THE CHEMBIOBANK AND EU-OPENSCREEN INITIATIVES IN CHEMICAL BIOLOGY: CURRENT STATUS AND CASE STUDIES

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The Chembiobank initiative in Spain (CBB, http://www.pcb.ub.cat/chembiobank/) has built a chemico-biological library and annotated database, which will be described. The library contains nearly 15.000 compounds both from commercial and from academic origins. The annotated database has been developed to ensure proper registration and searching of chemical structures, analytical chemistry data, logistics / compound management information, and virtual screening and experimental screening data associated with the Chembiobank library compounds.

Three case studies describing the Chembiobank workflow, structure and applications will be shown: a) how to profile compounds of academic origin towards targets of therapeutic interest; b) how to find new possible therapeutic applications for commercially available compounds (reprofiling); and c) how may the Chembiobank workflow help the medicinal chemistry community.

This Chembiobank initiative is coordinated with other Chemical Biology initiatives being developed in several European countries, in the ESFRI-funded European Research Infrastructure on Open Screening Platfoms, the EU-OPENSCREEN initiative (www.eu-openscreen.eu). This project is currently in its Preparatory Phase and its status and future implications and benefits for the medicinal chemistry community in Europe will be described.

MYCOBACTERIUM TUBERCULOSIS DNA GYRASE INHIBITORS AS NOVEL ANTITUBERCULARS

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Tuberculosis has become one of the most extended diseases around the world. Shortening of the current treatment as well as new drugs effective against increasingly appearing resistant strains are urgently needed. A new family of DNA Gyrase inhibitors with a different mode of action[1] to Fluoroquinolones, and therefore not cross-resistant, has been developed at GSK. Herein we present our progress in shaping these compounds into antituberculars.

Screening against *Mycobacterium tuberculosis* (Mtb) of a subset of compounds selected from the GSK Gyrase inhibitors collection enabled us to identify 7-substituted-1,5-naphthyridones (Figure 1, left) as a starting point. Variation of the substituents in position 7 had a significant impact on the activity and metabolic stability of the compounds. Incorporation of monocyclic aromatic moieties in the right-hand side of the molecule proved to be optimal for a selective anti-Mtb profile.

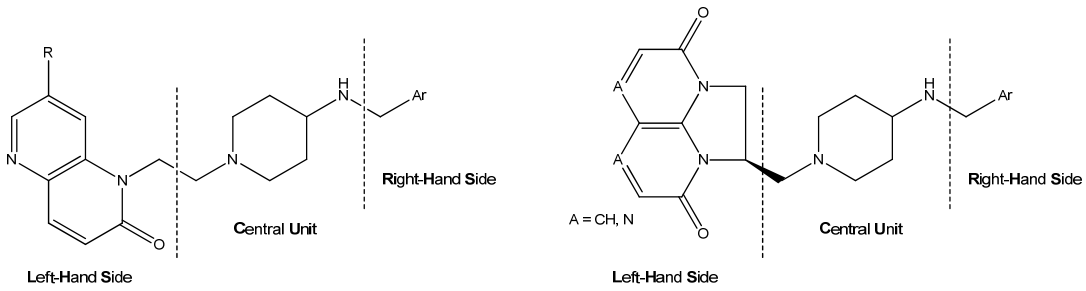

Figure 1

A potential cardiotoxicity liability related to hERG inhibition was initially encountered and a correlation with lipophilicity was observed. Optimization led us to a new series of more polar compounds having a 6,6,5-dione in the left-hand side (Figure 1, right). This family possesses a more balanced profile in terms of activity, metabolism and safety.

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NEW NEUROPROTECTIVE DRUGS INDANE DERIVATES

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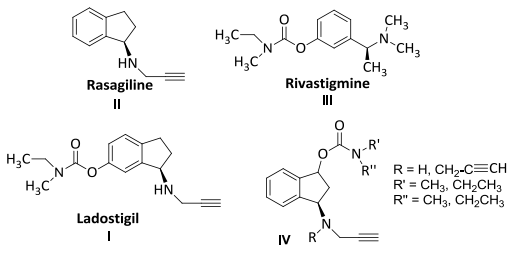
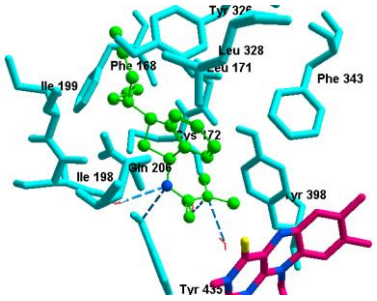
The use of dual action drugs is one of the strategies used the most in the design of drugs. These hybrid compounds contain two pharmacophores combined in a single molecule, aiming at bonding two types of different receptors.[1]

In the treatment of multifactorial neurodegenerative diseases, such as Parkinson's disease (PD), Alzheimer's disease (AD) and Amyotrophic lateral sclerosis (ALS), where multiple etiopathologies coexist, the use of dual inhibitors drugs has develop into a promising new therapeutic strategy. As an example, the neuroprotective drug **Ladostigil**,[2] **I**, [(*N*-propargyl-(3*R*) aminoindan-5-yl)-ethyl methyl carbamate], which combines in a single molecule the pharmacophoric groups of the **Rasagiline**, **II** (selective inhibitor of MAO_B) and the **Rivastigmine**, **III** (inhibitor of *acetyl* and *butyrylcolynestherase*) is being investigated for the treatment of PD and AD. Furthermore, this compound has demonstrated to partially revert the effect of neurodegenerative diseases through the induction of neurogenesis.[3]

Following a research line aiming at searching for new neuroprotective indane derivates,[4,5] we report here the synthesis, pharmacological evaluation and a theoretical approach to discover the behavior of this new type of ladostigil **IV** derivatives. In these compounds we have modified the position of the carbamate group to locate in the cyclopentane ring, position 3. Also, the carbamate group substituents have been modified and a second propynyl group on the nitrogen atom has been introduced.





Acknowledgements:
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THEORETICAL STUDY OF GSK-3*A*: NEURAL NETWORKS QSAR STUDIES FOR THE DESIGN OF NEW INHIBITORS USING 2D-DESCRIPTORS FOR CONSTRUCTION COMPLEX NETWORKS

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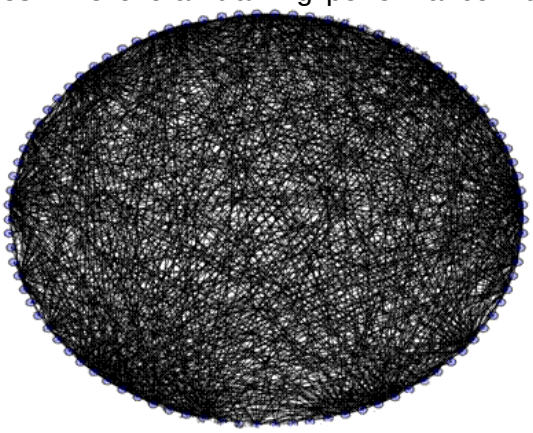
QSAR could play an important role in studying these GSK-3 inhibitors. For this reason we developed QSAR models for GSK-3β, LDA, ANNs and CT from more than 40000 cases with more than 2400 different molecules inhibitors of GSK-3β obtained from ChEMBL database server[1]; in total we used more than 45000 different molecules to develop the QSAR models. We used 237 molecular descriptors calculated with DRAGON software. The model correctly classified 1310 out of 1643 active compounds (79.7%) and 24823 out of 26156 non-active compounds (94.9%) in the training series. The overall training performance was 94.0%. Validation of the model was carried out using an external predicting series. In this series the model classified correctly 757 out of 940 (80.5%) active compounds and 14166 out of 14937 non-active compounds (94.8%). The overall predictability performance was 94.0%. In this work, we propose five types of non Linear ANN and we show that it is another alternative model to the already existing ones in the literature, such as LDA[2]. The best model obtained was RBF 166:166-402-1:1 which had an overall training performance of 94.2%. All this can help to design new inhibitors of GSK-3β. The present work reports the attempts to calculate within a unified framework probabilities of GSK-3β inhibitors against different molecules found in the literature. We used the mt-QSAR to predict the biological activity of 1000 selected GSK-3β inhibitors measured in three conditions, IC50 (nM), Ki (nM) and Selectivity, for Homo sapiens. As a result, the most important is the reduction of the large universe of GSK-3β inhibitors to a basic set of compounds in order to obtain the best candidates for the experimental assays.



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INDOL BASED DIARYLETHENES AS POTENT INHIBITORS OF TUBULIN POLYMERIZATION AND CYTOTOXIC AGENTS.

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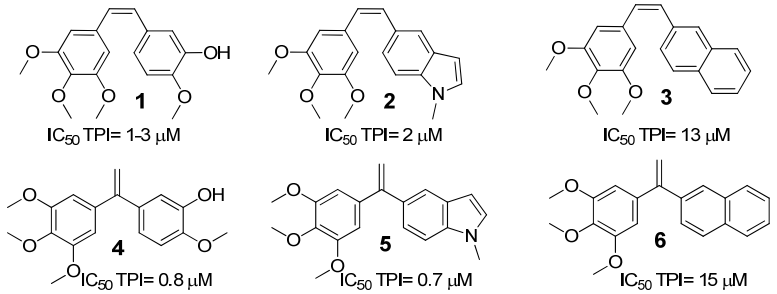
Combretastatins are natural products that are able to inhibit tubulin polymerization by binding at the colchicine-site. Combretastatin A-4 has been taken as structural reference to establish structure–activity relationships because of its highest cytotoxicity. Its phosphate derivative is now in clinical trials (phase II).[1] Replacement of the guaiacol ring by bicyclical systems such as naphthyl and indolyl rings has produced compounds that maintain cytotoxicity and tubulin polymerization inhibitory effects.[2]

One of the main problems associated with these compounds are the ready isomerization of the double bond of combretastatins to the inactive *trans* isomer. In order to avoid this drawback, we have recently synthesized 1,1-diarylethenes with phenyl rings having the best substitution patterns of combretastatins. They show equal or more potent inhibition of tubulin polymerization than their corresponding combretastatins.[3]



Another typical problem of this kind of compounds is their poor water solubility. Therefore, taking into account docking studies, we have designed and synthesized new substituted indole derivatives of isocombretastatins that incorporate different polar groups to the molecule.

Tubulin polymerization inhibitory and cytotoxic assays and solubility measurements will be presented. The effects of these structural modifications on the activity and the physicochemcal profile of this antimitotic family will be discussed.

Acknowledgements: We thank AECID (Spanish PCI-Mediterráneo D/033593/10), MCINN (SAF 2008-04242) and Junta de Castilla y León (SA090A06) for financial support.

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INTERACTIONS BETWEEN TRANSMEMBRANE DOMAINS OF BCL2 FAMILY MEMBERS

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The role of the Bcl-2 protein family is one of the most studied aspects in the apoptotic intrinsic pathway. The relevance of Bcl´s BH3 domain in defining the protein-protein interactions between different pro- and anti- apoptotic members has been extensively analyzed. However the role of the transmembrane domain (TM) in such interactions has not been yet defined. We are interested in the analysis of the contribution that the TM have to the oligomerization process. For this purpose, we have adapted the ToxR system, a genetic tool to monitor interactions of α-helical TMDs, to analyze homo- and hetero-oligomerizations between the TMDs of antiapoptotic (Bcl-2, Bcl-XL, Bcl-W and Mcl-1) and proapoptotic (Rambo,Diva, Bid and Bik) members.

Preliminary results indicate that TMDs from Bcl-2 members are prone to homo- and hetero-oligomerize. Different levels of interaction are observed depending of the Bcl-2 family members studied. The results from this study could contribute to a better understanding of how the equilibrium between members of the Bcl-2 protein family shifts from anti- to pro-apoptotic when subjected to specific stimuli.

NEW OXADIAZOLINE ANALOGUES OF COMBRETASTATIN AS ANTIMITOTIC AGENTS

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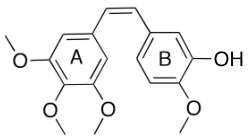
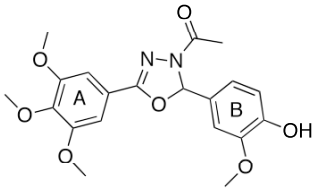
Combretastatins are natural products with antitumor activity. They act by binding to tubulin at the colchicine site, thus altering its function during cell division. Combretastatins are compounds with high cytotoxicity but have the disadvantage of their low aqueous solubility, leading to low bioavailability. Therefore, series of analogues have been designed to improve their water solubility without decreasing activity. 2,5-Diaryl-1,3,4-oxadiazolines[1] are polar analogues that differ from combretastatins in the bridge between the two aromatic rings (A and B). One of the requirements[2] of these compounds is that the bridge has 1-4 carbon atoms and the arrangement of the two aromatic rings are close but not in the same plane. 2,5-Diaryl-1,3,4-oxadiazolines are designed to adopt the optimal geometric conformation in the active site of the protein and keep the structural requirements.

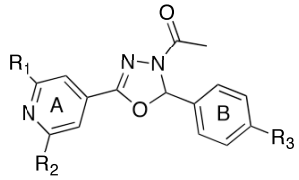


Combretastatin A-4

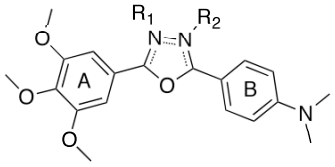


2,5-diaryl-1,3,4-oxadiazoline

New 2,5-Diaryl-1,3,4-oxadiazoline analogues have been designed and synthesized with the intention of increasing the aqueous solubility by replacing the trimethoxyphenyl ring with a disubstituted pyridine. Another used strategy was the replacement of the B ring with a dimethylaminophenyl ring.



R1, R2 = OMe, SMe
R3 = Me_2N, OMe



R1, R2 = CH_3CO

These compounds have been tested for cytotoxicity against different human tumour cell lines and for tubulin polymerization inhibitory activity (TPI).

Acknowledgments: L.A.V. aknowledges a predoctoral grant from the Universidad de Salamanca-Banco Santander. Financial support came from Spanish PCI-Mediterráneo D/033593/10, Spanish MCINN SAF2008-04242, Junta de Castilla y León SA067A09.

