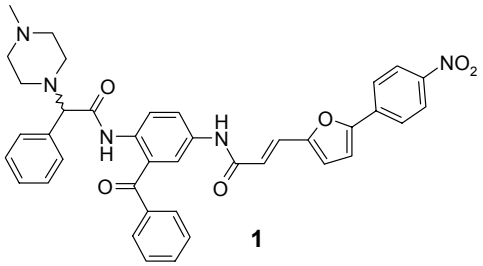
Martin Schlitzer[a], Katja Kettler[a], Jochen Wiesner[b], Mirko Altenkämper[a], Jacek Sakowski[a], Katrin Silber[c], Peter Haebel[c], Hans-Martin Dahse[d], Regina Ortmann[a], Hassan Jomaa[b], Gerhard Klebe[c]

[a]Department Pharmazie, Ludwig-Maximilians Universität München, Germany,
[b]Biochemisches Institut der Justus-Liebig-Universität Gießen, Germany,
[c]Institut für Pharmazeutische Chemie, Philipps-Universität Marburg, Germany,
[d]Hans-Knöll-Institut für Naturstoff-Forschung e.V., Jena, Germany

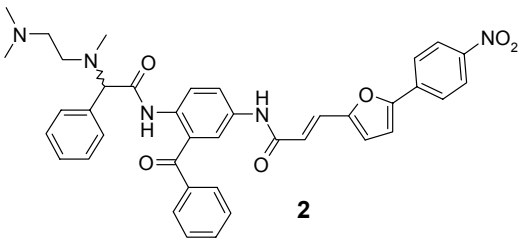
Malaria tropica, caused by the infection with *Plasmodium falciparum*, is one of the most important infectious diseases. Approximately 40% of the world population live in areas with malaria risk, and 2 to 3 million people die each year from malaria. Due to the increasing spread of parasites resistant against the common anti-malarials there is an urgent need for novel malaria therapeutics.

The development of farnesyltransferase inhibitors directed against *P. falciparum* has been generally accepted as a new strategy towards new drugs against malaria. Previously, we described benzophenone-based farnesyltransferase inhibitors with high *in vitro* anti-malarial activity but no *in vivo* activity.

Through the introduction of a methylpiperazinyl moiety we obtained the first farnesyltransferase inhibitors (e.g. **1**) for which *in vivo* anti-malarial activity was described [1]. Subsequently, a structure-based design approach was chosen to further improve the anti-malarial activity of this type of inhibitors.



**1**



**2**

Since no crystal structure of the farnesyltransferase of the target organism is available, homology modelling was used to reveal differences between the active sites of the rat/human and the *P. falciparum* farnesyltransferase. Based on flexible docking results, the piperazinyl moiety was replaced by open chain amines (e.g. the *N,N,N'*-trimethylethylenediamine moiety). This modification resulted in an inhibitor (**2**) with significantly improved *in vitro* and *in vivo* anti-malarial activity. Currently, this compound represents the farnesyltrasferase inhibitor with the highest anti-malarial activity known. Furthermore, inhibitor **2** displayed a notable increase in selectivity towards malaria parasites compared to human cells.

[1] Wiesner, J.; Kettler, K.; Sakowski, J.; Ortmann, R.; Katzin, A. M.; Kimura, E. A.; Silber, K.; Klebe, G.; Jomaa, H.; Schlitzer, M. Farnesyltransferase-Inhibitoren hemmen das Wachstum von Malaria-Erregern in vitro und in vivo. *Angewandte Chemie* **2004**, *116*, 254-257; Farnesyltransferase Inhibitors Inhibit the Growth of Malaria Parasites In Vitro and In Vivo. *Angew. Chem. Int. Ed.* **2004**, *43*, 251-254.

# BENZOPHENONE-BASED FARNESYLTRANSFERASE INHIBITORS DISPLAY HIGH ACTIVITY AGAINST TRYPANOSOMATID PARASITES

Mirko Altenkämper[a], Katja Kettler[a], Monica Esteva[b], Hans-Martin Dahse[c], Peter Haebel[d], Regina Ortmann[a], Esteban Bontempi[b], Gerhard Klebe[d] and Martin Schlitzer*[a]

[a]Department Pharmazie, Ludwig-Maximilians-Universität München, Germany;
[b]National Institute of Parasitology Buenos Aires, Argentina;
[c]Hans-Knöll-Institut für Naturstoff-Forschung e.V. Jena, Germany;
[d]Institut für Pharmazeutische Chemie, Philipps-Universität Marburg, Germany

New drugs are desperately needed against diseases caused by *Trypanosoma cruzi* and *Trypanosoma brucei* the causative agents of the African Sleeping Sickness and Chagas disease.
The WHO estimates that approximately 300.000 cases of African Sleeping Sickness occur annually in 36 countries. Nearly 16-18 million people in Latin America suffer chronically from Chagas disease. Most of the currently used drugs (nitrofuran- and nitroimidazole-derivatives) against these diseases are generally highly toxic and often ineffective because of the fast development of drug resistances in some cases.
Farnesyltransferase has been identified in several pathogenic parasites e.g. *T. brucei* and *cruzi* [1]. From the few farnesyltransferase inhibitors tested against trypanosomatids so far, only 4 have been assayed against *T. cruzi*.
We developed a new class of farnesyltransferase inhibitors based on a benzophenone scaffold [Fig.1] [2].

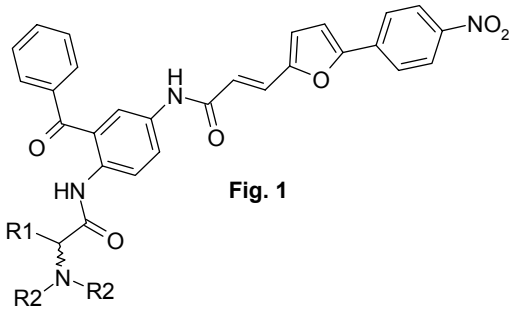



**Fig. 1**

Subject of this study was the efficacy of this type of compounds against *T. cruzi.* Some of our compounds displayed excellent activity against *T.cruzi in vitro* and *in vivo* while being not cytotoxic thus displaying high selectivity against *T.cruzi* in comparison to human cells.

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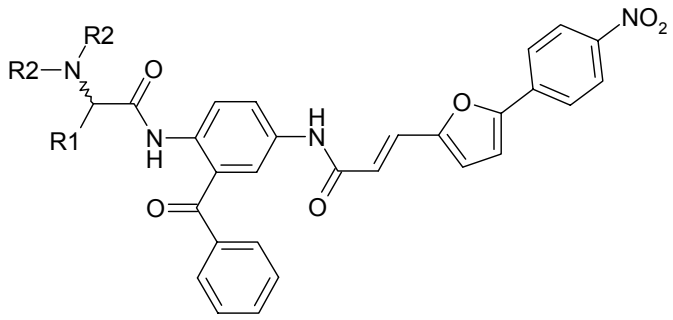
[1] Buckner, F. S. *et al.*, *J. Biol. Chem.* **2000**, *275*, 21870-21876.
[2] Schlitzer, M., *Curr. Pharm. Design* **2002**, *8*, 1713-1722.

# FARNESYLTRANSFERASE INHIBITORS AS NOVEL AGENTS AGAINST LEISHMANIASIS

Regina Ortmann[a], Katja Kettler[a], Mirko Altenkämper[a], Monica Esteva[b], Esteban Bontempi[b], Hans-Martin Dahse[d], Peter Haebel[c], Gerhard Klebe[c], Martin Schlitzer* [a]

[a]Department Pharmazie, Ludwig Maximilians-Universität München, Germany
[b]National Institute of Parasitology Buenos Aires, Argentina
[c]Institut für Pharmazeutische Chemie, Philipps-Universität Marburg, Germany
[d]Hans-Knöll-Institut für Naturstoff-Forschung e. V., Jena, Germany

*Leishmania* species are protozoan parasites for which adequate chemotherapies are not available. Because the presently used antimony compounds show significant toxic side effects novel agents are urgently needed.
Farnesyltransferase (FTase) catalyzes the posttranslational modification of numerous proteins which are involved in the intracellular signal transduction. FTases have been found in different pathogenous protozoa including *Leishmania* [1]. Therefore, FTase inhibitors are interesting candidates for new drugs against *Leishmania* parasites.
We tested a series of benzophenone based farnesyltransferase inhibitors against *Leishmania mexicana*.




R1 = aryl, alkyl
R2 = H, heterocycle, alkyl

Some of our derivatives showed excellent activity in the nanomolar range and high selectivity.

[1] Bruckner, F. S.; Eastman, R. T.; Nepomuceno-Silva, J. L.; Speelmon, E. C.; Myler, P. J.; Van Voorhis, W. C.; Yokoyama, K.; *Mol. Biochem. Parasit*. **2002**, *122*, 81 – 183.

# DESIGN OF NEW ALDOSE-REDUCTASE-INHIBITORS – A STRUCTURE BASED APPROACH

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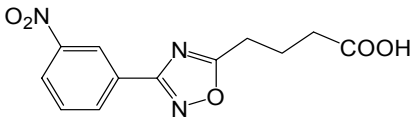
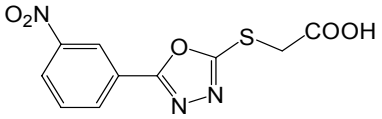
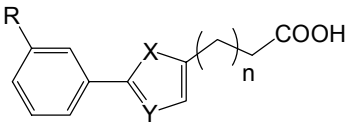
Diabetes mellitus is a universal health problem. The WHO estimates that 150 million people suffer from diabetes mellitus worldwide in 2005. A serious problem in the treatment of diabetes are the so-called long-term complications as neuropathy, retinopathy, nephropathy or cataract. The sorbitol accumulation has been proposed to be an important factor in their development. Catalyzing the NADPH-dependent reduction of glucose to sorbitol, aldose reductase is an important target for preventing these complications. Therefore, new aldose reductase inhibitors are strongly needed.
A structure-based design approach is an efficient way to create new aldose reductase inhibitors. Virtual screening based on the ultrahigh resolution crystal structure of the inhibitor IDD594 in complex with human AR, identified two compounds (**1,2**) with $IC_{50}$ values in the micromolar range, serving us as lead structures [1]. Based on the known interactions between the ligand and its binding pocket, we reduced the lead structures on the minimal structural requirements and developed practical synthetic pathways from commercially available compounds (**3**). The new synthesized compounds were assayed for their inhibition of AR, showing inhibitory activities in a low micromolar range. Additionally, based on flexible docking results, the alkylcarboxylic acid moiety was replaced by phenylacetic acid (**4**), improving the inhibitory activity.




**1 (BTB02809)**

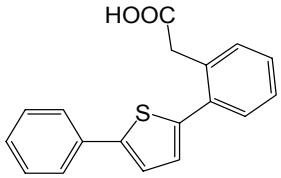



**2 (JFD00882)**




X = O, S Y = H n = 1, 2 | X = S Y = N n = 1 | X = N Y = S n = 1

**3**




**4**

[1] Kraemer, O. *et al*., *Proteins: Struct., Funct., Bioinf.* **2004**, *55*, 814-823.

# PREPARATION AND PRE-CLINICAL INVESTIGATION OF L-α-METHYLTYROSINE LABELED WITH IODINE -131 OR IODINE-123 (IMT-131I, IMT-123I)

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The amino acid α-methylotyrosine (IMT) radiolabeled with the iodine-131 or iodine-123 has been used in the diagnostics of recurrent brain tumors, in the planning of re-operation and/or external radiotherapy. The mechanism of IMT uptake is connected with the active transport of this amino acid to the rapidly proliferating tumor cells. From the clinical point of view the IMT-$^{123}$I is the radiopharmaceutical of choice due to the favorable physical characteristics of iodine-123. However, taking into account the high cost of imported iodine-123, the diagnostic investigations using IMT-$^{131}$I still present considerable clinical value.

The aim of our work was to indicate that both radiopharmaceuticals IMT-$^{131}$I and IMT-$^{123}$I can be prepared in a reproducible manner, retaining high in vitro and in vivo stability and thus can be a subject of preliminary clinical investigation.

For radiolabeling of IMT-$^{123/131}$I the electrophilic substitution reaction has been applied in the presence of iodogen. The walls of the reaction vial were covered with a film of iodogen and then 300μg of L-α-methylotyrosine dissolved in the boric buffer (pH 8.0) was added followed by iodine-131 or iodine-123 (111-3700 MBq) in carbonate buffer (pH =8.5). The reaction was carried out during 10 minutes. Then the reaction mixture was transferred on the Sephadex DEAE A-25 column and the column eluted with water. Purified radiolabel was collected in the first 5 fractions (1 ml each). For quality control of the labelling yield and radiochemical purity of the iodinated compounds the methods of HPLC and electrophoresis were employed. The investigations of biological distribution were carried out on Swiss mice and the abnormal toxicity test was performed according to Polish Pharmacopoeia VI.

Altogether 18 batches of IMT-$^{131}$I and 6 batches of IMT-$^{123}$I were prepared. The radiolabeling yield of IMT-$^{123}$I was at the level of 85-92%, and for IMT-$^{131}$I at the level of 92-98%. The radiochemical purity of both iodinated compounds was in the range of 99.5-99.9%. The shelf life of IMT-$^{123}$I was determined to be 10 hours from the date and hour of calibration of iodine-123, which is a consequence of its half-life (13.27 h). The shelf life of IMT-$^{131}$I was confirmed to be 7 days, when stored at $+4^0$C - $+8^o$C.

The preparation is not harmful in the dose of 4200MBq/70kg.

The final parameters of the two radiopharmaceuticals have been formulated:

Pharmaceutical form - $^{123/131}$I- IMT solution in 0.9% NaCl for injection,

Specific activity -10 - 36.6 mCi/mg (370-1357 MBq/mg)

Radioactive concentration > 95%, radionuclide purity > 98%

Both radiopharmaceuticals $^{123/131}$I-IMT fulfill the requirements of pre-clinical phase.

# [ $^{99m}$Tc] TRODAT-1 SYNTHESIS FOR DOPAMINE TRANSPORTER IMAGING

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[b] Jan Kochanowski University in Kielce 25 406 Kielce, Poland
[c]Institute of Organic Chemistry, PAS, Kasprzaka 44/52, 01-224 Warsaw, Poland

Technetium-99m, $^{99m}$Tc is the most commonly used radionuclide in clinical nuclear medicine for imaging procedures. $^{99m}$Tc is preferred to other radionuclides due to greater proton flux per unit of radiation dose delivered to the patent. Also there are several parameters as half life for imaging and cheapness which decided for its convenient using. Development technetium-99m labeled receptor specific imaging agent for studying the central nervous system is extremly useful for evaluation of brain function in normal vs. disease states. A several $^{99m}$Tc tropane derivative complexes was evaluated and characterized after injecting into rats [1]. Preliminary studies of $^{99m}$Tc a series of N-ethanethiol tropane complexes containing Tc$^{V}$O iminobis [ethanethiol] unit have been done. It appeared, however that SPET imaging in nonhuman was unsuccesful because of the low initial brain uptake. In search of selective and suitable ligand for chelating $^{99m}$Tc complexes as TRODAT-1 it was found that this compound can be useful as potential dopamine transporter imaging agent in patients with early stage of Parkinson's disease. In this study we describe a 4 step synthesis of TRODAT-1 from cocaine, prepared for easy labeling with technetium-99m.

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[1] Megalla SK.., Plosst K., Kung M-P., Chumpradit S., Stevenson DA., Kushner S.A., McElgin WTMozley PDKung HF.(1997) J.Med.Chem. 40, 9-17.

# RESTRICTED Arg-Trp(NPS) AND Trp(NPS)-Arg DERIVATIVES AS TRPV1 RECEPTOR CHANNEL BLOCKERS

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[1]Instituto de Química Médica (CSIC),Madrid, Spain
[2]Instituto Biología Molecular y Celular, Universidad Miguel Hernández, Alicante, Spain
[3]DiverDrugs, SL, Gavà, Barcelona, Spain

Vanilloid receptor-1 (TRPV1 or VR1) is a non-selective cation channel, predominantly expressed by peripheral neurons, which is known to play a key role in the detection of noxious painful stimuli, such as capsaicin, acid and heat. A growing body of evidence demonstrates the therapeutic potential of TRPV1 modulators, particularly in the management of pain [1]. Among TRPV1 antagonists, a series of Arg-rich peptides and several *N*-alkylglycines have recently been described as non competitive TRPV1 antagonists [2]. Similarly, we have found that the basic dipeptide derivative Arg-Trp(NPS) (**1**), previously identified as *in vivo* antinociceptive agents (icv) [3], is able to block the TRPV1 channel in the micromolar range. These compounds also block, but with less potency, the $Ca^{2+}$ influx through the NMDA receptor induced by glutamate. In order to obtain TRPV1 selective antagonists, we have now investigated the effect of the incorporation of conformationally constrained azetidine-containing Arg residues into compound **1** and its reverse sequence analogue Trp(NPS)-Arg.

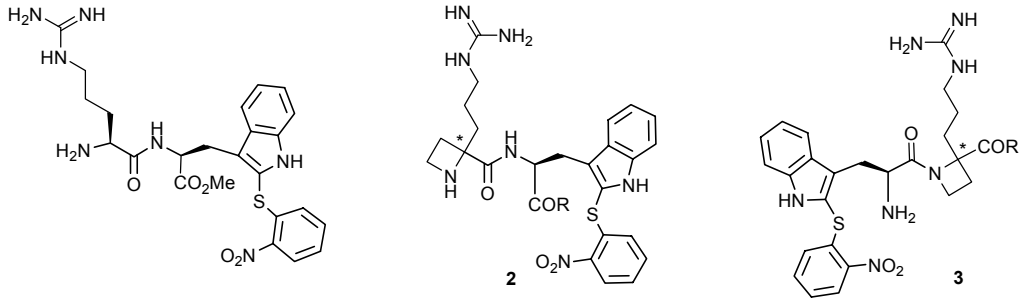



Figure 1

This contribution deals with the synthesis and biological evaluation, as NMDA and TRPV1 channel blockers, of compounds **2, 3** and related analogues (Figure 1).

[1] (a) Szallasi, A.; Blumberg, P.M. *Pharmacol. Rev.* **2002**, *118*, 110-121. (b) López-Rodríguez, M.L.; Viso, A.; Ortega-Gutiérrez, S. *Mini Reviews in Medicinal Chemistry* **2003***, 3,* 733-752. (c) Szallasi, A.; Appendino G. *J.Med.Chem.* **2004**, *47*, 2717-2723.
[2] (a) Planells-Cases, R. et al. *FEBS Lett*. **2000**, *481*, 131-136. (b) García-Martínez, C. et al. *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 2374-2379.
[3] (a) García-López, M.T. et al. *Peptides* **1986**, *7*, 39-43. (b) García-López M.T. et al. *J.Med.Chem.* **1987**, *31*, 1658-1663.

# 1-SUBSTITUTED 3-(ω-AMINOALKYL)-1*H*-INDOLE DERIVATIVES AS POSSIBLE σ LIGANDS.

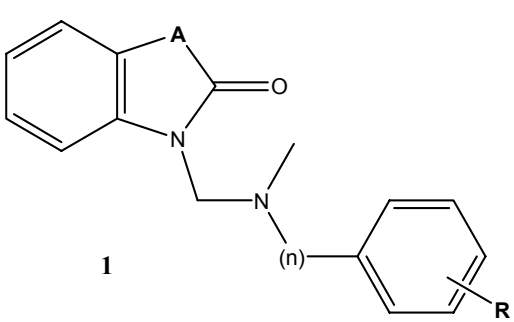
Maria Grazia Mamolo[a], Daniele Zampieri[a], Luciano Vio[a], Simona Collina[b], Ornella Azzolina[b], Chiara Florio[c]

[a]Department of Pharmaceutical Sciences – University of Trieste – Italy
[b]Department of Pharmaceutical Chemistry – University of Pavia – Italy
[c]Department of Biomedical Sciences (Pharmacology Sect.) - University of Trieste - Italy

Sigma (σ) 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. The interest for 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]. The $\sigma_2$ receptor agonists results in morphological changes and apoptosis in various cell lines, including breast tumor cells. Thus, $\sigma_2$ receptors may be involved in regulating cell growth and proliferation.

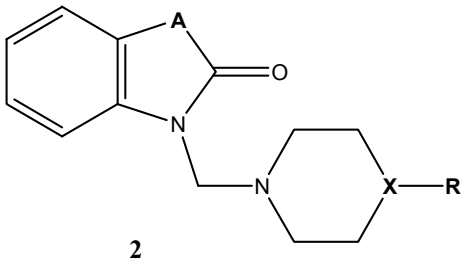
Several classes of structurally unrelated compounds interact with σ receptors, but only few $\sigma_2$ ligands are known.

With the aim to obtain new σ selective ligands, we synthesized some benzooxazol-2-one and benzooxazol-2-thione (**1** and **2)** derivatives.




**1**

A= O, S
n= 1,2,3
R= H, Cl, Br




**2**

A= O, S
X= CH, N
R= Ar, Ar-$CH_2$

All synthesized compounds will be tested for their σ receptors affinity.

[1] G. Ronsisvalle, O. Prezzavento, A. Marrazzo, F. Vittorio, E. Bousquet, R. Di Toro, S. Spampinato, *Eur. J. Pharm. Sci.*, 12 (2001) 277-284.
[2] K. W. Crawford, A.Coop, W.D.Bowen, *Eur. J. Pharmacol.*, 443 (2002) 207-209.

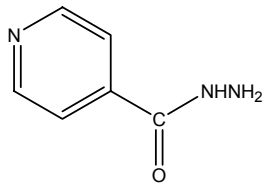
# SYNTHESIS AND ANTIMYCOBACTERIAL ACTIVITY OF 1,3,4-OXADIAZOL-2-ONE DERIVATIVES

Daniele Zampieri[a], Maria Grazia Mamolo[a], Luciano Vio[a], Giuditta Scialino[b], Elena Banfi[b]

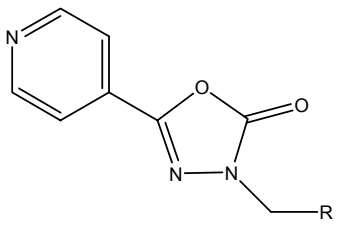
[a]Department of Pharmaceutical Sciences – University of Trieste, Italy
[b]Department of Biomedical Sciences (Microbiology Sect.) – University of Trieste, Italy

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 whit interesting antimycobacterial activity**:**



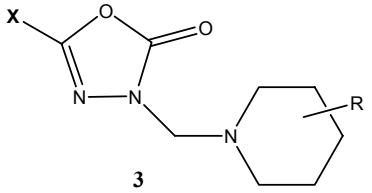

**1**




**2**

R= various cyclic amines

Trying to increase the antimycobacterial activity of the compounds **2**, we synthesized 3-(2, 3, and 4-methyl-piperidin-1-ylmethyl)-5-heteroaryl-3*H*-1,3,4-oxadiazol-




**3**

R= -$CH_3$

X=Ar, 2-,3-Pyr

2-one derivatives **3**, characterized by the presence of piperidine moiety:

We projected the substitution of the pyridin-4-yl ring with others heteroaryl rings maintaining the best pharmacophoric group in the 3- position of our precedent work [1].

[1] M.G.Mamolo, D.Zampieri, L.Vio – M.Fermeglia, M.Ferrone, S.Pricl – G.Scialino, E.Banfi; Biorg. Med. Chem (2005) in press.

# SAR EVALUATION OF CHIRAL *NAPHTYLORFINES* WITH ANTINOCICEPTIVE ACTIVITY

Ornella Azzolina[a], Simona Collina[a], Guya Loddo[a], Mariangela Urbano[a], Maria Grazia Mamolo[b], Daniele Zampieri [b], Luciano Vio [b], Annalisa Barbieri[c], Enrica Lanza[c], Victor Ghislandi[a]

[a]Department of Pharmaceutical Chemistry, University of Pavia, 27100 Pavia, Italy
[b]Department of Pharmaceutical Sciences, University of Trieste, 34100 Trieste, Italy
[c]Department of Experimental and Applied Pharmacology, University of Pavia, 27100 Pavia, Italy

Although many works concerning analgesia and opioid ligands have been recently published, the molecular basis of opioid action has not yet been completely elucidated [1]. The advance in molecular biology, synthetic methodology and molecular mechanics has made possible a deeper comprehension of the interaction mechanisms of opioid ligands with the different receptor subtypes. With the purpose to obtain analgesic compounds with reduced side effects, subtype selective ligands were designed and prepared. Nevertheless, this approach did not give the expected results and potent opioid analgesics without side effects are still unknown.
As an alternative approach, several attempts were made to identify non selective ligands of mu, kappa and delta receptors, characterized by different profiles of agonism and antagonism. Based on this approach, a non specific pharmacophore model for mu, kappa and delta receptors was proposed [2].
In the last years we dedicated a part of our efforts in the SAR of novel antinociceptive agents. The identified chiral lead compound, which belongs to a series of compounds named *naphtylorfines* shows a non subtype-specific binding with opioid receptors and an interesting *in vivo* analgesic activity (Hot Plate Test, in mice) [1]. Different derivatives of the lead compound were prepared in order to investigate the SAR of these *naphtylorfines*.
We considered the influence of both the structural features of the aliphatic and aromatic moieties, and of the stereochemistry of the tested compounds on their pharmacological properties.

In the present work, the synthesis, the analytical characterization and the configurational assignment of the new compounds is described. Biological results (affinity pattern of the compounds *versus* mu, kappa and delta receptors and *in vivo* evaluation of their antinociceptive activity) are discussed.

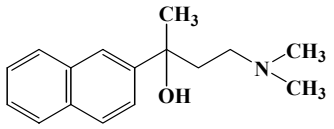
[1] Filizola, M.; Villar, H.O.; Loew, G.H. Molecular determinants of non-specific recognition of delta, mu and kappa opioid receptors. *Bioorg. Med. Chem.* **2001**, 9,69.
[2] Azzolina, O.; Collina, S.; Brusotti, G.; Rossi, D.; Linati, L.; Barbieri, A.; Lanza E.; Mennuni L.; Makovec F.; Alcaro S.; Ghislandi, V. XVI Convegno Nazionale Divisione Chimica Farmaceutica, SCI; Sorrento, settembre **2002**.

# RATIONAL APPROACHES TO THE DESIGN OF SELECTIVE SIGMA 1 LIGANDS

Ornella Azzolina[a], Simona Collina[a], Mariangela Urbano [a], Stefano Alcaro[b], Christian Lagner[c] and Thierry Langer[c]

[a]Department of Medicinal Chemistry, University of Pavia, 27100 Pavia, Italy
[b]Department of Pharmacobiological Sciences, University of Catanzaro, 88021 Roccelletta di Borgia (CZ), Italy
[c]Department of Pharmaceutical Chemistry, University of Innsbruck, A-6020 Innsbruck, Austria

In the present work the naphthylaminoalcohol (*R*/*S*)-**1** previously shown to exert moderate affinity for sigma1 receptors (Ki = 25 nM), was chosen as lead compound to develop novel sigma1 ligands using *Molecular Modeling* techniques.




**(R/S)-1**

Basing on pharmacophore sigma1 selective models of literature, proposed by Glennon [1] and Gund [1], we designed a virtual library of 171 arylamino analogues with diverse functionalities on the aromatic moiety and on the alkilamine side chain of (*R*/*S*)-**1**. Gund's additional electronegative site (O or S) did not map the O atom of (*R*/*S*)-**1**, thus suggesting to focus on alkanes and alkenes series of the designed library. Each compound of the library was subdued to conformational search with Monte Carlo method. We evaluated the match degree with both pharmacophore models and filtered the library. Interestingly, a shorter distance from amine center to the secondary hydrophobic site of (*R*/*S*)-**1** as well as of all *N*,*N*-dimethylamino derivatives suggested to enhance steric hindrance at the amino group. Further screening using Lipinski's rule-of-five, yielded 30 potential ligands. Subsequent selection by hand on the basis of chemistry compatibility and structural diversity gave rise to a 15-member small library. In this phase, we used a 3D pharmacophore model [3] for sigma1, developed with HypoGen module in Catalyst. Testing this five-point pharmacophore (4 hydrophobic and one positive ionizable features) against conformers of the 15 selected compounds, sustained the hypothesis to direct our synthetic work towards alkenes and alkanes series of compounds with a bulk substituent at the amino group.
Preliminary binding data, prompted us to synthesize new promising ligands bearing either β-naphthyl or 4-diphenyl aromatic moieties [4].

[1] Glennon, R., A.; Ablordeppey, S. Y.; Ismaiel, A, M.; El-Ashmawy, M. B.; Fischer, J. B.; Burke Howie, K.; *J. Med. Chem*. **1994**, *37*, 1214.
[2] Gund, T., M.; Floyd, J.; Jung, D.; *J. Mol. Graphics and Modelling* **2004**, *22*, 221.
[3] unpublished data.
[4] Collina, S.; Loddo, G.; Prezzavento, O.; Ronsisvalle, G.; Azzolina, O.. Synthesis and SAR of novel sigma 1 selective ligands. JMMC June 20-23, 2005; Vienna, Austria.

# SYNTHESIS AND SAR OF NOVEL SIGMA1 SELECTIVE LIGANDS

Simona Collina[a], Guya Loddo[a], Orazio Prezzavento[b], Giuseppe Ronsisvalle[b], Ornella Azzolina[a]

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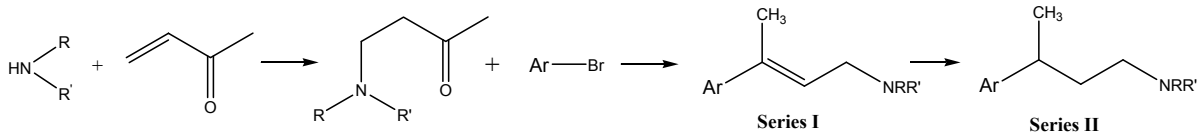
[b]Department of Pharmaceutical Sciences, University of Catania, 95125 Catania, Italy

A research on a series of naphthylalkylamines, previously synthesized to account for its possible involvement in opioid analgesia [1], indicated several dimethylaminoalkylnaphthalenes as novel potential ligands for sigma receptors.
Binding assays revealed that some compounds possess also affinity for $sigma_1$ receptors in nanomolar range.
These encouraging results led to the development of a small library [2] of new arylalkylamino analogues, characterized by suitable modifications of the aromatic moiety and of the alkylamine sidechain.
The proofing library for this approach was constructed as outlined in Scheme 1.

The β-aminoketones intermediates were prepared using Michael reaction, in which the methylvinylketone was allowed to react with the appropriate amines. Thus, the olefinic compounds (Series I) were obtained by nucleophilic addition of the aromatic anions to the β-aminoketones and quenching of the reaction with chloridric acid. Series II compounds were obtained by microwave assisted reduction of the corresponding alkenes.




**Scheme 1**

A careful evaluation of the $sigma_1$ binding data ranging from 0.78 to 229 nM provided interesting SAR observations.

[1] Azzolina, O.; Collina, S.; Brusotti, G.; Rossi, D.; Callegari, A.; Linati, L.; Barbieri, A.; Ghislandi, V. Chemical and biological profile of racemic and optically active dialkylaminoalkylnaphthalenes with analgesic activity. *Tetrahedron: Asymmetry* **2002**, *13*, 1073-1081
[2] Azzolina, O.; Collina, S.; Urbano, M.; Alcaro, S; Lagner, C; Langer, T. Rational approaches to the design of selective sigma1 ligands. JMMC June 20-23, 2005; Vienna, Austria.