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Validation of FtsZ protein as a new potential therapeutic target for the discovery and development of new antibacterial agents

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Emergence and spread of antibiotic-resistant strains of pathogenic bacteria have boosted an urgent need for new antibacterial agents with novel modes of action.[1] In this sense, FtsZ (Figure 1) "a widely conserved tubulin-like GTPase" has recently been proposed as an attractive target for antibacterial drug discovery due to its essential role in bacterial cell division.[2]

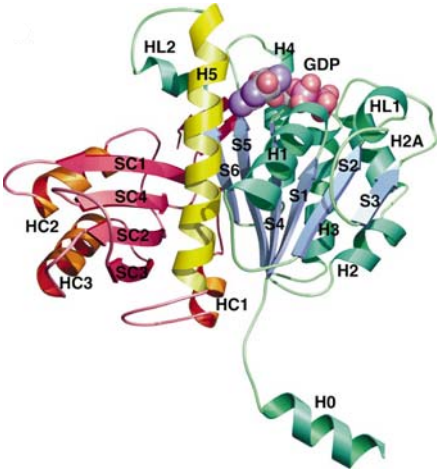
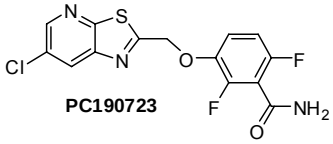


Figure 1. FtsZ from *M. jannaschii*.

Recently, new compounds that specifically target FtsZ and inhibit its function in bacterial division have been identified.[3] Among them, the most promising FtsZ inhibitor discovered so far, PC190723 that binds an alternative site different from the classical GTP binding site,[4] has shown potent activity both in vitro and in vivo against *Staphilococcus* but it is inactive against a range of Gram-positive and Gram-negative pathogenic bacteria. Hence, the development of new inhibitors of FtsZ able to act as broad spectrum antibacterials, needs still to be addressed and is the focus of the present work.



PC190723

Therefore, the main goal of this project is the discovery of FtsZ inhibitors targeting both binding sites, using two different strategies: the design of GTP-mimetics and virtual screening. In addition, design and synthesis of fluorescent derivatives of PC190723 is being carried out to obtain further information about the interactions of the inhibitors in this newly identified binding site, which will allow the rational design of new agents with improved antibacterial properties.

Acknowledgments: This work is supported by a grant from the Spanish Ministerio de Ciencia e Innovación (MICINN, SAF2010-22198). The authors thank MICINN for a predoctoral grant to M.A.

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SELENOCYANATES AND DISELENIDES: A NEW APPROACH IN LEISHMANIA TREATMENT

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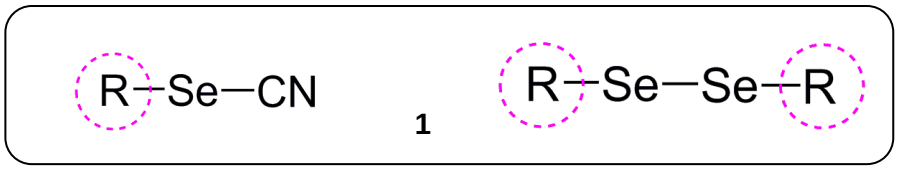
Leishmaniasis is a protozoan vector borne disease prevalent throughout the world and present in at least 88 countries. The World Health Organization (WHO) estimates that the disease results in 2 million new cases a year and that there are 12 million people currently infected worldwide[1].

Different studies recognised the trace element selenium as a new defense strategy against Leishmania infection[2] and after the work developed by our research group[3,4], we realize that selenocianate and diselenide groups are important to achieve potential compounds. We carried out the synthesis and biological evaluation of new organoselenium derivatives, according with these general structures (**1**):



1

R= Carbo cyclic, heterocyclic, mono, bi and polycyclic, aliphatic and aromatic groups.

All the synthesized compounds were subjected to *in vitro* screening against *L. infantum* promastigote and the most active were tested in amastigote model. In order to establish the selectivity index (SI) their cytotoxic effect was carried out against Jurkat and THP-1 cell lines.

Acknowledgements: The authors wish to express their gratitude to the Department of Education of the Navarra Government for fellowships granted to Y.B. and E.M. We thank the financial support from the Ministerio de Educación y Ciencia, Spain.

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SYNTHESIS AND EVALUATION OF SALICYLAMIDE AND SULFONAMIDE DERIVATIVES OF QUINOXALINE 1,4-DI-*N*-OXIDE AGAINST LEISHMANIANIASIS AND MALARIA

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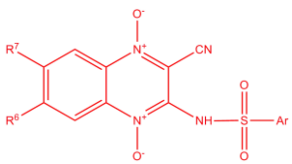
[f]*Institut de Recherche pour le Développement; UMR-152; Mission IRD Casilla 18-1209 Lima, Perú.*

Fourteen new 3-amino-1,4-di-*N*-oxide quinoxaline-2-carbonitrile derivatives (**1**,**2**) were synthesized and evaluated for their *in vitro* antimalarial and antileishmanial activity against *Plasmodium falciparum* and *Leishmania amazonensis*. Further computational studies were carried out in order to analyze graphic SAR and ADME properties, finding interesting results. They indicate that compounds with one halogenous group substituted in position 6 and 7 provide an efficient approach for further development of antimalarial and antileishmanial agents.



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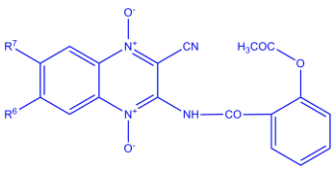

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ANTI-LEISHMANIAL AND CYTOTOXICITY EVALUATION OF POLYAMINE DERIVATIVES

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The natural polyamines spermidine and spermine, and their precursor diamine putrescine, are ubiquitous polycationic compounds that play important biological functions in cell growth and differentiation. In trypanosomatid protozoa that are causative agents of important human diseases such as leishmaniasis, polyamines have an additional role participating in the endogenous redox equilibrium.[1]

Current treatment of leishmaniasis is based on chemotherapy with some attempts at immunotherapy. However, the resistance to chemotherapics and the high toxicity and cost of second-line drugs reveal the urgent need of a search for novel chemotherapeutic agents with antileishmanial activity.[2]

In this study, we have investigated the leishmanicidal effect of a series of polyamine derivatives against promastigote forms of *Leishmania spp* (Figure 1). Cytotoxic properties have been evaluated against J774 macrophages. The discovery of some polyamines as potent anti-leishmanial compounds with high selectivity and their SAR analysis will be presented.

Figure 1

Acknowledgements: This work was supported by the Conselleria de Sanitat (Project AP158/10), the University CEU-Cardenal Herrerra (Project PRCEU-UCH18/10), Spanish Agency for International Cooperation and Development (A-024457/09), CONSOLIDER CSD2010-00065 and PROMETEO 2011/008. P. Bilbao-Ramos is fellow MAEC-AECID.

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SUBSTITUTED BENZENESULFONAMIDES AS POTENTIAL ANTIGIARDIAL AGENTS

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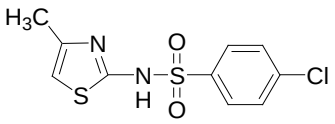
Giardia intestinalis (also known as *Giardia lamblia* or *Giardia duodenalis*) is a flagellate protozoan that causes giardiasis. The clinical features vary from acute or chronic diarrhea, malabsorption and weight loss. Giardiasis occurs worldwide with a prevalence of 20–30% in developing countries. The CDC estimates there are an upwards of 2.5 million cases of giardiasis annually.[1] Effective treatments include the nitroimidazoles tinidazole and metronidazole or the 5-nitrothiazole derivative nitazoxanide.[2] Nevertheless current drugs have considerable adverse effects. In addition, the widespread development of resistance by some parasites strains constitutes an important health problem.[3] In this context the study of new chemotherapeutic agents plays a fundamental role. Recently, in vitro antigiardial effect of a sulfonamide derivative has been shown.[4]

Herein our initial work on sulfonamides with antiprotozoal activity has been extended to *Giardia intestinalis*.[5] Among the tested sulfonamides, 16 compounds have shown more than 80-90% inhibition against *Giardia* growth with IC_{50} range between 15-0.5 µg/mL. Benzenesulfonamides **1** and **2** emerged as the most active compounds in the series (IC_{50} = 0.96 and 0.5 µg/mL, respectively) with a selectivity index (SI = CC_{50}/IC_{50}) higher than 100 and several folds more potent than the reference drug metronidazole. The potent activity and straightforward synthesis of sulfonamides **1** and **2** suggest that they are potential candidates for the development of more efficacious antigiardial agents.



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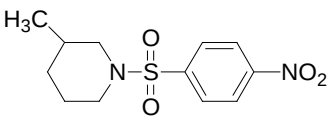

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Acknowledgements: This work was supported by the Conselleria de Sanitat (AP158/10), the University CEU-Cardenal Herrerra (PRCEU-UCH18/10), the Ministerio de Ciencia e Innovación (Programa CONSOLIDER-INGENIO CSD2010-00065) and the Spanish Agency for International Cooperation and Development (A-024457/09). P. Bilbao-Ramos is fellow MAEC-AECID.

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THE RECOGNITION OF *N*-GLYCANS BY PLANT LECTINS STUDIED BY STD-NMR

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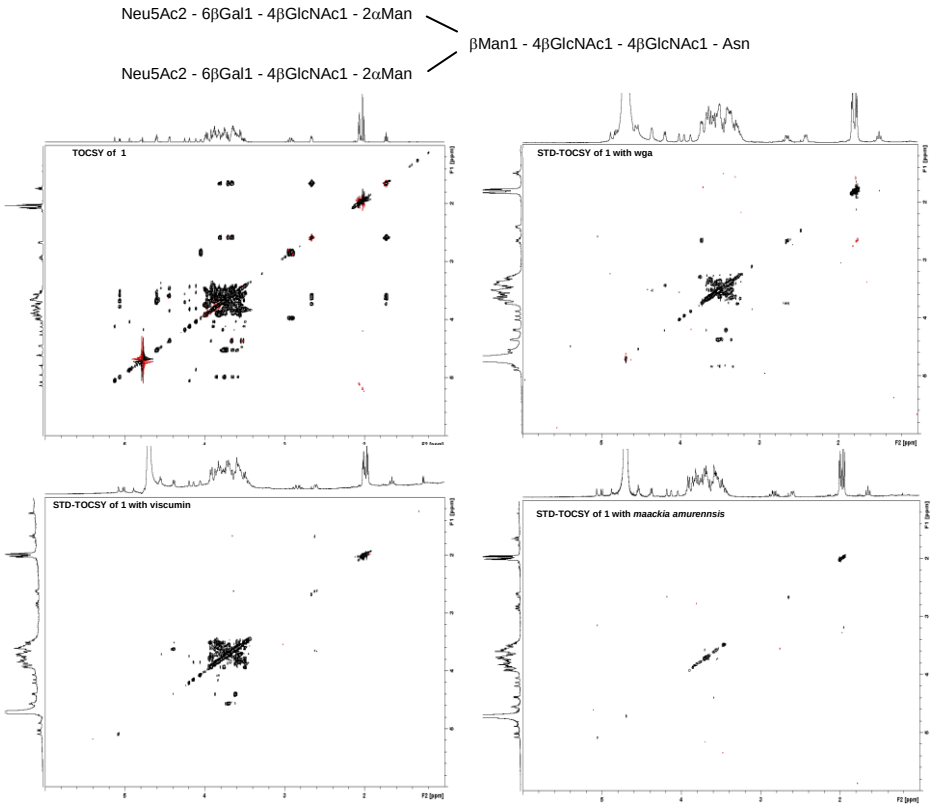
The recognition of carbohydrates by lectins (proteins that selectively recognize carbohydrates without enzymatic or immunological activity) is an ubiquitous event in living organisms. Indeed, they mediate a variety of key biological processes. *N*-glycosylation represents a highly diverse and intriguing protein modification, essential for the proper folding and / or function of the glycoprotein The recognition of these glycidic parts of glycoproteins are behind some essential and very distinct processes such as, for instance the ER quality control system for newly synthesized glycoproteins,[1] or the viral entry on hosts cells.[2] Understanding how this recognition takes place is a topic of major interest.

We have recently reported on the binding of the trisaccharide *N*-glycan core to the small plant lectin hevein.[3] In the current communication, we want to describe our recent results in the distinct recognition of high-mannose type of *N*-glycans by different plant lectins through STD NMR. Epitope mapping has been performed and the fine details of the interactions have been explained by careful analysis of the experimental NMR data.



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OPTIMIZING CRYSTAL SIZE AND HABIT OF BILASTINE

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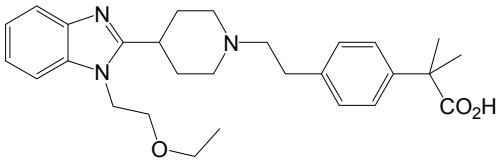
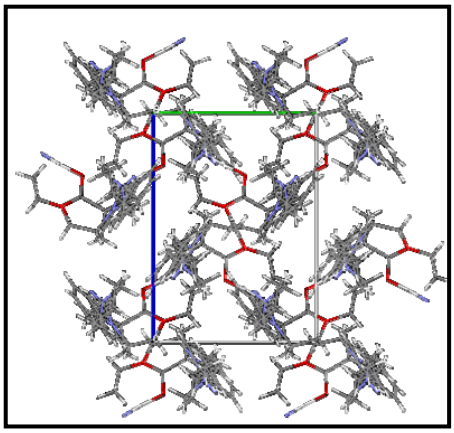
FAES FARMA, S.A., R&D and Innovation Department. Polymorphism and Salt Optimization C/ Máximo Aguirre, N. 14, 48940-Leioa (Vizcaya), Spain

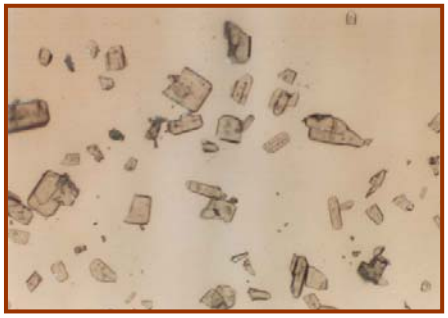
Differences in crystal size and morphology can affect drug bioavailability and processability at secondary manufacturing[1,2].



1

A crystallization study of Bilastine (Figure 1) was carried out and the effects of parameters such as solvent, temperature, stirring rate and concentration were studied. Crystal structure of the final product was characterized by powder and single crystal X-ray diffraction and morphology was observed by optical microscopy. This work enabled the development of a robust commercial process and batches from three different manufacturers have shown the same crystal size and habit.





Acknowledgements:

- This work was supported in part by the Ministry of Science and Technology of Spain (MITYC) and the Department of Industry, Commerce and Tourism of the Basque Government (INNOTEK-FEDER funding).
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DIPEPTIDYL-PEPTIDASE IV (DPPIV/CD26)-BASED PRODRUGS OF HYDROXY-CONTAINING COMPOUNDS

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We have developed a novel prodrug approach[1] that provides conjugates of therapeutic agents with a peptide moiety as a carrier where the conjugate [peptide]-[drug] is specifically cleavable by the endogenous dipeptidyl-peptidase IV enzyme (DPPIV), present on the surface of certain cells or in plasma. This enzyme, also known as CD26, belongs to a group of atypical serine proteases and cleaves X-Pro (or X-Ala) dipeptides from the N-terminus of a variety of natural peptides. We applied this strategy to a variety of drugs containing free amino groups through the direct coupled of di- or oligopeptide moiety $(Xaa\text{-}Pro)_n$ to the amino group *via* an amide bond.[2] With these conjugates it was possible to modulate the hydrolisis rate (half-life) and the physicochemical properties of compounds by modifying the nature and length of the peptide (di- or tetrapeptides) moiety.
This prodrug technology has been also applied to a hydroxy-containing compound, the antiviral drug Cf1743,[3] which exhibit a very low water solubility and poor oral bioavailability. In this hydroxy-containing drug conjugates [Xaa-Pro]-[connector]-[drug] that contain a dipeptide moiety (cleavable by DPPIV/CD26), an heterobifunctional connector [released by chemical or enzymatic hydrolysis of the ester bond] and the drug (*tripartate prodrugs*) were prepared and evaluated, showing a high improvement in water solubility together with an enhance oral bioavailability of the prodrugs *vs* the parent drug in mice.[4]

We now explore the viability of the tripartate prodrug approach activated by DPPIV/CD26 in hydroxy-containing drugs of different nature (primary, secondary, tertiary or aromatic hydroxyl groups). A broad variety of prodrugs have been designed, synthesized and evaluated for their pharmacokinetic properties including chemical and enzymatic stability (cleavage rates) and water solubility. The results indicated that the prodrugs are efficiently converted to the parent drug. Moreover, several of them showed markedly increased water solubility compared to the parent drug. Thus, the results support the wide applicability of our prodrug approach.

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SYNTHESIS AND BIOLOGICAL EVALUATION OF NOVEL CONFORMATIONALLY CONSTRAINED ANALOGUES OF HALOPERIDOL AS CNS AGENTS

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Schizophrenia is a complex mental disorder affecting approximately 1% of world population. For its treatment, classical (typical) neuroleptics, such as Haloperidol (**Figure 1**), are currently used but their use is limited due to their severe mechanism-related side effects, such us induction of acute extrapyramidal symptoms (EPS) and their inefficacy against the negative symptoms of the syndrome.[1]

The introduction of Clozapine (**Figure 1**) in therapeutics gave rise to a new generation of treatments for schizophrenia, called atypical antipsychotics, which add to the blockade of dopamine receptors a potent activity at serotonin ones.[2]

In this communication, we will describe our recent efforts to discover new templates for potential use as treatments for schizophrenia including a variety of different derivatives bearing a tetralone (**I, Figure 1**) or a tetralol core (**II**, **Figure 1**) which are able to antagonize receptors of the 5-HT_2 and D_2 families.[3]

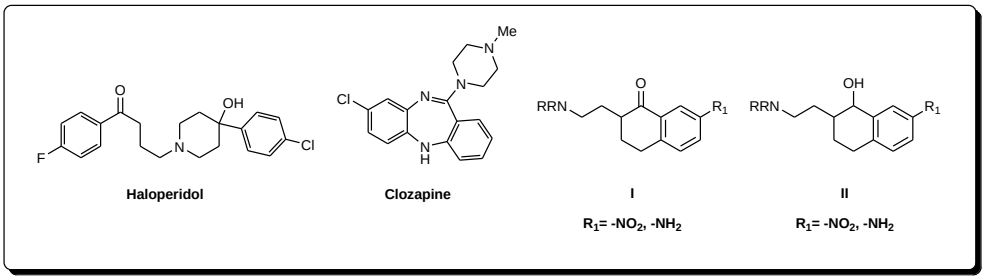

Figure 1

The synthetic routes and the binding affinities on human receptors implicated in schizophrenia of these new compounds will be further discussed in the presentation.

Acknowledgements: We would like to thank the Spanish *Ministerio de Ciencia e Innovación* for the financial support of this work (**Ref. SAF2009-13609-C04**).

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NEW PYRIDAZINOINDOLE DERIVATIVES AS MT_1 AND MT_2 AGONISTS: DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION

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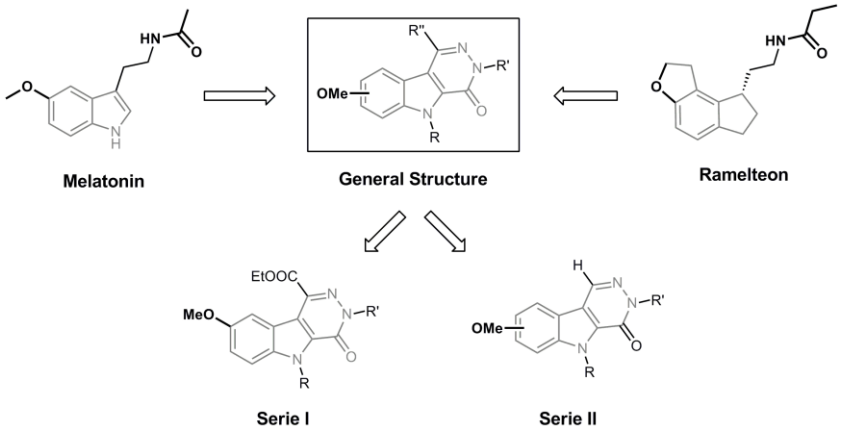
Insomnia is a widespread health problem. Recent studies estimated that 25-30% of adults in the general population experience occasional sleep problems. Moreover, 10% suffer from sleep disorders[1].

Melatonin is a pineal gland hormone involved in the regulation of sleep and circadian rhythms. Currently, the relation between melatonin and insomnia is an undeniable fact. This hormone acts through two GPCR receptors: MT_1 and MT_2.[2]

Pharmacotherapy is the most useful tool to treat insomnia. There are several sedative-hypnotic agents such as benzodiazepines and non-benzodiazepine drugs[2]. Due to benzodiazepine's adverse effects, new pharmacological strategies against insomnia have appeared in the last years.

A great deal of assays has shown that melatoninergic receptor agonists such as melatonin and ramelteon improve the sleep without including adverse effects[2]. Based on these studies and large experience in our research group[3], we decided to design and synthesize two novel pyridazinoindole series (I and II).



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LOOKING FOR NEW CHEMICAL DIVERSITY AGAINST MYCOBACTERIUM TUBERCULOSIS

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Tuberculosis remains a very important infectious disease causing significant morbidity and mortality today. In 2009, there were an estimated 8.9 -- 9.9 million incident cases of tuberculosis and 12 – 16 million prevalent cases of this disease. A total of 1..3 million deaths due to tuberculosis occurred in HIV negative people, and an additional 0.38 million tuberculosis deaths occurred in HIV-infected subjects. Furthermore, an estimated 250 000 (range, 230 000–270 000) had multidrug-resistant TB (MDR-TB), with bacillary resistance to at least isoniazid and rifampicin, were estimated to emerge in the same year, with around 5 -- 10% being extensively drug-resistant (XDR) tuberculosis. (Who report 2010)

There is an urgent need for new TB drugs, which can:

1. Target MDR or XDR strains;

2. Shorten treatment and

3. Be co-administered with HIV medications.

Nowadays, finding and developing new compounds active against MDR and XDR tuberculosis constitutes a main objective in the GSK anti-tuberculosis drug discovery portfolio. Both Target- and cell-based screens have being approached to identify new anti-TB compounds. The new chemical structure active against TB have two potential benefits in Drug Discovery:

1.- The new potential Lead for finding a anti-TB drug and

2.- The opportunity of finding a new biological target essential for this bacteria.

The TB DPU (GSK) aim with this initiative is to explore the greater compound diversity in the TB phenotypic assay. Then, finding Academic Institutions that are willing to share with us their libraries is a great opportunity because that chemical diversity is not found through commercial sources. In addition to that, these collaborations could be part of an approach to support the Tres Cantos Medicines Development Campus in its Open Innovation Strategy.

The TB DPU is looking forward to your comments about this initiative and finding opportunities for collaborations in this field.

DISCOVERY AND BIOLOGICAL EVALUATION OF NOVEL DECAHIDROQUINOLINAMIDES DERIVATIVES AS POTENT 11β-HSD1 INHIBITORS FOR THE TREATMENT OF GLAUCOMA.

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11β-Hydroxysteroid dehydrogenase type 1 (11β-HSD1) has attracted attention during the last few years due to its potential as a target for the treatment of metabolic syndrome and type 2 diabetes. However, 11β-HSD1 is also expressed in the basal cells of the corneal epithelium[1] and seems to be responsible for both the secretion and outflow of aqueous humor regulating intraocular pressure[2].

Therefore, 11β-HSD1 inhibitors have been of great interest and could provide a potential therapeutic target for lowering IOP in the treatment of glaucoma by topical administration.

We will disclose the discovery of a series of Decahidroquinolinamides (**1**), a novel class of 11β-HSD1 inhibitors[3] that shows potent and selective inhibition of both rabbit and human enzyme with excellent *in vivo* pharmacokinetics and ocular bioavailability.

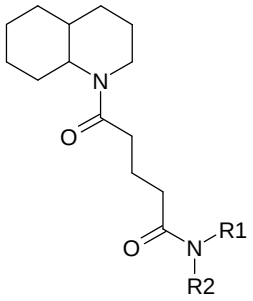

Figure (1)

1. Stokes et al*., Invest Ophthalmol Vis Sci.* **2000**, *41*, 1629-1638
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3. WO2010139827

SYNTHESIS OF NEW KYNURENAMINES UREA AND THIOUREA DERIVATIVES AS NITRIC OXIDE SYNTHASE INHIBITORS

M. Chayah, M. E. Camacho, M. D. Carrión, M. E. García, M. J. Pineda de las Infantas, L C. López-Cara, M. A. Gallo, A. Entrena, A. Espinosa

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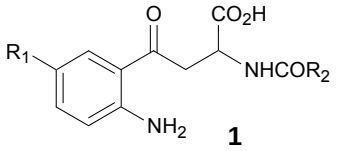
Nitric oxide (NO), which is produced from *L*-arginine by the nitric oxide synthase (NOS),[1] is a cell messenger with important regulatory functions in the nervous, immune and cardiovascular systems.[2] However, overproduction by nNOS or iNOS has been associated with neurodegenerative pathologies such as Parkinson's, Alzheimer's and Huntington's diseases[3] and chronic inflammatory diseases such as arthritis,[4] and septic shock.[5] Consequently, selective inhibition of these enzymes by means of synthetic derivatives constitute an interesting therapeutic objective.

Previously, we have described the synthesis of a series of kynurenine **1**[6] and kynurenamine **2**[7] derivatives as neuroprotective agents. Basing on these precedents and looking for new NOS inhibitors with structural relation with L-Arg, we have synthesized a family of urea and thiourea derivatives, represented by the general formula **3**, and we have started their biological evaluation.



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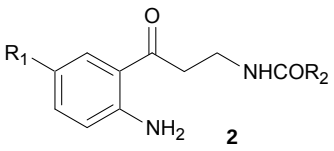

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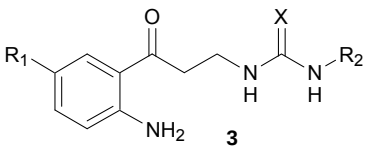

3

In this family, the modifications had been performed in the benzene ring (R_1) with electron-withdrawing (Cl), electron-donating (OCH_3) and electron-neutral (H), and in the urea substituent (R_2).

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Polymer-Drug Conjugates for the treatment of Familial Amyloidotic Polyneuropathy (FAP)

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Polymer Therapeutics are well-known as effective drug delivery systems with demonstrated clinical benefits since the 90's.[1] In particular polymer conjugates are considered new chemical entities capable to improve bioactive compound properties and decreasing their inherent limitations. Currently, a second generation of conjugates focused on improved structures, combination therapy or new molecular targets are needed to move this platform technology further.[2] Following these concepts, novel specific nanoconjugates for the treatment of neuropathological disorders are proposed in this study.

Familial Amyloidotic Polyneuropathy (FAP) is a neurodegenerative disorder characterised by systemic extracellular deposition of a mutated protein called transthyretin (TTR) as amyloid fibrils in several organs, mainly in the peripheral nervous system. This disease is characterised by an ascending sensorimotor polyneuropathy and progressive dysautonomia, becoming usually fatal 10 to 15 years after its onset. TTR has been proposed to trigger neurodegeneration through engagement of the Receptor for Advanced Glycation End products (RAGE). Prof. Saraiva et al. have discovered a specific peptidic sequence (named RAGE peptide) able to suppress TTR aggregate-induced cytotoxicity in cell culture[3] by means of TTRagg-peptide interaction, which impedes protein recognition by the receptor. This interaction is conserved across mouse and human species. Based on this finding, avoidance of TTR-aggregates cytotoxicity is a promising target for therapeutic propose in FAP treatment.

Due to the well-known limitation of specific peptide delivery *in vivo*, mainly due to a low stability and possible immunogenecity. Here we report, the PEGylation of RAGE peptide through two different types of linkage: peptidic and disulphide bond. Conjugates were biophysically characterised, also looking at conformation in solution, and its activity as TTR cytotoxicity inhibitors was studied. The results obtained allowed us to confirm that after the correct linker and conjugate conformational design, PEGylation of RAGE peptide can retain drug activity *in vitro*. A decrease in toxicity, immunogenicity and an enhancenment of peptide stability in blood is expected after PEGylation. This would offer the possibility to develop, for the first time, efficient macromolecular FAP inhibitors for clinical applications.

Acknowledgements: The authors would like to thank the Spanish Ministry of Science and Innovation (MICINN) (CTQ2007-60601, CTQ2010-18195, FPU grant (ref. AP2007-01665)), CIPF (Valencia, Spain) and Fundação para Ciencia e Tecnologia (FCT), Portugal.