# SYNTHESIS AND PHARMACOLOGICAL EVALUATION OF NEW 1-PHENYL-1,3,8-TRIAZASPIRO[4.5]DECAN-4-ONE DERIVATIVES AS NEW POTENTIAL SELECTIVE NOP LIGANDS

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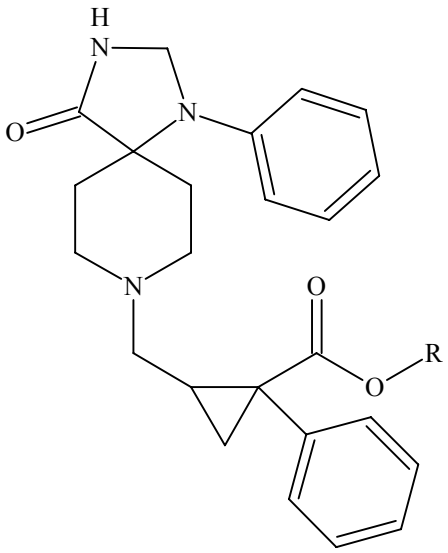
Recently reported as the fourth member of the opioid receptor family, NOP receptor [also called opioid receptor-like ($ORL_1$) or nociceptin receptor ($OP_4$)] is a G-protein-coupled receptor closely related to KOP, MOP and DOP opioid receptors. The endogenous ligand is a 17-amino acid neuropeptide, named nociceptin or orphanin FQ.
NOP receptor and nociceptin are widely distributed in the central nervous system as well as in the periphery and are involved in several physiological effects including nociception, attenuation of anxiety, modulation of learning and memory, stimulation of food intake, diuresis, hypotension and bradycardia, inhibition of reward pathways in drug addiction.
Thus, considering the number of modulatory effects in which nociceptin is involved, the synthesis of selective non-peptide NOP agonists or antagonists represent an interesting target for the development of novel therapeutics for several neurological conditions.
In this work we present the design, synthesis and preliminary pharmacological data of methyl, ethyl, propyl and isopropyl 2-[(4-oxo-1-phenyl-1,3,8-triazaspiro[4.5]dec-8-yl)methyl]-1-phenylcyclopropanecarboxylate enantiomers as new potential selective NOP ligands useful to provide new insights in the binding to this subtype of opioid receptor.




R = $CH_3$, $C_2H_5$, $C_3H_7$, i-$C_3H_7$

# COMPUTATIONAL STUDY OF PYRAZOLINE DERIVATIVES PROVIDED WITH POTENT AND SELECTIVE MONOAMINOXIDASE ACTIVITIES

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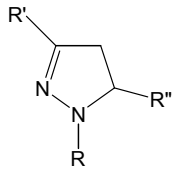
In this communication we present a computational study conducted with the aim to rationalize the structure-activity relationships of potent inhibitors of monoamine oxidases (MAO) based on the pyrazoline scaffold.




Most of the new synthesized compounds proved to be more reversible, potent, and selective inhibitors of MAO-A than of MAO-B.[1] This feature is particularly important for the development of new antidepressant and antianxiety drugs.
The 30 most active compounds show inhibitory activity on MAO-A in the range $8.6 \times 10^{-8}$ - $9.0 \times 10^{-9}$ M. Moreover, most of them are characterized by a Selectivity Index $_{\text{MAO-B/MAO-A}}$ in the range 10,000 - 12,000.
The computational work has been conducted developing new pharmacophore models for the pyrazoline derivatives following an approach previously reported.[2]

[1] Chimenti, F.; Bolasco, A.; Manna, F.; Secci, D.; Chimenti, P.; Befani, O.; Turini, P.; Giovannini, V.; Mondovi', B.; Cirilli, R.; La Torre, F. *J. Med. Chem*. **2004,** *47*, 2071.
[2] Gritsch, S.; Guccione, S.; Hoffmann, R.; Cambria, A.; Raciti, G.; Langer, T. *J. Enz. Inhib*. **2001,** *16,* 199.

# STRUCTURE-BASED PHARMACOPHORE MODELLING WITH LIGANDSCOUT

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Virtual screening together with computer assisted drug design have emerged as one answer to increasing economic pressure that forces the pharmaceutical industry to develop new drugs in a faster and more efficient way [1]. Structure-based drug design is usually tightly associated with docking, which bears the lack of computational efficiency for high-throughput virtual screening. We propose the creation of chemical feature based pharmacophore models suitable for virtual screening from experimentally determined 3D ligand-complex data.

The LigandScout program [2] provides an automated method for creating three-dimensional, chemical feature based pharmacophore models from structure data, as e.g. publicly available from the Protein Databank (PDB). In a first step, small molecule ligands are extracted and automatically analyzed in terms of chemical functionality and hybridization states including the assignment of hybridization states and bond orders. Second, from the interactions of the interpreted ligands with relevant surrounding amino acids, pharmacophore models reflecting functional and steric interactions are created, which can interactively be reviewed, modified and visualized.

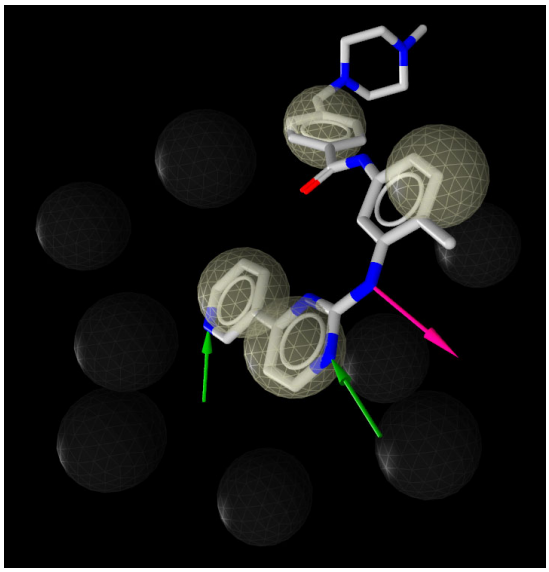

**Fig. 1:** Chemical function based 3D pharmacophore model automatically derived using LigandScout from PDB entry 1T46 with ligand STI-571 (Gleevec) and its alignment with the bio-active ligand conformation.

[1] H. Kubinyi. In Search for New Leads, EFMC - Yearbook 2003, 14-28.
[2] G. Wolber, T. Langer. LigandScout: 3-D Pharmacophores Derived from Protein-Bound Ligands and Their Use as Virtual Screening Filters *J. Chem. Inf. Model.,* **2005,** *45,* 160-169

# DESIGN, SYNTHESIS AND BIOLOGICAL ACTIVITY OF *N*- SUBSTITUTED INDOLES AND BENZIMIDAZOLES AS POTENTIAL HYPNOTIC DRUGS

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R&D Center, Ferrer Internacional S.A.,
Juan de Sada 32, E-08028 Barcelona, Spain

Among the known non-benzodiazepine hypnotic drugs, Zolpidem, Zaleplon and Indiplon have shown high affinity and selectivity on the α1 subunit of the GABA-A receptor [1-2]. Our group has performed pharmacophoric studies and ADMET-prediction to evaluate a virtual library of new molecules based on privileged structures [3]. Among these, we have synthesized a library of *N*-substituted indoles and a library of *N*-substituted benzimidazoles. Afterwards, in vitro screening and in vivo spontaneous motor activity in mice has revealed molecules with good in vitro affinities for the α1 receptor and potent in vivo induction of sedation.

[1] Peterson, E.N.; Benzodiazepine receptor pharmacology: new vistas. *Drugs Future* **1987**, *12*, 1043-1053.

[2] (a) George, P.; Rossey, G.; Depoortere, H.; Mompon, B.; Allen, J.; Wick, A. Imidazopyridines: towards novel hypnotic and anxiolytic drugs. *Il Farmaco* **1991**, *46*, 277-288. (b) Browne, L.J.; Shaw, K.J. New anxiolytics. *Annu. Rep. In Med. Chem.* **1991**, *26*, 1-10.

[3] DeSimone, R. W.; Currie, K. S.; Mitchell, S. A.; Darrow, J. W.; Pippin, D. A. Privileged structures: Applications in drug discovery. Combinatorial Chemistry and High Throughput Screening **2004**, *7*, 473-493.

# EXPLORATORY CHEMISTRY TOWARD THE IDENTIFICATION OF NEW CLASSES OF MDR REVERTERS

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Multidrug resistance (MDR) is a kind of acquired drug resistance of cancer cells and micro organisms to a variety of chemotherapic drugs that usually are structurally and mechanistically unrelated [1]. This kind of resistance is often referred to as classical MDR and 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 by using ATP as energy source. Based on the structure of MsbA protein, obtained from two different bacteria, several Pgp homology models have been developped [3-5]. From these and many other reports on the structure of extruding pumps, it appears that the recognition sites are large, flexible, rich of aminoacids able to establish a variety of interactions with substrates, in particular aromatic hydrophobic interactions. Information gathered on the structure of Pgp and sister proteins point to the existence of a large, polymorphous drug binding domain, where a variety of substrates and inhibitors can be accommodated in a plurality of binding modes [6].
Taking into account the emerging picture of Pgp recognition site, it can be predicted that flexible molecules, carrying a basic nitrogen flanked, at properly modulated distance, by two aromatic moieties, as is the case of verapamil [7-10] and pervilleines [11], will easily adapt to the recognition site and bind with high affinity.
To verify our hypothesis, we designed a new series of molecules having the general structure shown below, where H1 and H2 represent a variety of aromatic moieties found present in previously studied MDR reverters, while L is a linker of variable length carrying one or two basic nitrogen atoms.


The good results obtained, that will be presented together with the synthesis of the new molecules, can be considered the proof of concept of our approach.

[1] Mitscher, L.A. et al., *Med. Res. Reviews.* **1999**, *19,* 477-96. [2] Arzalos, A. et al. *Anticancer Res*. **1998**, *18*, 2937. [3] Stenham, D. R. et al., *FASEB J*., **2003**, *17*, 2287-2289. [4] Seigneuret, M. et al. *J. Biol. Chem.,* **2003**, *278*, 30115-30124. [5] Pleban, K. et al., *Mol. Pharmacol.,* **2005**, *67*, 365-374. [6] Yu, E.W. et al. *Science,* **2003,** *300*, 976-980. [7] Teodori, E. et al. *J. Med. Chem.,* 1999, *42*, 1687-1697. [8] Dei, S. et al. *Bioorg. & Med. Chem.*, 2001, *9*, 2673-2682. [9] Teodori, E., et al., *Med. Chem. Res.*, 2001, *10*, 563-576. [10] Dei, S. et al. *Bioorg. & Med. Chem.* **2005**, *13*, 985-998. [11] Mi, Q. et al., *Cancer Research,* **2001**, *61*, 4030-4037.

# SIDE CHAIN AZASTEROLS AS ERGOSTEROL BIOSYNTHESIS INHIBITORS

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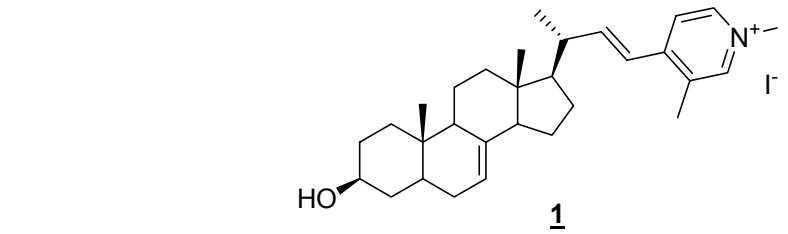
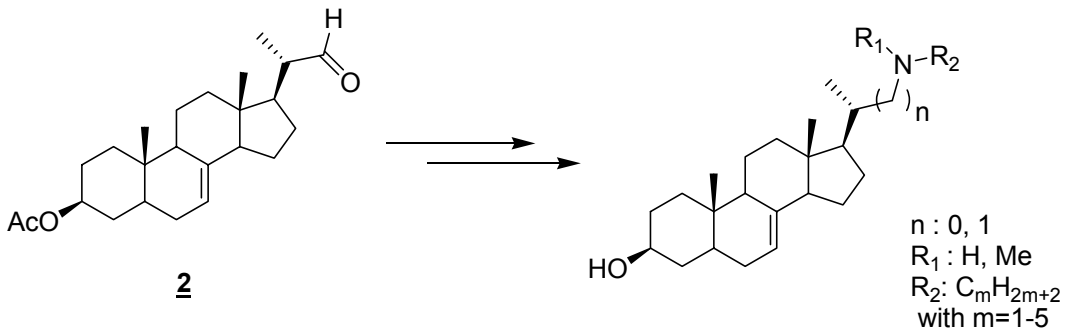
The increase in the incidence of fungal infections together with gradual rise in drug resistance highlight the need to find novel drugs. Amphotericine B and 5-fluorocytosine are the oldest ones [1]. Because of their pour selectivity and their great toxicity, new types of inhibitors of ergosterol biosynthesis have been developed: allylamines as inhibitors of squalene epoxidase, azoles as inhibitors of *C-14*α demethylase and morpholines as inhibitors of $\Delta^{14}$-reductase and $\Delta^{8}$,$\Delta^{7}$-isomerase [1,2].
Previous work on the synthesis of the marine steroid alkaloid plakinamine B **1** led to the identification of azasterols which inhibit the fungal enzyme *C-24* methyltransferase [3]. Taking these results in account, we decided to investigate structure-activity relationships in this new class of inhibitors. Starting from the aldehyde **2**, various aminosteroids were synthesised. They were obtained using reductive amination as crucial step. The so obtained compounds were screened for antifungal activityand their ability to inhibit selected enzymes in ergosterol biosynthesis.




**1**




**2**

[1] P.G Hartmann, D. Sanglard, *Current Pharmaceutical Design*, **1997**, 3, 177-208.
[2] D. Berg, M. Plempel, *Sterol Biosynthesis Inhibitors*, Wiley VCH, **1988**.
[3] Dissertation M. Gans, Ludwig-Maximilians University, Munich, **2003**.

# NOVEL 5-VINYL-3(2H)PYRIDAZINONES AS ORALLY ACTIVE ANTINOCICEPTIVE AGENTS

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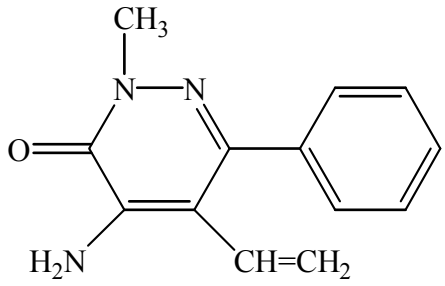
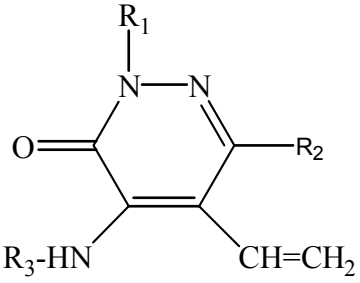
Two major groups of drugs are currently used for treatment of pain: non-steroidal antiinflammatory drugs (NSAIDs) and opioids. The first class shows side-effects like ulcerations, nephrotoxicity and platelet aggregation inhibition. Unwanted effects associated with the clinical use of opioids are: respiratory depression, tolerance, physical dependence and constipation. Thus, there is still the need to discover new pain-killers characterized by higher therapeutic index, in particular for treatment of neuropathic pain.
Pursuing our studies in the field of 4,5-functionalized pyridazinones as antinociceptive agents [1-4] we report here the synthesis of a novel series of compounds (1) structurally related to BB3 [1,2].




**BB3**




**1**

In the attempt to optimize the potency and the safety of the lead compound BB3, we introduced different alkyl chain at $R_1$, substituted phenyls and heteroaromatics at $R_2$ and alkyl(acyl) groups at $R_3$. The novel compounds were evaluated as antinociceptive agents both in writhing and in hot-plate tests by oral administration. Compound with $R_1$= n.butyl, $R_2$= Ph and $R_3$= Me, as well as the analog with $R_1$= Me, $R_2$= 4-pyridyl and $R_3$= H were the most interesting being able to reduce the writhes of 40% at the dose of 20 mg/kg p.o. and to induce a licking latency of 90-100% at the same dose in the hot-plate test.
SAR studies will be discussed in the occasion of the meeting.

References:
[1] Dal Piaz V., Giovannoni M.P. et al., *Eur. J. Med. Chem*., **1996**, 31, 65-70.
[2] Pieretti S., Dal Piaz V., Giovannoni M.P. et al., *Life Sci*., **1999**, 65, 1381-1394.
[3] Dal Piaz V., Vergelli C., Giovannoni M.P. et al., *Il Farmaco*, **2003**, 58, 1063-1071.
[4] Giovannoni M.P., Vergelli C., Ghelardini C., Dal Piaz V. et al., *J. Med. Chem*. **2003**, 46, 1055-1059.

# SYNTHESIS AND BIOLOGICAL ACTIVITY OF NOVEL PYRIMIDINE DERIVATIVES

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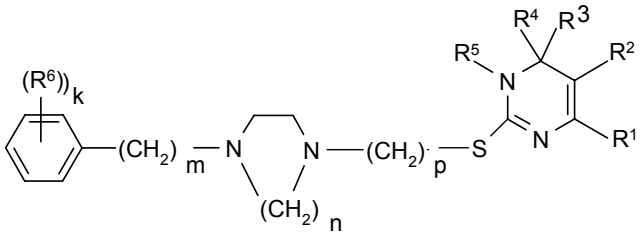
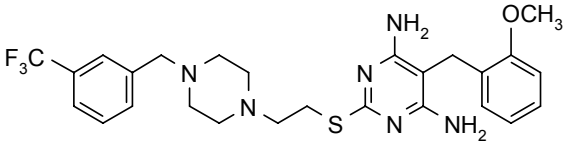
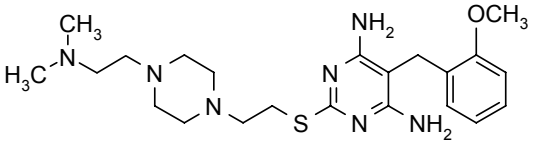
As a part of our research program new pyrimidine derivatives were synthesized **1.** These compounds were found to have high affinity for the 5-$HT_{2C}$ receptors and some of them proved to be selective over 5-$HT_{2A}$ receptors [1]. From our structure – activity relationship studies EGIS-8465, **2** was selected for further evaluation.



**1**



**2**



**3**

The very low biovailability in rats indicated high first pass metabolism of the compound. So we synthesized its analogues to avoid the enzimatic degradation. Among these molecules the "dimethyl-amino-alkyl" derivative **3** was the most effective in anxiolytic tests [2]. Synthesis and pharmacological results will be presented.

[1] WO 97/16429 patent application
[2] WO 01/00617 patent application

# REDUCTION REACTIONS OF DINITROIMIDAZOLE DERIVATIVES

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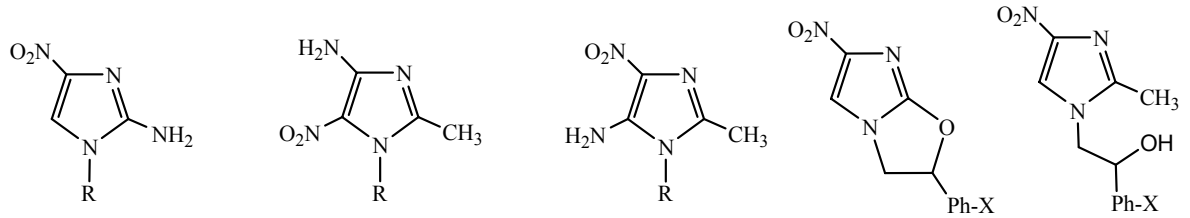
Nitroimidazole derivatives play a significant role in some of the therapies. Many of their analogues are used in the treatment of various infections, particularly as antifungal substances and radiosensitizers, as well as recently as antivirals.

Our earlier works presented syntheses of many N-substituted derivatives of 4,5-dinitroimidazoles and 4-(substituted amino)-5-nitroimidazoles [1]. Some of them were tested as to their pharmacological activity. Recent research shows that a few new compounds show high antioxidant activity.

Now, we describe some reduction reactions of 2,4-dinitro- and 2-methyl-4,5-dinitroimidazole derivatives, where the N-1 positions are substituted with one, two or three-carbon aliphatic chain or with phenacyl group. These starting nitroimidazole derivatives had been prepared according to methods described in the literature [2] and they were reduced with the excess of iron dust in glacial acetic acid, at room temperature or with sodium borohydride in boiling methanol. The reactions with iron led unexpectedly to products in which only one nitro group was reduced to amino function, but the second remained intact. N-Substituted 2,4-dinitroimidazole derivatives were reduced to respective 2-amino-4-nitroimidazoles (**1**). Reduction of appropriate 4,5-dinitroimidazoles led to two isomers 4-amino-5-nitro- (**2**) and 5-amino-4-nitro- (**3**), with predomination of the latter mentioned. Identification of these products was based on full spectral analysis and in some cases on X-ray crystallography. The borohydride reduction was performed for N-phenacyl derivatives only. In the case of 2,4-dinitroimidazole, a part from the reduction of carbonyl group we observed also substitution of nitro group at the C-2 position with newly formed hydroxyl group. Compounds with imidazooxazole structure (**4**) are the only products of this reaction. Use of appropriate 4,5-dinitroimidazoles led to obtaining the respective alcohol derivatives (**5**) with the lack of 5-nitro group. Structures of these compounds were confirmed by Jones oxidation reaction and comparison of products with the original ones, which were described earlier [3].




**1** **2** **3** **4** **5**

where: X=H, p-Cl; R=$CH_3$, $C_2H_5$, $CH_2CH(OH)CH_2Cl$, $CH_2CH(OH)CH_2Br$, phenacyl or (p-Cl)-phenacyl

[1] L. Zaprutko et al., *Monatsch. Chem.,* **134**, 1145-1150 (2003); [2] L. Zaprutko et al., *Pharmazie*, **44**, 81-84 (1989); [3] D. Skwarski et al., *Pol. J. Chem*, **57**, 551-553 (1983).

Acknowledgements: This work is partially financed by Grant No. 2 PO5F 039 27 from the KBN.

# MICROWAVE ASSISTED SYNTHESIS AND FRAGRANCE PROPERTIES OF HETEROCYCLIC ANALOGUES OF JASMONE

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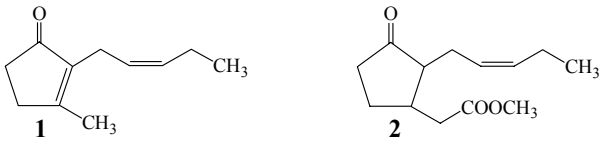
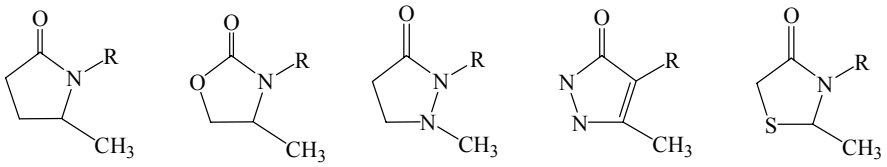
*cis*-Jasmone **1** and methyl jasmonate **2**, belong to jasmonoids group, are 2,3-disubstituted, five-membered cyclic ketones. They are the most important representatives of natural jasmine fragrances which were isolated from jasmine flowers. Jasmonoids not only exhibit characteristic fragrance properties but also play a key role as phytohormones in plants.




In the present investigation, microwave assisted synthesis and olfactory properties of some heterocyclic analogues of jasmone were described. New analogues are based on heterocycles with carbonyl function e.g. oxazolidinone, pyrrolidinone, pyrazolidinone, pyrazolinone and thiazolidinone cycles. Almost all compounds were successfully prepared under microwave irradiation and the results of those microwave accelerated syntheses were compared with classical, thermally initiated reactions in solvent. Preparation of heterocyclic compounds was performed in two steps: condensation and/or alkylation, which were conducted according to two alternative microwave procedures, without solvent (solvent-free conditions) or with small amount of polar, ecological friendly solvent e.g. ethanol. Reactions under microwave irradiation were more efficient and much faster.




R = -*n*-pentyl or -*n*-2-pentynyl

Many of obtained compounds have shown interesting and specific odor which had similar, but essentially different, note to floral, typical jasmine odor of *cis*-jasmone.

# NEW METHOD OF SIMULTANEUOS DATERMINATION OF $pK_a$ AND $log\ k_w$ EMPLOYING *pH*-GRADIENT HPLC

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*pH* gradient reversed-phase HPLC consists of a programmed increase during the chromatographic run of the eluting power of the mobile phase with regard to ionizable analytes. On the analogy of the conventional organic modifier gradient RP HPLC, in the *pH* gradient mode, the eluting strength of the mobile phase increases due to its increasing (with acid analytes) or decreasing (with basic analytes) *pH*, whereas the content of organic modifier is kept constant. A strict theoretical model is proposed to determine $pK_a$ values based on the retention data employing a *pH* gradient RP HPLC run. The $pK_a$ data so obtained are discussed in relation to the concentration of methanol in the mobile phase, the type of stationary phase, and the duration of the gradient.

The approach applied is demonstrated to provide, along with the $pK_a$ data, also the chromatographic lipophilicity parameter, $log\ k_w$. The $pK_a$ values determined by the *pH* gradient method are related to the respective data obtained conventionally in a series of isocratic experiments. A close similarity of the two types of chromatographically determined $pK_a$ data is demonstrated. The HPLC-derived $pK_a$ parameters correlate to the literature $pK_a$ values ($^{w}{}_{w}pK_a$) determined by titrations in water. The chromatographically derived and the reference $pK_a$ values are not identical, however. That is probably due to the effects on the chromatographic $pK_a$ of the specific sites of interactions with analytes on the surfaces of the HPLC stationary phases. Nonetheless, the proposed *pH* gradient HPLC method may supply in a fast and convenient manner comparable acidity parameters for larger series of drug candidates, including those available in only minute amounts, without need of their purification, and also when the compounds are provided as complex mixtures, like those produced by combinatorial chemistry.

# NEW CYTOTOXIC TRITERPENEQUINONE DERIVATIVES

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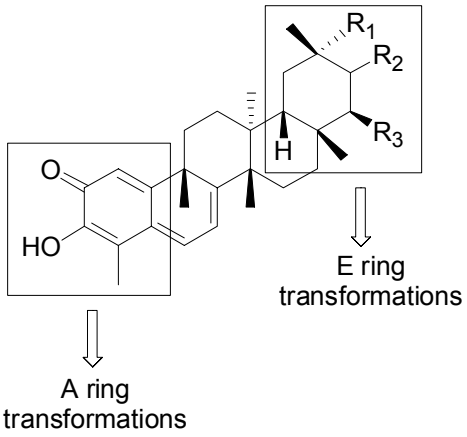
Driven by on interest in bioactive metabolites present in *Celastraceae* species used in South American folk medicine [1], we carried out the phytochemical study of *Cheiloclinium hippocratioides*. We isolated a set of nine novel sesquiterpene-triterpene hetero Diels-Alder adducts together with several triterpenequinones which turned out to be the main secondary metabolites [2].
Because of the cytotoxic activity of the triterpenequinones, we decided to evaluate the influence on the antitumor activity of some modifications on the triterpenemethide skeleton. We carried out transformations on the A and E hydrophilic rings, modifying the type and number of hydrogen bond donors and acceptors present in these rings.
The results achieved and the structure-activity relationships that can be inferred from the available data will be discussed in this communication.

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# 3D-QSAR AND PHARMACOPHORE MODELING OF NAPHTHOQUINONE DERIVATIVES WITH CYTOTOXIC ACTIVITY IN HL60 CELL LINES.

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Catalyst HypoGen pharmacophore modeling approach and 3D-QSAR comparative molecular similarity indices analysis (CoMSIA), were employed on a set of 51 naphthoquinones tested in HL-60 leukaemia cell lines. The aim was to identify pharmacophores and to outline structural requirements of these naphthoquinones as antitumoral agents. Quantitative chemical functions based on pharmacophore models were generated using the HypoGen algorithm, which is implemented in the CATALYST program. The best output hypothesis consists of three features: two hydrogen bond acceptor (HBA) and one hydrophobic (Hy). The 3D-QSAR modeling afforded predictive models with consistently high values of both leave-one-out cross-validated R2(0.967) for the training set and predictive R2(0.999) for the test set. The results of both modeling approaches were sensitive to the selection of the training and test sets used for model development and validation.

# SYNTHESIS AND CYTOTOXIC ACTIVITY OF *bis*-PYRANO-1,4-BENZOQUINONES

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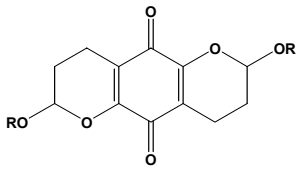
The pyrano-1,4-benzoquinone core occurs in many natural products and pharmaceutically important compounds[1]. Some representative examples[2] are isoasterriquinone, irisquinone, geldenamicyn and dactyloquinones. These wide range of biological applications have stimulated considerable interest in evolving newer synthetic methods for the construction of benzoquinone derivatives[3].
This communication describes an efficient method to synthesize *bis*-pyran-benzoquinones based on a domino three-components reaction, from easily available starting materials. The results of cytotoxic activity of the benzoquinones synthesized will be also presented.

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# OPTIMISATION AND PHARMACOLOGICAL EVALUATIONS OF 4-HETEROARYL-2-PHENYLAMINO-PYRIMIDINE CDK INHIBITORS

Janice McLachlan,[a] Christopher Meades,[a] Gary Griffiths,[a] Laura Ingram,[a] Carol Midgley,[b] Mokdad Mezna,[a] Wayne Jackson,[a] Campbell McInnes,[a] Mark Thomas,[a] George Kontopidis,[a] Robert C. Jackson, Peter M. Fischer and Shudong Wang.[a]

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Cyclin-dependent kinases (CDKs) are key regulators of cell cycle progression. These enzymes are activated by the formation of periodic complexes with cyclins, proteins that are present at specific stages of the cell cycle. Activities of CDK2, CDK4 and CDK6 are required for cell-cycle entry and passage into S-phase, where DNA replication takes place. CDK1 and CDK2 activities are essential for cells to pass through S-phase into G2-phase and mitosis. Cyclin-CDKs are frequent targets of genetic alterations in human cancers, either directly or as consequences of mutations that lead to deregulation of their kinase activity. CDK9 is another member of the CDK family and its activating subunits are members of the cyclin T and cyclin K families. CDK9/cyclin T1 is responsible for the activating phosphorylation of the carboxy-terminal domain (CTD) of RNA polymerase II (RNAPII), the key mediator of RNA transcription. The requirement for RNAPII function for constitutive expression of anti-apoptotic genes in order to maintain the transformed state of cancer cells suggest that chemical agents targeting RNAPII function may constitute a new class of anti-cancer agents. CDK2, 7 and 8 have also been implicated in modulation of RNAP elongation by phosphorylation of the CTD of RNAPII. We have designed and synthesized a number of potent 4-heteroaryl-2-phenylamino-pyrimidine selective mechanistic prototype CDK inhibitors. Many of them not only display considerable anti-proliferative activity in tumor cells *in vitro*, but also possess favorable pharmaceutical profiles. The discovery chemistry, biology and optimization of pharmaceutical properties of these compounds will be presented.

# CICLOPENTADIENE AS SCAFFOLD FOR THE SYNTHESIS OF NEW ACTIVE COMPOUNDS

Concepción Álvarez, Concepción Pérez , Rafael Peláez and Manuel Medarde

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Modern drug discovery takes advantage of the ideas and methodologies of combinatorial chemistry. The search of new lead compounds and the improvement of their pharmacological activities, requires accessible supporting structures to introduce a high variability through the attachment of diverse substituents. To complete this goal, base structures named "scaffolds", with geometrically defined attachment points, are required.

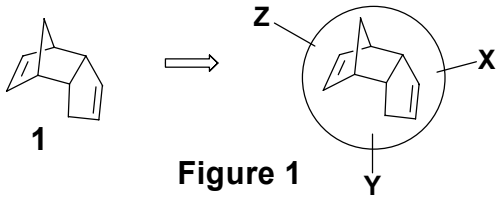
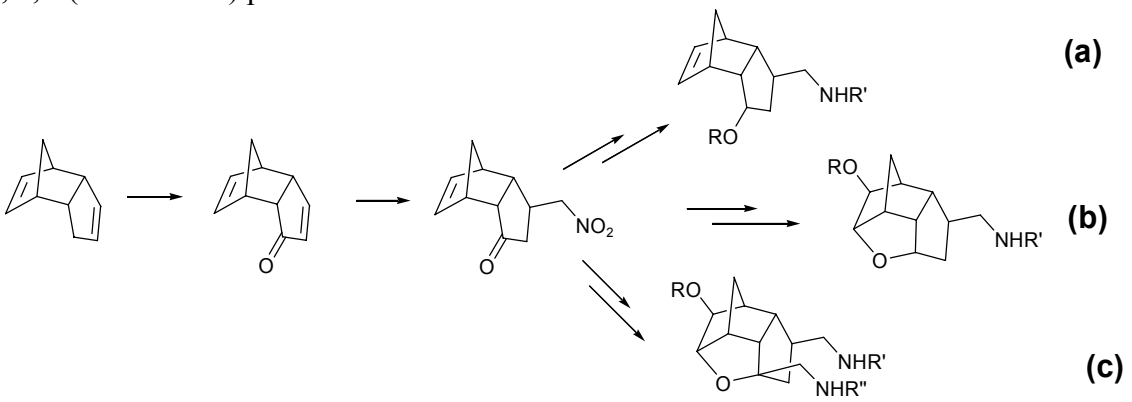



Figure 1

We have recently initiated a research line directed at the preparation of simple analogues of active diterpenoids[1], requiring the preparation of convex rigid core-substructures containing attachment points to introduce substituents with defined orientations. Dicyclopentadiene, a well know molecule finding application in the search of new polymeric materials, appears to fulfil these requirements, presenting two double bonds to introduce the functionalities and the convex rigid structure. Following this reasoning, we intend to prepare derivatives from dicyclopentadiene which contain the X, Y and/or Z points of attachment (**Figure 1**).

In this communication we present the initial transformations of dicyclopentadiene, producing three base structures with the X,Y (Scheme 1-**a**), or the X,Z (Scheme 1-**b**) or the X,Y,Z (Scheme 1-**c**) points of attachment.




**Acknowledgements:** Financial support came from JCyL (073/02) , MEC (CTQ2004-00369) and MCYT (PPQ2001-1977). CAS also thanks to MCYT a fellowship position.

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# SYNTHESIS AND STRUCTURE-ACTIVITY RELATIONSHIPS OF NOVEL ANTICANCER OXALIPLATIN ANALOGUES

Markus Galanski,[a] Ladislav Habala,[a] Alexey A. Nazarov,[a] Michael A. Jakupec,[a] Afshin Yasemi,[a] Susanna Slaby,[a] Nikolai Graf von Keyserlingk,[b] and Bernhard K. Keppler[a]

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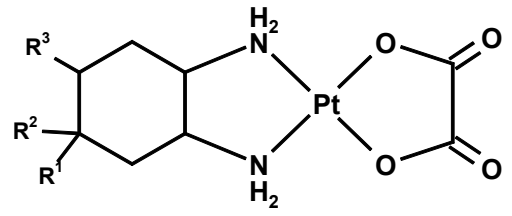
Oxaliplatin, (*trans-R,R*-cyclohexane-1,2-diamine)oxalatoplatinum(II), has recently been approved for combination chemotherapy of metastatic colorectal cancer. Based on the assumption that the steric demand and/or the lipophilicity of the cyclohexane ring are structural requirements for the specific pharmacological properties of oxaliplatin, derivatization of the cyclohexane ring might result in a marked effect on antitumor activity. Following this concept and in order to explore structure-activity relationships, a series of new oxaliplatin analogues has been synthesized and characterized [1, 2, 3]. Their *in vitro* antitumor activity in comparison to oxaliplatin has been tested in different cancer cell lines.




| $R^1$ | $R^2$ | $R^3$ |
|---|---|---|
| $CH_3$ | H | H |
| $CH_2CH_3$ | H | H |
| $CH_2CH_2CH_3$ | H | H |
| $C_6H_5$ | H | H |
| $C(CH_3)_3$ | H | H |
| $CH_3$ | $CH_3$ | H |
| $CH_3$ | H | $CH_3$ |

Compared to oxaliplatin, potency is increased in subsets of cell lines, particularly in leukemia and some colon carcinoma cells, by introduction of small substituents (methyl, ethyl). Within a panel of five colon carcinoma cell lines, the activity profile of the 4,4-dimethyl-substituted complex most closely resembles that of oxaliplatin, while that of the *cis*-4,5-dimethyl-substituted complex contrasts sharply.

Acknowledgement: The support of the FWF and COST is gratefully acknowledged.

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# LOW PH IN SOLID TUMORS - SELECTIVE ACTIVATION OF ANTICANCER PLATINUM COMPLEXES

Kristof Meelich[a], Markus Galanski[a], Vladimir B. Arion[a], Michael A. Jakupec[a], Petra Schluga[a], Christian G. Hartinger[a], Nikolai Graf v. Keyserlingk[b], and Bernhard K. Keppler[a]

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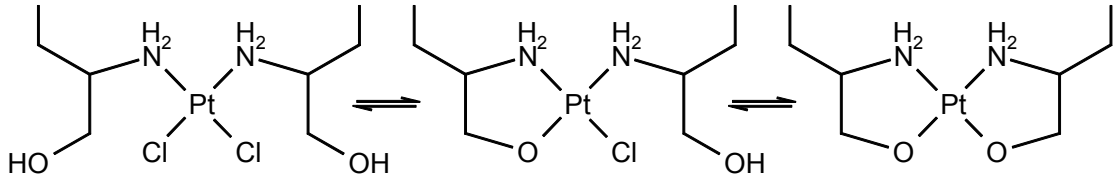
Since the successful introduction of cisplatin, a wide spectrum of platinum compounds has been synthesized and tested [1]. Nevertheless, only a limited number made their way into clinical trials, just three of them have been approved for a worldwide use. The low selectivity of most anticancer agents still appears to be a major problem; severe side effects or ineffective treatment are the results. The search for a more selective and tumor targeted therapy was the stimulus for the design of pH sensitive platinum complexes. It is known that most solid tumors display increased hypoxia, which results in a decrease of pH (5.5 - 7.4). The acidic environment, which is usually a problem for weak base organic drugs, could advantageously be used for the introduction of pH sensitive agents, such as aminoalcoholato platinum(II) complexes.




These substances show a pH-driven reversible intramolecular ligand exchange reaction in aqueous solution. At pH (7.4) ring-closed species are formed, which display a significantly low reactivity. Also a low cytotoxicity of the mentioned ring-closed forms could be found. On the other hand, the ring-opened complexes, which are formed at lower pH, as found in tissues of solid tumors, are far more reactive. Respectively a much stronger cytotoxic effect was observed at pH 6. Two aminoalcoholato complexes have been studied in detail: Synthesis, characterization, chemical behavior, and cytotoxicity are described. In both cases, the ring closed species appear to be remarkably stable substances, also in aqueous solution under physiological pH and chloride ion concentration [2].
These interesting results provide evidence that the concept of administration of rather unreactive drugs and activation under acidic pH conditions, can be realized.

Acknowledgement: The support of the FWF and COST is gratefully acknowledged.

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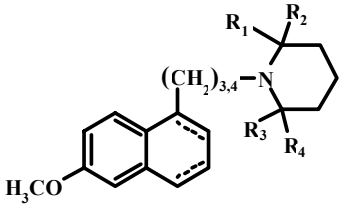
# BINDING SELECTIVITY FOR SIGMA RECEPTOR SUBTYPES TRIED BY NOVEL DIMETHYLPIPERIDINE LIGANDS

Francesco Berardi, Savina Ferorelli, Maria Paola Pedone, Nicola Antonio Colabufo, Marialessandra Contino, Roberto Perrone and Vincenzo Tortorella

Dipartimento Farmacochimico, Università di Bari, via Orabona, 4, 70126 Bari, Italy

On the basis of present knowledge, σ (sigma) receptors are almost two subtypes of intracellular binding sites related to signal modulating function involving $Ca^{++}$ release from endoplasmic reticulum. They are localized in high density in the brain, heart, spleen, kidney, liver and in endocrine, immune and reproductive tissues, suggesting their involvement in various modulating activities. Furthermore, they are overexpressed by several cancer cell lines. Cloning of human $\sigma_1$ receptor pointed out a moderate homology with a yeast $\Delta_8$-$\Delta_7$ sterol isomerase (SI) involved in ergosterol biosynthesis. Therefore, it has been hypothesized that $\sigma_1$ receptor may belong to the family of human SI.
Although no specific σ ligand has reached the market, the $\sigma_1$ agents are thought to be useful in the treatment of several central nervous system disorders and deficits, including schizophrenia, pain, memory and cognitive deficits, Alzheimer's disease, neurodamage and cocaine abuse. $\sigma_2$ Agents have been found to promote cell apoptosis and inhibit p-glycoprotein expression in some tumour cell lines. An important role can be played by σ ligands in tumour diagnosis through P.E.T. (Positron Emission Tomography) analysis.
Several high-affinity σ ligands are known, that are rather selective for $\sigma_1$ receptor subtype, while very few ligands display only a moderate selectivity for $\sigma_2$ receptor subtype. Since a decade we have been dealing with the synthesis and biological evaluation of a number of novel σ receptor ligands, with the aim to explore structure-affinity/activity relationships and prepare lead compounds selective for each σ subtype receptor.
A recently prepared series of *N*-[6-methoxytetralin(and -naphthyl)alkyl]methylpiperidines demonstrated high to very high affinity toward $\sigma_1$ receptor and only moderate affinity toward $\sigma_2$ receptor. Exploring the effect of methyl position on the piperidine ring, 3,3-dimethylpiperidine derivatives displayed the lowest $\sigma_2$ affinity, but yet considerable affinity toward SI. In a new effort to extend our investigation on σ receptor subtype selectivity, we prepared a series of piperidine analogues bearing more methyl substituents in α-position relative to N-atom, in order to cause some steric hindrance.




$R_1$, $R_2$, $R_3$, $R_4$ = $CH_3$, H

Binding assays on $\sigma_1$ and $\sigma_2$ receptors and SI site are in progress. Preliminary results showed dramatic changes in $\sigma_1$ and $\sigma_2$ receptors affinities, while less ones in SI affinities. These findings proved the importance of piperidine N-atom and its neighbourhood in binding selectively σ receptors.

# PSEUDOLARIC ACID B, A NOVEL MICROTUBULE-DESTABILIZING AGENT THAT CIRCUMVENTS MULTI-DRUG RESISTANCE PHENOTYPE AND EXHIBITS ANTITUMOR ACTIVITY *IN VIVO*.

Vincent K.W. Wong[1,2], Pauline Chiu[1,2], Stephen S.M. Chung[3], Larry M.C. Chow[4], Y.Z. Zhao[4], Burton B. Yang[5], Ben C.B. Ko[1,2].

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[2]Open Laboratory of Chemical Biology of the Institute of Molecular Technology for Drug Discovery and Synthesis
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Pseudolaric acid B (PAB) is the major bioactive constituent in the root bark of *pseudolarix kaempferi* that has been used as an anti-fungal remedy in traditional Chinese medicine. We purified PAB to apparent homogeneity and showed that it exhibits potent growth inhibition towards a panel of cancer cell lines (average $IC_{50}$=1 μM). We found that PAB induces cell cycle arrest at $G_2$-M transition through the microtubules destabilization mechanism, leading to apoptosis. In addition, polymerization of purified bovine brain tubulin was dose-dependently inhibited by PAB, suggesting that tubulin is the direct target of PAB. However, PAB did not displace [$^3$H] colchicine or [$^3$H] vinblastine from tubulin binding in competition binding assays, suggesting that PAB interacts with tubulin through a novel binding site. Most importantly, PAB circumvents multi-drug resistant mechanism, displaying remarkable potency in P-glycoprotein overexpressing cells and we demonstrated that PAB inhibit tumor growth effectively *in vivo* using murine xenograft tumor model. Furthermore, we also found that the blood vessel density of the PAB-tumor was significantly reduced, suggesting that, similar to other microtubule-destabilizing compounds, PAB also exhibits anti-angiogenic activity *in vivo.*

# FIRST DUAL $M_3$ ANTAGONISTS / PDE4 INHIBITORS FOR THE TREATMENT OF COPD

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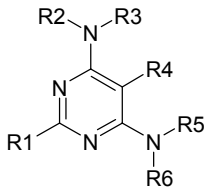
COPD is a chronic, progressive and poorly reversible condition characterized by impaired expiratory outflow and abnormal inflammatory response of the lungs to noxious particles and gases. COPD is one of the most common chronic diseases worldwide, it affects 4-6 % of people older than 45 and is predicted to be the third leading cause of death by 2020. Cigarette smoking is by far the most important risk factor for the development and progression of the condition.[1] There are currently no drug therapies able to slow down the progression of the disease. Patients are commonly treated with drugs developed for asthma but it proves to be quite inefficient as both inflammatory processes differ markedly.[1, 2] Bronchodilator drugs are the current mainstay of treatment for symptoms relief. Anticholinergic bronchodilators, particularly selective muscarinic $M_3$ antagonists, are currently the preferred choice for the symptomatic management of COPD. However, although bronchodilators are quite effective to improve symptoms, they do not address the underlying chronic inflammation or the changes in airway structure. Among the new anti-inflammatory agents currently being developed, PDE4 inhibitors proved to be very efficient in attenuating the responses of various inflammatory cells through their ability to elevate cAMP levels. Although new generation's compounds, currently in Phase III for the treatment of COPD, have been shown to significantly improve lung function of patients, their bronchodilating effect remains rather limited. Therefore, the combination of selective muscarinic $M_3$ antagonism with selective PDE4 inhibition may lead to a new class of drugs combining both bronchodilating and anti-inflammatory properties.
We present here the discovery and optimization of the first family of dual $M_3$ antagonists and PDE4 inhibitors as potential new drugs for COPD treatment.[3] Full details of synthesis and SAR around 4,6-diaminopyrimidine derivatives are given together with some interesting pharmacological properties of selected leads. We show that an optimal balance between activities, physicochemical properties and selectivity profile can be reached by the proper choice of substituants.

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# RUTHENIUM PYRAZOLE COMPLEX: POTENT ANTI-CANCER AGENT WITH ANTIANGIOGENIC PROPERTIES

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[b]Department of Biology and Chemistry, City University of Hong Kong, Tat Chee Avenue, Kowloon, Hong Kong SAR, China

Ruthenium-based compounds are recently receiving considerable interest as novel anti-cancer drug candidates due to the versatile oxidation states and diverse coordination chemistry of ruthenium. $[Ru(C_3N_2H_4)_4Cl_2]Cl$, a ruthenium-based compound with pyrazole as ligand was newly synthesized. It was shown to be cytotoxic toward a series of carcinoma cell lines (HeLa, HepG2, Hep3B, QGY-TR50, MCF-7 and HCT-8) with $IC_{50}$ at micromolar concentration based on MTT assay. Its mode of anti-cancer action was further investigated and its anti-angiogenic ability was tested through *in vitro* studies of our ruthenium-based compound on endothelial cell functions, which is necessary for angiogenesis to develop. Pretreatment of human umbilical vascular endothelial cells (HUVEC) with our ruthenium-based compound can inhibit *in vitro* capillary-like tube formation in a three-dimension matrix gel in a dose-dependent manner.

# A NEW CLASS OF POTENT ANTI-HIV AGENTS: OXOVANADIUM(IV) PORPHYRIN COMPLEXES

Suk-Yu Wong,[a] Raymond W.-Y. Sun,[a] P.-Y. Chung, [b] C.-L. Lin[b] and Chi Ming Che*[a]

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Study on the use of vanadium as potential therapeutics for anti-diabetes is well-known for decades.[1] Currently, potential utility of vanadium complexes on anti-HIV was discovered.[2, 3] Oxovanadium(IV) porphyrin complexes (**1a–e)** were synthesized and characterized. Their anti-HIV properties were evaluated by HIV-1 p24 antigen assay. In summary, compound **1a** has demonstrated excellent solution stability against glutathione reduction and comparable potency as the clinically used AZT in inhibiting HIV-1 replication in Hut/CCR5 cells.

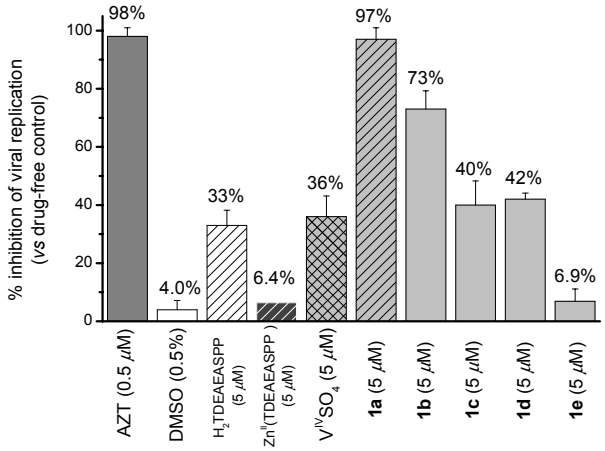


**Fig. 1** Percentage inhibition of HIV-1(BaL) replication in Hut/CCR5 cells (7 days) by oxovanadium(IV) porphyrins and related complexes

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# STRUCTURE-ACTIVITY HYPOTHESIS IN THE DESIGN OF NEW AMPA RECEPTOR ANTAGONISTS

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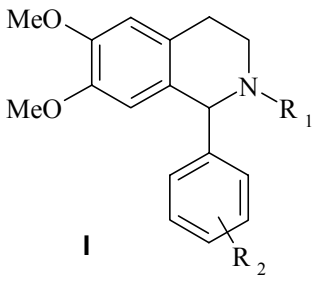
In the past, serendipity played an important role in the discovery of new drugs. Nowadays it has been demonstrated that the search for new drug candidates may be more efficient by establishing biological or structure-activity hypotheses and/or selecting certain scaffolds and substituents. Our research group, long since involved in designing new anticonvulsant agents, has recently developed a predictive pharmacophore hypothesis which led to the discovery 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline derivatives as a new class of noncompetitive AMPA receptor antagonists [1-2].

AMPA receptor is one of the three subtypes of glutamate receptor ion channels and its antagonists have been reported as agents useful in the prevention and treatment of a variety of neurological diseases such as epilepsy [3-4].

In this work, the module HypoGen/Catalyst has been used to perform SAR-based hypothesis generation and to derive 3D pharmacophore models with quantitative predictive ability in terms of anticonvulsant activity. Several tetrahydroisoquinolines (**I**) characterized by different efficacy against seizures in DBA/2 mice were thus selected as the training set.




**I**

The HypoGen-generated hypothesis, consisting of five features (two hydrogen bond acceptors, two hydrophobic features, and one hydrophobic aromatic part), showed high correlation coefficient (r=0.919) and predictive power.

The obtained model was validated using an external test set of anticonvulsant agents. The results of our study have been useful to improve the understanding of the structure-activity relationships and furnished interesting suggestions to design new ligands able to interact with AMPA receptor complex in a noncompetitive fashion.

[1]Barreca ML, Gitto R, Quartarone S, De Luca L, De Sarro G, Chimirri A. "Pharmacophore Modeling as an Efficient Tool in the Discovery of Novel Noncompetitive AMPA Receptor Antagonists". *J Chem Inf Comput Sci*, **43** (2), 651-655 (2003)

[2] Gitto R, Barreca ML, De Luca L, De Sarro G, Ferreri G, Quartarone S, Russo E, Constanti A, Chimirri A. "Discovery of a Novel and Highly Potent Noncompetitive AMPA Receptor Antagonist". *J Med Chem*, **46** (1), 197-200 (2003)

[3] De Sarro G, Gitto R, Russo E, Ferreri Ibbadu G, Barreca ML, De Luca L, Chimirri A. "AMPA Receptor Antagonists as Potential Anticonvulsant Drugs". *Current Topics in Medicinal Chemistry*, **5** (1), 79-84 (2005)

[4]Gitto R, Barreca ML, De Luca L, Chimirri A. "New trends in the development of AMPA receptor antagonists". *Expert Opin Ther Pat*, **14** (18), 1199-1213 (2004)

# HIV-1 INTEGRASE INHIBITORS: PHARMACOPHORE MODELING AND RATIONAL DRUG DESIGN

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Human immunodeficiency virus type 1 (HIV-1) encodes three enzymes which are required for viral replication: reverse transcriptase, protease, and integrase (IN).

HIV-1 IN has emerged as an attractive target for antiviral therapy, because it plays a key role in stable infection and a known functional analog is lacking in the human host [1]. The integration reaction is carried out in two steps (3'-end processing and strand transfer) and divalent cations such as $Mn^{2+}$ or $Mg^{2+}$ are required for the catalytic activity.

Although a wide variety of compounds have been reported as IN inhibitors, drugs active against this enzyme have not as yet been approved by the FDA.

To date, β-diketo acid (DKA) analogues represent the major leads in the development of anti-HIV-1 IN drugs, seeing that the only two IN inhibitors undergoing clinical trials belong to this family.

For the above reasons and as a continuation of our work in this research field [2-4], our idea was to generate a simple 3D pharmacophore model that could correctly predict the activity of compounds belonging to the DKA class.

On the basis of the statistically most significant hypothesis, we designed and synthesized new potential DKA IN-inhibitors containing a benzylindole skeleton.

The biological results of the IN inhibitory activity confirmed the strength of our rational approach and suggested that our 3D QSAR model can be useful and predictive in designing new compounds.

In particular, our ongoing work is to use the quantitative pharmacophore as 3D query for the identification of new potential IN inhibitors in large 3D databases of molecules.

[1] Anthony NJ. HIV-1 integrase: a target for new AIDS chemotherapeutics. Curr Top Med Chem 2004;4 (9):979-90.

[2] Barreca ML, Lee KW, Chimirri A, Briggs JM. Molecular dynamics studies of the wild-type and double mutant HIV-1 integrase complexed with the 5CITEP inhibitor: mechanism for inhibition and drug resistance. Biophys J 2003;84 (3):1450-63.

[3] Barreca ML, Rao A, De Luca L, Zappala M, Gurnari C, Monforte P, De Clercq E, Van Maele B, Debyser Z, Witvrouw M, Briggs JM, Chimirri A. Efficient 3D database screening for novel HIV-1 IN inhibitors. J Chem Inf Comput Sci 2004;44 (4):1450-5.

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# NEW 2,3-BENZODIAZEPINES AS NONCOMPETITIVE AMPA RECEPTOR ANTAGONISTS

Silvana Grasso[a], Maria Zappalà[a], Giovanna Postorino[a], Alessia Pellicanò, Nicola Micale[a], Carlo De Micheli[b], Giovanni Grazioso[b], Frank S. Menniti[c]

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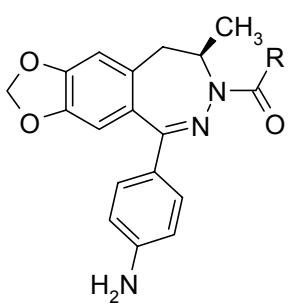
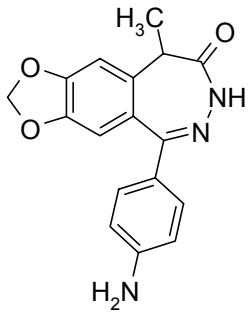
The discovery of GYKI 52466 as the prototype of the noncompetitive AMPA receptor antagonists endowed with anticonvulsant and neuroprotective properties, induced wide-ranging research activities focused on 2,3-benzodiazepines [1]. Highly active analogs of GYKI 52466 have been found such as 3,4-dihydro-3-*N*-methylcarbamoyl (GYKI 53655) and 3,4-dihydro-3-*N*-acetyl (GYKI 53405) derivatives. In particular, the 4-*R* enantiomer of GYKI 53405 was chosen as a drug candidate and is now in clinical trial as LY 300164 (Talampanel) [2].

On these basis we planned the synthesis and resolution of 3,5-dihydro-5-methyl-7,8-methylenedioxy-4*H*-2,3-benzodiazepin-4-one (±)-**1**. The enantioselective interaction of (±)-**1** with the 2,3-benzodiazepine binding site of the AMPA receptor complex was demonstrated by the difference in affinity in favour of the *S*-(-)-**1** enantiomer with respect to the racemate.

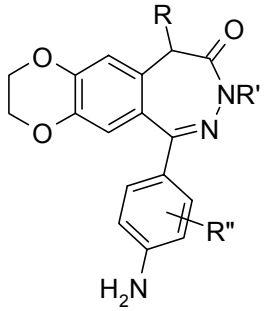
Furthermore, we designed new 3,5-dihydro-7,8-ethylenedioxy-4*H*-2,3-benzodiazepin-4-ones (**2**), in order to check how the replacement of the dioxole nucleus with the dioxane moiety affects the AMPA antagonist activity. Binding data and functional assays indicate a selective antagonism at the AMPA receptor complex higher than that displayed by GYKI 52466.




Talampanel




**1**




**2**

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# SYNTHESIS AND ANTITUMOR STUDIES OF HYDRAZONES DERIVED FROM MONOSUBSTITUED 2-ACETYLPYRIDINES AND 2-HYDRAZINO-1-METHYLBENZIMIDAZOLE:

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Institute of Pharmacy, Pharmaceutical Chemistry Department, Innrain 52a, University of Innsbruck,
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In the course of the development of novel hydrazones as potential antitumor agents, we have found that 1-methyl-2-benzimidazolyl hydrazone derived from 2-acetylpyridine (compound EPH 116) exhibits potent cytotoxic activity ($IC_{50}$ = 0.004-0.018 μM) *in vitro* against a pannel of human tumor cell lines. [1] EPH 116 was also found to be a potent inducer of apoptosis in Burkitt´s lymphoma cells compared to camptothecin. Furthermore, EPH 116 inhibited the growth of CXF 280 colon tumor xenografts in nude mice in a dose dependent manner. In view of this promising antitumor activity we have synthesized several analogues of EPH 116 in which various positions of the 2-acetylpyridine ring is substituted by electron withdrawing or donating groups. The antiproliferative activities of these agents were studied in a pannel of human tumor cell lines (Burkitt´s lymphoma, Hela cervix carcinoma, HT-29 colon carcinoma, hydroxyurea-resistant and multidrug resistant KB cell lines). The activities were compared to that of EPH 116. The following conclusions could be drawn: i) All the compounds are potent inhibitors of the proliferation of Burkitt´s lymphoma cells ($IC_{50}$ = 0.001-2.02 μM). ii) 2-Acetylpyridines bearing electron donating substituents are highly cytotoxic to HeLa ($IC_{50}$ = 0.0003-0.183 μM) and HT-29 ($IC_{50}$ = 0.003-0.14 μM) cells compared to those bearing electron withdrawing groups ($IC_{50}$ = 0.165-17.56 μM). The 6-methyl-2-acetylpyridine analogue EPH 135 inhibited the growth (60%) of HTB 1773 cells transplanted in nude CD1 mice at doses of 60 mg/kg/b. wt. However due to problems of solubility, a MTD was not reached.
In Burkitt´s lymphoma cells, two-fold $IC_{50}$-concentrations of two novel hydrazones derived from 2-acetyl-6-phenyl-pyridine (EPH 355) and 2-acetyl-4-dimethylaminopyridine (EPH 362) induced 60 and 81 % apoptosis respectively. On the contrary, two-fold $IC_{50}$-concentrations of hydroxyurea and camptothecin induced 6.5 and 10 % of apoptosis respectively. The synthesis and structure-activity relationships of this class of novel antitumor agents will be presented.
Financial support was provided by the Austrian Science Foundation (FWF), project No. P12384-MOB.

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# NEW INHIBITORS OF CANCER RELEVANT PROTEIN KINASES

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Frank Totzke,[b] Michael Kubbutat,[b] Daniel Vitt,[a] Christoph Schächtele [b]

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[b] ProQinase GmbH, Breisacher Str. 117, D-79106 Freiburg, Germany

4SC AG and ProQinase GmbH collaborate to identify a new generation of protein kinase inhibitors as the basis for the development of new anticancer drugs.

Protein kinases play a pivotal role in the regulation of cellular functions. These include processes like cell growth and division, cell differentiation and cell death, but also many other cellular activities. Several oncogenes are pathologically modified genes which in their proto-oncogenic form encode for protein kinases involved in normal, physiological regulation of cell growth and division. Tumor progression involves (1) cell proliferation/cell cycle control, (2) regulation of programmed cell death (apoptosis) and cell survival, (3) tumor angiogenesis, and (4) tumor metastasis. In each of these processes, certain protein kinases play a key role.

The intention of the project is the identification of monospecific protein kinase inhibitors, which preferentially inhibit one protein kinase causatively involved in tumor progression, but also so-called multi-target protein kinase inhibitors, which inhibit at least two different protein kinases playing a role in two or more different molecular mechanisms of tumor progression.

ProQinase provides disease relevant protein kinase targets using its integrated technology platform, including cell based assays and in vivo models.

New hit structures were identified using 4SC's proprietary virtual High Throughput Screening technology, 4SCan®: Out of a virtual library of 5 Mio compounds, ATP competitive ligands of the desired protein kinases were selected, resulting in a hit rate of 16 % in the enzyme assay. Modification of selected hit compounds by Medicinal Chemistry led to the identification of new classes of selective inhibitors of cancer relevant protein kinases with low nanomolar activity, e.g. on Aurora and VEGF-R2 kinases. Compounds showing this selectivity profile are expected to inhibit cell proliferation and tumor angiogenesis at the same time.

# MICROWAVE-ASSISTED SYNTHESIS OF SUBSTITUTED 2(1*H*)-QUINOLONES AS MAXI-$K^+$ CHANNEL OPENERS

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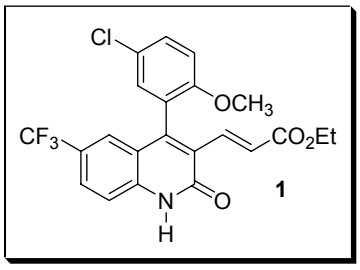
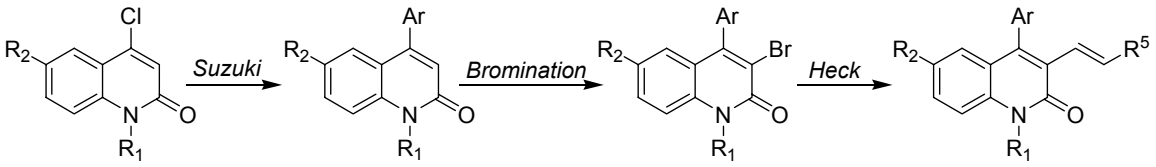
The erectile dysfunction is known as a persistent inability of a man to achieve and/or maintain erection sufficient for a satisfactory sexual performance. According recent studies more than 30 million man in the United States are affected. There are many different treatments but most widely used in the last years are such well-known drugs as Viagra®(Sidenafil) and its new analogs –Vardenafil and Tadalafil. In the recent few years some new chemical substances, known as maxi-$K^+$channel openers [1], have been studied by Bristol-Myers Squibb and reported as a potential new drug candidates, e.g. 2(1H) quinolone **1** [2].

Here we present a case study, involving a novel, stepwise, and diversity generating synthesis of some of these biologically active substituted quinolones, using a sequence of up to six microwave-assisted reaction [3] steps. Two of the key transformations involve rapid microwave-assisted Suzuki arylation on the C-4, and Heck olefination on the C-3 position. Scale-up and details of the reaction processing issues will be discussed.




1) Steers, W.D., *Rev.Urology*, **2002,** 4, 17-25
2) Hewawasam, P. et al., *J.Med.Chem.,* **2003,** 46, 2819-2822.
3) Kappe, C.O., *Angew.Chem.Int.Ed.,* **2004**, 43, in press.