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CHEMICAL MODULATION OF CELLULAR SIGNALLING ROUTES RELEVANT TO THE CONTROL OF APOPTOSIS

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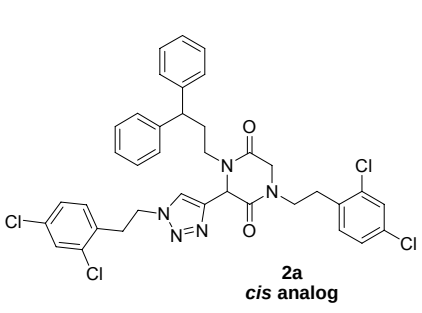
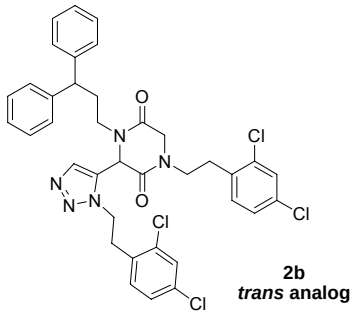
Apoptosis is a biological process relevant to human disease stated that is regulated through protein-protein complex formation. The apoptosome is a multiprotein complex that is of interest for the development of apoptotic modulators.[1] We have previously reported a peptidomimetic compound bearing a 3-substituted-piperazine-2,5-dione moiety as potent apoptotic modulator.[2]

Structural studies of this compound have shown the presence of *cis/trans* isomers of the exocyclic tertiary amide bond in slow exchange, which should be of high relevance for off-target interaction in front of the biological target.[3] This information encouraged us to mimic those isomers through an isosteric replacement of the amide bond by a 1,2,3-triazole moiety (1,4- and 1,5-disubstituted triazole to mimic the *cis* and *trans* isomers, respectively).



2a
***cis* analog**



2b
***trans* analog**

The syntheses of these restricted analogs were carried out using the Ugi multicomponent reaction[4] followed by an intramolecular cyclization. The full NMR analysis (including ^{1}H-^{15}N correlations at natural abundance) of these compounds has led us to the unambiguous characterization of the corresponding substitution patterns. Finally, the results on the inhibitory activity of these compounds have provided highly useful information for improving the inhibition of apoptosome.

We acknowledge the financial support from MICINN (Grans SAF 2008-00048 and BIO2007-60066), the fellowship to M.C. from CSIC JAE program and Esteve S.A. for the SEQT award.

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INTRODUCTION OF AMINES TO GET ANTIPARASITIC ACTIVITY ON NEW ANTIMALARIAL DRUGS

Jose M. Coteron, María J. Chaparro, Beatriz Díaz, Esther Fernández, Mariola Gordo, Laura de las Heras, María Marco, Esther Porras, Elena Sandoval, Pilar Ventosa and José M. Fiandor.

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Malaria is one of the major disease problems of the developing world. The most virulent malaria parasite is *Plasmodium falciparum* which is the cause of hundreds of millions of cases of malaria and about 1 million deaths each year, mostly in African children.

Falcipain-2 and falcipain-3, *P. falciparum* cysteine proteases, are involved in different processes at the erythrocytic cycle of the malaria parasite, including host hemoglobin hydrolysis, which is thought to be essential for the development of the *P. falciparum* parasite.

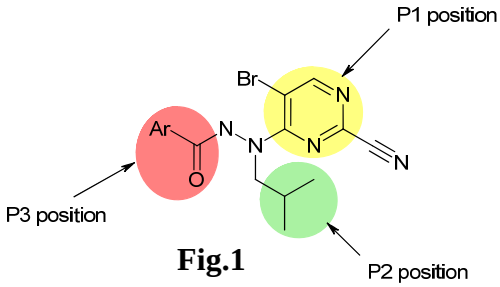


Fig.1

A new class of falcipain inhibitors[1] based on a heteroarylcarbonitrile scaffold was discovered, and subsequently optimized through SAR studies on the different structural areas of the chemical scaffold named P1, P2 and P3, as shown in **figure 1**, affording extremely potent inhibitors against falcipain-2 and falcipain-3 although lacking whole-cell activity.

After trying different approaches, antiparasitic activity was achieved with the introduction of basic amines like N-methylpiperazine at P3 position, as exemplified in **table 1**. Improvements of >300-fold in whole-cell activity were observed for compounds **b** and **d** over derivatives **a** and **c** respectively. In summary, excellent enzymatic inhibitors lacking whole-cell activity were transformed into excellent enzymatic and whole-cell inhibitors through introduction of a basic amine in their structure.

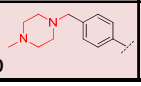
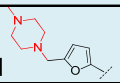
Ar	IC_{50} FP2(nM)	IC_{50} FP3(nM)	*PfIC50 (nM)*	Ar	IC_{50} FP2(nM)	IC_{50} FP3(nM)	*PfIC50 (nM)*
a 	0.7	54.7	>10000	c 	1.4	38.7	>10000
b 	<0.5	20.7	29	d 	<0.5	4.6	16.7

Table 1

[1] Coterón et al. *J.Med Chem. 2010, 52, 6129-6152*

NEW 3-D MODEL OF CFTR PROPOSES CONDUCTING STATE CONFORMATION & TARGET FOR CHANNEL BLOCKERS

J. Dalton[a], O. Kalid[b], J. Villà-Freixa[a], N. Ben-Tal[b].
[a] *Ciències Experimentals i de la Salut, Universitat Pompeu Fabra, Barcelona, Spain.*
[b] *George S. Wise Faculty Of Life Sciences, Tel Aviv University, Israel.*

Several models of Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) have been published so far, all derived by homology to the bacterial transporter Sav1866. While these models mostly agree with experimental data relating to overall CFTR structure, all present the channel in an outward-facing conformation that does not correspond to the conducting state. Here, a new 3-D model of CFTR has been developed that is in its "channel-like" conducting state, derived by a unique modeling approach combining multiple-template homology modeling and Rosetta refinement. In contrast to those previously published, the model is in agreement with expected channel properties such as pore shape and dimensions, relative solvent accessibility of pore residues and experimentally derived pairwise distances. The model also allows for an exploration of the interaction of anionic open channel blockers within the pore, revealing a common binding mode at the lower vestibule dominated by ionic interactions with K95 in agreement with experimental data, as well as interactions with W1145 and R352. The binding-site structure and binding-mode hypothesis have been further validated with a virtual screening experiment, showing known blockers significantly enriched compared to a random set of drug-like compounds. In addition, a model of mutant F508del CFTR has been derived from the *wt*, with both proteins subjected to molecular dynamics (MD) simulations. These reveal a destabilizing effect of the F508 deletion, in agreement with experimental data. Modeling and MD suggest previously unaddressed salt-bridge interactions that may be important for structural stability, as well as identifying pore-lining residues that likely take part in Cl^- conductance. As a consequence, the model may provide an improved structure-based framework for the design of CFTR modulators as potential Cystic Fibrosis therapeutics and channel blockers as potential anti-diarrheals.

SMALL-MOLECULE ACTIVATION OF PROCASPASE-9 AS A NOVEL ANTICANCER STRATEGY

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Apoptosis, or programmed cell death, is one of the physiological processes in charge of removing unwanted or extensively damaged cells from the body, in order to regain homeostasis. This process is executed by a family of cystein-proteases called caspases. In normal non-apoptotic cells, these hydrolytic enzymes are kept as inactive zymogens, named procaspases. There are two main pathways of apoptosis, the extrinsic and the intrinsic. Caspase-9 (C-9) is the apical caspase of the intrinsic pathway of apoptosis. This process is activated by cellular stressful conditions as DNA damage.

It has been widely described in the literature that the vast majority of cancers posses halted apoptosis mechanisms; thereby the reestablishment of such endogenous mechanisms is a therapeutic approach of great interest. In this study, a screening of chemical libraries was performed in order to find specific procaspase-9 activators, these small-molecules were characterized in vitro and in cell models. From the initial screening, four small-molecule activators were found who share several molecular and structural features. It was demonstrated in defined cell models that these compounds specifically activate procaspase-9, and hence, the intrinsic apoptotic pathway. The specificity of the identified compounds was demonstrated in experiments where the in cell expression of procaspase-9 was inhibited by means of siRNA. Structural characterization studies in silico suggested that the binding site of these compounds is in the vicinity of the active site of the enzyme and also allowed the identification of key residues for binding. Such predictions were corroborated using site directed mutagenesis.

NEW CATHEPSIN INHIBITORS TO EXPLORE THE FLUOROPHILIC PROPERTIES OF THE S_2 POCKET OF CATHEPSIN B. DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION

Santos Fustero,*[a,b] Vanessa Rodrigo,[b] María Sánchez-Roselló,[b] **Carlos del Pozo**,[a] Joaquín Timoneda,[c] Maxim Frizler,[d,e] Mihiret T. Sisay,[d,f] Jürgen Bajorath,[f] Luis P. Calle,[g] F. Javier Cañada,[g] Jesús Jiménez-Barbero,*[g] and Michael Gütschow*[d,e]

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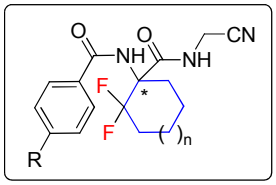

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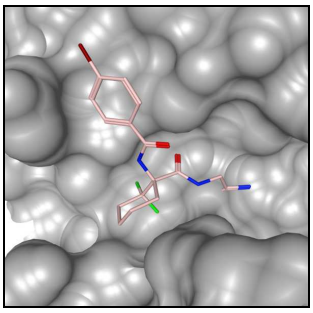
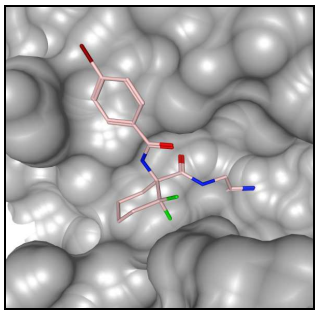
Based on β,β-difluorinated 1-amino-1-cyclopentane- and cyclohexane carboxylic acid scaffolds, a small library of new dipeptide nitriles was generated. The inhibitory activity of these derivatives against the human cathepsins B, K, L, and S was evaluated. Almost all of them inhibited cathepsins B and K, and were inactive against cathepsins L and S. To elucidate the orientation of the fluorinated face relative to the protein structure, molecular modeling studies of representative inhibitors in the active site of cathepsin B were performed. In the cases of the (*R*)-configured eutomers, the fluorine atoms are directed to the S^2 pocket, whereas in the cases of the (*S*)-configured distomers, the fluorinated face is solvent-exposed. The orientation of the enzyme-bound ligand was confirmed by NMR measurements, using transferred NOE (trNOE) and Saturation Transfer Difference (STD) experiments. On the basis of these findings, the fluorophilic nature of the S^2 pocket of cathepsin B could be demonstrated for the first time.[1]



Left: Modeled complex of (*R*)-**1a** inside the active site of Cat B.

Right: Modeled complex of (*S*)-**1a** inside the active site of Cat B.



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DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION OF NEW DISELENIDE COMPOUNDS AS ANTIPROLIFERATIVES AGENTS

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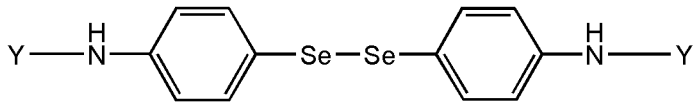
It is well known that cancer represents a major public health problem, as it is the second cause of death worldwide. Recent epidemiological, experimental and clinical studies have identified selenium as a chemopreventive agent against cancer, acting through mechanisms related to apoptosis, cell cycle and oxidative stress[1].The dose and chemical form in which the compounds are administered are critical for its biological activity. Organoselenuim derivatives are better tolerated and exhibited greater antitumor activity than inorganic forms. Among this group the diselenide function has emerged strongly in recent years, acting through different mechanisms.

In order to continue and complete the investigation previously done by our group in selenium derivatives[2,3], we report the synthesis and biological evaluation of novel diselenide compounds according to the general structure **1** , where specific groups to modify both volume and polarity are introduced without changing the molecular symmetry.



1

Novel selenocompounds were tested against PC-3 (prostate cancer) cell line, and two of them proved to be more cytotoxic than *cis*-platinum and taxol, drugs commonly employed in cancer theraphy. These compounds were also tested against four tumoral cell lines: CCRF-CEM (leukaemia), HTB-54 (lung cancer), HT-29 (colon cancer) and MCF-7 (breast cancer).The results evidence promising antiproliferative activities for diselenide compounds.

Acknowledgements: M.Díaz acknowledges the Association of Friends of the University of Navarra for a PhD Grant and the project funding by Ministerio de Ciencia e Innovación.

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NEMATICIDAL ACTIVITY OF NOVEL SELENOCOMPOUNDS

Enrique Domínguez Álvarez [a,c], Ylenia Baquedano [a], Esther Moreno [a], Marta Díaz [a], Carmen Sanmartín [a], Juan Antonio Palop [a], María Font [b], Lalla A. Ba [c], Claus Jacob [c].

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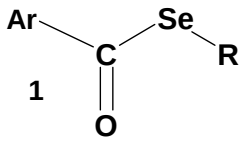
Scientific literature indicates that sulfur compounds such as alkyl sulfides and polysulfides possess antitumoral, antioxidant and antimicrobial activity [1]. Besides, abovementioned sulfur derivatives are also known to be nemotoxic agents [2]. On the other hand related structures with selenium instead of sulfur show an equal or improved behaviour in cancer cells and in ROS removal [3].

Within this context and based on the known parallelism in chemical properties and biological effects between sulfur and selenium organic molecules, we have ascertained whether the organoselenium derivatives designed and synthesized in Pamplona as antineoplasics with the alkyl selenide motif have also antiparasitic properties for being nematicides, with the aim to broaden the knowledge and range of the possible biological applications of these selenium molecules.



In this way around forty derivatives with CO-Se chemical bonds have been isolated. In general formula **1 Ar** is an aromatic or heteroaromatic ring with or without substituents, and **R** the second substituent, which can be, among others: CH_2COOH, CH_2COOCH_3, CH_3, CH_2CONH_2 or CH_2COCH_3 [4,5].

A selection of the aforementioned selenium compounds has been tested as nemotoxics in *Steinernema feltiae* in Saarbruecken. According to results obtained so far, all the structures tested show a higher toxicity against these worms than thiabendazole, the reference compound used in our study. Furthermore, certain molecules exhibit a DL_{50} in the micromolar range; therefore they can be considered as very promising novel nematicides with potential practical applications.

Acknowledgements: Authors wish to express their gratitude to the *Ministerio de Ciencia e Innovación* of the Government of Spain for financial support, and thank the ADA (*Asociación de Amigos de la Universidad de Navarra*) and Bancaja for the award of grants.