# SYNTHESIS OF 2,4-DIMETHYL-8-[2'-(2H-TETRAZOL-5-YL)-BIPHENYL-4-YLMETHYL]-5,8-DIHYDRO-6H-PYRIDO[2,3-D]PYRIMIDIN-7-ONE (TASOSARTAN) AND APPLICATION OF MICROWAVE ASSISTED ORGANIC SYNTHESIS

Klaus Gerdes[a], Jennifer M. Kremser[b], C.O. Kappe[b] and Ulrich Jordis[a*]

[a]Institute of Applied Synthetic Chemistry, Vienna University of Technology, Getreidemarkt 9/163 1060 Vienna;
[b]Institute of Chemistry, Karl Franzens Universität Graz, Heinrichstrasse 28, 8010 Graz
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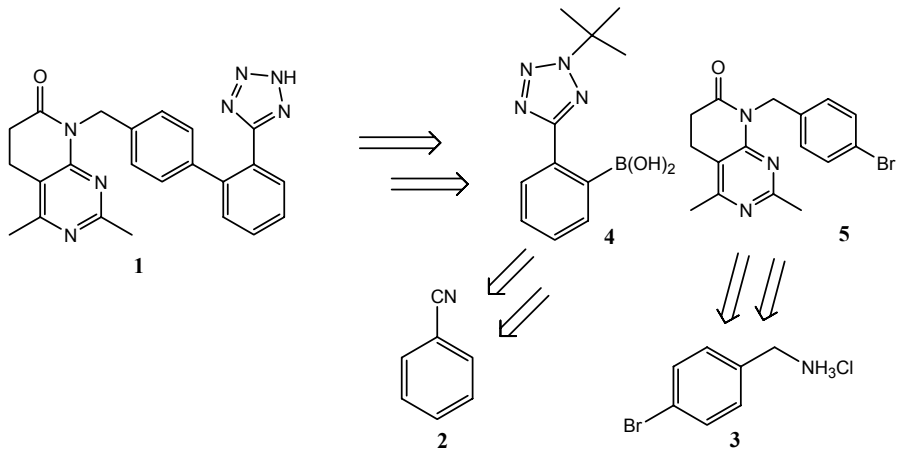
**Introduction:** The improved synthesis of 2,4-Dimethyl-8-[2'-(2H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-5,8-dihydro-6H-pyrido[2,3-d]pyrimidin-7-one (tasosartan) **1** on multigram-scale is described. Tasosartan is an angiotensin II antagonist for potential treatment of hypertension. Due to unresolved safety issues its NDA application was withdrawn in 1998. However, Tasosartan and its metabolites are still of interest in genomic and pharmaceutical studies [1]. Here the synthesis was developed in context of the DrugMatrix[2] genomics project.

**Chemistry: 1** was synthesized according to literature known procedures [3,4] starting from 4-bromobenzylamine hydrochloride **2** and benzonitrile **3** in a convergent strategy, and the eight step synthesis was optimized using different reagents and applying microwave assisted organic synthesis (MAOS) [5] in several steps. The key step of the synthesis is the palladium catalysed coupling of boronic acid **4** with bromide **5**. For this reaction MAOS optimizations were first carried out on small scale, and then transferred to large scale MAOS using the newly developed Synthos 3000 from Anton Parr. The poster will disclose details of this optimization process.




Literature: [1] Jack D. *Drug News and perspectives,* **2000**, 13, 121-124. [2] http://www.iconicpharm.com/products/products_main.html. [3] Ellingboe J. W., Antane M., Nguyen T., Collini, M. D., Antane S., Bender R., Hartupee D., White V., McCallum J., *J. Med. Chem.*, **1994**, 37, 542-550. [4] Ellingboe J. W., Nikaido M., Bagli J. F., EP, 539086, **1993**. [5] For a recent review see: Kappe C.O., *Angew. Chem. Int. Ed.* **2004**, 43, 6250-6284.

# SOLID PHASE SUPPORTED SYNTHESIS OF ALKYL DITHIOCARBAMATES

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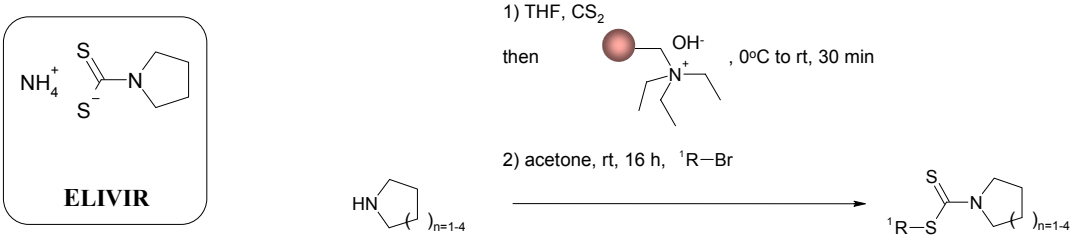
Pyrrolidine dithiocarbamate (ELIVIR) is reported to show antiviral activity against several kinds of viruses (human rhinovirus, enterovirus, influenzavirus) causing respiratory infections in humans [1, 2]. Thereby, the efficacy against more than only one type of virus hints for a more general mechanism of action affecting directly host cell processes. Agents of this type might represent a powerful tool in the fight against viral pandemics and the latent threat by animal viruses being on the mend to become adapted to humans (e.g. avian influenza).




However, in terms of better physiological availability and a possible oral application as well as due to potential toxicology issues, the quest for alternative compounds featuring the same or even an improved activity than ELIVIR is of interest. The alkylation of the free dithiocarbamate carbamate moiety is considered to be a first, promising approach along this line [3]. Herein, we report a robust procedure which allows alkylation of dithiocarbamates with a wide range of alkyl bromides in a parallel manner. Thereby, the dithiocarbamate is directly formed on the polymer support which is later used as a polymer reagent in the alkylation. The anticipated alkyl dithiocarbamates result in very good to excellent purities and yields and were submitted without further purification for biological screening.

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[2] Gaudernak, E.; Seipelt, J.; Triendl, A.; Grassauer, A.; Küchler, E.; *Journal of Virology*, 76, *12*, 6004-6015, **2002**.
[3] Osei, S.; Bakare, O.; *Solution Phase parallel Synthesis of a Twelve-Member Library of Dithiocarbamate Ester Analogs of Pyrrolidine*, Poster Presentation, Department of Chemistry, Howard University, Washington, USA.

# SYNTHESIS OF DEUTERATED ANALOGS OF PHARMACEUTICALS

Petr Knesl and Ulrich Jordis*

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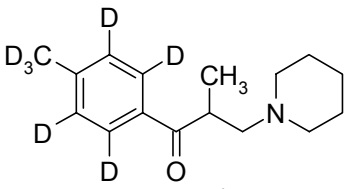
We will report report the synthesis of deuterated analogs of tolperison $d_7$ (1), formoterol $d_6$ (2) and BYK 170424 $d_4$ (3). These compounds were synthesized both for further pharmacological studies with potentially improved pharmacokinetic as well as pharmacodynamic properties in comparison to the non-deuterated compounds [1] as well as standards suitable for LC/MS/MS determination in biological samples.

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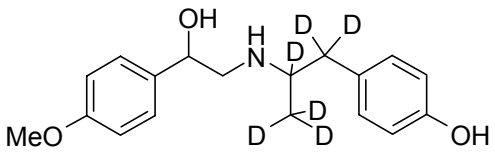


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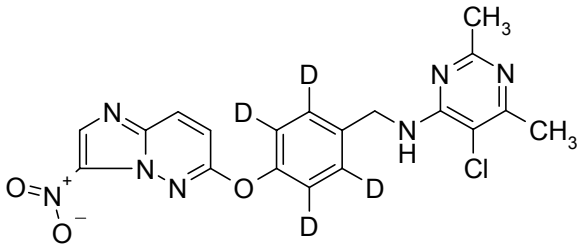


3

[1] Alken R.G., Stabingis T, WO 02088100 (2002)

# STRUCTURE – ACTIVITY STUDIES OF NOVEL AMIDINE ANALOGUES OF MELPHALAN

Anna Bielawska[a], Krzysztof Bielawski[a], Katarzyna Sosnowska[b], Wojciech Miltyk[a], Tomasz Anchim[c]

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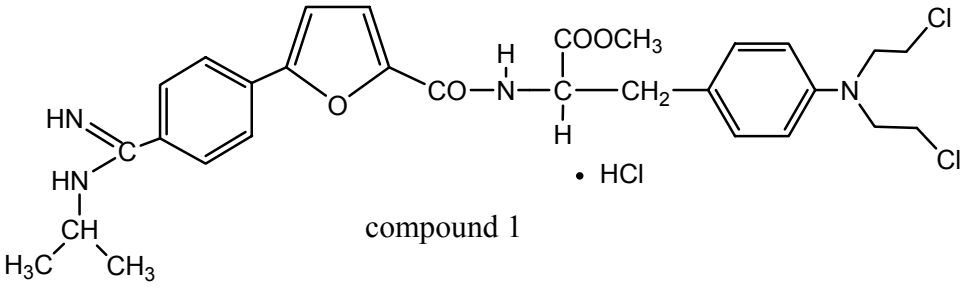
A number of novel amidine analogues of melphalan were synthesized and examined for cytotoxicity in breast cancer cell cultures. Evaluation of the cytotoxicity of these compounds employing a MTT assay and inhibition of [$^3$H]thymidine incorporation into DNA in both MDA-MB-231 and MCF-7 breast cancer cells demonstrated that these compounds were more active than melphalan. Data from the ethidium displacement assay indicated that these compounds bind in the minor groove of DNA and show moderate specificity for AT base pairs. To test whether cytotoxic properties were related to topoisomerase action, the most potent compound **1** was evaluated in a cell-free system. Compound **1** inhibited the catalytic activity of both topoisomerases I and II at a concentration of 120 and 20 μM, respectively. This suggests that DNA binding may be implicated in the cytotoxicity of these bisamidines, possibly by inhibiting interactions between topoisomerase II and their DNA targets.




compound 1

Molecular mechanic studies were carried out using the AM1 method, in order to generate a set of representative low-energy conformations for one representative amidine analogue of melphalan, i.e., compound **1**. Molecular dynamics approach was used to examine the structure of complexes formed between the d(CGCGAATTCGCG)$_2$ and compound **1**. The resulting structures of the ligand-DNA model complexes and their energetics were been examined. It was predicted that the compound **1** should have a decreased affinity for the minor groove of AT-rich regions in comparison to furamidine, netropsin and distamycin. From the energetic analysis it appears that van der Waals and electrostatic interactions are more important than specific hydrogen bonds in stabilizing the compound **1** - duplex complexes.

# SYNTHESIS AND CYTOTOXIC PROPERTIES OF NOVEL ALKYLATING DERIVATIVES OF L-PROLINE

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[b]Department of Pharmaceutical Technology, Medical University of Białystok, Surawska 2, 15-422 Białystok, Poland
[c]Department of Gynecological Endocrinology, Medical University of Białystok, Skłodowskiej 24A, 15-276 Białystok, Poland
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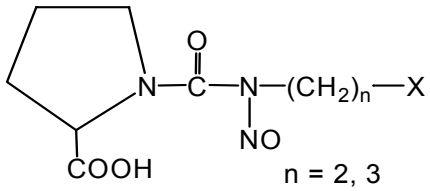
New alkylating derivatives of L-proline were synthesized as a prodrugs susceptible to the action of ubiquitously distributed, cytosolic imidodipeptidase – prolidase [E.C.3.4.13.9]. Although prolidase [E. C. 3. 4. 13. 9] is found in normal cells, substantially increased levels are found in some neoplastic tissues [1-4]. Because prolidase evokes ability to hydrolyse imido-bond of various low molecular weight compounds coupled to L-proline, we hypothesized that coupling of L-proline through imido-bond to an alkylating moiety 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 [1-4].




These compounds were used as substrates for prolidase activity assay. They were found as good substrates for prolidase, however with weak susceptibility. Their susceptibility were comparable to the well-known endogenous prolidase substrate, glycyl-L-hydroxyproline. We have compared several aspects of pharmacological actions of the alkylating derivatives of L-proline in MCF-7 and MDA-MB 231 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 these compounds were more active than carmustine and chlorambucil. New alkylating derivatives of L-proline were compared for their effects on collagen and DNA synthesis in breast cancer MCF cells. Increased ability of these compounds to suppress the protein synthesis, compared to chlorambucil and carmustine, was found to be related to an inhibition of prolidase activity and expression.

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# POTENTIAL HIV-1 INTEGRASE INHIBITORS: MOLECULAR STRUCTURES OF SELECTED QUINOLINE DERIVATIVES

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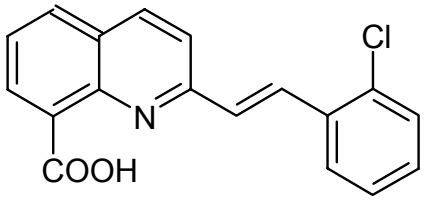
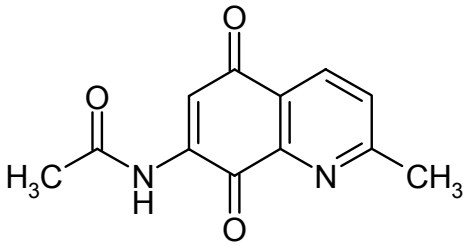
Integrase is the third main HIV enzyme, the first two: reverse transcriptase and protease are used as targets in Highly Active Antiretroviral Therapy (HAART). HIV-1 integrase (HIV-1 IN) is the enzyme that inserts the viral DNA into the host chromosome. There is no mammalian counterpart for that protein, which makes it an attractive target for antiviral drug design [1]. The combination therapy suppresses the replication of HIV-1 and makes the virus level undetectable in the plasma but still the virus exists in peripheral-blood mononuclear cells [2]. This means that discovery of new therapeutic agents is necessary to eradicate HIV-1 infection.

In our experiments we obtained single crystals of compounds, which were postulated to be potential inhibitors of HIV-1 IN.




(1)




(2)

The diffractometer KappaCCD was used for the intensity data collection (MoKα radiation), the phase problem was solved with direct metods.

Both molecules, (1) and (2), are approximately planar. The crystal packing of (1) is dominated by intermolecular non-classical C-H…O hydrogen bonds and short-contact interactions. In (2) the only interactions seem to be π-π stacking of the quinoline rings and week hydrogen bonds.

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# DESIGN, SYNTHESIS, AND BIOLOGICAL EVALUATION OF A SMALL-MOLECULE INHIBITOR OF THE HISTONE ACETYLTRANSFERASE GCN5

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Histone proteins are basic components of the eukaryotic chromatin [1]. They contain a DNA-interacting globular domain and a more flexible N-terminal region, which is a target for several posttranslational modifications. These include the acetylation of lysine residues and the methylation of lysine and arginine residues as well as the phosphorylation of serine hydroxyl functions and the attachment of an ubiquitin group [2]. The "histone-code hypothesis" is based on the assumption that these modifications create a specific substitution pattern on the histone tails. This pattern is readable by regulatory proteins, which connect the histone code with fundamental cellular processes like activation or repression of transcription. To date, acetylation is the best studied histone modification. It has been shown that the adjustment of a specific acetylation balance on the histone *N*-termini within a particular gene region is the result of a highly regulated interplay of selective histone acetyltransferases (HATs) and histone deacetylases (HDACs) [1].
For decoding the histone code a carefully directed influence on fine-tuning these processes through the development of low-molecular-weight and cell-permeable inhibitors is of extraordinary importance. Furthermore new inhibitors may open up new possibilities for treatment of pathological diseases like cancer [1]. In contrast to the HATs, several small molecule inhibitors of the HDACs are known, and some of them are in clinical trials [3]. Recently, anacardic acid was identified in a broad screening of plant extracts with antitumor activity as the first small-molecule inhibitor of the HAT p300 [4].
Here, we describe the development and biological evaluation of the first small-molecule inhibitor, structurally based on the α-methylene-γ-butyrolactone motif, of the human histone acetyltransferase Gcn5, a prominent member of the GNAT family with high preference for histone H3 as a substrate. The affinity of this inhibitor to the Gcn5 enzyme is comparable to that of the natural substrate H3 and provides an excellent starting point for the study of structure–activity relationships [5].

[1] M. Biel, V. Wascholowski, A. Giannis, *Angew. Chem.Int. Ed.* **2005**, in press.
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# A NEW GENERATION OF PROGESTERONE RECEPTOR POSITIVE BREAST CANCER THERAPEUTICS: SYNTHESIS AND BIOACTIVITIES

Claudia Hödl[a], Katrin Raunegger[a,b], Wolfgang S.L. Strauss[b], Reinhard Sailer[b], Olaf Kunert[a], Christoph Seger[c], Rudolf Steiner[b], Ernst Haslinger[a], H. Wolfgang Schramm[a]

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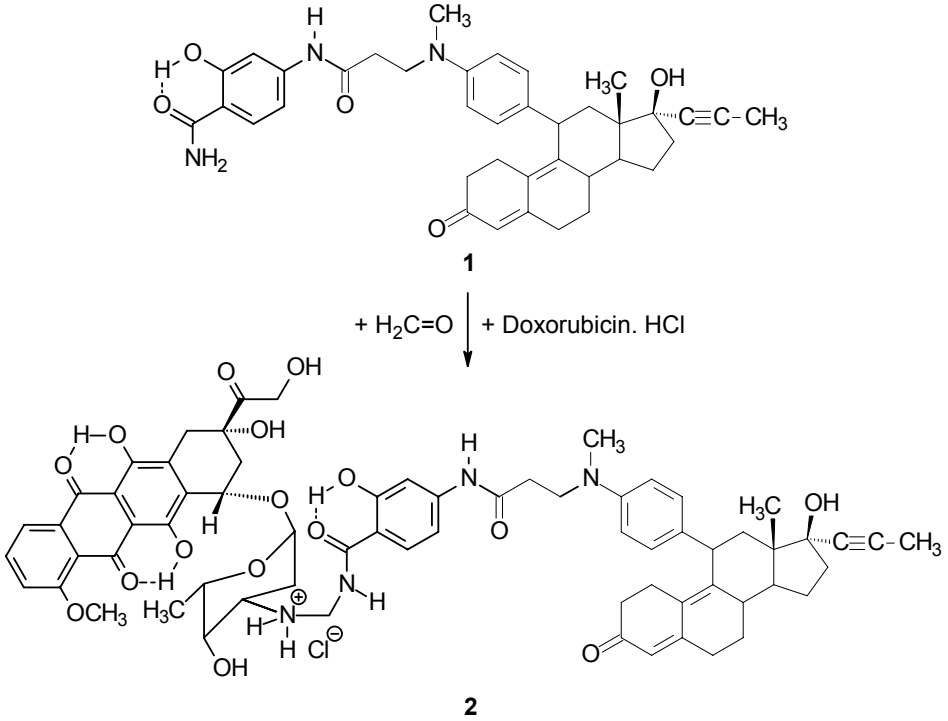
We did show recently [1,2] that functionalized mifepristone derivatives are shuttled into the nucleus of a progesterone receptor (PR) cancer cell line. To allow treatment of PR positive breast cancer tumors, combining the receptor selective anti-progestine mifepristone with well established tumor therapeutics seems promising. In this contribution, we present an approach to functionalize mifepristone (1) with doxorubicin and will discuss the assessed in vitro activities of the reaction product 2.




**1** **2**

[1] Hödl, C., Strauss, W.S.L., Sailer, R., Seger, Ch., Steiner, R., Haslinger, E., Schramm, H.W., (2004) A Novel High Affine, and Fluorescent Progesterone Receptor Antagonist. Synthesis and in Vitro Studies. Bioconjugate Chem. **15**, 359-365

[2]Hödl, C., Strauss, W.S.L., Sailer, R., Kunert, O., Seger, Ch., Steiner, R., Haslinger, E., Schramm, H.W., (2004) Development and Bioactivities of Novel Progesterone Receptor positive Breast Cancer therapeutics; Eur. J. Pharm. Sci. **23** (Suppl. 1), 33.

# SYNTHESIS AND BIOLOGICAL STUDIES OF A NEW CHROMOPHORE - STEROID CONJUGATE FOR THE PROGESTERONE RECEPTOR

Katrin Raunegger[a,b], Claudia Hödl[a], Wolfgang S.L. Strauss[b], Reinhard Sailer[b], Olaf Kunert[a], Sonja Sturm[c], Rudolf Steiner[b], Ernst Haslinger[a], H. Wolfgang Schramm[a]

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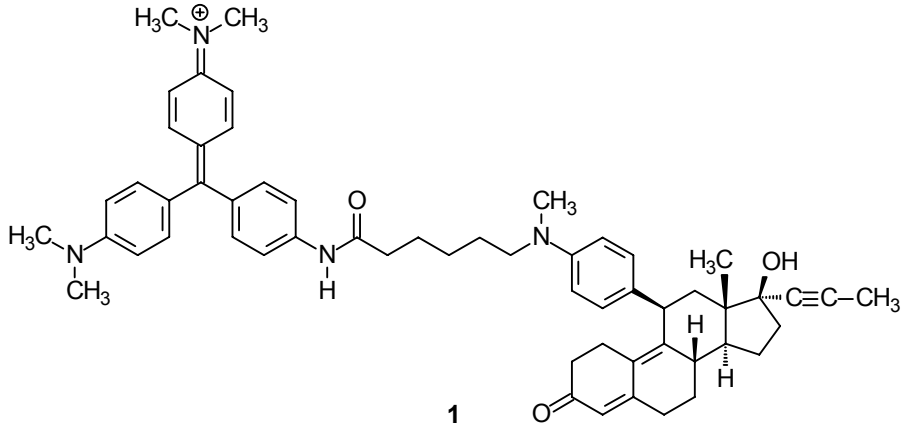
The progesterone receptor (PR) is a member of the nuclear receptor superfamily of ligand-dependent transcription factors. It mediates the effects of progesterone in various hormone-dependent tissues and plays a crucial role in normal breast development and in breast cancer. In some breast cancer cells PR overexpression was observed.
Chromophore-assisted laser inactivation (CALI) [1] is considered to be a powerful technology for acute protein inactivation in living cells. However, CALI has several limitations that restrict wider biological applications, mainly due to the use of antibodies for target recognition. To circumvent these limitations small molecule-based CALI (smCALI) was developed, where binding to the target protein is mediated by a synthetic molecule.
The PR was selected as target protein of interest, with reference to our previous work [2] demonstrating that functionalized mifepristone derivatives are shuttled into the nucleus of PR positive cancer cells.
The triphenylmethane-steroid-conjugate **1** was synthesized, using an activated carboxylate moiety on the steroid scaffold as linkage partner for the chromophore [3] and its biological activities, e.g. antiprogestagenic activity, was evaluated in appropriate cellular test systems.



**1**

[1] Jay D.G., Sakurai T. (1999) Biochim. Biophys. Acta 87452, M39-M48
[2] Hödl C., Strauss W.S.L., Sailer R., Seger C., Steiner R., Haslinger E., Schramm H.W. (2004) Bioconjugate Chem. 15, 359-365
[3] Raunegger K., Hödl C., Strauss W.S.L., Sailer R., Kunert O., Seger C., Steiner R., Haslinger E., Schramm H.W. (2004) Eur. J. Pharm. Sci. 23 (Suppl.1) 31.

# HIT&LEAD GENERATION BEYOND HTS

Konrad H. Bleicher

F. Hoffmann-La Roche, Basel

High throughput screening has developed to a well established methodology within the early drug discovery phase of both big pharmaceutical companies as well as specialized small biotechs. In fact most of the research projects where chemistry is initiated deliver the first starting points from massive random screening of large compound inventories. The luxury of hit lists and compound clusters identified *via* HTS are highly appreciated in the chemistry community. Nevertheless screening capacity is not unlimited. Although the actual testing phase might be very short (>100K/day) and the costs per data point fairly low (0.1-1US$) protein preparation, assay development, compound purchasing and logistics etc. are all adding to the HTS overhead. In addition, increasing compound inventories, constantly upcoming novel targets and requested selectivity screening campaigns are setting clear limits for this approach.
This presentation will discuss complementary technologies which allow the initiation of chemistry programs either in addition to the HTS route or without any random screening method.
Two case studies in the area of G-protein coupled receptors will be disclosed where focused compound libraries have been designed and generated either using the 'privileged structures approach' for the identification of novel NK-1 receptor ligands or 'virtual screening' for the generation of CB-1 receptor inverse agonists. The impact of focused libraries in a chemogenomics program will be further discussed.

# EXTRACTION AND SYNTHESIS OF MONOGALACTOSYL AND MONOGLUCOSYL DIACYLGLYCEROLS ISOLATED FROM EUPHORBIACEAE.

Francesca Cateni[a], Paolo Bonivento[b], Fabiana Frausin[c], Giuseppe Procida[b], L. Gabrielli Favretto[a], Vito Scarcia[c], Marina Zacchigna[a], Jelena Zilic[a]

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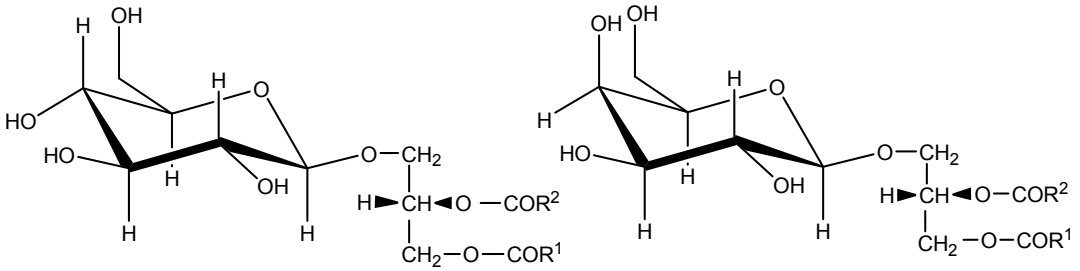
Monogalactosyl and monoglucosyl diacylglycerols (MGDG), a class of glycolipids, which were identified as anti-inflammatory active components in various species of Euphorbiaceae, were synthesized [1,2]. In particular, monogalactosyl diacylglycerols are the major constituents of the chloroplast membrane in the plant kingdom and have attracted much attention in recent years because of their biological activities, such as anti-tumor-promoting activity, DNA polymerase inhibition, activity of violaxanthin de-epoxidase in liposomes and haemolytic activity.



$R^1$, $R^2$ = Acyl residue

Besides, it was recently shown that glycoglycerolipid analogues have a promising inhibitory effect on Epstein-Barr virus early antigen (EBV-EA) activation induced by the tumour promoter 12-O-tetradecanoylphorbol-13-acetate (TPA). Thus, we focused our studies on in vitro structure-activity relationships in an effort to gain some insight into the action mechanism. In particular, analogues of both the glucose and the galactose series, bearing the some acyl residues and a short-medium length fatty acid acyl chain at the 3-position of the glycosyl-glycerol skeleton, shorter than chains present in natural compounds, have been synthesized and have shown an interesting in vitro inhibitory activity against KB and IMR-32 cell lines.

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# COMPARATIVE CONFORMATIONAL ANALYSIS OF INDOLICIDIN AND ITS ANALOGUES BY SIMULATED ANNEALING

Balázs Leitgeb[a], Ádám Kerényi[a], and Gábor Rákhely[a,b]

[a]Institute of Biophysics, Biological Research Centre of the Hungarian Academy of Sciences, P.O. Box 521, H-6701 Szeged, Hungary
[b]Department of Biotechnology, University of Szeged, P.O. Box 521, H-6701 Szeged, Hungary

Indolicidin is an antimicrobial tridecapeptide isolated from bovine neutrophils, and it has a remarkable primary structure containing five Trp-s, three Pro-s and three basic residues (Arg, Lys) [1]. This peptide has a broad spectrum of antimicrobial activity against Gram(+) bacteria, Gram(-) bacteria and fungi, as well as it exhibits haemolytic and antiviral activities. The aim of this study was to compare the different structural properties of indolicidin and its *enantio*-, *retro*- and *retroenantio*-analogues, because these analogues showed approximately the same antibacterial activity as the parent peptide [2]. The conformational analysis of the investigated peptides containing *trans* Xxx-Pro peptide bonds were performed using simulated annealing (SA) method. The distributions of the conformers obtained by SA were represented in Ramachandran plots, in which the preferred conformational regions were identified. Furthermore, to characterize the backbone conformations, pseudo-torsion angle maps were constructed, in which the pseudo-torsion angles defined by four consecutive $C_\alpha$ atoms were depicted. The *g(+)*, *g(-)* and *trans* rotamer populations of the amino acid side-chains were also determined. For all peptides, the occurring secondary structural elements were examined, and poly-proline II helical segments were observed along the entire sequence of molecules. Based on the distributions of the values of $\Phi$ and $\Psi$ torsion angles, the largest conformer populations were determined. Using the ranges of torsion angles characteristic to the largest populations, the poly-proline II helix-like structures were identified in certain parts of the molecules. In the case of indolicidin and its analogues, the different intramolecular interactions (H-bonds, aromatic-aromatic interactions and proline-aromatic interactions) were also investigated, which can play a role in the stabilization of various conformers.

[1] Selsted, M. E.; Novotny, M. J.; Morris, W. L.; Tang, Y.-Q.; Smith, W.; Cullor, J. S. (1992) *J. Biol. Chem.* **267**, 4292-4295.
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# IMPROVEMENT OF THE PHARMACOLOGICAL PROFILE OF COX-II ACTIVE RESVERATROL-ANALOGUES

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[a]Department of Medicinal and Pharmaceutical Chemistry
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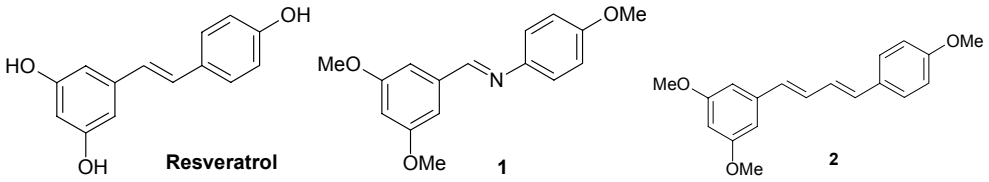
Resveratrol shows a variaty of pharmacological effects like a moderate inhibition of both COX-I and COX-II with a selectivity index of about 1. In our work the effect of resveratrol was studied in isolated smooth muscle preparations (terminal ilea, aortic- and arteria pulmonalis-rings) and compared to the effect of two newly synthesized derivatives **1** and **2** (Fig. 1).

Fig.1:




The relaxing effect of the compounds was measured using the method described by Reiter [1]. Aortic- and arteria pulmonalis-rings were precontracted by 90 mmol/l KCl, terminal ilea by 60 mmol KCl. Resveratrol concentration-dependently reduced contractility of vascular smooth muscle (aorta, n = 5, $EC_{50}$ = 145 µmol/l; arteria pulmonalis, n = 6, $EC_{50}$ = 126 µmol/l). The most potent effect was seen in terminal ilea (n = 5, $EC_{50}$ = 46.4 µmol/l). Compound **1**, a methoxy derivative of resveratrol with nitrogen in the molecule building an imine, lacked of any effects on the preparations studied. Only in terminal ilea it exerted a weak relaxing effect. Compound **2**, a methoxy derivative with an additional ethenyl group in the stilbene scaffold, did not cause any effect in smooth muscle preparations.
Former studies showed that molecules **1** and **2** had an selective inibitory effect on COX-II in µmolar concentration. Therefore we conclude that changes in the molecular structure lead to compounds with an improved COX-II-inhibition and a complete loss of the vasodilatory effect.

[1] Reiter M (1967) Bioassay of inotropic agents on the isolated papillary muscle. Drug Res 17:1249-1253.

# SYNTHESIS AND BIOLOGICAL EVALUATION AS PDT/BNCT AGENTS OF Zn(II)- AND Si(IV)-PHTHALOCYANINE DERIVATIVES BEARING BORON CLUSTERS

Donata Dei[a], Francesca Giuntini[b], Daniele Nistri[a], Giacomo Chiti[a], Clara Fabris[a], Elisabetta Friso[b], Giulio Jori[b], Paolo Colautti[c] and Gabrio Roncucci[a]

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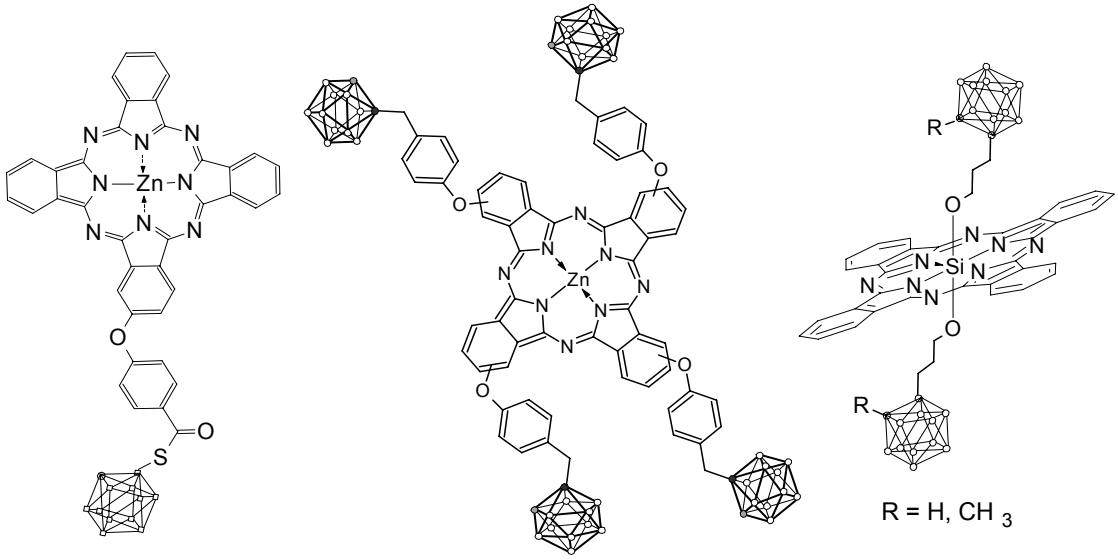
Photodynamic therapy (PDT) [1] and boron neutron capture therapy (BNCT) [2] are two binary therapeutic modalities, which are currently under investigation for the treatment of several kinds of malignancies. Both of them rely on the interaction of two relatively harmless factors: a photo- or radio-sensitising compound and an external radiation. PDT treatment consists in loading the target cells with a photosensitizer that is able to generate highly reactive species (mainly singlet oxygen) upon irradiation with light of the appropriate frequency. Similarly, BNCT is based on the interaction of the non radioactive $^{10}B$ nucleus and a thermal neutron. Due to their selectivity of accumulation in tumor over many normal tissues, phthalocyanines have recently been proposed as boron carriers to target tumoral tissue in BNCT treatment [3].