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A LACTOSE DERIVATIVE AS DIVALENT LIGAND FOR GAL3 AND MMP12. INTERACTION STUDY OF A TERNARY COMPLEX BY NMR AND MOLECULAR MODELLING.

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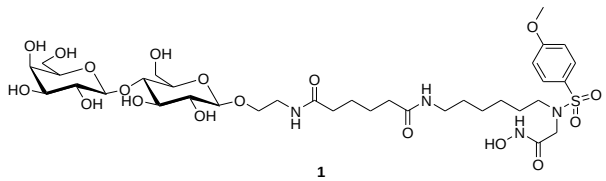
Galectins are a structural related family of animal proteins defined by having at least one characteristic carbohydrate recognition domain (CRD).[1] They bind carbohydrates in a variety of biological processes offering numerous possibilities as source of new drugs. In particular, Galectin-3 has been demonstrated to be implicated in cell growth, apoptosis, inflammation, angiogenesis, invasion and metastasis.[2] Additionally, there have been a number of enzymatic processes involving metalloproteinase inhibition which plays an important role in cancer and inflammatory diseases. Matrix Metalloproteinases (MMPs) overexpression or wrong modulation is also related to cancer,[3] they mediate the breakdown of connective tissue and are therefore targets for therapeutic inhibitors.[4] With these features in mind, we present here a new ligand **1** designed for targeting both class of proteins. The interaction of this lactose derivative with Galectin 3 and Matrix Metalloproteinase 12 has been studied by NMR from both the persective of the ligand (STD and trNOE)[5] and the proteins (^{1}H-^{15}N HSQC chemical shift mapping).[6] These experimental results together with molecular modelling allow us to describe a 3D model of the interaction.



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ANTIANGIOGENIC AND ANTI-CANCER POLYMER-DRUG CONJUGATES IN COMBINATION THERAPY

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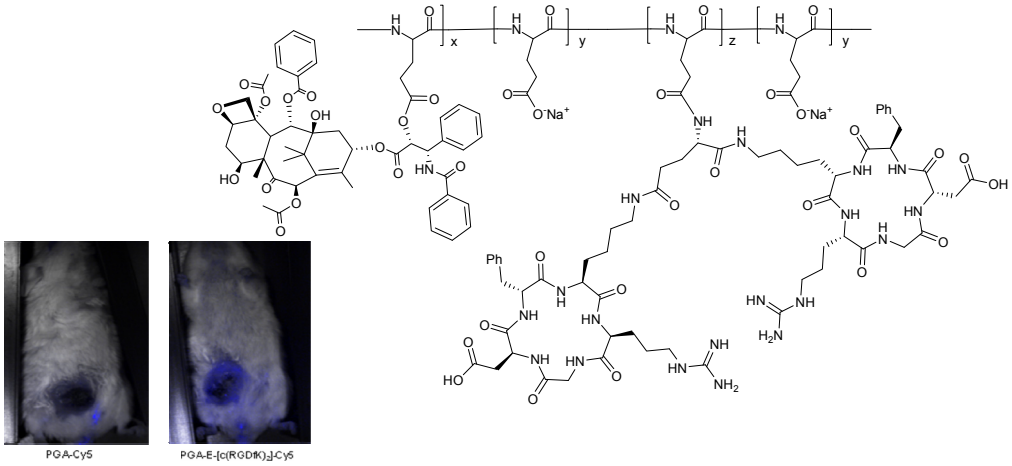
Angiogenesis, new capillary blood vessel growth from pre-existing vasculature, is a critical factor in cancer progression. Therefore, anti-angiogenic therapy, alone or in combination with conventional cytotoxic therapy, may be a promising therapeutic approach[1]. Paclitaxel (PTX) is a potent cytotoxic hydrophobic drug that exhibits anti-angiogenic effects at low dose; however, its use is limited by severe side effects.

In order to overcome these drawbacks, a polyglutamic conjugate of PTX has been designed and synthesized, (PGA)-PTX-E-[c(RGDfK)$_2$] [2]. This nanodrug passively targets the tumor tissue exploiting the enhanced permeability and retention effect (EPR). The cyclic RGD peptidomimetic plays a dual role both, as an antiangiogenic agent and additionally offering active targeting to the $\alpha v\beta 3$ integrin, which is overexpressed in tumor endothelial and epithelial cells. In this communication, the physicochemical characterization of the conjugate, as well as the *in vitro* and *in vivo* experiments that proves its biological activity will be discussed.



Acknowledgements: The authors would like to thank the Spanish Ministry of Science and Innovation (MICINN) (CTQ2007-60601, CTQ2010-18195, EUI2008-03905) and Centro de Investigación Príncipe Felipe (Valencia, Spain).

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NOVEL AMINATED COLCHICINE-SITE LIGANDS

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Combretastatin A4, is a potent cytotoxic agent that strongly inhibits the polymerization of tubulin by binding to the colchicine-binding site of the β-tubulin subunit. Microtubules are built up by polymerization of αβ-tubulin dimers, and are involved in a wide range of cellular functions such as cell division where they are responsible for mitotic spindle formation and chromosomal separation.

Colchicine is the first drug that is known to bind tubulin, and the colchicine binding site has been characterized from a complex with colchicine and a stathmin-like domain. CA-4 is a good structural template for the design of related compounds. More soluble derivatives such as CA-4 phosphate sodium salt and the amino acid hydrochloride salt AVE8062 have been evaluated. They show potent activities to disrupt vasculature and to reduce significantly the tumor blood flow [1].

We are now developing a research project aimed at synthesizing new families of microtubule destabilizing agents [2]. In this communication, we will describe the preparation and the evaluation of new aminated compounds belonging to the isocombretastatin and combretastatin families (figure 1). The introduction of nitrogen atoms in the B ring and the amino group as substituent could enhance efficacy and polarity.

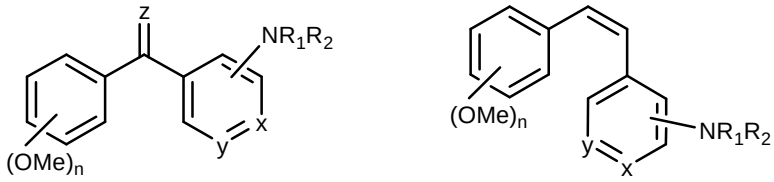

Fig.1. Families of designed Isocombretastatins and Combretastatins.

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Polymer Therapeutics as Hypoxia Inducible Factor (HIF) inhibitors

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The development of intratumoural hypoxia is a universal hallmark of rapidly growing solid tumours, which leads to epigenetic and genetic adaptation of clones, increased invasiveness and metastasis, and confers resistance to current therapies. In animal models HIF-1 overexpression is associated with increased tumour growth, vascularisation and metastasis, whereas HIF-1 loss of function has the opposite effect, thus validating HIF-1 as a target[1].

We report here the first examples of Polymer Therapeutics synthesised with the aim of inhibiting Hypoxia Inducible Factor (HIF). Four compounds were selected from a large family of similar molecules known as Stilbenes; Diethylstilbestrol (DES), Bisphenol A (BISPA), Dienestrol and Hexastrol. These are non-steroidal molecules with structural similarities to oestrogen, and of which DES and BISPA have previously been reported for HIF-1α inhibition.[2,3] These molecules were incorporated into a poly(ethylene glycol) (PEG) based polyacetal system using a acid-catalysed reaction of selected diols with divinyl ethers without the need for biodegradable linkers.[4,5] These systems were found to be in a Mw range 24000-36000g/mol and pdi ~1.4. These polymers are stable over long periods of time at neutral pH such as is found in blood plasma, but hydrolytically cleaved under acid conditions (such as those found in lysosomes or the extracellular fluid of some tumours) yielding the free drug. Additionally, the advantages of the polymer conjugation include that an otherwise water insoluble drug can be solubilised and that the polymer size can take advantage of the EPR effect leading to tumour targeting.[6] The polymers have little to no toxicity and therefore provide an opportunity to parentally administer higher drug dosage.

To study selected molecules, and their polymer conjugates in targeting the HIF pathway, we used a stable luciferase reporter gene developed in HeLa cells (HeLa 9xHRE-LUC), which was also used to analyse their cytotoxicity. HPLC data showed ~30% drug release after 48h at pH5.5. This was reflected in the cells with inhibition of HIF up to 50%. Whilst the polymers were non-toxic and exhibited HIF inhibiting properties, the free drugs had associated cytotoxicity.

Acknowledgements: The authors would like to thank the MICINN (CTQ2007-60601, CTQ2010-18195, EUI2008-03904), Instituto de Salud Carlos III (PI08-1255) and CIPF(Valencia, Spain).

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A NEW FAMILY OF "MODIFIED" NUCLEOSIDE ANALOGUES AS POTENTIAL ANTIVIRAL INHIBITORS

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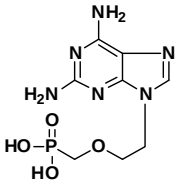
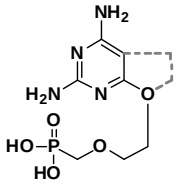
Recently, a new structural subclass of acyclic pyrimidine nucleoside phosphonates (ANP) named as PMEO-DAPym (2) was identified.[1,2] Unlike other ANPs, these derivatives are recognized by HIV-1 RT as a purine base instead of a pyrimidine base and incorporated opposite to thymine (in DNA) or uracil (in RNA). PMEO-DAPym represents a prototype compound of a novel class of pyrimidine acyclic nucleoside phosphonates with potential antiviral or antimetabolic properties.[3]

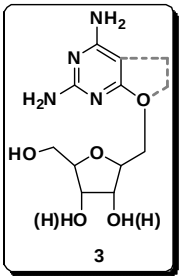
We are currently working on the design and synthesis of new "nucleos(t)ides" analogues of PMEO-DAPym (3) where the acyclic alkyl chain has been replaced by an intact furanose ring. These compounds would allow us to study whether the corresponding nucleoside analogues could be converted into triphosphates by cellular kinases and incorporated into DNA as a purine nucleotide. The peculiar structure of these novel nucleosides may represent a new family of antiviral inhibitors with eventually lower drug resistance levels.



PME-DAPym (1)



PMEO-DAPym (2)



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Acknowledgements: The Spanish MICINN is acknowledged for financial support (SAF2009-13914-C02-01).

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NUCLEASE ACTIVITY AND ULTRASTRUCTURAL EFFECTS OF SULFONAMIDES WITH ANTILEISHMANIAL AND TRYPANOCIDAL ACTIVITIES

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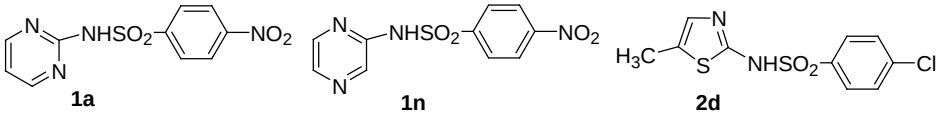
Trypanosoma cruzi and *Leishmania spp.* are the causative agents of a number of human and animal diseases. However, no vaccines or safe chemotherapy are available.[1] As a part of our ongoing research in the design and synthesis of new antikinetoplastid candidates, herein we report the in vitro efficacy of a series of sulfonamides against epimastigotes of *T. cruzi* and promastigotes of *Leishmania spp.* To elucidate the probable mechanism of action, the DNA interaction of selected sulfonamides was investigated by nuclease activity assays. In addition, the cellular targets of these compounds in treated parasites were also analyzed by transmission electron microscopy. The most active compound **1a** displayed significant in vitro activity against *T. cruzi* epimastigotes and *Leishmania spp.* promastigotes without toxicity to J774 macrophages. Our findings show that the efficacy of compound **1a**, previously seen in murine leishmaniasis caused by *L. infantum,*[2] extends to *L. braziliensis*, *L. guyanensis, L. amazonensis* and *T. cruzi*. Selected sulfonamides **1a**, **1n** and **2d** showed nuclease activity in the presence of copper salt. Mechanistic data reveal the involvement of a redox process. Evidence for the formation of reactive oxygen species (ROS) responsible for DNA strand scission is provided. Electron microscopic analysis of *Leishmania infantum* promastigotes treated with compounds **1a, 1n** and **2d** shows an overall cellular disorganization effect which is mainly addressed to DNA bearing structures such as the nucleus, mitochondria and kinetoplast. These ultrastructural changes along with the nuclease activity corroborate the antiprotozoal effect of these sulfonamides which seems to be mainly addressed against both nuclear and extranuclear DNA.



Acknowledgements: This study has been financed by grants: AECID (A-024457/09), Conselleria de Sanitat-Generalitat Valenciana (AP116/09, AP158/10), University CEU-UCH (PRCEU-UCH18/10) and Ministerio de Ciencia e Innovación (CONSOLIDER-INGENIO CSD2010-00065). Bilbao is fellow MAEC-AECID. We thank the UAH Microscopy Facility.

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DEVELOPMENT OF MOLECULAR PROBES FOR THE STUDY OF 5-HT$_{1A}$ RECEPTOR

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In spite of the big efforts that academia and industry are making, there is a significant decrease in the number of new drugs. In order to solve this pharma innovation gap we do not only need to identify new drugs but also it is crucial to validate/identify new therapeutic targets. In this sense, functional proteomics and activity-based protein profiling have emerged as powerful chemical strategies to improve our knowledge of native biological systems. This approach has been successfully applied to the study of different enzyme families related to pathologies.[1] However, the superfamily of G protein-coupled receptors (GPCRs) remains to be addressed, though it represents the 50% of the druggable genome.[2]

In this context, we have focused our efforts on the 5-HT$_{1A}$ receptor (5-HT$_{1A}$R) that is one of the most important GPCR therapeutic targets. Herein, we report the development of a set of labeled ligands targeting this receptor, based on our previous experience.[3] Among the synthesized compounds, fluorescent derivative UCM-120[4] (K_i = 2 nM) and biotin-derivative UCM-122 (K_i = 4.7 nM) have been identified as high-affinity ligands that enable direct visualization of the 5-HT$_{1A}$R in cells. In addition, dual probes that combine benzophenone and biotin or a fluorophore in the same molecule are being evaluated for covalent binding and affinity pull-down of target proteins. These results provide the basis for further development of these derivatives as probes for serotonin 5-HT$_{1A}$R in an approach that we are further extending to other GPCRs.