As a part of our current interest in the synthesis of phthalocyanines for biomedical applications, we undertook the synthesis of Zn(II)- and Si(IV)-phthalocyanine derivatives bearing one to four boron clusters, with the purpose of developing radio/photosensitizing agents for the treatment of tumors, by means of a combination of BNCT and PDT. *In vitro* and *in vivo* studies concerning the biological and photophysical activity of such compounds are also reported.

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[2] Soloway, A. H. et al. *Chem. Rev.* **1998,** *98*, 1515-1562
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# INVESTIGATION OF MULTIPOLAR INTERACTIONS IN THE ACTIVE SITE OF THROMBIN

Eliane Schweizer, Anja Hoffmann-Röder, Jacob Olsen, Kaspar Schärer, and François Diederich

Labratorium für Organische Chemie der Eidgenössischen Technischen Hochschule, ETH Hönggerberg, 8093 Zürich, Switzerland

Due to its rigidity and well-defined binding mode, the enzyme thrombin represents an interesting target to investigate multipolar interactions occurring upon binding of the tricyclic inhibitors developed in the *Diederich* group [1]. With regard to the surprising results obtained during the fluorine scan in the D and S1 pockets, which lead to the discovery of a new type of orthogonal interactions between the fluorine atom and a backbone carbonyl group [2], we became interested in studying related interactions of fluorine, hydroxy and methoxy substituents, respectively, in the oxyanion hole as well as in position 4 of the benzyl ring reaching into the D pocket of the active site (figure 1). For the latter analysis we performed also the synthesis of the bromo and chloro derivatives, additionally envisaging the comparison with the iodo substituted compound.

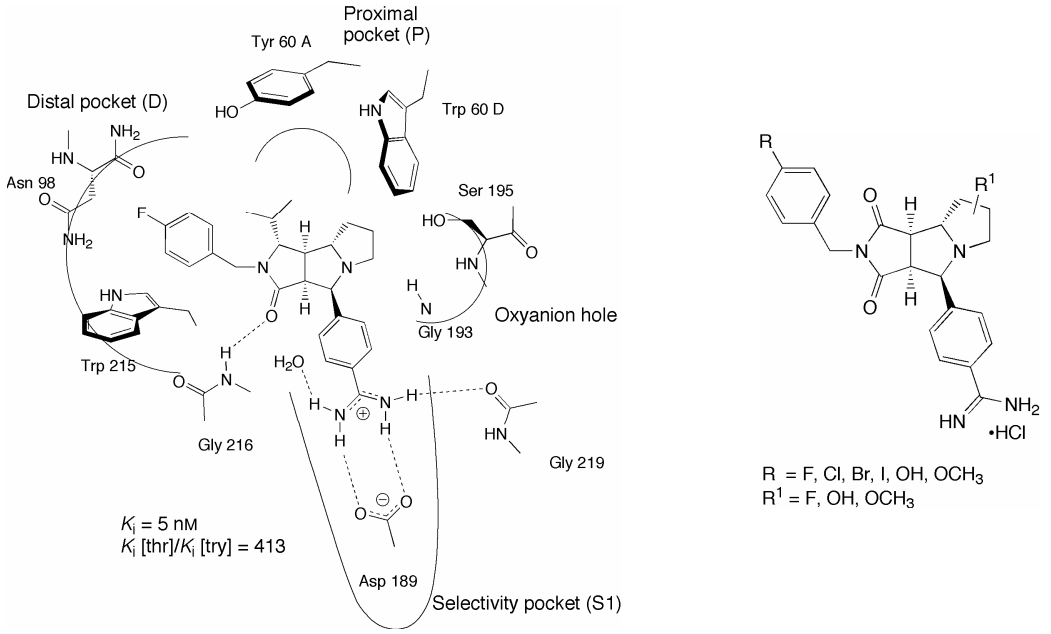



**Figure 1:** *Our most potent tricyclic inhibitor bound in the active site of thrombin (left) and the substitution pattern for the investigation of the multipolar interactions (right).*

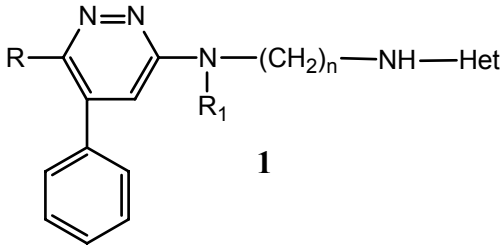
[1] U. Obst, V. Gramlich, F. Diederich, L. Weber, D. W. Banner, *Angew. Chem.* **1995**, *107*, 1874-1877; *Angew. Chem. Int. Ed.* **1995**, *34*, 1739-1742.
[2] J. A. Olsen, D. W. Banner, P. Seiler, B. Wagner, T. Tschopp, U. Obst-Sander, M. Kansy, K. Müller, F. Diederich.

# PYRIDAZINE DERIVATIVES AS NOVEL ACAT INHIBITORS

Arianna Gelain[a], Daniela Barlocco[a], Lucio Toma[b] , Byoung-Mog Kwon[c], Tae-Sook Jeong[c], Young-Sook Kim[c]

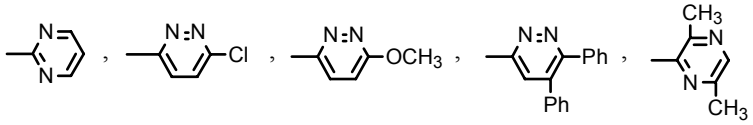
[a]Istituto di Chimica Farmaceutica e Tossicologica, Università di Milano, v.le Abruzzi 42 20131 Milano, Italy;
[b]Dipartimento di Chimica Organica, Università di Pavia, v. Taramelli 10, 27100 Pavia, Italy;
[c]Korea Research Institute of Bioscience & Biotechnology, 52 Uen-Dong Yusung-Ku,Taejeon 305-600, Korea

Acyl-CoA:cholesterol acyl transferase (ACAT) is an intracellular enzyme that catalyses the esterification of free cholesterol in various tissues; it represents a good target in the treatment of hypercholesterolemia and coronary disease.
As a part of our ongoing studies on pyridazine derivatives as ACAT inhibitors [1-4] we synthesized a new series of derivatives of general formula **1** :



**1**

n = 6-8 ; R = H, Ph ; $R_1$ = H, $(CH_2)_6CH_3$ ;

Het = 

The present communication describes the synthesis and the pharmacological evaluation of the title derivatives. Their activity was tested toward ACAT extracted from rat liver microsomes. At a final concentration of 100 μg/mL, the most significant compounds showed inhibition ranging between 80 and 95%.

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References:
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# SYNTHESIS OF 3,6-DIAZABICYCLO[3.1.1]HEPTANES AS NOVEL LIGANDS FOR THE OPIOIDS RECEPTORS.

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Crude opium has been used since antiquity as an analgesic. In modern medicine, only the purified opium alkaloids are commonly employed. Among these, morphine remains one of the most valuable analgesics for relief of severe pain. However, they can cause severe side effects such as respiratory depression, constipation, vomiting, and, moreover, their chronic use results in tolerance and dependence.

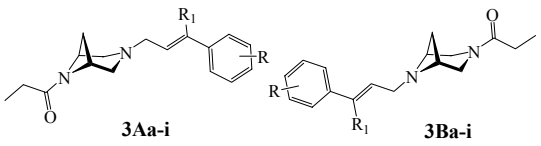
Opioids interact with three major opioid receptor types (μ, δ and κ) and while each of the opioid receptors is associated with analgesia, side effects have been attributed to non specific recognition of subtype receptors. Selective ligands are therefore desirable to reduce adverse side effects. *N*-3(8)-Arylpropenyl-*N*-8(3)-propionyl-3,8-diazabicyclo[3.2.1]octanes **1A,B**, belonging to the bridged diazabicyclic systems, are potent analgesics selectively labelling μ-opioid receptors [1].

Compounds bearing a $NO_2$ or a Cl groups at the *para* position of the arylpropenyl moiety were reported to elicit reduced physical dependence and tolerance compared to morphine.

Moreover, results on *N*-3(9)-arylpropenyl-*N*-9(3)-propionyl-3,9-diazabicyclo[3.3.1]nonane congeners **2A,B** provided evidence that the endopropano bridge, present in these analogues instead of endoethane of DBO series, had a major impact on the ability of the compounds to interact with opioid receptors [2]; in general $N_9$-arylpropenyl-substituted DBN derivatives **2B** exhibited markedly higher binding affinities than that of the isomeric ones **2A**. Molecular modeling studies established that the DBN diaza skeleton in its chair-chair favoured conformation oriented the propano bridge towards *N*-3 and this would result in an unacceptable steric bulk around this atom with reduced μ-receptor interaction of the corresponding derivatives.

On the basis of these considerations, it was of interest to evaluate the affinity towards opioid receptors of 3,6-diazabicyclo[3.1.1]heptanes (DBH), which, having an endomethano bridge, represent a further diazabicyclic variant of **1** and **2** characterized by increased rigidity.

Accordingly, representative 3-arylpropenyl-6-propionyl DBH (**3Aa-i**) and their 6-arylpropenyl-3-propionyl isomers (**3Ba-i**) have been synthesized and tested *in vitro* towards opioid receptors.




| 3A, 3B | a | b | c | d | e | f | g | h | i |
|---|---|---|---|---|---|---|---|---|---|
| R | H | 4-$NO_2$ | 2-Cl | 3-Cl | 4-Cl | H | 4-Cl | 3,4-$Cl_2$ | H |
| $R_1$ | H | H | H | H | H | $CH_3$ | $CH_3$ | $CH_3$ | Et |

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# SYNTHESIS AND CYTOTOXIC ACTIVITIES OF 3-(5-PHENYL-[1,3,4]OXADIAZOL-2-YL)-1*H*-BENZO[g]INDOLE AND RELATED COMPOUNDS

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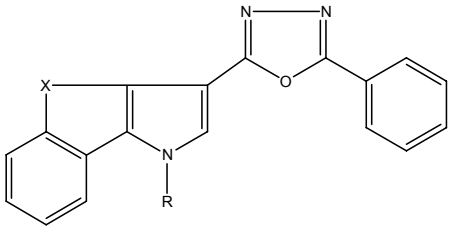
Cancer is the second leading cause of death in industrialized nations. Cancer chemotherapy commonly involved the use of cytotoxic agents that destroy rapidly dividing cells. Within the past decade, advances in our understanding of the cell cycle have presented new targets that may allow the development of more selective chemotherapeutic agents able to target only cancer cells. Despite this progress, cytotoxic agents will remain a mainstay in cancer chemotherapy for the near future [1].
In this context we have recently synthesized a series of compounds of general structure (**1**) [2]. Among them the compound **1a** (X= CH=CH, R = H, $R_1$= 5-phenyl-[1,3,4]-oxadiazole) was evaluated *in vitro* by the National Cancer Institute (NCI Bethesda) against 60 tumor cell lines derived from nine cancer cell types. Biological results showed a very interesting antitumor activity in particular against leukemia, colon and breast cancer.




**1**

X = $CH_2$, $CH_2CH_2$, CH=CH
R = H, Alk

On the bases of these results, we have extended our investigation on other series of structurally related compounds, in which the oxadiazolyl moiety was modified. The activity of the new compounds was evaluated on colon cancer cells, a predictive model of antitumor activity. The chemistry and the biological data will be discussed in the poster.

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# DESIGN AND SYNTHESIS OF NEW DERIVATIVES OF α-SUBSTITUTED AMIDES OF γ-PHTHALIMIDOBUTYRIC ACID WITH POTENTIAL GABA-ERGIC ACTIVITY

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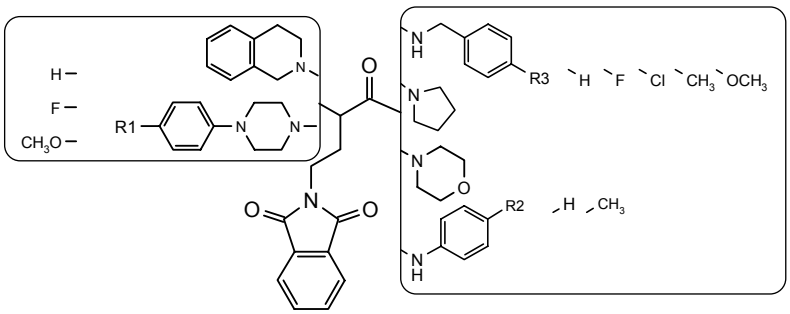
Department of Physicochemical Drug Analysis; Laboratory of Pharmacobiology, Faculty of Pharmacy, Medical College, Jagiellonian University, Kraków, Poland

4-Aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the central nervous system, and exerts its action through three classes of GABA receptors. $GABA_A$ receptor agonists can be useful in certain neurological and psychiatric disorders as antiepileptic, myorelaxant, antinociceptive, anxiolytic and hypnotic agents. It was proved that among different chemical classes of compounds with GABA-ergic activity, appropriate gabaamides act as a full agonists of $GABA_A$ receptors [1]. Progabide, which structurally belongs to 4-aminobutyramides group is well known antiepileptic drug which functiones as a GABA prodrug.

Searching for a novel ligands of the GABA binding site of GABA-A receptors, we have prepared a series of α-substituted amides of γ-phthalimidobutyric acid. These phthalimide series may be considered as analogues of GABA prodrugs, in which the amino function is converted into an imido group and the acid group is changed into an amido group.




The newly synthesized compounds were tested *in vitro* in [$^3$H]muscimol binding assay (as indicates of GABA-A receptor affinity). Among investigated compounds the most active was *N*-(4-methylbenzylamide) of 2-(4-phenylpiperazine)-4-phthalimidobutyric acid with $IC_{50}$ = 8.1 ± 2.8 μM value. Preliminary anticonvulsant *in vivo* tests of these compounds i.e. a maximal electroshock (MES) test, a subcutaneous metrazole (scMet) induced seizures, and a rotorod toxicity (Tox) assay on mice were employed.

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Acknowlegements: We thank Dr J. P. Stables for anticonvulsant testing and to the Anticonvulsant Screening Project (ASP), National Institute of Neurological Disorders and Stroke, NIH, Bethesda, Maryland USA.

# STRUCTURE-ACTIVITY RELATIONSHIPS AND COMPUTATIONAL STUDIES ON PDE$_4$ INHIBITORS

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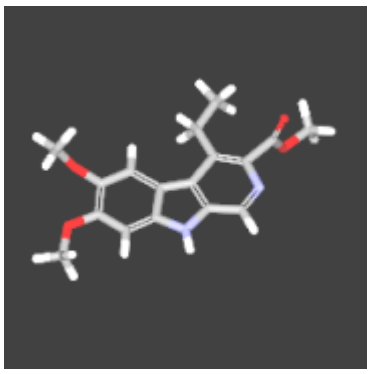
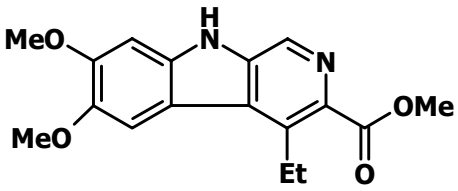
Cyclic AMP controls a wide variety of cellular functions. As the only means of degrading this second messenger is through the action of cyclic nucleotide phosphodiesterases (PDEs), these enzymes provide a key regulatory system [1]. Interest in the potential use of isoenzyme selective phosphodiesterase inhibitors has increased in recent years. In particular, the type 4 family of phosphodiesterases (PDE 4) is comprised of enzymes characterized by their specificity for cAMP hydrolysis [2]. The aim of this present work is to clarify the binding mode of PDE inhibitors by computational studies of new derivatives structurally related to DMCM. Infact a successful synthesis of various β-carboline was carried out and appear to be very attractive models for new PDE 4 inhibitors, with potential uses in the treatment of asthma, chronic pulmonary obstructive disease and some autoimmune disease [3]. In order to identify how selective inhibitors with different chemical structures bind to the similar catalytic pockets of PDEs we performed specific studies to try to define the binding site of the protein, the position and shape of which was used in docking calculations. Some of these synthesized compounds were selected for further biological and pharmacological evaluations of autoimmune and inflammatory diseases.






**DMCM**

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# SYNTHESIS AND IN-VITRO ANTITUMOR ACTIVITY OF 1-[(INDOL-1-YL)PROP-1-YN-1-YL]PHTHALAZINES AND RELATED COMPOUNDS

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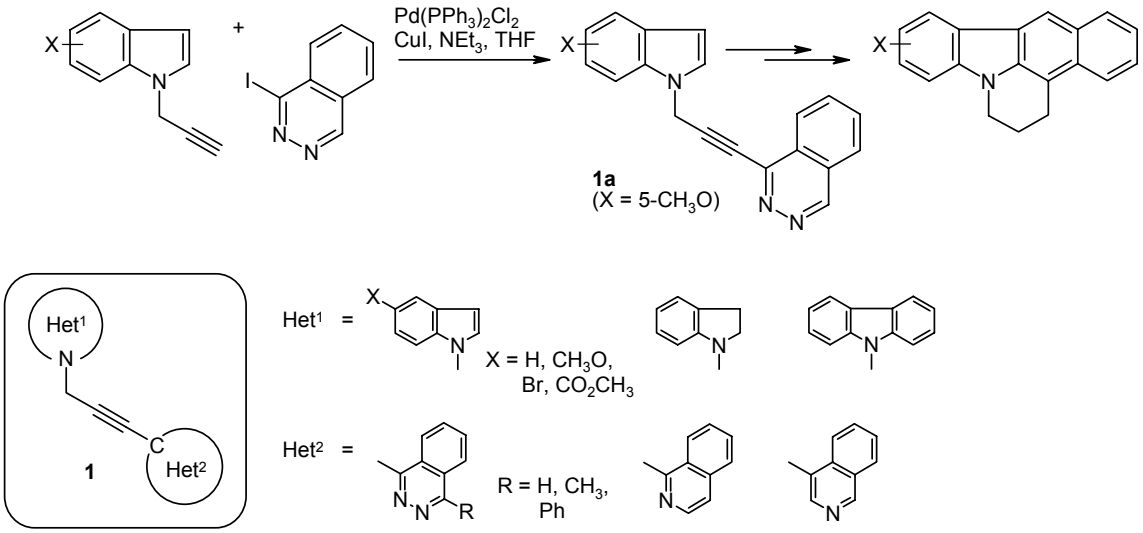
The phthalazine derivative, 1-[3-(5-methoxy-1*H*-indol-1-yl)prop-1-yn-1-yl]phthalazine (**1a**), had been prepared recently as a key intermediate in the synthesis of pentacyclic analogs of the antitumor alkaloid, *ellipticine* [1], via a pathway involving an intramolecular inverse-electron-demand Diels-Alder reaction of indolylalkyl-substituted diazines [2]. In the course of a routine screening, compound **1a** was found to exhibit growth inhibitory activity towards several human tumor cell lines.

Based on the concise synthesis of **1a**, a focused library of compounds featuring the same propyne motif with one electron-rich and one electron-deficient hetarene attached, was now made available. For all new compounds of type **1**, the key step in the reaction sequence is a Sonogashira cross-coupling of an appropriate *N*-propargyl-substituted indole, indoline or carbazole, respectively, with an iodo- or bromohetarene. In the context of this work, also an improved synthesis of 1-iodophthalazine [3] was developed.

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# MARINE ORGANISMS AS A SOURCE OF THERAPEUTIC AGENTS: NEW BIOACTIVE METABOLITES ISOLATED FROM THE MARINE SPONGE *PLAKORTIS ZYGGOMPHA*

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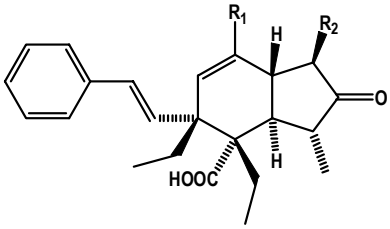
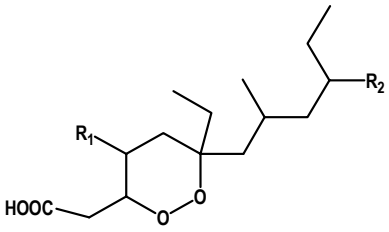
Recent developments in the applied field of natural products make it obvious that nature provides chemicals, secondary metabolites, of impressive complexity. It is generally accepted that these natural products offer new potential for human therapy. Organisms of choice useful for such exploitation live in the marine environment. Among them, marine sponges (phylum: Porifera), sessile filter feeders, have a long evolutionary history (600-500 million years ago) during which they could develop a chemical defence system to fight successfully against other invading organisms.

As part of our ongoing search of new biologically substances from marine organisms, we undertook the study of the sponge *Plakortis zyggompha* (order Homosclerophorida, family Plakinidae) collected around the Martinique island in 2002.

*Plakortis zyggompha*




Spiculoic acids (**1**)




Cyclic peroxides (**2**)

After a biological primary screening, this organism showed interesting pharmacological activity and led to the isolation of news compounds belonging to two different original polyketide families: the spiculoic acids (**1**) and the cyclic peroxides (**2**) [1]. The new spiculoic acids family with an uncommon spiculan skeleton was recently described by Andersen *et al.* in 2004 [2].

The structural determination of these new compounds based on 1D and 2D NMR studies and mass spectral determinations is presented, as well as the results of their biological activities evaluation. An interpretation of their relative stereochemistry on the basis of their biogenetic pathway is also proposed.

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# NEW ENDOMORPHIN ANALOGUES WITH 2`,6`-DIMETHYL-Tyr AND e-β-MePhe SUBTITUTIONS

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Endomorphins (endomorphin-1: H-Tyr-Pro-Trp-Phe-$NH_2$ and endomorphin-2: H-Tyr-Pro-Phe-Phe-$NH_2$) are the likely endogenous ligands of μ-opioid receptors [1]. These peptides showed high affinity and selectivity for μ-opioid receptors in rat and mouse brain homogenates and in recombinant μ-opioid receptors in direct and indirect radioreceptor binding assays. In order to improve their biological activity we designed and synthesized new analogues with unnatural amino acids such as 2',6'-dimethyltyrosine (Dmt), in position 1 and eritro-β-methylphenilalanine (e-β-MePhe) in position 4. Competitive radioreceptor binding assays indicated that four endomorphin analogues had high affinity for μ- and δ-opioid receptors in mouse brain membranes. (Dmt-Pro-Trp-(2S,3S)-β-MePhe-$NH_2$ $K_{i\mu}$ = 0.97 nM $K_{i\delta}$ = 12.53 nM, Dmt-Pro-Trp-(2R,3R)-β-MePhe-$NH_2$ $K_{i\mu}$ = 7.47 nM $K_{i\delta}$ = 145 nM, Dmt-Pro-Phe-(2S,3S)-β-MePhe-$NH_2$ $K_{i\mu}$ = 0.75 nM $K_{i\delta}$ = 35.48 nM, Dmt-Pro-Phe-(2R,3R)-β-MePhe-$NH_2$ $K_{i\mu}$ = 3.08 nM $K_{i\delta}$ = 76.40 nM). Some Dmt-endomorphin analogues stimulated [$^{35}$S]GTPγS binding in rat brain membranes, but these showed mixed μ agonist /δ antagonist properties using known specific μ and δ antagonists. Preliminary results supported these findings in recombinant cell membranes expressing the human μ- or human δ-opioid receptors. We found that two analougs were better analgetics than the analogous Dmt-endomorphins by the tail-flick tests. (Dmt-Pro-Trp-(2S,3S)-β-MePhe-$NH_2$ $A_{50}$ = 0.026 nmol, Dmt-Pro-Trp-Phe-$NH_2$ $A_{50}$ = 0.134 nmol, Dmt-Pro-Phe-(2S,3S)-β-MePhe-$NH_2$ $A_{50}$ = 0.138 nmol, Dmt-Pro-Phe-Phe-$NH_2$ $A_{50}$ = 0.64 nmol).

Supported by grant of NKFP 027/2001.

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# NEW ENDOMORPHIN-2 DERIVATIVES CONTAINING TRITIUM LABELLED 2-AMINOCYCLOPENTANECARBOXYLIC ACID AND 2-AMINOCYCLOHEXANECARBOXILIC ACID

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Endomorphin-1 (H-Tyr-Pro-Trp-Phe-$NH_2$) and endomorphin-2 (H-Tyr-Pro-Phe-Phe-$NH_2$) were revealed in the 90s as opioid ligands which can bind selectively to the μ-receptor[1]. Although their stability against proteolytic enzymes is bigger than that of the other endogenous opioid peptides for example enkephalins, the half-life of these endogenous peptides is short to produce long-lasting analgesia. To obtain a proteolyticly more stable analogues with high specificity to the μ-receptor we have synthesized endomorphin-2 analogues containing 2-aminocyclopentenecarboxylic acid (H-Tyr-ΔACPC-Phe-Phe-$NH_2$) and 2-aminocyclohexenecarboxylic acid (H-Tyr-ΔACHC-Phe-Phe-$NH_2$) based on a Boc-strategy using racemic cis-ACPC and cis-ACHC by solid phase peptide synthesis. The diastereomeric peptides were separated by RP-HPLC. The configuration of the ΔACPC and ΔACHC in the peptides was determined by RP-HPLC after the hydrogenation of all peptide isomers with comparison of earlier made standard peptides (H-Tyr-(1S,2R)ACPC-Phe-Phe-$NH_2$), (H-Tyr-(1R,2S)ACPC-Phe-Phe-$NH_2$ H-Tyr-(1S,2R)ACHC-Phe-Phe-$NH_2$) and (H-Tyr-(1R,2S)ACHC-Phe-Phe-$NH_2$,).

Previous studies have shown that the (1S,2R)ACPC and the (1S,2R)ACHC containig derivatives bind with higher affinity and specificity to the μ-receptor than the (1R,2S)ACPC and the (1R,2S)ACHC containing isomers [2]. The suitable dehydro derivatives were tritiated using tritium gas in the presence of $PdO/BaSO_4$ catalyst. We have got two saturated derivatives containing tritium in the ring of prolin mimetics. The crude tritiated peptides were purified by RP-HPLC using radioactive detector. First we have studied the binding properties of $^3$H-(1S,2R)ACHC-endomorphin-2 derivative in rat brain membrane. The ligand showed much higher affinity to the μ-receptor than to the δ- or to the κ-receptors.

The binding studies of $^3$H-(1S,2R)ACPC-endomorphin-2 derivative are in progress. We hope that these results will help us to better understand the structural requirements of opioid binding.

Supported by OTKA T04514 and RET 08/2004.

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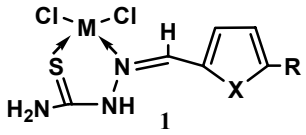
# CYTOTOXICITY OF METALLIC COMPLEXES OF HETARYLTHIOSEMICARBAZONES

Luba Ignatovich, Ilze Sleiksha,Vitalijs Romanovs, Olga Starkova, Irina Shestakova, and Edmunds Lukevics

Latvian Institute of Organic Synthesis, Aizkraukles 21, Riga LV1006, Latvia

Si- and Ge-containing metal complexes of thiosemicarbazones **1** have been prepared.




**1**

R = H, Cl, Me, $Me_3Ge$, $Et_3Ge$, $Me_3Si$, $Et_3Si$, $PhMe_2Si$, $Ph_2MeSi$, 1-Me-silacyclopentane, 1-Me-silacyclohexane, 1-(2-thiosemicarbazonomethyl-5-hetaryl)-1-sila(germa)cycloalkyl;
X = O, S; M= Cu, Pd

Cytotoxicity of **1** on four tumour cell lines has been studied. Copper (II) complexes exhibited higher cytotoxic activity than corresponding Pd (II) derivatives.

# 2-PHENYL-PYRROLO[2,3-h]QUINOLIN-4-ONES AS NOVEL SELECTIVE ANTIMITOTIC AGENTS

Venusia Gasparotto[a,b], Gianfranco Chiarelotto[a], Vincenzo Pezzi[c], Ignazio Castagliuolo[b] and Maria Grazia Ferlin[a]

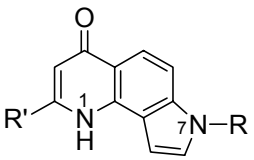
a) Department of Pharmaceutical Sciences, b) Department of Histology, Microbiology and Medical Biotechnologies, University of Padova, c) Department of Pharmaco-Biology, University of Calabria, Italy.

In our search for new potential anticancer drugs, we designed and synthesized a series of tricyclic compounds containing the antimitotic 2-phenyl-azaflavone chromophore fused [1] to a pyrrole ring in a pyrroloquinoline structure. A multi-step synthesis was performed via an amino-indole intermediate cyclized to a pyridinone structure using Conrad-Limpach or Gould-Jacobs conventional reactions.

Compounds **8**, **18**, **19**, **22**, **23**, **25** and **26**, when tested against a panel of fourteen human tumor cell lines, showed poor *in vitro* cytotoxic activity ($IC_{50} \geq 50$ μM), whereas **20, 21** and **24** showed a significant activity ($IC_{50}$ ranging from 0.7 to 50 μM). Steroid hormones sensitive ovary, liver, breast and adrenal gland adenocarcinoma cell lines displayed highest sensitivity ($IC_{50}$ values ranging from 0.7 to 8 μM).

Compound **24** blocked cells in the $G_2$/M phase of the cell cycle and induced a significant increase in apoptosis cell death, as assessed by Flow Cytometry analysis and detection of cytoplasmic histone-associated DNA fragments. Compounds **20**, **21**, **24** proved to alter the microtubule assembly and stability displaying a microtubule cytoplasmic network similar to the Vincristine caused one, as observed by Immunofluorescence Microscopy analysis.

In addition, these tree most active compounds, when subjected to the tritiated water release assay, exhibited high inhibitory effects on Aromatase activity, that can be considered the reason to explain the exerted selectivity against estrogen-sensitive tumor cell lines.




**18-26**

| | R | R' |
|---|---|---|
| 18 | H | $CH_3$ |
| 19 | H | tienyl |
| 20 | H | phenyl |
| 21 | $CH_3$ | phenyl |
| 22 | H | p-$NO_2$-phenyl |
| 23 | H | m-$NO_2$-phenyl |
| 24 | H | m-$OCH_3$-phenyl |
| 25 | H | m-$NH_2$-phenyl |
| 26 | H | m-$NH_2$-phenyl. HCl |

Finally, *in vivo* administration of compound **24** to Balb/c mice inhibited growth of a syngenic hepatocellular carcinoma by 67%.