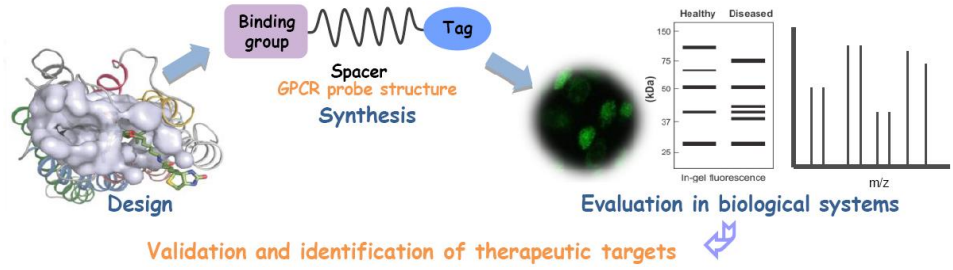

Figure 1. Strategy for the development of chemical probes targeting GPCRs.

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CARD-CARD INTERACTIONS: PEPTIDES INHIBITORS OF APOPTOSIS AND INFLAMMATION

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The CARD (Caspase recruitment domain) domain is present in a large number of proteins[1]. Initially the CARD domain was recognized as part of the caspase activation machinery. CARD-CARD interactions have a role in apoptosis as responsible for the Apaf-1 mediated activation of procaspase-9 in the apoptosome[2]. CARD-containing proteins mediate the inflammasome-dependent activation of the pro-inflammatory caspase-1[3]. More recently new roles for CARD-containing proteins have been reported in signalling pathways associated to immune responses[4]. The functional role of CARD-containing proteins and CARD domains in coordinating apoptosis, inflammatory and immune responses are yet elusive. We have explored the putative crosstalk between apoptosis and inflammation by analyzing the modulatory activity on Apaf-1/procaspase-9 interaction and on the inflammasome-mediated caspase-1 activation of CARD-derived polypeptides. To this end we have analyzed the activity of individual recombinant CARD domains and rational designed CARD-derived peptides.

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DESING AND SYNTHESIS OF 3,4-DIHYDROQUINAZOLIN-2(1*H*)-ONE AND -THIONE DERIVATIVES AS NITRIC OXIDE SYNTHASE INHIBITORS

M. E. García, M. E. Camacho, M. D. Carrión, M. Chayah, M. J. Pineda de las Infantas, L C. López-Cara, M. A. Gallo, A. Entrena, A. Espinosa

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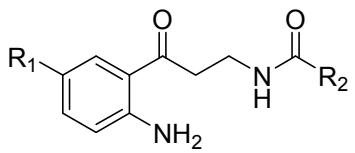
The physiological roles of nitric oxide (NO) have been extensively studied in recent years.[1] In mammalian systems NO is produced by a two-step oxidation of the terminal guanidine group of L-arginine, by nitric oxide synthase (NOS).[2] Three distinct isoforms of this enzyme have been identified: nNOS, eNOS, and iNOS.[3] An NO overproduction by nNOS is implicated in chronic neurodegenerative pathologies[4] such as Huntington's disease, or amyotrophic lateral sclerosis. Over expression of iNOS has been implicated in a number of inflammatory diseases,[5] for example, septic shock and rheumatoid arthritis. Moreover, a reduced NO production by eNOS may cause hypertension and atherosclerosis.[6]

Looking for new nNOS and iNOS inhibitors with structural relation with the main brain metabolite of melatonine, the N-acetyl-5-methoxykynurenamine **1** (R_1 = OCH_3, R_2 = CH_3)[7] we have synthesized and evaluated several families of rigid analogue compounds **2**, that include 4,5-dihydro-1*H*-pyrazoles (X = CH_2)[8].



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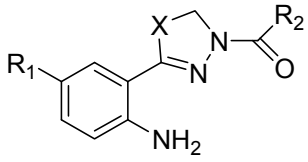

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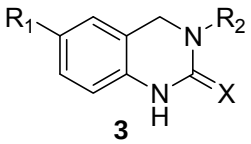

3

Herein we report the synthesis of a new family of 2,3-dihydroquinazolin-2(1*H*)-one and -thione derivatives **3**, where the 2-NH_2 group of the benzene ring is making a cycle, that restricts the conformational freedom, to evaluate their inhibitory activity.

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INFLUENCE OF SOLUTION CONFORMATION OF NOVEL DES-POLYACETALIC SYSTEMS ON THEIR THERAPEUTIC OUTPUT AS ANTICANCER NANOMEDICINES.

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The design of improved polymeric carriers to be used in the next generation of polymer therapeutics is an ongoing challenge. There is an urgent need to move away from heterogeneous, undefined carriers and to develop biodegradable systems with well defined polymer structures, which can better exploit EPR-mediated tumour targeting[1] and control the drug release in a specific site of action. Within this context, we previously designed pH-responsive polyacetalic systems, tert-polymers, where a drug with the adequate diol-functionality was incorporated within the polymer mainchain. The synthetic, non-steroidal oestrogen, diethylstiboestrol (DES) clinically used for the treatment of prostate and breast cancer was chosen as model drug.[2]

In order to improve the properties of this *tert*-polymer, novel polyacetalic systems as *block-co*-polymers, with well-defined structure have been obtained. This second generation polyacetals allowed higher drug capacity than the *tert*-polymer, greater rate of DES release at acidic pH and due to its controlled amphiphilic character readily formed micelle-like structures in solution. These features result in an enhancement of conjugate therapeutic value in selected prostate cancer cell models. Exhaustive physico-chemical characterisation focusing on nanoconjugate solution behaviour and using advanced techniques, such as, pulsed-gradient spin-echo NMR (PGSE-NMR) or small-angle neutron scattering (SANS), has been carried out in order to demonstrate this hypothesis. Clear evidence has been obtained of significantly different conformation in solution for both polyacetals. These results clearly demonstrate that an adequate control on molecular or supramolecular conformation in solution with polymer therapeutics is a key issue to achieve the desired therapeutic output.[3]

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PERMEABILITY AND BIODISPONIBILITY OF NEW ANTIPARASITIC AGENTS

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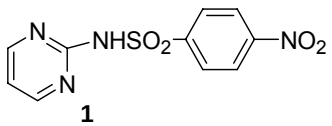
Protozoa of the order Kinetoplastida are the causative agents of a number of human and animal diseases including leishmaniasis (*Leishmania spp*.).[1] These infections have a large disease burden. However, few therapeutic agents are currently available. Moreover, many of them produce adverse side effects, in certain cases with high toxicity, require inconvenient routes of administration, long-term treatments and show low activity in immunosuppressed patients. In addition, the widespread development of resistance by some *Leishmania* strains to antimonial compounds, constitutes an important health problem.[2] Therefore there is an urgent need for the discovery of new therapeutics displaying leishmanicidal activities.

It is well-known that the sulfonamide pharmacophore is an important structural core in medicinal chemistry that shows a broad spectrum of pharmacological activities. Thus in vitro antileishmanial and trypanocidal effects of compounds containing the sulfonamido moiety have been shown.[3] However, a limited number have been tested in a murine animal model and neither of them displayed significant *in vivo* activity.

Our research group has obtained and characterized compounds containing the sulfonamido moiety and their leishmanicidal effect has been assessed *in vitro* and *in vivo*. Among the compounds evaluated, sulfonamides **1** and **2** showed *in vivo* activity in a Balb/c mice model of *L*. *infantum*.[4] The next step, whose results will be presented in this congress, was to study the permeability of these compounds across the intestinal barrier and to determine their biodisponibility. The excellent preclinical results that we have obtained give us green light to continue the studies with these molecules.



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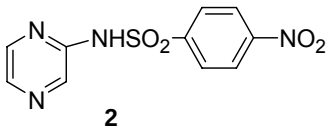

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DEVELOPMENT OF NEW LIGANDS FOR THE VALIDATION OF THE LYSOPHOSPHATIDIC ACID RECEPTOR LPA_1

Inés González-Gil, Debora Zian, Henar Vázquez-Villa, Silvia Ortega-Gutiérrez, Mª Luz López-Rodríguez

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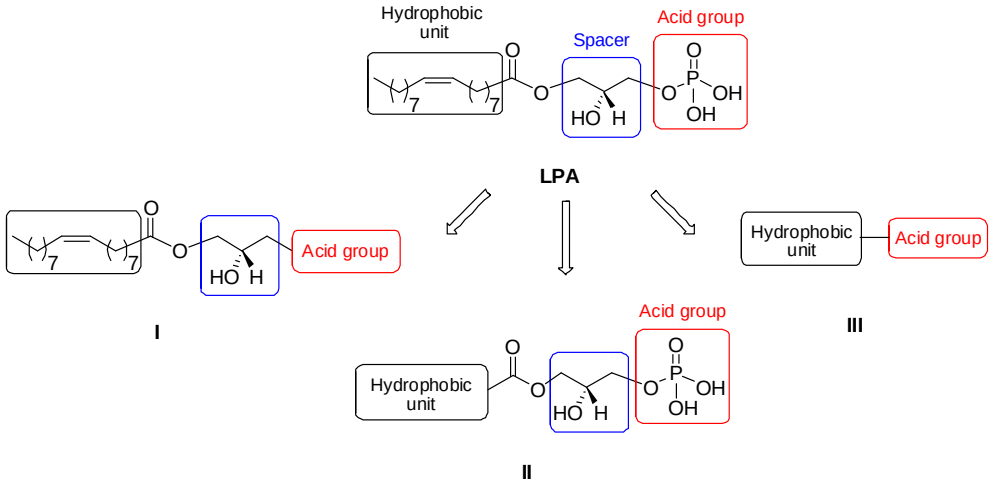
Validation of therapeutic targets is nowadays a prior objective, as the need of new targets to face unmet clinical needs is constantly increasing.[1] In this aspect, G protein-coupled receptors (GPCRs), which constitute around 50% of the druggable genome, stand out as a suitable family for the development of new drugs.[2] Among them, former orphan Edg2 receptor has been recently characterized as the lysophosphatidic acid (LPA) receptor of type 1 (LPA_1R). Given the key role of LPA in the central nervous system,[3] the need of selective and high affinity ligands of LPA_1R is critical for the validation of this receptor.

Herein, we present the design, synthesis and biological evaluation of three series (**I**-**III**) of new compounds based on the structure of the endogenous ligand LPA with the objective of identifying new LPA_1R ligands. These results should provide the basis for further biological studies to enlighten the role of LPA_1R in human physiology.



Acknowledgements: This work has been supported by grants from the Spanish Ministerio de Ciencia e Innovación, (MICINN, SAF2010-22198) and Comunidad Autónoma de Madrid (S-SAL-249-2006). The authors thank MICINN for a predoctoral grant to I.G-G.

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BILASTINE POLYMORPHIC FORMS (I, II AND III) UNDER STRESS CONDITIONS [ICH Q1A (R2)]: POLYMORPH I, THE SELECTED SOLID FORM

Lorena González, Carmen Pumar, Maravillas Bordell, Gonzalo Canal, Rosa Urzay, Karmele Sustacha, Ana Gonzalo, MªLuisa Lucero

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Selection of appropriate crystalline forms is a matter of great concern at early stages of a new chemical entity (NCE) development. Rationale for the choice of polymorphs is based on relevant aspects such as bioavailability, activity, efficacy, safety, manufacturing process and stability. Current practices conclude that the most stable polymorphic form of a drug substance is used to formulate due to its lowest potential for conversion among different polymorphs. Therefore, stability is an integral part of polymorphism studies.

Stability under forced conditions focus on the effect of temperature and humidity, photolysis, acid and base hydrolysis and oxidation. In this study, photostability of bilastine was thoroughly investigated on samples subjected sequentially to confirmatory and stress conditions in compliance with ICH Q1B.

Bilastine is a novel antihistaminic agent of great efficacy and safety, which has diverse crystalline forms (F96221BM1, F96221BM2 and F96221BM3). Behaviour of these different polymorphic forms was evaluated searching for degradation mechanisms and potential degradation products.

Degraded samples of bilastine polymorphic forms were generated after direct exposure to light providing an overall illumination of not less than 1.2 million lux · hours and an integrated near- ultraviolet energy of not less than 200 watt · hours / square meter (Confirmatory conditions) and an overall illumination of not less than 3×1.2 million lux · hours and an integrated near- ultraviolet energy of not less than 3×200 watt · hours / square meter (Stress conditions).Studies were performed at controlled temperature (25ºC).

Stability samples were peerly examined at the aid of HPLC (UV/vis), RX Difraction, IR and DSC techniques. Under these stress conditions, potential degradation products (oxidative route) were found solely for F96221BM3. This polymorphic form differed from the one used for manufacturing Bilaxten® at industrial scale, F96221BM1, which is the most stable candidate.

The study provided some relevant conclusions regarding F96221BM1 stability under extreme light exposure. Such conclusion had been previously made in other stability studies, (i.e. regarding bilastine 20 mg tablets and included in the marketing authorisation for Bilaxten). Furthermore, former studies on stress testing in solution also confirmed excellence of F96221BM1 in terms of stability.

Polymorph F96221BM1 exhibited great stability whereas Polymorph 2 and 3 showed a major liability at the sight of performed stability studies (See Photostability and Stress in solution studies).

Acknowledgements: This work was supported in part by the Department of Industry, Commerce and Tourism of the Basque Government (INNOTEK-FEDER funding).

DEVELOPMENT OF CHEMICAL PROBES FOR THE STUDY OF SEROTONIN 5-HT$_6$ RECEPTORS

Juan A. González-Vera, Henar Vázquez-Villa, Bellinda Benhamú, María L. López-Rodríguez and Silvia Ortega-Gutiérrez

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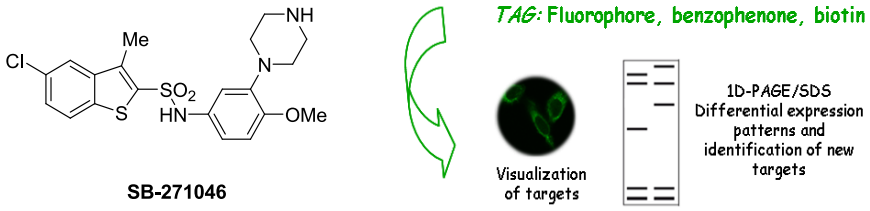
Development of compounds that enable direct observation of sub-fractions of the proteome has become of paramount importance in the quest of understanding biological processes at the systems level. In this context, activity-based protein profiling can be considered as one of the major contributions. This approach has been successfully applied to the study of different enzyme families.[1] However, the superfamily of G protein-coupled receptors (GPCRs), which account for more than the 50% of the druggable genome,[2] remains to be addressed. Among the different families of GPCRs, we have focused on the serotonin receptors, particularly on the 5-HT$_6$ subtype, due to their therapeutic potential for the treatment of neuropsychiatric disorders.[3]

Herein we present our results concerning the synthesis and evaluation of novel labeled ligands for the study of 5-HT$_6$ receptors. Our strategy encompasses the selection and tagging of the known high affinity 5-HT$_6$ antagonist SB-271046 (K_i = 1 nM)[4] with fluorescent, photoactivatable and/or affinity tags. Fluorescent ligands were synthesized by attachment of the corresponding fluorophore (dansyl or lissamine) to different positions of SB-271046. Synthesized probes have shown both good fluorescent properties and high binding affinities, which enable direct visualization of the h5-HT$_6$ receptors in cells.[5] In addition, probes which combine benzophenone and biotin or a fluorophore in the same molecule are being evaluated for covalent binding and affinity pull-down of target protein. These strategies should contribute to optimize the therapeutic exploitation of known or new members of the GPCR superfamily by providing valuable information about their location or level of expression.