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# THE PARALLEL SYNTHESIS AND CYTOTOXIC EFFECT OF CARBOCYCLIC DISTAMYCIN ANALOGUES

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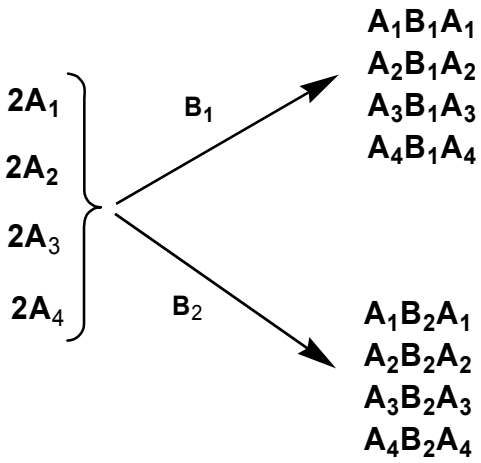
The natural antibiotics, netropsin and distamycin, be characterize by high selectivity of bond of regions DNA rich in the steam A - T, as well as the activity both antineoplastic, as and antiviral [1]. The model of binding netropsin and distamycin with B-DNA became the inspiration to searches of compounds with similar DNA-compound interaction.

In our investigations we concentrated on variously the put segments of benzene, uniting it inter the se and from some heterocyclic segments. We chose 4 different aromatic amines $\mathbf{A_1}$-$\mathbf{A_4}$ (2-aminothiazole, 2-amino-2-nitropyridine, 2-aminopyridine and 3-nitroaniline), which were acylated using terephtaloyl chloride ($\mathbf{B_1}$) and isophtaloyl chloride ($\mathbf{B_2}$). This procedure led to obtainment the trimmers **ABA**, analogues of distamycin. All of them were investigated and showed antiproliferative and cytotoxic effects in the standard cell line of mammalian tumour MCF-7.




Scheme 1. Parallel synthesis of analogues of distamycin.

This work was founded by grant KBN No. 2P05F 017 27.

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# DISCOVERY OF NOVEL ORALLY ACTIVE, NON-COVALENT DPP-IV INHIBITORS

Meritxell López-Canet, Sonja Nordhoff, Silvia Cerezo-Gálvez, Achim Feurer, Oliver Hill, Barbara Hoffmann, Victor Giulio Matassa, Christian Rummey, Meinolf Thiemann, Holger Deppe and Paul John Edwards[a]

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Inhibition of the serine protease dipeptidyl peptidase-IV (DPP-IV) leads to increased levels of the incretin hormones glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP) which play a central role in insulin release and glucose homeostasis. The use of DPP-IV inhibitors as potential therapeutic agents for the treatment of type 2 diabetes has been receiving increasing attention in recent times. Many of the early DPP-IV inhibitors have been designed as irreversible or reversible covalent substrate analogues, amongst them Novartis' phase III clinical compound Vildagliptin (LAF237). The received opinion for identification of potent DPP-IV inhibitors for a long time included the requirement of a serine trap within the inhibitor structure. However, more recently progress has been reported in this field culminating in the discovery of non-covalent DPP-IV inhibitors, such as Merck's phase III compound MK-0431 being the most advanced of this cohort.

Herein, we present two novel series of non-covalent DPP-IV inhibitors. After screening of Santhera's fragment collection, two β-phenylethylamine fragments were uncovered as weak DPP-IV inhibitors (DPP-IV $IC_{50}$ 37 and 40 μM). By the x-ray structure of one of these inhibitors complexed with porcine enzyme, we discovered an unexpected non-substrate like reverse-binding mode for this DPP-IV inhibitor. This discovery laid the foundation for the design of several novel DPP-IV inhibitor series, two of which are reported here. The medicinal chemistry optimization process ultimately led to potent non-covalent small molecule DPP-IV inhibitors ($IC_{50}$ sub-10 nM), that are highly selective over related DPP-like enzymes, and exhibit oral bioavailability. Inhibition data for DPP-IV, selectivity data, efficacy data in ob/ob mice and an x-ray structure showing the non-substrate like reverse-binding mode are given here.

# XANTHONE DERIVATIVES: NEW INSIGHTS IN ANTITUMOUR ACTIVITY AND RELATED ACTIVITIES

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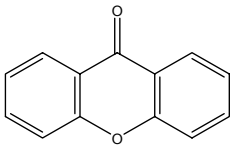
The xanthone is an important tricyclic framework in many compounds documented as antitumor agents.



Xanthone

Naturally and synthetic xanthones have been reported by our group as having antiproliferative effect on human tumour cell lines and lymphocytes [1,2] and modulatory activity on protein kinase C (PKC) [3-5]. In this communication are presented the recent improvements on these activities, based on molecular modifications, chiral resolution and drug delivery systems, as well as the scavenger effect on peroxyl radical and the effect on NO and ROS production. The most promising xanthone derivatives for the following activities will be displayed: (i) inhibition of the *in vitro* growth of human cancer cell lines, MCF-7 (breast), NCI- H460 (non-small cell lung), SF-268 (central nervous system) and UACC-62 (melanoma);(ii) inhibition of NO and ROS production; (iii) antiproliferative effect of human T-lymphocytes; (iv) *in vivo* modulatory activity on isoforms -α, -βI, -δ, -η and -ζ of PKC.

The improvement of biological performance of some xanthones by incorporation on new formulations (nanospheres and nanocapsules) is also presented. Additionally, the tumor cell growth inhibitory effect of some enantiomeric xanthonolignoids is demonstrated to be enantioselective.

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# EFFECTS OF TWO ABIETANES ON MCF-7 BREAST CANCER CELL LINE

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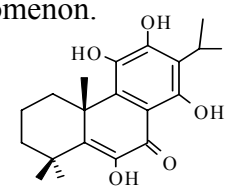
A study on the effect of coleon U (**1**) and 7α-acetoxy-6β-hydroxyroyleanone (**2**), isolated from *Plectranthus grandidentatus*, on the metabolic activity of MCF-7 cells is now presented. We have evaluated if their tumour growth inhibitory effect was related with apoptosis. **1** and **2** had shown a potent growth inhibitory effect on different human tumour cell lines, including the breast cancer cell line MCF-7 [1].

The metabolic activity of MCF-7 cells was evaluated by MTT assay after exposition to a range of concentrations (0.02 to 50 μM) for different periods of time (12, 24 and 48 h). MCF-7 cells viability was measured by trypan blue exclusion assay. Fragmentation of the genomic DNA was evaluated by the *in situ* Cell Death Detection Kit Fluorescein-TUNEL assay (Boehringer Mannheim, Germany). Cell morphological analysis was accessed by fluorescent microscopy after DAPI staining.

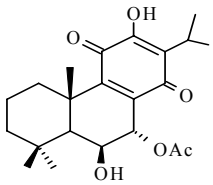
The metabolic activity of MCF-7 cells was analysed based on their capacity to reduce MTT. At concentration below 3 μM compounds **1** and **2** did not affect significantly cellular metabolic activity (≥ 80 %) even after 48 h exposition. Only concentrations above 3 μM and expositions of 48 h caused an abrupt loss of activity (<50 %). Treatments of 12 and 24 h affected differently the metabolic activity of MCF-7 cells. While compound **2** did not affect significantly this activity**,** compound **1** increased the capacity of cells to reduce MTT**.** Further studies are needed to elucidate the cause of this unexpected increase MTT reduction capacity of cells.

MCF-7 cells were exposed to **1** or **2** (6.4 μM and 5.5 μM, respectively) for 48 h and evaluated for apoptosis. Treatment with coleon U (**1**) was associated with an increase of cells with abnormal nuclear condensation when compared with untreated control cells, but they still presented high values of viability (> 80%). This nuclear alteration was associated with DNA fragmentation, characteristic of apoptotic cells, when stained with TUNEL assay. The number of apoptotic cells reached 24.8 % after coleon U (**1**) treatment *versus* 4% on control cells. Treatment with compound **2** did not show an increase of apoptotic cells. These results suggest that coleon U exerts their growth inhibitory effect against MCF-7 cell line through the involvement of apoptosis while no relation could be established between compound **2** and this phenomenon.

**Acknowledgements**. FCT (Madalena Pedro grant SFRH/BD/1456/2000 and Cristina G-Marques grant Praxis XXI/BD/18046/98; I&D 226/94 and 8/94), POCTI (QCAIII) and FEDER for financial support. NCI, USA, for providing MCF-7 cells.




**1**




**2**

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# PRENYLATED XANTHONIC DERIVATIVES: SYNTHESIS, STRUCTURE ELUCIDATION AND INHIBITION OF GROWTH OF HUMAN TUMOUR CELL LINES

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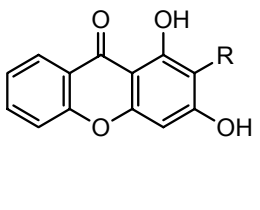
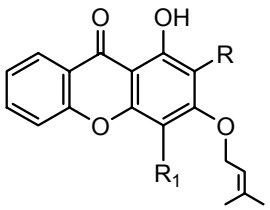
Recently we have investigated the effect of several hydroxy and methoxyxanthones on the *in vitro* growth of human tumour cell lines [1]. In order to improve the antitumor activity we have synthesized new prenylated derivatives, so in this work we describe the synthesis, structure elucidation and biological activity of nine xanthones **1-9**. [2-4]. Xanthones **1** and **2** were used as building blocks for prenylation, being compounds **3** and **4** obtained from **1** and **5**, **6, 7** and **8** from compound **2**. The cyclic derivative **9** was obtained from the prenylated xanthone **4** [4].

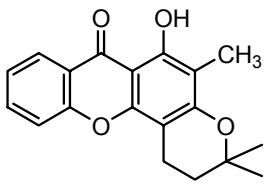
Structures were established by IR, UV, MS and NMR ($^{1}H$, $^{13}C$, HSQC and HMBC). The effects of the compounds on the *in vitro* growth of four human tumour cell lines: MCF-7 (breast), NCI- H460 (non-small cell lung), SF-268 (central nervous system) and UACC-62 (melanoma) were evaluated. Compounds **6, 7** and **9** were found to be selective and highly potent against the MCF-7 cell line.




**1** R=$CH_3$
**2** R=H




**3** R=$CH_3$, $R_1$=3,3-dimethylallyl
**4** R=$CH_3$, $R_1$=H
**5** R=H, $R_1$=H
**6** R=H, $R_1$=1,1-dimethylallyl
**7** R=H, $R_1$=3,3-dimethylallyl
**8** R=3,3-dimethylallyl, $R_1$=H




**9**

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FCT (I&D 226/94), FEDER, POCTI and for the PhD grant to Raquel Castanheiro (SFRH/BD/13167/2003) and Madalena Pedro (SFRH/BD/1456/2000).

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# IN SILICO INSIGHTS INTO DNA-BINDING OF RUTHENIUM(II)-ARENE ANTICANCER COMPOUNDS

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The discovery of cisplatin as an anticancer drug led to considerable interest in metallopharmaceuticals. Problems remain associated with their use, including general toxicity, drug resistance and low selectivity. Recently, organometallic ruthenium(II) arene complexes showed there potential to overcome this drawback. Rational design requires a detailed understanding of structure-property relationships at an atomistic level.

We performed classical MD and mixed QM/MM Car-Parrinello MD simulations[1] to rationalize the binding mode of two series of anti-cancer ruthenium(II) arene complexes to double-stranded DNA (dsDNA). The bifunctional RAPTA [Ru($\eta^6$-arene)$X_2$(PTA)] (**1**)[2] (PTA=1,3,5-triaza-7-phosphatricyclo [3.3.1.1] decane) and the monofunctional [Ru($\eta^6$-p-cymene)Xen] (**2**)[3-4] series of compounds were both bound to the dsDNA sequence d(CCTCTG*G*TCTCC)/d(GGAGACCAGAGG), where G* is guanosine that binds to the ruthenium compounds through its N7 atom. As reference, the same sequence was also simulated without any drug and in its canonical, unperturbed B-DNA form.

The local and global structural modifications of dsDNA upon complexation were analysed in detail. In particular the induced "bending angle" of dsDNA, which is thought to be a trigger for apoptosis, is investigated in depth.
The differences of the DNA-interaction-properties between the two series of compounds as well as with respect to the canonical B-DNA are discussed and linked to experimental observations. In particular, an atomistic description of a Watson-Crick base-pair break upon binding of **2** to dsDNA is proposed, that has been recently suggested on the bases of experimental results.[5]

Fundamental differences between binding of **1** or **2** to single stranded DNA (ssDNA) or dsDNA are rationalized.[6]

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# DEVELOPMENT OF A QSAR MODEL AND SYNTHESIS OF A CNS-FOCUSED LIBRARY

Rafael Gozalbes[a], Boryeu Mao[b], Frédérique Barbosa[a], Eric Nicolaï[a], Dragos Horvath[a,c] and N. Froloff[a]

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We have designed a diverse CNS-focused library based on the structure/CNS-oriented activity relationships of marketed drugs, clinical candidates and reference compounds and derived on hand of the data from our BioPrint® database (an overall of 2500 compounds profiled in house in more than 170 in vitro assays: receptors, enzymes, ion channels, cellular functional tests and in vitro ADMET assays) [1, 2].

A total of 978 compounds from BioPrint were classified such as "CNS-active" (193 compounds) or "CNS-inactive" (785 compounds), and a Linear Discriminant Analysis (LDA) was performed on a randomly chosen training set of 665 compounds in order to generate a CNS-QSAR model. LDA is a pattern recognition method providing a classification model based on the combination of variables that best predicts the category or group to which a given compound belongs. Independent variables in this study were Cerep 3-D pharmacophoric descriptors (Fuzzy Bipolar Pharmacophoric Autocorrelograms). Compounds with positive and negative LDA values correspond to "predicted CNS-active" and "predicted CNS-inactive" respectively.

The resulting QSAR model was applied to the test set of 313 structures, and it was able to correctly classify 80% of both CNS-active and inactive compounds. Furthermore, when the model was applied to an external set of 545 structures from the Merck Index, the ratio of CNS-active compounds witnessed a significant enrichment among the predicted actives, since 79.8 % of them (170 from 213 predicted actives) were correctly classified.

We have used this model to select and synthesize a library of CNS-focused compounds. In order to further guarantee the ability of these compounds to pass the blood-brain barrier, several important physicochemical parameters have been predicted as well using our predictive QSAR models derived from BioPrint® data, such as LogD at pH=7.4 and Caco-2 apical-to-basolateral permeability. Empirical filters (Lipinski rule-of-5, PSA) were also applied. Finally a set of more than 2000 compounds with predicted LDA > 0 and optimal physicochemical parameters were selected for synthesis. Amongst the final set of compounds, several original chemotypes have been identified.

[1] Krejsa, C.M. *et al*: "Predicting ADME properties and side effects: the BioPrint approach". *Curr. Opin. Drug Discov. Devel.* **2003**, *6 (4)*, 470-480.
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# SYNTHESES AND BINDING STUDIES OF NEW 4-(*N*-BENZYLAMINO)PIPERIDINE DERIVATIVES AND RELATED COMPOUNDS AS POTENTIAL ANTIDEPRESSANT DRUGS WITH DUAL AFFINITY FOR SEROTONIN AND NOREPINEPHRINE TRANSPORTERS

Aurelio Orjales, Ramón Mosquera, Luisa Alonso-Cires, Inés Tapia, Pedro Luis López-Tudanca, Luis Labeaga and Ana Innerárity

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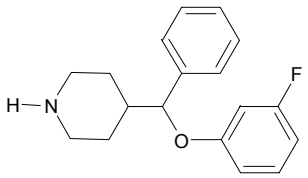
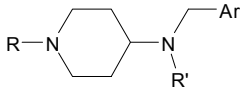
As a result of our research program to obtain new, efficient and fast-acting antidepressant drugs [1], F-98214-TA was found to display a dual binding profile with very high affinity values for serotonin transporter (SERT) and norepinephrine transporter (NET) ($K_i$ = 1.9 nM and $K_i$ = 13.5 nM respectively).




F-98214-TA

We have prepared series of new compounds having a 4-(*N*-benzylamino)piperidine moiety according to a chemical program based on the previously reported F-98214-TA as lead compound. They have been evaluated for SERT, NET, dopamine transporter (DAT) and $\alpha_2$, 5-$HT_{1A}$ and 5-$HT_{2A}$ receptors.
We have synthesized new arylmethylaminopiperidines which display high affinities for SERT and NET.




A critical, remarkable and common structural feature of these compounds is the necessary requirement of a secondary amine (R=H) for binding to SERT. Whenever the nitrogen atom of the piperidine ring is substituted the compounds lack affinity for this transporter.

Acknowledgement: this work was funded in part by the Ministry of Science and Technology of Spain (PROFIT 2000-2003) and the Department of Industry, Commerce and Tourism of the Basque Government (INTEK 2002).

[1] Orjales, A.; Mosquera, R.; Toledo, A.; Pumar, M.C.; García, N.; Cortizo, L.; Labeaga, L.; Innerárity, A.; Syntheses and Binding Studies of New [(Aryl)(aryloxy)methyl]piperidine Derivatives and Related Compounds as Potential Antidepressant Drugs with High Affinity for Serotonin (5-HT) and Norepinephrine (NE) Transporters. *J. Med. Chem.* **2003**, 46, 5512-5532.

# NOVEL 4-AMINOPYRIMIDINE DERIVATIVES AS SELECTIVE COX-2 INHIBITORS

Aurelio Orjales, Ramón Mosquera, Luis Labeaga, M. Teresa Núñez, Roberto Olivera and Beatriz López

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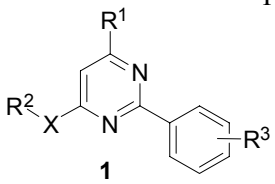
After some trisubstituted pyrimidines were described as selective COX-2 inhibitors [1], we have synthesized a series of pyrimidines **1**, which have shown also high selectivity as COX-2 inhibitors. In an effort to evaluate the influence of substituents on **1** regarding COX-2 inhibitory activity and selectivity, a new series of pyrimidines with different substituents on $R^3$ and X were prepared.




**1**

X = O, S, SO, $SO_2$, NH, NMe, NEt

$R^3$ = 4-SMe, 4-SOMe, 4-$SO_2$Me, 3-$SO_2$Me, 4-$SO_2NH_2$, 4-$NO_2$, 4-$NH_2$, 4-$NHSO_2$Me, 4-$OSO_2$Me

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

Analysis of the *in vitro* COX-1 and COX-2 inhibitory activity data permitted to draw the following conclusions:

a) Potency widely varies with the chemical nature of X. When X = S, NH, NMe products with good to medium potency are obtained. On the other hand, linking $R^2$ through an oxy or aminoethyl bridge (X = O or NEt) led to compounds with no significant COX-2 inhibitory activity.
b) Introduction of a SO or $SO_2$ linking bridge dramatically decreases COX-2 inhibitory activity.
c) The presence of $SO_2CH_3$ or $SO_2NH_2$ at $R^3$ is essential for significant COX-2 inhibitory activity.
d) Location of the polar group $SO_2$Me at *meta* position of the 2-aryl moiety moderately affects activity. To the best of our knowledge this finding has no precedent in the literature.
e) When assayed *in vitro*, sulfamoyl substituted derivatives exhibited a substantial decrease in activity against human blood COX-2 compared to results of the assay against the commercial purified enzyme.

From this study, 4-aminopyrimidine **1e** ($R^1 = CF_3$, X = NH, $R^3$ = 4-$SO_2CH_3$, $R^2$ = Bn) was identified as a more potent and selective COX-2 inhibitor (COX-2 $IC_{50}$ = 71.0 nM and selectivity index COX-1/COX-2 = 1,408) than rofecoxib. Further pharmacological evaluation and preliminary results of cytotoxicity will be presented somewhere else.

Acknowledgement: this work was funded in part by the Ministry of Science and Technology of Spain (PROFIT 2000-2003) and the Department of Industry, Commerce and Tourism of the Basque Government (INTEK 2002). B. L. was granted by the Ministry of Science and Technology of Spain and the European Social Fund (Programa Torres Quevedo).

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# SEARCH FOR SELECTIVE ANTAGONISTS AT $A_1$-ADRENORECEPTOR SUBTYPES: WB-4101 RELATED COMPOUNDS

Cristiano Bolchi,[a] Roberta Budriesi,[b] Alberto Chiarini,[b] Laura Fumagalli,[a] Marco Gobbi,[c] Pierfranco Ioan,[b] Barbara Moroni,[a] Marco Pallavicini,[a] Chiara Rusconi,[a] Ermanno Valoti[a]

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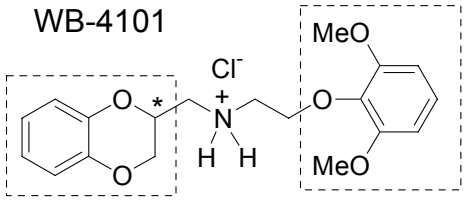
The development of subtype selective $\alpha_1$ ligands is intensively pursued in order to obtain more effective and safer agents for the treatment of cardiovascular pathologies such as hypertension and arrhythmia, but also and particularly of benign prostatic hyperplasia (BPH) and lower urinary tract symptoms (LUTS). One of the oldest and most potent $\alpha_1$ antagonists is represented by WB-4101, a 2-aminomethyl-1,4-benzodioxane derivative which is slightly selective for $\alpha_{1A}$ and, to a minor extent, for $\alpha_{1D}$-ARs with respect to $\alpha_{1B}$-AR and 5-$HT_{1A}$ serotoninergic receptor. Many structural modifications of WB-4101 have been done to improve both affinity and selectivity [1-4]. Some evidences, resulting from mutagenesis and docking studies, suggest that the benzodioxane moiety and the 2,6-dimethoxyphenoxy residue of WB-4101 are, respectively, involved in conferring $\alpha_{1a}$ selectivity and high $\alpha_1$ affinity. Consistently with these findings, our recent researches have demonstrated that removal of one or both *ortho*-methoxy substituents adversely affects the affinity for the three $\alpha_1$-AR subtypes, but not that for the 5-$HT_{1A}$ receptor [3]. On the basis of these indications, we synthesized a number of *S* and *R* analogues of WB-4101, characterized by different substitutions at the benzodioxane and/or phenoxy fragment, in order to modulate and, hopefully, to improve the activity and selectivity profile of the parent compound. In particular, we considered derivatives with benzodioxane 8-substituted with F [4], Cl, OH or OMe [4] or fused with a cyclohexane to give a tetrahydronaphthodioxane polycycle [2]. On the other hand, 2,6-dimethoxyphenyl residue was replaced by *ortho* methoxy substituted 1-naphthyl [2] or biphenyl systems. Finally, hybrid structures were designed combining some of the above modifications. After binding assays, which demonstrated the better $\alpha_{1a}$, $\alpha_{1b}$, $\alpha_{1d}$ and 5-$HT_{1A}$ affinity of the *S* enantiomers, these latter were tested in functional assays on isolated tissues, finding that almost all were able to discriminate among the $\alpha_1$-AR subtypes.

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# DEVELOPMENT OF INHIBITORS OF THE MOLECULAR CHAPERONE HSP90

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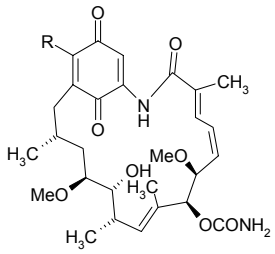
In the 90th of the last century it could be shown, that heat-shock proteins or chaperones can protect proteins from unfolding and aggregation caused by cell stress. HSP90 is an ATP-dependent chaperone protein essential for the maturation and activity of a diverse group of proteins involved in signal transduction, cell cycle regulation and apoptosis. It was observed, that blocking of HSP90 can interrupt regulatory mechanism of the cell. Tumour cells suffer from cell stress, caused by immune system or anti tumour therapy, so heat-shock proteins like HSP90 show an increased activity. Therefore antagonists of HSP90 seem to be good anticancer candidates. The ATPase activity can be inhibited with some selectivity by various antibiotics such as geldanamycin **1a** and its derivatives (see figure 1). Recent studies with geldanamycin derivatives suggest that cancer cells are particularly sensitive to HSP90 inhibition, because HSP90 has different conformations in healthy and tumour cells [1].




R
1a -$OCH_3$
1b -$NHCH_2CH{=}CH_2$

**Figure 1: Structures of geldanamycin 1a and 17-AAG 1b**

As naturally derived structures the benzoquinone ansamycins like geldanamycin are difficult to synthesise. Therefore it seems to be useful to develop small, easy to synthesise molecules as HSP90-binders.

X-ray investigations show that HSP90 inhibitors bind to the ATP-binding site at the N-terminus of HSP90. A seven-stranded beta sheet forms the backbone of the protein and four alpha helices are arranged such they form a compact cavity in which resides the ATP binding site (see figure 2) [2].

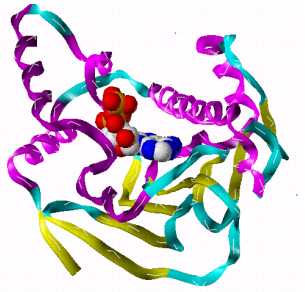
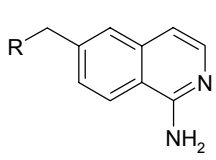


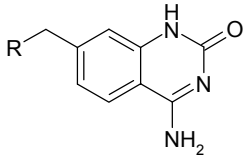
**Figure 2: Structure of the ADP/ATP- binding site of HSP90 with an ADP bounded inside**

Starting from the published structures of both unliganded protein and HSP90-complexes with a variety of inhibitors, we used Molecular Modelling methods to design isoquinolin-1-ylamin **2** and 4-amino-1H-quinazolin-2-one derivatives **3** as potential new HSP90 binders (see figure 3).

As the next step, we will synthesise the predicted structures by a modified Heck reaction. The binding affinity of the structures to HSP90 will be tested in a malachite green ATPase assay. Promising candidates will be tested for their antitumour activity by the NCI in an in vitro human tumour cell line test [3].




2




3

**Figure 3: Structures of the possible new HSP90 binders**

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# A HYBRID SOM/FUZZY ARTMAP METHODOLOGY FOR THE PREDICTION OF ECOTOXICITY IN PHENOLS USING MOLECULAR DESCRIPTORS AND MODES OF TOXIC ACTION

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Toxicity modelling is a challenging task, mainly due to the variability in experimental endpoints and the scarcity of public data. A variety of QSAR models have been reported in the literature, some of them based on the selection of structural analogues, which produce QSAR models that are dependent on the assumption that compounds of the same "chemical class" behave in a similar toxicological manner. However, the correct identification of a chemical class is not an easy task. An alternative approach is to develop QSAR models based on the concept of modes of toxic action (MOA) instead of using chemical classes.

The aim of this study is to derive a descriptor-based classification of 220 phenols grouped into four MOAs (153 polar narcotics, 18 respiratory uncouplers, 27 pro-electrophiles and 23 soft electrophiles). The MOA data set of Shüürmann et al.(2003)[1] is used. The goodness of the SOM model is assessed by comparing the resulting classifier with other well-known classification models from the literature.

The methodology developed proceeds in a two steps procedure. First, in a preprocessing stage, redundant descriptors are removed and the best set of descriptors to predict the toxicity is found. Second, in the modelling stage, all the compounds are classified according to their MOA and a Fuzzy ARTMAP based QSAR model is developed for each class.

The good performance of the proposed integrated SOM-fuzzy ARTMAP approach is assessed by comparing predicted and measured toxicity log $1/IGC_{50}$ (mmol/L) data.

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# A CHEMOMETRIC APPROACH TO PREDICT $A_1$ AGONIST EFFECT OF ADENOSINE ANALOGUES

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Many selective agents have been developed until now for the $A_1$ receptor subtypes and some of these seem promising as potential therapeutic agents in the treatment of Parkinson's disease, cognitive deficits, schizophrenia and epilepsy [1], however there are currently no $A_1$ adenosine agonists in clinical development. In this context, as a prosecution of our researches in understanding the structural basis of ligand-$A_1$ receptor interactions [2], we have focused our interest in identifying the molecular properties responsible for affinity toward $A_1$ AR by means of quantitative structure-activity relationships (QSAR). As is well known, in these methods correlations are derived between experimentally determined binding affinities and a number of different descriptors, which should encode for the thermodynamics of binding of a set of ligands. The base assumption in fact is that a correlation exists between the enthalpy of binding of a congeneric series of molecules (with similar size and flexibility) and their molecular properties. A set of selective $A_1$ agonists was thus considered. For the prediction, the structural information encoded by a large number of molecular descriptors for topological, electronic, geometric and polar surface properties was taken into account together with atomic charges on those specific positions of the adenosine skeleton highlighted as important by previous structure-activity relationships studies. In addition, calculated receptor-ligand binding energies for the selected compounds were included among variables. The versatile chemometric package PARVUS [3] was subsequently applied to handle such information, discarding all non-informative descriptors and extracting meaningful QSAR models. The results obtained with many both linear and non-linear approaches converge in the selection of few parameters, which result highly informative for the prediction of the biological response. This “a priori” evaluation strategy could be a useful tool in the screening of large libraries of compounds and in the rational design of new selective adenosine agonists.

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# MODELLING THE INTERACTION OF STEROID RECEPTORS WITH ORGANIC POLYCHLORINATED COMPOUNDS

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The organic polychlorinated compounds like dichlorodiphenyltrichloroethane (DDT) with its metabolites (p,p'-DDT, o,p'-DDT, p,p'-DDE and p,p'-DDD), polychlorinated biphenyls (PCBs) and, more recently, polybrominated diphenyl ethers (PBDEs) are present in atmospheric particulate as persistent contaminants. They have been recognized to have detrimental health effects both on wildlife and humans acting as endocrine disrupters chemicals (EDC) due to their ability of mimicking the action of the steroid hormone and thus interfering with hormone response. They are responsible of a long list of very serious human and animal health problems, including cancers, infertility, osteoporosis, depression, cardiovascular diseases and deformities of the reproductive organs [1, 2]. There are several experimental evidences that they bind and activate human steroid receptors [3], however molecular data of the interaction of these compounds with biological targets are still lacking. In order to better understand the ability of various EDC to interact with the receptor hormone binding pocket, we have simulated by docking approach the molecular models of the complexes between some DDT and PCB derivatives and estrogen (hER$\alpha$), progesterone (hPR) and androgen (rAR) receptors. The results of our investigation allow to describe a wide pattern of interactions between EDC ligands and steroid receptors, suggesting that their action can be brought about by a possible combined effect.

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# COMPUTER AIDED PROPERTY BASED DRUG DESIGN

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Poor oral bioavailability, high clearance, low solubility and formulation difficulties often are responsible for the failure of active compounds in a modern drug design process. All these pitfalls are closely related to physicochemical properties. A key property is the most basic and most acidic pKa value, respectively.

The most reasonable approach to calculate pKa values is based on Hammet/Traft equations, which characterize the influence of substituents on the pKa value of a known scaffold. These equations are therefore an ideal starting point for property based drug design.

An example is given, how to modify an active scaffold, to receive a series of structures with more desirable physico-chemical properties, such as solubility or LogD. Substituents are chosen from a database and connected to a specified position. The results are analyzed graphically by PSA and Lipinski's "rule of five" properties, to select the most promising candidates for synthesis.

# 2-AMINOBENZENSULFONAMIDES DERIVATIVES AS ALLOSTERIC MODULATORS OF AMPA/KAINATE RECEPTORS

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Recent studies indicate that compounds that reduce the AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptor desensitization could improve impaired synaptic functions associated with learning and cognition pathology and can be useful in the treatment of attention disorders in children as well as in senile dementias, including early stages of Alzheimer disease. Among these compounds, (±)IDRA21 (7-chloro-3-methyl-3,4-dihydro-2H-1,2,4-benzothiadiazine 1,1-dioxide) has attracted particular interest for its ability to act as cognitive enhancing agent in normal young and aged rhesus monkeys when given orally in low (0,5-10 mg/Kg) doses. Despite the high potency in modulating AMPA receptor function in vivo of (±)IDRA21, its potency in vitro is low with an $EC_{50}$ close to 100 μM. Since (±)IDRA21 has been administered orally in the in vivo pharmacological tests, studies on the stability of the drug in acidic condition similar to that of stomach are clearly matter of interest. Hydrolysis of IDRA21 at different pH and temperature was studied by a chromatographic procedure. The results have been shown that IDRA21 undergoes rapid hydrolysis in acidic solution to 2-amino-5-chlorobenzensulfonamide and acetaldehyde.
Moreover microdialysis experiments have been demonstrated that in vivo IDRA21 hydrolyses to give 2-amino-5-chlorobenzensulfonamide which was present at high levels in the brain for at least six hours after oral administration of IDRA21 itself. Subsequently electrophysiological experiments have been demonstrated that 2-amino-5-chlorobenzensulfonamide posses in vitro a biological activity similar to that of IDRA21.
Taking 2-amino-5-chlorobenzenesulfonamide as lead compound, a series of 2-aminobenzensulfonamides with different substituent at benzene ring and amino groups has been prepared and studied for their activity as allosteric modulators of kainate-activated currents in primary cultures of cerebellar granule neurons.
Some terms were powerful potentiators of kainate-activated currents, with activity close to that of IDRA21. These compounds could be good candidates as new therapeutic agents for the treatment of cognitive deficits.

# DETERMINATION OF ENANTIOMERIZATION ENERGY BARRIERS BY ENANTIOSELECTIVE STOPPED-FLOW HPLC

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An on-column stopped-flow high-performance liquid chromatography (sfHPLC) procedure using a chiral stationary phase (CSP) has been developed for the determination of rate costants and free energy barriers of enantiomerization of 3,4-dihydro-1,2,4-benzothiadiazine 1,1-dioxide type compounds. After initial separation of the enantiomers in the first section of the column, the flow was stopped and the resolved species allowed to enantiomerize on-column. From this conversion, which could be determined from the enantiomeric ratios at different enantiomerization times, kinetic rate constants and free energy barriers of enantiomerization were calculated [1,2].

Several chiral 3,4-dihydro-1,2,4-benzothiadiazine 1,1-dioxide type compounds different substituted to the benzene ring and to C(3) chiral carbon atom were prepared and an enantioseparation HPLC method was developed in reversed phase mode employing cellulose tris (3,5-dimethylphenylcarbamate) as CSP (Chiralcel OD-R) [1]. It turned out that, during chromatography in aqueous solvents, a rapid enantiomer interconversion occurred resulting in pronounced peak coalescence phenomena.

The enantiomerization rate costants and the corresponding energy barriers were calculated by the sfHPLC procedure developed [2]. The results showed that substitution in C(3), C(6) and C(7) position of the benzothiadiazine ring exert a dramatic influence on free energy barriers of enatiomerization.

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# APPLICATION OF ROBUST REGRESSION IN QSAR MODELING

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Partial Least Squares Regression is routinely employed in QSAR/QSPR modeling. A number of studies focus on ruggedizing QSAR modeling in experimental design [1], in variable selection [2][3] and in model validation [4]. Partial Least Squares regression is frequently employed using the SIMPLS [5] and NIPALS [6] algorithms. However, these are sensitive to outliers in the dataset. The resulting regression may be skewed towards bad leverage points, whilst the regression accuracy still seems acceptable.
To circumvent the risk of biased results caused by outliers, robust statistical approaches have been developed. These provide estimates for statistical parameters (e.g. covariance matrix), that are not susceptible to the influence of outliers. Furthermore, outliers can clearly be identified.
Hundreds of molecular descriptors are available to map the biological/chemical interface. To discard redundant information contained in the descriptor-set and improve the model accuracy descriptor selection methods are used [2][3]. Descriptor selection techniques are especially important in PLS modeling since regression models increase their fitting capability on increasing the number of variables, even if these are random and are not related to the problem [7].
Here, we present QSAR/QSPR models for public available datasets, generated by classical and robust PLS regression [8] for a variety of variable selection approaches.
Our aim is to investigate how outlier detection methods and variable selection influence each other and to compare the robustness and predictive power of derived PLS models. From these results we will propose an approach to derive QSAR models, based on a cleaned, homogenized set of compounds, with better predictive performance and justified model validation.

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# TRIAZENE DERIVATIVES ENDOWED WITH ANTIPROLIFERATIVE AND ANTIVIRAL ACTIVITIES

Michele Tonelli[a], Caterina Canu[a], Bruno Tasso[a], Vito Boido[a], Fabio Sparatore[a], Bernardetta Busonera[b], Gabriella Collu[b], Giuseppina Sanna[b], Paolo La Colla[b], Roberta Loddo[b]

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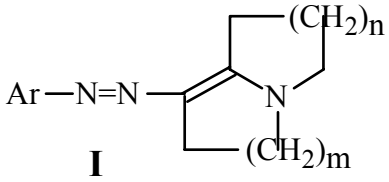
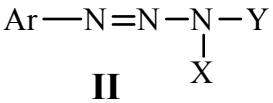
The importance of triazene derivatives as antitumor agents is well known [1], one example being represented by 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide (DTIC, dacarbazine). The triazene moiety may also be embedded in a cyclic structure, as in the acyltriazene prodrug temozolomide, which *in vivo* gives rise to 5-(3-methyl-1-triazeno)imidazole-4-carboxamide (MTIC). Many other substances have been developed, in which the heterocyclic part of dacarbazine has been replaced by variously substituted benzene rings.
As part of a vast research program aimed at the synthesis of new antiproliferative and/or antiviral compounds, we have recently described [2] a series of aryl/heteroarylazoenamines corresponding to the general structure **I**:




**I**

Ar = aryl, heteroary

These compounds show significant activity on a variety of $RNA^+$ and $RNA^-$ viruses at non cytotoxic concentrations.
Since arylazoenamines may be considered as vinylogues of aryltriazenes, we deemed interesting to broaden our studies to include a selected set of substances represented by the general formula **II**:




**II**

Ar = aryl, heteroaryl
X = H, alkyl,dialkylaminoalky
Y = aryl, heteroaryl, alkyl

Some of the compounds tested so far were cytotoxic for MT-4 cells, while others showed a selective, although not very potent activity against viruses representative of the *Flaviviridae* and *Paramyxoviridae* families.

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# CoMFA 3D-QSAR STUDIES ON BENZAZOLE DERIVATIVES AS EUKARYOTIC TOPOISOMERASE II INHIBITORS

Ilkay Yildiz, Ozlem Temiz-Arpaci, Betul Tekiner-Gulbas, Tugba Ertan, Esin Aki-Sener, Ismail Yalcin

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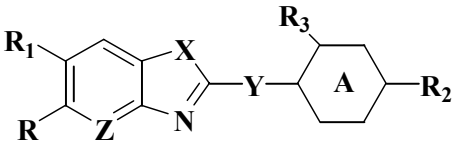
Since the activity of topoisomerases is essential for several cellular processes such as replication, transcription, and chromosome condensation, investigation of the inhibitory activities of eukaryotic topoisomerases is widely used in anticancer drug development. Topo II is the target for some of the most active anticancer drugs such as etoposide, teniposide, and doxorubicin used in the treatment of human malignancies [1-3]. Therefore selective Topoisomerase II inhibitors have attracted much attention in recent times in the design of new antitumor compounds.

Many pharmacological studies have resolved receptor active/binding sites using numerous computational 3D-quantitative structure-activity relationship (3D-QSAR) techniques [4].

In this study, 3D-QSAR studies have been performed on a series of benzazoles (**Figure**) that act as eukaryotic topoisomerase II inhibitors [5], using C*o*mparative Molecular Field Analysis (CoMFA) [6] with partial least squares (PLS) fit. The analysis was carried out on 23 analogues of which 16 were used in the trainig set and the rest considered for the test set. These studies produced reasonably good predictive models with high cross-validated and conventional $r^2$ values in all the three cases.




**X**|= O, NH, S; **Y** = –, $CH_2$, $CH_2O$, $CH_2S$, $C_2H_4$;
**Z** = CH, N; **A**= Phenyl, Cyclohexyl, Cyclopenthyl;
**R** = H, $NH_2$, $NO_2$, $CH_3$, Cl, $COOCH_3$; $\mathbf{R_1}$ = H, $NO_2$;
$\mathbf{R_2}$ = H, Cl, $CH_3$, $C_2H_5$, $NH_2$, $NHCH_3$, $OCH_3$, $OC_2H_5$;
$\mathbf{R_3}$ = H, $CH_3$, $OCH_3$

**Figure**

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# SYNTHESIS AND ANTIFUNGAL ACTIVITY OF SOME NEW 5-ETHYLSULPHONYL-2-SUBSTITUTEDBENZOXAZOLES

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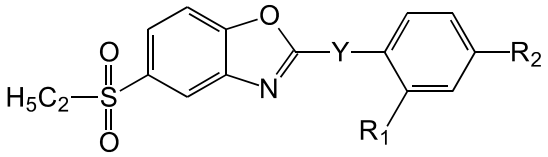
The prevalence of systemic fungal infections has increased significantly during the past decade. This increase is due to greater use of broad-spectrum antibiotics, immunosuppressive agents. Therefore, the theraphy with antifungal agents and the research of the new effective compounds are getting important [1-3].

Biologically active benzoxazoles have been known since long time and they were seen that position 2 is decisive for the biological activity, whereas position 5 determines the intensity of their activity [4-7]. So that we synthesized some novel 5-ethylsulphonyl-2-(substitutedphenyl-substitutedbenzyl-benzoxazole derivatives (Formula). The *in vitro* antifungal activity of the compounds was determined against *C. albicans* and *C. krusei* in comparison with standard drugs. Antifungal results indicated that the synthesized compounds possessed a broad spectrum of activity with a MIC values 50-12.5 µg/ml against the tested yeasts. Furthermore antifungal activity of the compounds not being more potent than the compared control drug miconazole.




$R_1$= H, Cl, $R_2$= H, Cl,Br, $CH_3$, $C_2H_5$, $C(CH_3)_3$, $NO_2$ Y= $CH_2$, -

**Formula**

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# SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF SOME NEW THIAZOLYL THIAZOLIDINEDIONE DERIVATIVES

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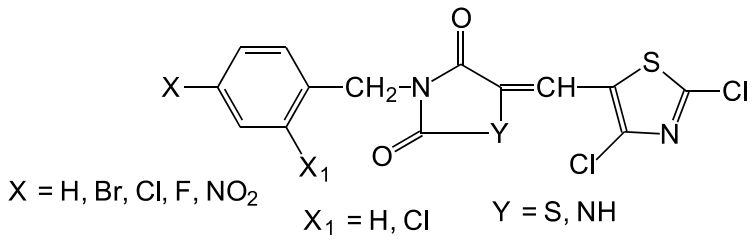
The presence of a thiazolidine ring in penicillins and related derivatives was first recognition of its occurence in nature [1]. Thiazolidine derivatives are reported to show variety of biological activities. Depending on the substituents, this heterocycle can induce different pharmacological properties such as antibacterial, antifungal [2], antidiabetic [3], cardiotonic [4], anticonvulsant [5], cyclooxygenase and lipoxygenase inhibitory [6]. Thiazoles have been reported to possess antibacterial [7], antifungal [8] activities, too. It has been established that introduction of arylidene moieties at different positions of the thiazolidine ring enhanced antimicrobial activity [2, 9]. In view of the antimicrobial property of the above pharmacophores, some novel thiazolyl thiazolidinedione derivatives have been synthesized as seen in below Formula.

Chemical structure of the compounds have been elucidated by their IR, $^1$H NMR, Mass and elementary analysis data. The synthesized compounds were tested for their antifungal and antibacterial activities in vitro. All the compounds was found active against used microorganisms.




Formula

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# SYNTHESIS AND ANTIDIABETIC ACTIVITY OF SOME NEW 5-(4-OXO-4H-CHROMEN-3-YL-METHYLENE)-THIAZOLIDINE-2,4-DIONES

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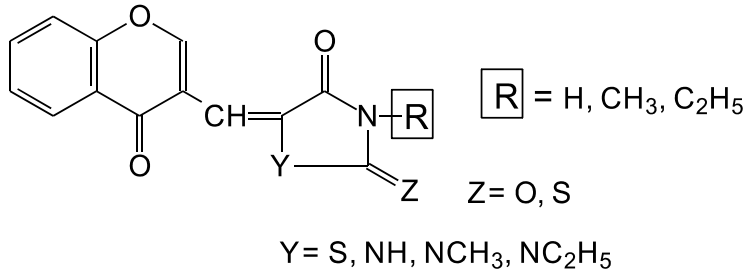
Type 2 diabetes is one of the most common metabolic diseases still lacking fully effective therapy and characterized by abnormalities of insulin secretion and by insulin resistance of major target tissues [1, 2]. 2,4-Thiazolidinediones (2,4-TZDs) are a new class of antidiabetic agents that improve peripheral insulin resistance in type 2 diabetic patients [3, 4].

The chromone moiety forms the important component of pharmacophores of a number of biologically active molecules of synthetic as well as natural origin and many of them have useful medicinal applications [5]. In this study, we describe further modifications of the 2,4-TZD derivatives containing chromone ring (Formula). The structural evaluation of the compounds were based on the various spectral data. The synthesized compounds are under investigation for their insulinotropic activities in INS-1 cells.




Formula

**References**

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# IRON SALEN COMPLEXES AS NOVEL TUMOR THERAPEUTICS: CYTOTOXICITY, DNA CLEAVAGE AND ACCUMULATION STUDIES

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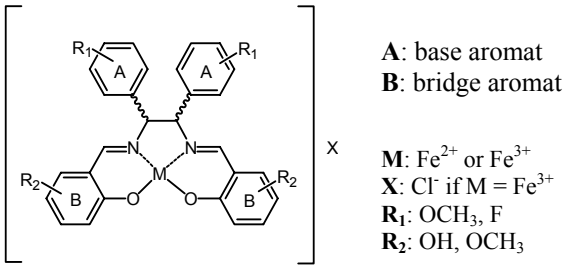
We synthesized [N,N´-bis(salicylidene)-1,2-diarylethylenediamine]iron(II) and -iron(III) complexes (Fig. 1) in order to develop these compounds as antitumor agents. Similar to the glycopeptide antibiotic Bleomycin, under physiological conditions these iron compounds are expected to catalyse the generation of reactive oxygen species, which subsequently oxidize DNA and RNA, leading to its cleavage up to total degradation.
The introduction of a hydroxyl group into the two salicylidene moieties creates a hydroquinone substructure which can interact with the iron redox system and in this way spontaneously generate free radicals needed for the oxidatitive DNA cleavage.
DNA cleavage properties of the hydroxylated diarylsalen complexes were confirmed *in vitro* using plasmid DNA. In this assay, chinonic salen complexes with hydroxyl groups in position 3 resp. 5 showed higher DNA degradation activity than complexes with the hydroxyl group in position 4. Iron(III)-complexes without hydroxyl groups at the base aromats (B in Fig. 1) only cleaved DNA in presence of a reducing agent.
To determine the antiproliferative activity, we measured the growth inhibition on MCF-7 and MDA-MB-231 breast cancer cell lines. In the cytotoxicity test, iron salen complexes showed a 10-fold higher efficiency on MDA-MB cells compared to MCF-7 cells, despite the significantly lower intracellular uptake.
In both cases the accumulation rate and the influence on cell growth strongly depended on the ligand structure. Complexes with fluorine substituents were taken up to a higher extend than complexes with methoxy groups. And iron salen compounds with the ligand in *d,l* configuration exerted higher cytotoxic activity than their *meso* configured analogues despite their decreased cell uptake.




**A**: base aromat
**B**: bridge aromat

**M**: $Fe^{2+}$ or $Fe^{3+}$
**X**: $Cl^-$ if M = $Fe^{3+}$
**$R_1$**: $OCH_3$, F
**$R_2$**: OH, $OCH_3$

**Figure 1:** Iron diarylsalen complexes

# MODULATION OF THE CONFORMATIONAL BEHEVIOR OF A β(25-35) BY INTERACTION WITH TWO POTENT β-SHEET BREAKER PEPTIDES

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The major components of neuritic plaques found in Alzheimer disease (AD) are peptides known as amyloid β-peptides (A-β-peptides). The A-β-(1-42) is the most prone to aggregation and is produced in larger quantities.
A-β-(25-35), sequence GSNKGAIIGLM, is a synthetic derivative of amyloid β-peptide, that is highly toxic and forms fibrillar aggregates typical of β-amyloid. Like the A-β-(1-42), A-β-(25-35) undergoes a conformational transition from a soluble, alpha-helical form to aggregated fibrillary β-sheet structures which are neurotoxic (1). Since it retains both the physical and biological properties of A-β-peptides it can be used as a suitable model of full-length peptides, for testing inhibitors of aggregation and toxicity. The design of inhibitors of aggregation is one of the strategies to overcome the Alzheimer disease. Many oligopeptides have beta-breaking activity since they are able to disaggregate the beta fibrils. The pharmacological profile of β-sheet breaker peptides can be improved to produce compounds with drug-like properties that might offer a new promise in the treatment of Alzheimer's disease.
Recently a 5-amino acid β-sheet breaker peptide (iAβ5p), end-protected, was shown to have the ability to induce a dramatic reduction in amyloid deposition in two different transgenic Alzheimer's models (2). Furthermore one peptide analog of iAβ5p containing a methyl group introduced at the nitrogen atom of one amide bond showed increased farmacocinetic and farmacodynamic characteristics (3). The ability of iAβ5p and of its N-methyl- derived analog to affect the conformation of A-β-(25-35) was investigated by Circular Dichroism and NMR spectroscopies.
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# IMMOBILIZED ARTIFICIAL MEMBRANE (IAM) CHROMATOGRAPHIC RETENTION FACTORS OF STRUCTURALLY DIVERSE DRUGS AS A MEASURE OF LIPOPHILICITY.

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Immobilized Artificial Membrane Liquid Chromatography is gaining interest in Drug Design, since it combines the simulation of cell membranes partitioning with rapid measurements. While the solid phase surface in IAM chromatography simulates the phospholipid bilayer, the mobile phase models the aqueous environment surrounding the cells. In order to comply better with this physiological environment, phosphate-buffered saline (PBS) is usually used as the mobile phase. In the present study the IAM retention factors ($logk_{wIAM}$) of a large number of structurally diverse drugs were determined at pH 7.4 and 5.0. At pH 7.4 both PBS and morpholinepropane soulphonic acid (MOPS) were used as aqueous components in the mobile phase in the aim to investigate the effect of buffer in retention. MOPS is usually used for the assessment of lipophilicity by reversed phase HPLC and due to its zwitterionic character it is consider not to interfere with the solutes and the stationary phase. The use of MOPS significantly increased the retention of the protonated bases as a result of a stronger contribution of electrostatic interactions. IAM retention was compared to octanol-water partitioning. The effect of ionization on retention was found to be less pronounced than expected and $logk_{wIAM}$ at pH 7.4 were better correlated with logD than with logP. Measurements at pH 5.0 also did not produce significant changes in $logk_{wIAM}$ in comparison to measurements at pH 7.4. $logk_{wIAM}$, logP, logD, as well as reversed phase $logk_w$ values of the investigated drugs were further analysed by multivariate statistics. For this purpose various molecular descriptors expressing bulk, electronic properties, hydrogen bonding capability and flexibility were calculated using Hyperchem/Chemplus and MSI-Cirius software packages. Principal Component Analysis confirmed the better similarity of $logk_{wIAM}$ to logP than to logD. PLS models revealed a significantly lower contribution of hydrogen bond interaction in IAM retention especially at pH 5.0, in comparison to octanol-water partitioning.

# SELECTIVE AND POTENT 3-AMINO-2-PYRIDINONE ACETAMIDE THROMBIN INHIBITORS INCORPORATING HETEROBICYCLIC P1-ARGININE MIMETICS

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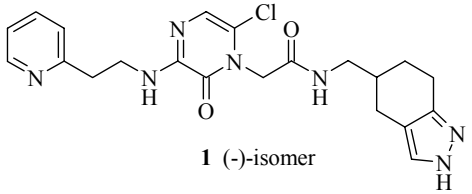
The major goal of pharmaceutical chemists dealing with development, synthesis and optimisation of new thrombin inhibitors is to convert *in vitro* active and selective inhibitors into *in vivo* orally bioavailable anticoagulants with the proper pharmaco-dynamical and pharmacokinetical profile which would enable one- or twice daily dosing and which would have advantages of safety and efficiency in comparison with classical and novel parenteral anticoagulants being used in therapy. The development of novel thrombin inhibitors containing weakly basic arginine side chain mimetics was coupled with the desire to overcome the limitations imposed by the amidine and guanidine moieties, whose high basicity reduces both bioavailability following peroral application, and selectivity for thrombin against trypsin. According to our structure activity relationship studies, we were interested in preparing a series of 2-(3-amino-6-methyl-2-oxo-2*H*-pyridin-1-yl)acetamide and 2-(3-amino-6-chloro-2-oxo-2*H*-pyrazin-1-yl)acet-amide template-based thrombin inhibitors incorporating novel, weakly basic, partially saturated, heterobicyclic arginine side chain mimetics at the P1 part of the inhibitor [1-7]. We prepared a series of highly selective and potent thrombin inhibitors represented by inhibitor **1** with the Ki value for thrombin of 40 nM and more that 2400-fold selectivity against trypsin and studied the influence of chirality on thrombin inhibitory potency [5-8].



**1** (-)-isomer

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# MOLECULAR DYNAMICS STUDY OF MUTANT AND WILD-TYPE HIV-1 PROTEASE TOWARDS RITONAVIR: INSIGHTS INTO THE MECHANISMS OF DRUG RESISTANCE

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Chemotherapies against HIV-1 are limited by mutations of the viral enzymes, which are targets for many commercial drugs. To understand the basis of drug resistance, particularly of the HIV-1 protease (PR), molecular dynamics (MD) simulations of the wild-type HIV-1 protease and three model mutant structures (V82F, I84V and V82F/I84V) complexed with ritonavir, a widely used drug, were carried out in explicit aqueous solution. The mutations V82F, I84V and V82F/I84V lower the binding affinity of ritonavir by a factor of 0.8, 11.2 and 700, respectively [1]. Analysis of two nanosecond MD trajectories of the simulated systems reveals the difference in ritonavir structure and flap region of double mutant complex. Simulations show significant differences of P1' subsite and side chain of Phe82 in double mutant complexes that reduce the VdW contact between HIV-1 PR and ritonavir. In flap region, we found that V82F/I84V mutant's flap open a few Ångstroms more than the wild-type's flap for chain B during the simulation time. The flap opening in V82F/I84V complex reduces the affinity of a water molecule (WAT211) which is important for maintaining the active conformation of HIV-1 PR, because this water molecule forms four hydrogen bonds with oxygen atoms of ritonavir and with flap region in both chains [2]. In addition, these mutations decrease the stability of hydrogen bonds between HIV-1 PR and ritonavir and alter the extent of VdW interactions. These MD studies support the experimental data and clearly explain the effect of substitution of Val82 by Phe and Ile84 by Val, leading to saquinavir resistance.

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# THEORETICAL INVESTIGATION ON STRUCUTRE AND FUNCTION OF ODORANT RECEPTORS

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Humans possess approximately 300 intact odorant receptors. They are integral membrane proteins belonging to family A of the large superfamily of G-protein-coupled receptors (GPCRs). With this repertoire, humans can distinguish between numerous chemical diverse odorants. To explain this enormous discriminating power, knowledge of the three dimensional structure and mapping of ligands to specific receptors are necessary. Due to experimental difficulties with membrane proteins up to date only the crystal structure of bovine rhodopsin [1] from family A has been solved. Therefore, homology-modeling techniques on the human odorant receptors, particularly hOR17-4 and hOR17-40 were applied using rhodopsin as template. These two receptors are excellent targets because hOR17-4 selectively binds canthoxal while hOR17-40 is solely activated by the structural related helional. The models were built using various modeling approaches and the ligand-binding site of each receptor was identified with specialized search programs. The location of these putative binding sites corresponds well to binding pockets found in other GPCRs. To analyze the nature of the active site, docking studies were performed with known ligands and structurally related molecules. Most of the key residues involved in odorant recognition are located in the space formed by the transmembrane helices 3, 5 and 6. These findings are consistent with studies performed on other odorant receptors. By comparing the docking results of helional, which exclusively activates hOR17-40 [2] and the structurally related canthoxal which only activates hOR17-4 [3] we were able to explicitly assign ligand specificity to structural features in the binding site. These results shed light on how the olfactory family has evolved the ability to recognize such a large variety of distinct chemical structures.

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# AZAPEPTIDOMIMETICS OF MYD88 CONSENSUS PEPTIDE : SYNTHESIS AND PRELIMINARY STUDY ON THEIR EFFECTS ON IL-1-INDUCED NF-ΚB ACTIVATION

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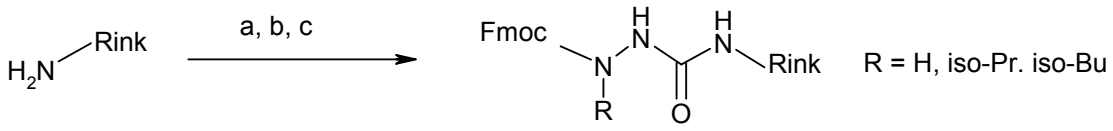
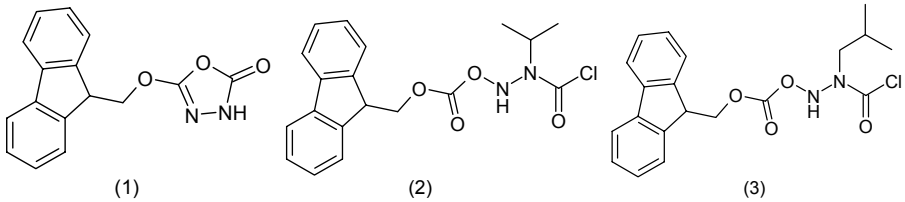
MyD88 plays a crucial role in the signalling pathways triggered by IL-1 and Toll-like receptors (TLR) in various phases of innate host defence. A crucial event in this signalling pathway is represented by dimerization of MyD88, which allows the activation of the downstream transcription factor NF-κB [1].

This work describes the synthesis of a number of azapeptidic compounds [2] structurally related to the MyD88 TIR domain consensus peptide RDVLPGT [3], and some evidence of their inhibitorial activity on IL-1 induced NF-κB activation.

The azapeptides were synthesized by Fmoc solid phase method on Rink-amide resin, using fosgene solution in toluene for azapeptide bond formation.




**a)** DMF, t.a., 30 min b) Piperidine 30% in DMF, t.a., 10 min c) (1) or (2) or (3), $CH_2Cl_2$, t.a., 90 min.




(1) (2) (3)

The aza-building blocks synthesis from tert-butyl-carbazate for the incorporation of aza-Gly, aza-Val and aza-Leu is also described.

The effect of these compounds on inhibition of IL-1-induced NF-κB activation was monitored by RGA (Reporter Gene Assay). Experimental data on some active azapeptides are also reported.

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# NEW CARBOXAMIDE DERIVATIVES OF THE BENZO[*b*]TIOPHENE 1,1-DIOXIDE AS POTENTIAL ANTINEOPLASTIC AGENTS

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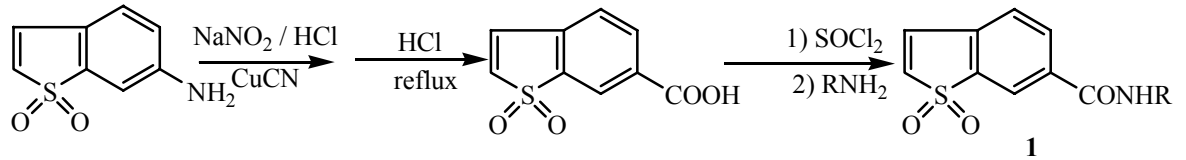
The benzo[*b*]thiophenesulphonamide (BTS) 1,1-dioxide derivatives have been recently reported as a new class of potential antineoplastic agents.[1] These compounds induce reactive oxygen species overproduction and apoptosis in tumour cells[2], and a correlation between their cytotoxic activities and ability to inhibit a tumour-associated NADH oxidase of the plasma membrane has been described.[3] In order to look for more selective antitumour compounds we extended our study to related *N*-substituted 6-benzo[*b*]tiophenecarboxamide 1,1-dioxides (**1**). These carboxamide derivatives were obtained from the 6-aminobenzo[*b*]thiophene 1,1-dioxide by known procedures: Nitrosation of the amino derivative, subsequent treatment with cuprous cyanide, and then acid hydrolysis of the corresponding nitrile to give the 6-benzo[*b*]tiophenecarboxylic acid 1,1-dioxide. This was treated with thionyl chloride and amines to yield the corresponding carboxamides **1** (35-75%).




**1**

R = **a** -H
**b** -$C_6H_4$-*p*-$OCH_3$
**c** -$C_2H_4C_6H_4$-*p*-$OCH_3$
**d** -$C_4H_9$

The cytotoxic activity of **1a-d** was tested against a panel of six human tumour cell lines representative of different types of solid tumours and leukaemias. All of these compounds showed a strong cytotoxic activity against the selected cell lines, with $GI_{50}$ values ranging from 0.002 to 7.85 μM. Compound **1b** was the most active among them the tested compounds, with $GI_{50}$ values of 0.002 (HTB-54), 0.047 (K-562), 0.76 (MEL-AC), 0.35 (HT-29), 0.004 (CCRF-CEM) and 0.003 (HeLa) μM. Selective assays are now in progress and they will be reported in due course.

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# ANALYSIS OF STABILITY OF COMPLEXES OF ISONICOTINIC ACID HYDRAZID DERIVATIVES WITH BETA-CYCLODEKSTRIN

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System containing isonicotinic acid N'-[4-(4-chlorobenzylidene)-5-oxoimidazolidin-2-yl]-hydrazid and beta-cyclodekstrin have been analyzed to find the possible mechanism of creation and stabilization of complexes. Experimental attempts failed to create stable systems. Theoretical analysis could be helpful in explaining factors originating instability of the complexes, and propose modifications in experimental procedures. Docking scheme was performed to find the most probable conformations of possible complexes and was followed by molecular dynamics simulations. To compare obtained results the whole procedure was also repeated for compounds found to create stable complexes with cyclodextrin and having similar structure motives to analyzed anti-tuberculosis agent.

# DESIGN AND SYNTHESIS OF NOVEL DNA BINDERS

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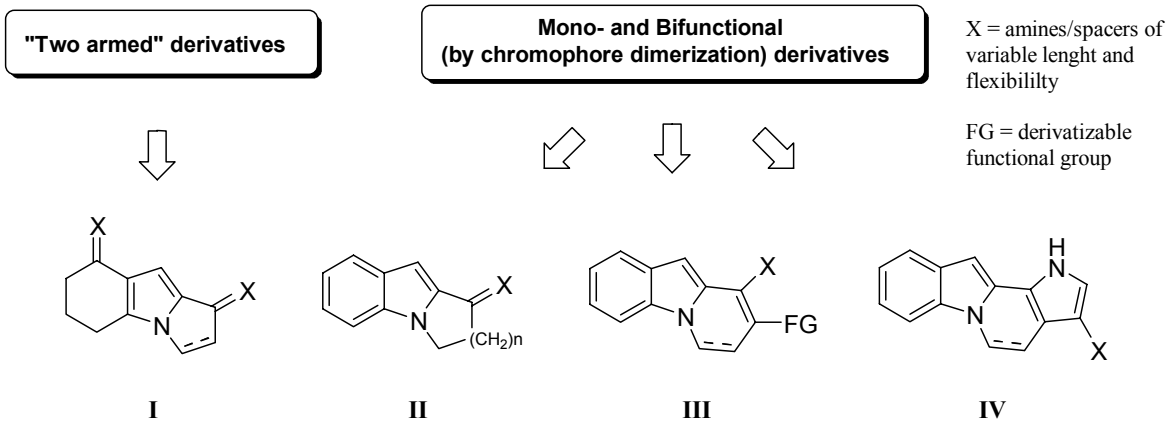
Design and development of nucleic acid targeted drugs is a challenging enterprise but real breakthroughs have been made in recent years [1]. Since DNA plays a fundamental role in normal cellular physiology and pathophysiology, it represents one of the most important molecular target of several chemotherapeutic drugs. In this context, molecular recognition of DNA by polycyclic heterocycles having a planar structure bearing appropriate side chains have been widely investigated.
In the course of our work aimed at developing novel heterocycles of pharmaceutical interest [2], we designed and synthetized several templates as potential substrate in drug design. In particular, by adopting different strategies, we obtained a set of condensed ring systems (**I**-**IV**) as versatile structural platforms to be functionalized as possible DNA-interactive agents by intercalation and/or reversible enzyme inhibition.