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MODELING DEVELOPMENT AND BIOPHYSICAL VALIDATION OF NEW HEPARANASE HITS

Silvia Mosulén[a], Rafael Gozalbes[a], Rodrigo J. Carbajo[a], Antonio Pineda-Lucena[a]

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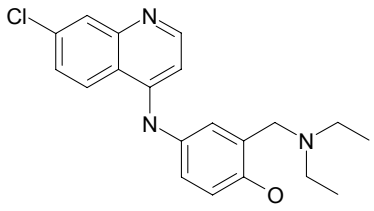
Heparanase is a key enzyme involved in the dissemination of metastatic cancer cells[1]. In this study we have used a combination of *in silico* techniques and experimental methods with the aim to find new inhibitors for this protein target. A structure model of heparanase from sequence homology was used to dock 27 known heparanase inhibitors and a commercial collection of drugs and drug-like compounds. The docking scores thus obtained were combined with those of a pharmacophore model recently published in which we used the same sets of chemicals[2]. Compounds were then ranked according to their theoretical heparanase binding ability, and the highest positioned commercial drugs were then selected for further experimental evaluation.

Biophysical methods (NMR and SPR) were applied to assess experimentally the interaction of the top ranked ligands with heparanase. Binding specificity was evaluated via competition experiments, using a known inhibitor of heparanase (suramine). Three of the selected drugs were shown to bind to the active site of the protein and their K_D values determined. Among them, the antimalarial drug amodiaquine presented low micromolar affinity towards the protein, and was singled out for the implementation of a medicinal chemistry campaign based on its chemical scaffold. Other known antimalarials and commercial compounds chemically related to amodiaquine were also explored against heparanase using the above biophysical methods. A subset of fourteen 4-arylaminoquinolines from a global set of 249 analogues of amodiaquine was extracted by application of the *in silico* models, a QSAR solubility prediction model[3] and chemical diversity analysis. Some of these compounds showed heparanase inhibition at micromolar ranges.



Amodiaquine

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THE ATP NON-COMPETITIVE CDK2/CYCLIN A INHIBITOR NBI1 SENSITIZES ERLOTINIB-RESISTANT CANCER CELLS TO THE COMBINATION TREATMENT

Tatiana Guevara[a,b], Mónica Sancho[a], Enrique Pérez-Payá,[a,b] Mar Orzáez[a]

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Abnormalities in the expression level of epidermal growth factor receptors (EGFRs) have been found in a wide range of cancers, including lung, breast and colon carcinomas.
A number of EGFR inhibitors have been developed such as monoclonal antibodies and low-molecular weight TK inhibitors, which compete with ATP to block the receptor catalytic domain (e.g. trastuzumab, cetuximab, gefitinib, erlotinib, lapatinib, erlotinib, AG1478). Currently, these agents have an established therapeutic role in non small cell lung, colorectal, pancreatic, breast and head and neck cancers. In particular, erlotinib has been approved by the FDA for the treatment of different cancers. However, several resistances have now been described in particular in breast cancer.

Molecular pathways are highly interconnected and the appearance of compensatory mechanisms to promote cancer cell survival after therapy represents the major limitation of molecular-targeted therapies. Combination therapies are thus emerging as an added value for cancer treatment.
Provided that in an EGFR resistant-setting defects on the nuclear translocation of the cyclin-dependent kinase inhibitor p27KIP have been described and increased activity of CDK2 has been detected, combination of CDK and EGFR inhibitors has been explored to define the basis of new combination therapies.
Current chemical inhibitors of CDKs are mostly directed to the ATP-binding site of CDKs. They have the ability to block neoplastic cell proliferation and induce apoptosis but have low specificity, which increases the occurrence of unwanted side effects.
We have previously reported the identification of an hexapeptide, NBI1, which inhibits the kinase activity of the CDK2/cyclin A, through its binding to cyclin A [1]. NBI1 does not compete with ATP nor with the CRS (substrate recruitment site).
Here we comparatively analyze the use of ATP competitive (R-roscovitine) and non-competitive (NBI1) CDK inhibitors in combination with EGFR-inhibitors (erlotinib) in both erlotinib-resistant and –sensitive breast cancer cell lines. The analysis of the molecular mechanism of action of the different drug combinations suggests that the use of CDK ATP non-competitive inhibitors and in particular CDK2/cyclin A inhibition provides re-sensitization to EGFR-inhibitors by apoptosis induction via caspase-10 activation.

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MINOCYCLINE INHIBITS CELL DEATH AND DECREASES MUTANT HUNTINGTIN AGGREGATION BY TARGETING APAF-1

Andrés E. Herrera[a], Mar Orzáez[a], Anna Gortat[a], Rodrigo J. Carbajo[b], Antonio Pineda-Lucena[b], Enrique Pérez-Payá[a, c] and Mónica Sancho[a].

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Minocycline (7-dimethylamino-6-dimethyl-6-deoxytetracycline) is a second-generation tetracycline that can cross the blood-brain barrier and has anti-inflammatory and neuroprotective effects. The potential of minocycline as a drug for treating Huntington's disease (HD) has been studied however, the molecular mechanism underlying the neuroprotective properties of minocycline remains elusive. In this study, we tested the hypothesis that a principal cellular target of minocycline is Apaf-1, a key protein in the formation of the apoptosome, a multiprotein complex involved in caspase activation. Minocycline binds to Apaf-1, as shown by nuclear magnetic resonance spectroscopy, and inhibits apoptosome activity *in vitro* and in *ex vivo* models. As a consequence minocycline-treated cells as well as Apaf-1 knock-out cells are resistant to the development of mutant huntingtin-dependent protein aggregation.

DESIGN AND SYNTHESIS OF RRE-Rev INTERACTION INHIBITORS OF HUMAN INMUNODEFICIENCY VIRUS TYPE-1 (HIV-1)

Ignacio Ibáñez,[a] Luis González,[b] Silvia Catalán,[a] José Gallego,[b] Santos Fustero [a,b]

[a]*Centro de Investigación Príncipe Felipe (CIPF), Valencia, España.* [b]*Universidad Católica de Valencia, España,* [c]*Universidad de Valencia, Valencia, España.*

HIV remains a global health problem of unprecedented dimensions.[1] Many efforts in this field have given rise to an important library of drugs capable to inhibit the virus life cycle, reverse transcriptase and viral protease inhibitors mostly. For that reason, discover new targets and effective molecules capable to inhibit the HIV life cycle is being considered the *state-of-the-art* in Medicinal Chemistry nowadays. The HIV-1 genomic RNA contains an asymmetric internal loop (RRE) with a major groove widened by several non-canonic base pairs. This loop is recognized by the viral protein Rev through an arginine-rich α-helix motif (Rev_{34-50}) (Figure 1).[2] Since the RRE-Rev interaction has an essential role in viral replication, those ligands capable of binding the RRE loop with high affinity, specificity and blocking its interaction with Rev represent useful leads for designing new anti HIV-1 agents.

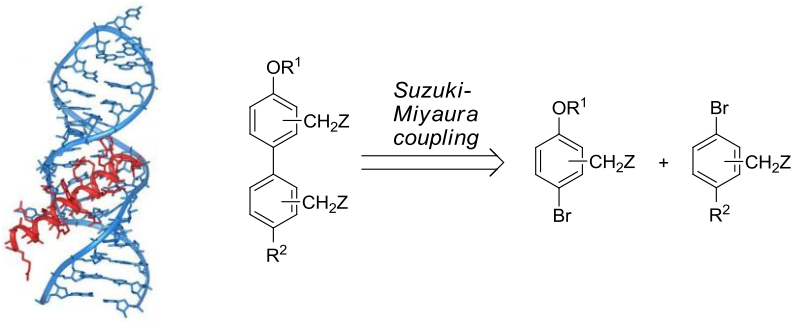

Figure 1. *Three-dimensional structure of the complex between internal loop RRE and* Rev_{34-50} *α-helix. Retro synthetic approach to the aim scaffolds (Z = functional group)*

We have designed a series of aromatic ligands (Figure 1) that mimics the spatial distribution of the Rev_{34-50} side chains in their complex with RRE. For doing that, we have taken into account the three dimensional structure of the complex as well as molecular modeling techniques and *docking* calculations.

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MODULATION OF ANTIOXIDATIVE DEFENSE ENZYMES IN A HUMAN HEPATOCARCINOMA CELL LINE BY SELENOIMIDOCARBAMATES

Elena Ibáñez[1,*], Iranzu Lamberto[1], Iosu Jiménez[1], Beatriz Romano[1], Daniel Plano[1], Alfonso Calvo[2], Juan Antonio Palop[1], Carmen Sanmartín[1], Lutz Schomburg[3]

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[3] *Institute for Experimental Endocrinology, Südring 10, CVK, Charité - Universitätsmedizin Berlin, 13353-Berlin, Germany*

The essential micronutrient selenium (Se) exerts its biological effects mainly through enzymatically active selenoproteins whose functions are related to stress response and might be closely linked with the growth of cancer cells. In the present study we evaluate the activity of various antioxidant and redox proteins under the supplementation of previously reported methyl- and benzylselenoimidocarbamates synthesized by our group[1] in a hepatic carcinoma cell line with the aim of finding novel chemopreventive drugs. Our results indicate that the cellular effects are highly dependent on the substitution of the selenoalkyl moiety and the bulk of the heteroaromatic ring. Compounds with a selenomethyl moiety, may be metabolized into methylselenol, which itself or through its incorporation in selenoproteins could be the responsible for the antiproliferative activity, triggering a stronger antitumoral activity than their benzyl analogues, prossibly through thioredoxin reductase 1 (TrxR1) inhibition. Moreover, when the size of the heteroaryl group was increased to a 3-fused-ring system a total loss of activity occurred. Selenomethyl derivatives **2a** and **8a**, as TrxR1 inhibitors, emerge as promising candidates in the context of anticancer research focused on developing novel cancer therapeutics. Moreover, non-cytotoxic inducers of selenoprotein P (SelP) biosynthesis and TrxR1 activity, as benzyl derivatives **1b**, **2b** and **4**–**8b,** could be considered as promising candidates for age-associated neurodisorders prevention.

Acknowledgements: The authors wish to express their gratitude for the financial support from the Ministerio de Educación y Ciencia, Spain. We thank the Department of Education of the Navarra Government for fellowships granted to E.I. and I.J. This work has been partially funded by UTE project CIMA.

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SYNTHESIS AND BIOLOGICAL ACTIVITY OF NOVEL 1,4-SELENOSUBSTITUTED PHTHALAZINESARIAL.

Iosu Jiménez[a], Iranzu Lamberto[a], Elena Ibañez[a], Beatriz Romano[a], María Font[a], Juan Antonio Palop[a], Ignacio Encío[b], Carmen Sanmartín[a]

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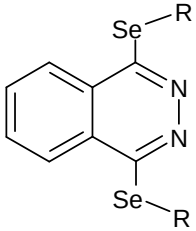
A series of 1,4-selenosubstituted phtlazines have been designed in order to combine the biological effects showed by the heterocycle ring and selenium compounds separately. Cytotoxicity, VEGFR-2 inhibition, and DNA bisintercalation are the main therapeutic activities1,2 observed for pthalazinic derivatives; while organic and inorganic selenium compounds have been used in medicine due to their chemoprotective3 and cytotoxic4 properties. The principal paths that selenium derivatives use as chemoprotectors consist in: (1) stimulate the activity of NK cells, lymphocytes and macrophages; (2) detoxification of free radicals and reactive oxygen species by redox processes[3]; (3) enhance the activity of DNA repair enzymes. Cytotoxic effect is based in the activation of apoptosis: (1) methylseleninic acid and Se-methylselenocystein enhance the proteolysis of casapases zymogens; (2) selenium was found to be effective in the suppression of cell proliferation by the production of G1 cell arrest cycle and mitochondria-mediated apoptosis[4]; (3) some selenocompounds produce overexpression of transmitting death signals like Fas ligand.



The synthesis of selenoderivatives was performed by aliphatic nucleophilic substitution of different benzyl and alkyl halides with 1,4-diselenolphtlazine.

All products are being tested as cytotoxic and cytostatic agents against threehuman cancer cells: HTB-54 (lung carcinoma), MCF-7 (breast adenocarcinoma).

Acknowledgements: The author wish to express his gratitude to the Navarra Govermment for the award of a grant.

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BIOLOGICAL EVALUATION OF THE ANTITUMOUR ACTIVITY OF IMIDOSELENOCARBAMATES 3g AND 3n

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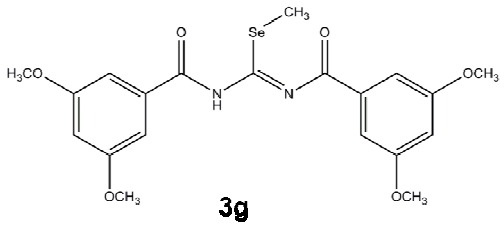
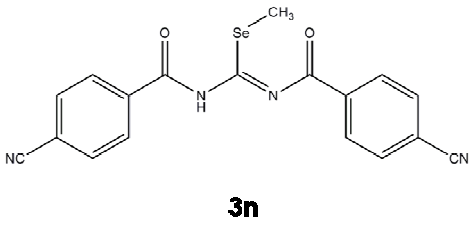
Cancer is becoming an increasingly significant disease worldwide[1]. It's well known that the trace element selenium (Se) appears to have cancer preventive properties based on a converging body of evidence from epidemiologic, clinical and experimental studies[2,3]. Although the mode of anticancer action of Se is not fully understood yet, several mechanisms, such as antioxidant protection by selenoenzymes, specific inhibition of tumor cell growth by Se metabolites, modulation of cell cycle and apoptosis, and effect on DNA repair have all been proposed[4].