**I** **II** **III** **IV**

Herein, we report the synthesis of these new tricyclic and tetracyclic heteroaromatic systems and a first series of their derivatives as well as results of viscosimetry tritration and molecular dynamic studies performed to investigate a possible DNA-binding mode of some model compounds.
Also, preliminary antiproliferative activity and other biological properties of these compounds are currently under investigation and will be presented.

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# SEARCHING FOR NOVEL HIV-1 INTEGRASE INHIBITORS BY 3D-DATABASE VIRTUAL SCREENING

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Despite significant advances in antiretroviral therapy, the search for new anti-AIDS drugs continues in the attempt to develop drugs capable of overcoming toxicity and resistence. HIV-1 integrase (IN) has emerged as an attractive and validated target for antiretroviral agents because of its crucial role in the viral replication processes [1]. The addition of an IN inhibitor to existing components of combination regimen is expected to improve the therapeutic outcome. Substantial progress has been made in understanding the structure and function of HIV-1 IN and application of that information to the rational design of IN inhibitors [2]. In particular, IN is a testable target because rapid and sensitive assays exist for ascertaining enzymatic activity and, even if there is currently no solved structure for the full-length enzyme, several crystal and NMR structures are available for use in rational structure-based drug design.

As more IN inhibitors are entering clinical trials [3], it is important to develop diverse chemical classes of selective inhibitors. In this context, together with other traditional strategies, several computational approaches have been performed and the large number of successful applications demonstrates their utility in the modern drug discovery paradigm [4]. Among them, virtual screening is one of the most interesting computational tools for rapid discovery of putative lead structures containing different chemical scaffolds. This method is used with the goal to detect molecules in compound libraries in order to increase the hit rate in subsequent biological assays [5].

Aim of this study was to develop a new virtual screening strategy to be used for the discovery of new potential pharmacophores for further chemical developments. In this context, starting from the available crystal structures of the HIV-1 IN catalytic core domain (PDB codes 1QS4 and 1BIS), we first selected a pool of known inhibitors in order to define putative pharmacophore criteria. Then we screened a database of commercially available compounds in a hierarchical fashion, using fast 2D filters, 3D pharmacophore searches, and protein-ligand docking. So far, a preliminary study has been performed and work is in progress to extend this virtual screening method in terms of scoring functions, databases, and target structures. Also, enzyme assays for a first set of selected compounds are under investigation and preliminary results will be presented.

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# 2-{3-[2-(4-CHOROPHENYL)ETHOXY]-PHENYLTHIO}-2-METHYLPROPANOIC ACID, A NEW FIBRATE-LIKE COMPOUND WITH ANTIDIABETIC AND HYPOLIPIDEMIC ACTIVITY

Natalina Dell'Uomo,[a] Emanuela Tassoni,[a] Tiziana Brunetti,[a] Pompeo Pessotto,[b] Ferdinando Maria Milazzo,[b] Anna Floriana Sciarroni,[b] Francesco De Angelis,[c] Alessandro Peschechera,[b] Maria Ornella Tinti,[a] Paolo Carminati,[d] Fabio Giannessi.[b]

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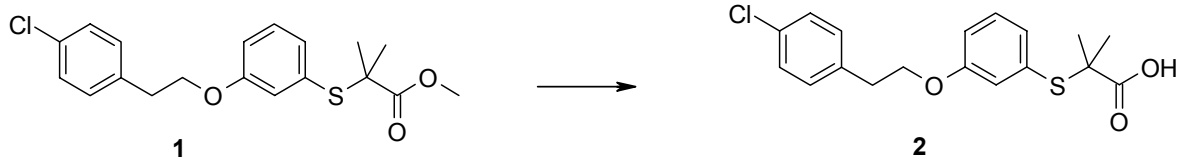
We report the synthesis, *in vitro* characterization and *in vivo* activity as hypolipidemic and antidiabetic, of a new fibrate-like compound **(1)** and of its corresponding acid **(2)**.

*In vitro* transactivation tests for activity as PPAR ligands were performed on mouse receptors transfected into monkey kidney COS-7 cells (for PPAR$\alpha$), and into mouse embryo NIH-3T3 fibroblasts (for PPAR$\gamma$). **1** proved a more potent PPAR$\alpha$ activator with respect to reference marketed fibrates, fenofibrate and bezafibrate, resulting in a subtype-selective PPAR activator. We found that ester **1** was promptly converted into **2** *in serum* and *in vivo*, and the latter showed dual activation of PPAR$\alpha$ and PPAR$\gamma$. As PPAR$\gamma$ isoform is involved in the mediation of antihyperglycemic activity, the antidiabetic activity of **2**, and indirectly of **1**, might be explained, at least in part**,** in terms of PPAR$\gamma$ activation.

In *db/db* diabetic mice **2** was able to maintain the best glycemic control and insulin-sensitizing activity, based on glucose and insulin levels. HDL cholesterol levels were increased by treatment with both **1** and **2** but not with rosiglitazone, while triglyceride levels were lowered by all the compounds. The undesirable increment of body weight observed with rosiglitazone was not observed in animals treated with **2** or **1,** as with the reference fibrates. Compound **1** was evaluated also in other models of hyperlipidemias and PPAR$\alpha$ activation: mice fed with a cholesterol-rich diet, and transgenic mice expressing human Apolipoprotein A-I.




**1** **2**

The results obtained indicate **2** (ST2518) as a promising candidate for further investigation and preclinical development as insulin-sensitizing antidiabetic and hypolipidemic agent.

# RAPID IDENTIFICATION OF A 5-$HT_{2c}$ ANTAGONIST: A SYNERGIC APPROACH BASED ON MEDICINAL AND COMPUTATIONAL CHEMISTRY

Fabrizio Micheli, Giorgio Bonanomi, Steve Bromidge, Anna Checchia, Federica Damiani, Caroline Goodacre, Dieter Hamprecht, Albert Jaxa-Chamiec, Frank King, Peter Lovell, Alessandra Pasquarello, Marilisa Rinaldi, Luca Tarsi, Giovanna Tedesco, Silvia Terreni, Martyn Wood

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Anxiety disorders are among the most common psychiatric illnesses affecting both adults and children causing a remarkable social impact.
Serotonin (5-hydroxytryptamine, 5-HT) is known to play an important role in anxiety (1) through actions mediated by a wide family of receptors, including the 5-$HT_2$ receptors (2).
Evidence supporting a role for 5-$HT_2$ receptors in anxiety arises mainly from studies showing that drugs acting as agonists at the 5-$HT_{2C}$ receptor present anxiogenic effects in clinical and experimental forms of anxiety.
In particular, the observation that m-chlorophenylpiperazine (mCPP), a functionally selective agonist at the 5-$HT_{2C}$ receptor, induces anxiety states in patients and in animal models (3), has promped different research lines aimed at developping 5-$HT_{2C}$ receptor antagonists for the treatment of anxiety disorders.
Our approach to potent and selective 5-$HT_{2c}$ antagonist compounds based on a synergic Medicinal and Computational chemistry strategy will be reported.

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# SYNTHESIS AND ANTIFUNGAL ACTIVITY OF NEW 3(5)-METHYL-5(3)-(2-THIOPHENYL AND -2-FURANYL)-1*H*-1-R-4-CYANOPYRAZOLES

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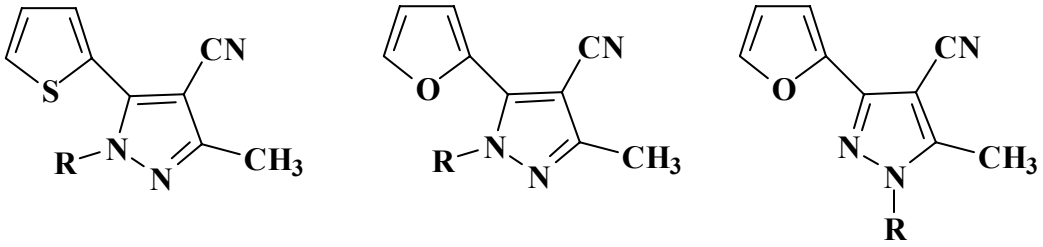
We have reported that 4-nitrosopyrazoles derivatives displayed *in vitro* potent antifungal activity at no cytotoxic concentrations and that some of these compounds were 4 times more potent than Amphotericine B and Fluconazole respectively against *Cryptococcus neoformans.* We reported also that the absence of NO group or its replacement with $NO_2$ or $NH_2$ groups gave compounds devoid of antimycotical activity. [1-2]
With the final aim to gain a deeper insight into the mechanism of action, we studied the *in vivo* metabolism of some 4-nitrosopyrazoles in rat liver and a rapid metabolization, with formation of the corresponding amines, was observed. [3]
These findings and need for novel antifungal agents lead us to synthesize and to investigate the antifungal activity of title compounds in which the 4-NO group was replaced with 4-CN group having, these last, similar steric and electronic features, but different routes by which may be metabolised *in vivo*.
Synthesis, SAR, *in vitro* and *in vivo* bilogical test of title compounds will be reported.




**a:R=H; b:R=$CH_3$**

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# SYNTHESIS AND SAR OF 2-CARBOXYLIC ACID INDOLES AS INHIBITORS OF PLASMINOGEN ACTIVATOR INHIBITOR-1

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Plasminogen activator inhibitor-1 (PAI-1) is a member of the serine protease inhibitor (serpin) gene family and is the principal inhibitor of tissue plasminogen activator (tPA) and urokinase plasminogen activator (uPA*) in vivo*. These serine proteinases convert plasminogen, an inactive zymogen, to the active enzyme plasmin, which digests fibrin clots by degrading insoluble fibrin molecules to small soluble fragments. In the acute setting, PAI-1 stored in platelet α-granules can be released upon platelet activation, resulting in significant local concentrations and the resistance of platelet-rich thrombi to lysis. In healthy individuals, PAI-1 expression is low, but it is elevated significantly in a number of diseases, including deep vein thrombosis, atherosclerosis, and type II diabetes. We have synthesized and evaluated a novel series of 2-carboxylic acid indole based inhibitors of PAI-1. Systematic modification of the N-1 position, the 5-position, and the 2-carboxylic acid of the indole scaffold resulted in the identification of several compounds that showed good potency against PAI-1. The design, synthesis and binding activity of 2-carboxylic acid indoles will be presented.

# SYNTHESIS AND BIOLOGICAL EVALUATION OF NEW 2-AMINO-SUBSTITUTED BENZOTHIAZOLES

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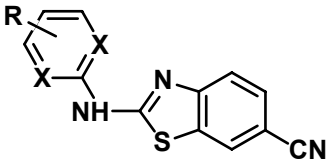
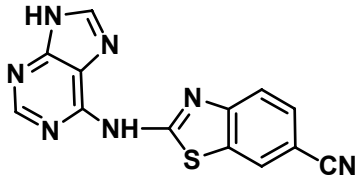
Over the past decade development of small molecule Src family kinase inhibitors has been an area of major pharmaceutical interest [1]. A large number of benzothiazole derivatives are of considerable biological and chemical interest [2]. 2-Amino-substituted benzothiazoles are reported as inhibitors of Src family kinases [3].
We prepared in multistep synthesis a series of new 2-amino substituted benzothiazoles **1-8** from 4-aminobenzonitrile. All compounds were characterized by IR, 1H- and 13C-NMR spectroscopy and elemental analysis.




**1-7**




**8**

**R= -CN; X= -C**
**-$CH_3$; X= -N**
**-Cl; X= -N**
**-H; X= -N**

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# DESIGN AND SYNTHESIS OF NOVEL POTENTIAL LIGANDS FOR ENDOTHELIN RECEPTORS

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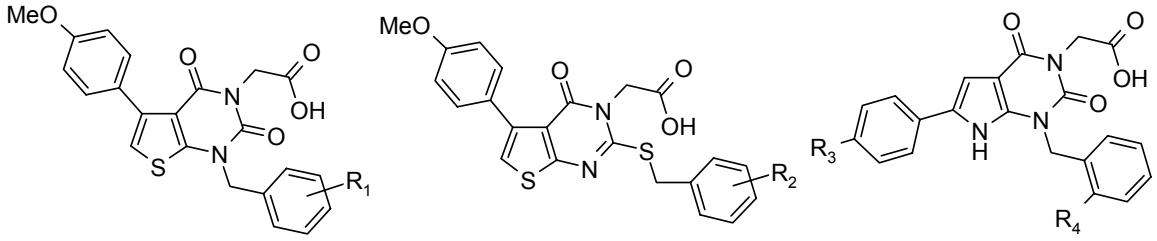
The endothelin (ET) system has been extensively studied over the last several years for its key role in physiological functions in normal tissue, acting as modulators of vasomotor tone, tissue differentiation, development, cell proliferation and hormone production and for its involvement in a variety of pathological conditions such us high blood pressure, pulmonary hypertension, acute myocardial infarction, congestive heart failure, renal failure and atherosclerosis. ETs comprise a family of three small peptides (ET-1, ET-2, ET-3) that exert their activities via specific seven-transmembrane, G-protein coupled receptors. To date two receptor subtypes, $ET_A$ and $ET_B$, have been identified and cloned. Several literature reports indicate that blocking the ET receptors is a novel and powerful therapeutic approach for the treatment of the aforementioned diseases. In the last years, our research group had been involved in the synthesis of novel ligands for endothelin receptors [1-2].




Recently Cho et al. reported the synthesis of some thieno[2,3-*d*]pyrimidine-3-acetic acid derivatives as a new class of endothelin receptor ligands [3]. We now report the synthesis of novel thienopyrimidine and pyrrolopyrimidine derivatives as new ligands for endothelin receptors. Binding assays for the synthesized compounds were performed on recombinant human endothelin receptors ($ET_{Ah}$ and $ET_{Bh}$) expressed in CHO-K1 cells. Complete binding profile for these new ligands will be reported at the symposium.

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# PLASMEPSIN II INHIBITION AND ANTIPLASMODIAL ACTIVITY OF PRIMAQUINE-STATINE "DOUBLE-DRUGS"

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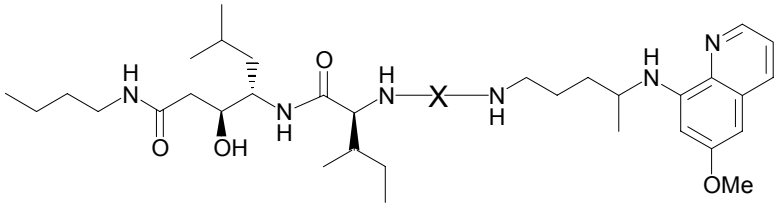
The diffusion of *Plasmodium falciparum* (*Pf*) strains resistant to traditional drugs is a major health problem, given the high mortality and morbidity rate of malaria in endemic countries. Inhibition of plasmepsin II (PLMII) is considered a valid strategy for the search of new drugs. PLMII inhibitors, including molecules with a statine-based core, have been developed: they possess low Ki values against the enzyme (nM order) but their effectiveness in killing the parasite is limited ($IC_{50}$ range 2-20 μM). [1]
We developed statine-based inhibitors of PLMII with the characteristics of double drugs. [2] Several compounds were synthesized using statine as PLMII inhibitor bound to primaquine by means of different linkers. The compounds were tested *in vitro* for anti-PLMII and antiplasmodial activity against chloroquine-sensitive (D10) and chloroquine-resistent (W2) strains of *Pf*. All compounds inhibited PLMII in a nanomolar range ($IC_{50}$ 0.5-400 nM). When tested for antiplasmodial activity, $IC_{50}$ ranging between 0.2 – 5.0 μM were obtained. In conclusion, the newly synthesized compounds possess anti-PLMII activity greater than the statine-based molecules previously reported. The antiplasmodial activity is significantly improved, as well. A correlation was found between the inhibition of PLMII and the antiplasmodial activity, suggesting that parasite death is due to the inhibition of haemoglobin digestion by PLMII. Systematic modification of the linker indicate that the antiplasmodial activity is linearly correlated with calculated logP.

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# INHIBITION OF BACE-1 BY HYDROXYETHYLAMINE AND HYDROXYETHYLSULFIDE ISOSTERIC REPLACEMENTS

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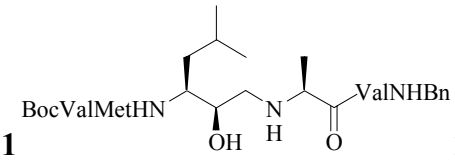
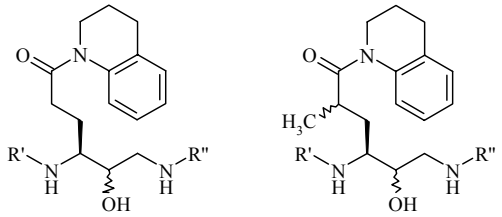
An abnormal extraneuronal deposition of the polypeptide β-Amyloid Peptide (Aβ) has an important role in the development of Alzheimer desease (AD). Aβ derives from the cleavage of a transmembrane protein APP by two proteases BACE-1 (β-site APP cleaving enzime) and γ-Secretase. BACE-1 inhibition, causing a reduction in the formation of the γ-Secretase substrate from which is obtained Aβ, is a promising therapeutic approach to prevent AD progression. BACE1 is a membrane-bound aspartyl protease and potent inhibitors have been synthesized.[1] In order to obtain new inhibitors we have synthesized as epimeric mixtures two structures containing the hydroxyehtylamine (HEA) and the hydroxyethylsulfide (HES) isosters. These molecules resulted BACE-1 inhibitors with nanomolar $IC_{50}$ for the HEA mimetic ($IC_{50}$=120 nM) and micromolar $IC_{50}$ for the HES compound ($IC_{50}$=1.85 μM).[2] Following these promising data stereoselective synthesis of the HEA and the HES transition-state mimetics were conducted and biological evaluation has shown that while the HES shows the usual *anti* stereopreference of aspartyl proteases the HEA *syn* isoster **1** resulted 100 times more active than the *anti* epimer ($IC_{50}$=14 nM vs $IC_{50}$=1.57 μM).




**1** $IC_{50}$=14 nM (BACE-1)

These compounds were also tested on BACE-2, murine BACE, Pepsin and Cathepsin D. Molecular modelling studies have suggested that the HEA *syn* epimer has a binding mode different from the published crystal structure of the hydroxyethyl isoster.[3]
Aiming to reduce the peptidic character of these compounds we have also modified the P region of the HEA isoster by introducing a large hydrophobic residue in $P_1$ that should interact also with the $S_3$ pocket. This modification was introduced based on the observation that the S region of BACE is characterized by a very large hydrophobic pocket spanning the $S_1$-$S_3$ subsites.

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# SYNTHESIS OF ANALOGUES OF GALLOCATECHIN-3-GALLATE AS INHIBITORS OF METALLOPROTEINASE-9 ACTIVITY AND GENE EXPRESSION

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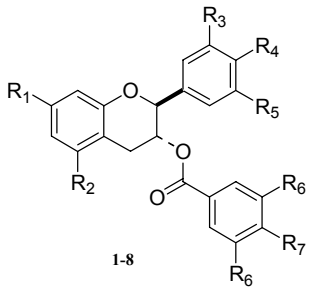
Overexpression of metalloproteinase-9 (MMP-9) leads to an excessive breakdown of extra cellular matrix in many pathological conditions including cancer invasion and metastasis, and atherosclerotic plaque rupture. Catechins have been shown to modulate MMP-9 gelatinolytic activity and gene expression [1]; in particular, gallocatechins decreased MMP-9 secretion, following a decrease of MMP-9 promoter activity and mRNA levels [2]. For a preliminary structure-activity relationship study, 7 analogues of (±)-gallocatechin-3-gallate (GCG) selectively deprived of hydroxyl groups were synthesized by and tested on MMP-9 activity and secretion by murine peritoneal macrophages. When tested at 10 μM, the effect on MMP-9 activity was dependent on the number of hydroxyl groups on rings A and D, (±)-GCG being the most active compound (82 % inhibition). Conversely, the effect on MMP-9 promoter activity and secretion was enhanced by complete deprivation of the hydroxyl groups; compound **8**, which has no substituents, was the most active at this regard. For (±)-GCG, (±)-robidanol-3-gallate and compound **8**, the down regulation of gene expression was mirrored by decreased enzyme secretion (as shown in the table). These results supply new insight into the ability of catechin analogues to act at the transcriptional level, suggesting that the structural requirements for enzyme inhibition are different from those for gene expression.




1-8

| Compound | MMP-9 secretion | MMP-9 promoter activity |
|---|---|---|
| (±)-gallocatechin-3-gallate | 28.8 ± 2.1 | 38.3 ± 14.1 |
| (±)-robidanol-3-gallate | 31.6 ± 3.4 | 47.7 ± 14.2 |
| **8** | 68.0 ± 1.1 | 62.0 ± 13.7 |

% inhibition for the most active compounds at 10 μM

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# APPLICATION OF MiPhaK ROUTINE TO GENERATE QSAR MODELS FOR PROPAFENONE TYPE P-GYLCOPROTEIN INHIBITORS

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The ATP-driven multidrug efflux pump P-glycoprotein (P-gp) is increasingly recognised as major limiting factor for bioavability and brain uptake. Additionally, its overexpression in tumour cells leads to resistance to a broad variety of divers natural product toxins. Vice versa, inhibitors of P-gp are proposed to resensitise multidrug resistant tumour cells. Due to a sort of promiscuity in the binding interaction of P-gp with ligands, the use of rational drug design approaches results rather difficult. Therefore, we used a novel computational routine, MiPhaK [1], that calculates a set of new descriptors by molecular surface estimation. These descriptors are determined from the calculated energy of interaction between the molecule and a probe atom around molecular or solvent accessible surface. They relate to pharmacophoric features such as molecular electrostatic potential, H-bond donor/acceptor capability and hydrophobicity of the whole molecule.

Our *in house* dataset of 131 propafenone type P-gp inhibitors served as training set for QSAR model generation. In total 70 descriptors were used, including ten co called classical molecular descriptors such as molecular weight, volume, polar/apolar surface area etc. We performed partial least squares analysis (PLS) – implemented in MOE - to correlate the biological activity, expressed as log(1/EC50), with the calculated properties. Validation was carried out by Leave-one-out cross-validation procedure (LOO) and by external prediction of 50 P-gp inhibitors of the propafenone type, that did not contribute to the establishment of the model. Both methods led to excellent results with a squared correlation coefficient of $R^2$=0.73 (LOO) and 0.71 (external) and a cross-validated correlation coefficient of $Q^2$=0.77 (LOO) and 0.73 (external). Hence, the use of MiPhaK descriptors for ADME profiling is suposed to be a valuable tool for multispecific targets.

[1] Meniconi, M.; Costantino, G.; Macchiarulo, A.; Pellicciari, R.. QSAR and Comb. Sci 2004. Submitted.

# SIMILARITY-BASED DESCRIPTORS (SIBAR): A TOOL FOR SAFE EXCHANGE OF CHEMICAL INFORMATION?

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Recently we published the sucessful application of a set of new descriptors on similarity values, denoted as SIBAR-descriptors (Similarity Based SAR). These descriptors are based on calculation of similarity (on basis of euclidian distances) for each compound of the data set to each compound of a reference set, using common descriptors. These euclidian distances ( = similarity values) are then further used for QSAR-studies. Both the reference set as well as the descriptors used for calculating the SIBAR-values are tailored to the specific QSAR-problem. Best results have been obtained when targeting ADMET-problems. In any case it needs the knowledge of the reference set to retrieve the corresponding descriptors. Assuming that only the descriptors for calculating the SIBAR-values, but not the structures of the reference compounds are available, it should be impossible to trace back the chemical structure of the original compounds of the training set.

For this, compounds GPV0005, Diazepam and Estriol were used as query compounds to search for similar compounds in a large database. For the description of the structures three different descriptor sets were used. The 32 VSA-descriptors [1], a set of ADME-realted descriptors (weight, TPSA, SMR, SlogP, APOL, number of rotable bonds and the number of H-bond acceptors and donors) and a set of autocorrelations vectors of the PETRA-descriptors [2]. The reference compounds needed for applying SIBAR were a set of 20 highly diverse compounds from the SPECs-library. As target database a compound collection was build up with more than 1.5 million compounds via merging the databases from ChemDiv, SPECS, Maybridge, and Enamine. Additionally, the database was spiked with 200 compounds similar to the query structures.

In the first run the euclidian distances for the three reference compounds to each compound of the database were calculated and the first 20 similar compounds for the three reference structures were extracted from the database. Subsequently, the step was repeated using the SIBAR-approach.

The results show, that, using only the euclidian distance, the top ranked compounds retrieved by the similarity search are structurally very closed to the reference compounds. Doing the additional step of calculating the SIBAR-values the number of similar structures retrieved is decreased.

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[1] Chemical Computing Group www.chemcomp.com

[2] Molecular Networks www.mol-net.de

# INVESTIGATION OF VOLATILE COMPOUNDS IN *TAGETES* SPECIES

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*Tagetes patula* L. and *T. lucida* L. are native to the warmer parts of America, but now it is cultivated world-wide. These plants synthetise many bioactive compounds, that can be used by the agriculture and medicine: biocid polyacetylenes with thiophene structure, antibacterial and antifungal essential oil, carotinoids and flavonoids.

We aimed to study the essential oil production of the genetically transformed hairy root cultures of *T. patula* and intact *T. patula* and *T. lucida* plants. Hairy roots were cultivated in liquid Gamborg's B5 medium (in 500 mL Erlenmeyer flasks filled with 100 mL medium) on rotary shaker (140 rpm) at 23±2 ºC in dark. *In vitro T. lucida* plants were cultivated on solid Murashige and Skoog medium supplemented with 2 % sucrose under light conditions.

The occurrence of volatile compounds was researched by GC and GC-MS. The essential oil was produced by steam distillation in a Clevenger apparatus for 3 hours. The content of oil was measured gravimetrically. The analysis was carried out by a Finnigan MAT GCQ mass spectrometer with ion trap analyzer. A capillary colmumn (column: 30 m × 0,25 mm ID × 0,25 µm film thickness MDN-5S) was used to fractionate the samples. The electron impact method was used to ionize the fractions. The carrier gas was helium. Samples were injected with a split ratio of 1: 62. The identification of the compounds was done by comparing the retention time, retention index and the recorded spectra with spectra known from literature and spectra of authentic standards.

The main essential oil components of the intact plants were found to be 'classical' terpenoids while the hairy roots and *intact* roots of *T. patula* and *in vitro T. lucida* roots poduced aromatic sulphurated thiophene structures.

The main constituent of *T. patula* capitula was β-caryophyllene (50,25 %) and the leaves had high concentrations of terpinolene (20,70 %).

The aerial parts of *intact* and *in vitro T. lucida* had the phenylpropanoid methyl chavicol (83,97 % and 71,96 % respectively) as the main oil compound.

The main volatile component of *T. patula* hairy roots and *intact* roots was 5-(3-buten-1-ynyl)-2,2'-bithienyl (BBT) of 28.87%. Other sulfurated compounds were also identified, such as α-terthienyl (15.51%) and 5-(4-acetoxy-1-butynyl)-2,2'- bithienil (BBTOAc) of 11.55%.

*T. lucida in vitro* root oil was also rich in thiophenes: BBT (9,41 %) and BBTOAc (12,20 %). These thiophenes are the main secondary metabolites of the intact roots, and can be found even in the essential oil of the intact plants. It is interesting to note that α-terthienyl was not detected in the essential oils of *T. lucida.*

# STUDIES ON THE ANTHRAQUINONE PRODUCTION BY *RUBIA TINCTORUM* HAIRY ROOT CULTURES

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European madder (*Rubia tinctorum* L.) is a perennial plant and a member of the family Rubiaceae. It is a source of a natural dye, it produces a variety of anthraquinone pigments in its roots and rhizomes. The main components are di- and trihydroxy-anthraquinones and their glycosides: ruberythric acid (alizarin-primeveroside), alizarin, pseudopurpurin, purpurin, rubiadin, munjistin, lucidin-primeveroside [1]. These substances show some bactericidal and spasmolytic activity and facilitate the loosening of kidney concrements containing calcium and magnesium phosphates. In this connection, madder is used in medicine. Some components show mutagenic activity (lucidin), but purpurin has some inhibitory effect on bacterial mutagenicity [2].

We have investigated the growth and anthraquinone content of the genetically modified hairy root cultures. The hypocotyl of *Rubia tinctorum* was infected by *Agrobacterium rhizogenes* (strain R-1601). After the elimination of bacteria, the hairy roots were cultured on liquid Gamborg B5 [3] and HMS [4] media in Erlenmeyer flasks.

For determination of anthraquinones, the lyophilized tissue samples were extracted with MeOH using an ultrasound device. After evaporation, the athraquinone glycosides were hydrolysed with HCl. The hydrolysate was purified by solid phase extraction. The purified samples were analysed by HPLC method. Alizarin and purpurin were identified with the use of external standards. Investigating the cultures growing on different media we found, that the production of purpurin was more than threefold higher (2.93 mg/g) than that of alizarin (0.87 mg/g) on HMS medium, while on B5 medium the alizarin production was slightly higher (1.12 mg/g).

Crude MeOH extracts were fractionated by flash chromatography method. Fractions were investigated by HPLC. Anthraquinone glycosides and aglycones were separated and ruberythric acid, lucidin-primeveroside, munjistin, pseudopurpurin and lucidin were detected via UV spectrum analysis.

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# SYNTHESIS AND BIOLOGICAL EVALUATION OF SOME NOVEL PYRAZOLINES FROM 1-(3-METHYL-4-HYDROXY PHENYL)-2-PROPEN-1-ONE AND 1-(3-METHYL-4-HYDROXY PHENYL-FURFURYL-2-PROPEN-1-ONE.

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The reaction of *o*- cresol with acetic anhydride in the presence of zinc chloride led to the formation of 3-methyl-4-hydroxy acetophenone (I).Condensation of compound (I) with some aromatic aldehydes in methanolic KOH yielded the corresponding chalcones (IIa-k). These corresponding chalcones were reacted with thio- semicarbazide to yield N-thio carbomoyl pyrazolines (IIIa-k). Similarly the reaction of (IIa-k) with phenyl hydrazine in acetic acid led to the formation of phenyl pyrazolines (IVa-k).The newly synthesized heterocyles were characterized on the basis of their chemical properties and spectroscopic data. The newly synthesized compounds were tested for anti-bacterial and anti-fungal activities.

**Key words**: pyrazoline ,anti-bacterial , anti-fungal activity

# ANTIPSYCHOTIC EFFECT OF GLYCINE REUPTAKE INHIBITION, INDUCED BY ORG-24461 A GLYCINE TRANSPORTER-1 TYPE BLOCKER

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The dopaminergic hyperactivity theory as the neurochemical basis of schizophrenia has been generally accepted for a long time, supported by clinical observations that dopamine receptor antagonists can attenuate the symptoms of the illness. Since the discovery that phencyclydine (PCP, a non-competitive NMDA receptor antagonist) has been able to induce symptoms of schizophrenia in human patients and it can be used as „schizophrenic agent" in animal experiments, NMDA receptor hypofunction became a new theory in the pathophysiology of schizophrenia. Though potential neurotoxicity excludes the use of direct glutamatergic agonists, facilitation of glutamatergic activity can be achieved by increasing the co-agonist glycine concentration around the NMDA receptors [1]. Glycine is released from glial cells via the glycine transporter-1 (GlyT1) and specific inhibitors of GlyT-1 can increase glycine concentration in the vicinity of NMDA receptors [2]. Org-24461 is an N-methylglycine derivative of fluoxetine, synthesized conveniently in two steps starting from fluoxetine.

Org-24461 inhibited glycine reuptake in vitro in rat brain synaptosomal preparation without having affinity to serotonergic, adrenergic and dopaminergic receptors. Behavioral tests suggest its preferential effect on the negative symptoms of shizophrenia, it inhibited PCP-induced hyperlocomotion in mice, PCP-induced social withdrawal and EEG-desynchronization in rats. On the other hand, it was ineffective to alleviate positive-like symptoms, like D-amphetamine-induced hyperlocomotion or apomorphine-evoked stereotypy or climbing. These results indicate the therapeutic usefulness of GlyT-1 inhibitors in antipsychotic treatment even in the amelioration of the often therapy resistant negative symptoms of schizophrenia.

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