Among the growing list of seleno-compounds with desirable anticancer activity, we previously reported the synthesis of various imidoselenocarbamates with significant in vitro antiproliferative activity against human prostate cancer cells PC-3 [5]. To further characterize the antitumour activity of these compounds, here we extend the evaluation of the antiproliferative action of two of them, 3g and 3n (**1**), to a panel of four human cancer cell lines (CCRF-CEM, HTB-54, HT-29 and MCF-7) and one non-malignant cell line (184B5). We also analyze the ability of 3g and 3n to induce apoptosis in CCRF-CEM and MCF-7 cells, as well as their effect on mitochondrial events in MCF-7 cells.



3g



3n

1

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SYNTHESIS AND BIOLOGICAL EVALUATION OF NOVEL 2-(3´, 4´, 5´- TRIMETHOXYBENZOYL)-3-ARYL/ARYLAMINO BENZO[b]TIOPHENE AS ANTIPROLIFERATIVE AGENTS

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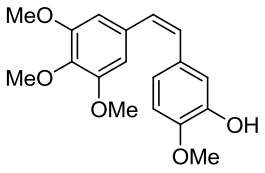
Cancer is one of the major causes of death in the world. Although many advances have been made in the treatment and management of the disease, the existence of chemotherapy-resistance means there is still a great need to develop new strategies and drugs for its treatment.

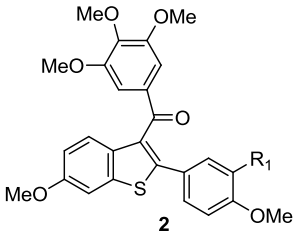
The biological importance of microtubules in mitosis makes them an interesting target for the development of anticancer agents. Such compounds would ideally be characterized by relatively simple structures and be easy to prepare in a cost-effective way. Among such compounds, the benzo[*b*]thiophene molecular skeleton is the core structure of a series of derivatives with general structure **2** described by Pinney et al. [1].

The aim of the research presented in this communication is the presentation of a flexible, concise and highly convergent protocol for the preparation of two new series of derivatives characterized by the presence of a substituted aryl or arylamino moiety, respectively, on the 3-position of the 2-(3',4',5'-trimethoxybenzoyl) benzo[b]thiophene core. The 3',4',5'-trimethoxyphenyl group on the 2-benzoyl moiety was kept unchanged because it is the characteristic structural requirement for activity in a numerous inhibitors of tubulin polymerization, such as colchicine, combretastatin (CA-4) and podophyllotoxin.

All of the compounds showed a considerable growth inhibitory effect against a panel of five human cancer cell lines.



Combretastatin A-4 (CA-4), **1**



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DUAL AChE/MAO-B INHIBITORY AGENTS: A NEW APPROACH TO THE TREATEMENT OF NEURODEGENERATIVE DISEASES

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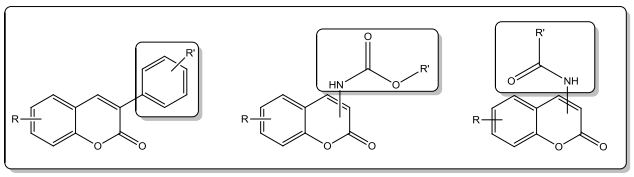
Alzheimer's disease is the most prevalent of the neurodegenerative diseases, followed by Parkinson's disease.[1] The development of effective neuroprotective therapies that slow down or stop disease progression in the earliest stages is one of the main goals of the researchers in this area. As pathologies with multiple pathogenic factors, in the last years the therapeutic sources have been focus in the multitargeting strategies. Acetylcholinesterase (AChE) and monoamino oxidase B (MAO-B) are actively involved in these pathologies. Therefore, dual AChE and MAO-B inhibitors are interesting chemical structures. Coumarins are an important family of natural and/or synthetic compounds that occupy an important place in the realm of natural products and organic chemistry.[2] Some synthetic 3-arylcoumarins already proved to be very potent and selective MAO-B inhibitors.[1,3] Based on these



results, and with the aim of finding dual inhibitors, we design, synthesized[4] and evaluated new series of differently substituted coumarins as potential AChE, MAO-A and MAO-B inhibitors. The introduction of either amide or carbamate groups under the coumarin moiety was the strategy to improve the dual pharmacological activity of the synthesized derivatives. The synthetic routes and experimental results will be reported in the communication.

Acknowledgements: Thanks to the Spanish Ministry (PS09/00501) and to Xunta da Galicia (PGIDIT09CSA030203PR and INCITE09E2R203035ES). M.J.M. thanks Fundação para a Ciência e Tecnologia for the PhD grant (SFRH/BD/61262/2009). S.V.R. thanks to Ministerio de Educación y Ciencia for the PhD grant (AP2008-04263).

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INTRACELLULAR CONTROLLED RELEASE OF SILICA MESOPOROUS MATERIALS CONTAINING "POLISACCHARIDE" MOLECULAR GATES

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In the present work, the synthesis of new capped silica mesoporous nanoparticles for on-command delivery applications is described. The gate-like functional hybrid systems consisted of nanoscopic MCM-41-based materials functionalized on the pore outlets with different "saccharide" derivatives and a dye contained in the mesopores[1]. A hydrolyzed starch product Glucidex 47 as saccharides was selected. The mesoporous silica nanoparticle S1 containing the grafted starch derivative was synthesized. Delivery studies in pure water in the presence of pancreatin were carried out for S1. S1 showed very low release in the absence of enzyme, but displayed cargo delivery in the presence of the corresponding enzyme. Moreover, nanoparticles of S1 were used to study the controlled release of the dye in intracellular media. Cell viability assays using HeLa and LLC-PK1 cells indicated that S1 nanoparticles were devoid of unspecific cell toxicity. The endocytosis process for S1 nanoparticle internalization in HeLa cells was confirmed, and the anchored starch was degraded by the lysosomal enzymes. Furthermore, a new mesoporous silica nanoparticle functionalized with Glucidex 47 and loaded with a cytotoxic, S1-DOX, was developed. The cell viability with S1-DOX decreased due to the internalization of the nanoparticle, enzyme-dependent opening of the saccharide molecular gate and the consequent release of the cytotoxic agent. As far as the authors know, this is the first example of enzyme-induced in-cell delivery using capped silica mesoporous nanoparticles.

Acknowledgements: The authors wish to express their gratitude to the Spanish Government (Projects MAT2009-14564-C04-01, MAT2009-14564-C04-04 and BIO2007-60066) and the Generalitat Valencia (Projects PROMETEO/2009/016 and PROMETEO/2010/005) for support. L.M. is grateful to the Generalitat Valenciana for a VALI+D postdoctoral contract. A.B. is thankful to the Universitat Politècnica de València for a postdoctoral contract. E.A. is grateful to the CIBER-BBN for a post-doctoral grant. We thank Roquette for the Glucidex samples. We would like to thank the CIPF confocal microscopy service for technical support.

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NEW RING AS A MODEL FOR ANTICANCER DRUGS: DESIGN AND SYNTHESIS.

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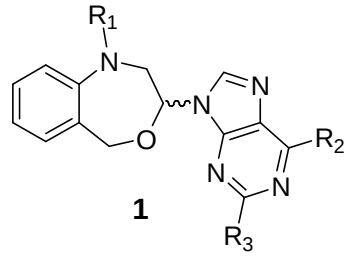
Cancer is the second cause of mortality in developed countries. Although research has increased the understanding of the cell and the molecular biology of cancer, the current treatment against cancer, such as chemotherapy, lacks of the specifity needed to destroy tumour cells without causing several adverse effects.[1] Between the structures synthesized by our Group, 9-(1,2,3,5-tetrahydro-4,1-benzodioxepine-3-yl)-9*H*-purines (**1**) have been the most active[2] compounds against the MCF-7 cell line (0.33-0.86 µM). Furthermore, the studies by the microarray technology show that the main molecular targets of these compounds are proapoptotic genes with protein kinase activity such as GP132, ERN1 or RAC1, which prevent the metastasic progression.[3]

The aim of our research is to develop new antitumoural compounds, with specific anticancer activity against apoptotic genes and genes involved in the control of the cell cycle. To this effect, we have designed the synthesis of new purine derivatives. To increase the antitumoural activity, the synthesis of eight-membered rings has been carried out with the objective of increasing the lipophilicity of this target molecules, in contrast to **1**.



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Compounds	IC_{50} (µM)
R_1= SO_2-C_6H_4-pNO_2; R_2=Cl; R_3=H	0.355 ± 0.011
R_1= SO_2-C_6H_4-oNO_2; R_2=Cl; R_3=H	0.383 ± 0.027

Acknowledgements: This study was supported by the Instituto de Salud Carlos III through the project PI10/00592, and the Ministerio de Ciencia e Innovación through the project SAF-2010-18263. The work was supported by a PhD grant of the Ministerio de Educación.

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NOVEL QUINAZOLINE AND PYRIDO[2,3-*d*]PYRIMIDINE DERIVATIVES AS ANTIPROLIFERATIVE, CYTOTOXIC AND CELL DEATH INDUCER AGENTS

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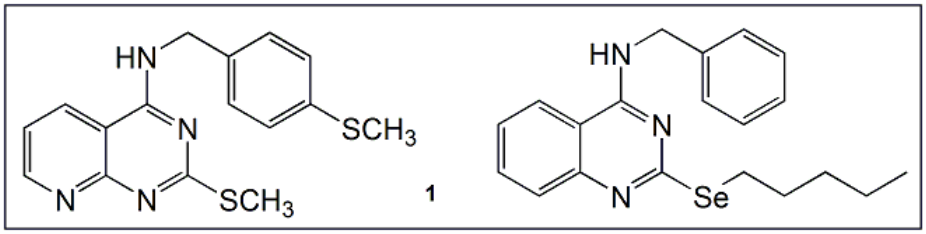
The disease of cancer has been ranked as a major health burden [1]. There are diverse biological profiles shown by the quinazoline and pyrido[2,3-*d*]pyrimidine nuclei: anti-inflammatory, anticonvulsant, antibacterial and antihypertensive, and their anticancer activity [2-3] is one of the most promising aspects.

Continuing with the investigations of our group [4-7], the synthesis and biological evaluation of novel quinazoline and pyrido[2,3-d]pyrimidine derivatives were carried out. Two compounds (**1**) were chosen as lead compounds:



The results show that the lead compounds exhibit a strong antiproliferative and cytotoxic activity against all of the cell lines tested, inducing time and concentration-dependent cell death in MCF-7 cells without modifications of the cell cycle.

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STRUCTURAL CHARACTERIZATION BY NMR OF NOVEL KINASE INHIBITORS

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Deregulated kinase activity is a frequent cause of disease, in particular cancer, wherein kinases regulate many aspects that control cell growth, movement and death. Drugs that inhibit specific kinases are being developed to treat several diseases. Staurosporine is a natural product that binds to many kinases with high affinity, though with little selectivity.

In this work, novel indolocarbazoles designed by mixing structural motifs of natural products staurosporine and rebeccamycine were prepared by combinatorial biosynthesis, including altered glycosylation patterns.[1] The novel analogues showed potent, subnanomolar, yet selective inhibition against a panel of kinases.[2]

The structures of these compounds were elucidated by Nuclear Magnetic Resonance (NMR) making use of cryoprobe technology, that proved essential in this case due to the low quantities of product available. ^{1}H-^{13}C heteronuclear 2D experiments enabled the assignment of all proton and carbon resonances while NOESY and TOCSY 2D experiments allowed the unequivocal identification of the different sugar-moieties attached to the indolocarbazole ring.

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NEW HISTONE DEACETYLASES INHIBITORS AS ANTICANCER DRUGS

Sonia Panadero Fajardo[a], Mavys Tabraue Chávez[a], José F. Domínguez Seglar[a], Paloma de la Cueva Batanero[b], Ana M. Sánchez López, Ana Isabel Sáez Castillo[b], José Antonio Gómez Vidal[a]

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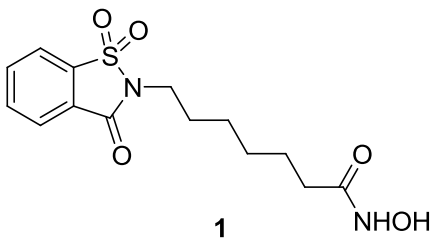
Histones are alkaline proteins found in eukaryotic cell nuclei which allow DNA organization into structural units called nucleosomes. The amino-terminal tails of the histones are subject to post-translational modification which can be covalently modified by acetylation, methylation, phosphorylation, etc.[1]

Histone deacetylases (HDACs) and histone acetyl transferases (HATs) determine the pattern of histone acetylation. Histone acetylation is associated with an increased in transcriptional activity while histone deacetylation is associated with the repression of gene expression. A misbalance in both enzyme activities can explain a disorder in cell proliferation and influences the differentiation process in normal cells.[2]

A large number of HDAC inhibitors have been synthesized in the last few years, some of which have demonstrated anti-proliferative activity against a number of solid and hematological tumors. However, one of the most established classes of these inhibitors is based upon the hydroxamic acid moiety, which is a widely recognized zinc-binding group. In 2006, suberoylanilide hydroxamic acid (SAHA) was approved by the US Food and Drug Administration for the treatment of advanced cutaneous T-cell lymphoma[3].

The synthesis and evaluation of new HDAC inhibitors for their use as anticancer agents have been one of the most important successes in our research. The structural modification of the compound MTC-124 (**1**) with antiproliferative activity (IC_{50} = 0.25 µM on isolated enzyme) has been carried out. This compound is included within a wide family of HDAC inhibitors developed by the Research Group BIO-250, which led to the application of two patents[4]. The synthesized derivatives were subsequently evaluated over three cell lines (MCF7, SF268, H460) at the Coordination Center of the Andalucia Tumor Bank Network.



1

Acknowledgements: Research Project funds from the Junta de Andalucía, entitled "Caracterización de la actividad antitumoral de nuevos inhibidores de las histonas desacetilasas" (P06-CTS-1407).

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OPTICALLY ACTIVE 1,3,4,4-TETRASUBSTITUTED β-LACTAMS: SYNTHESIS AND EVALUATION AS TUMOR CELL GROWTH INHIBITORS

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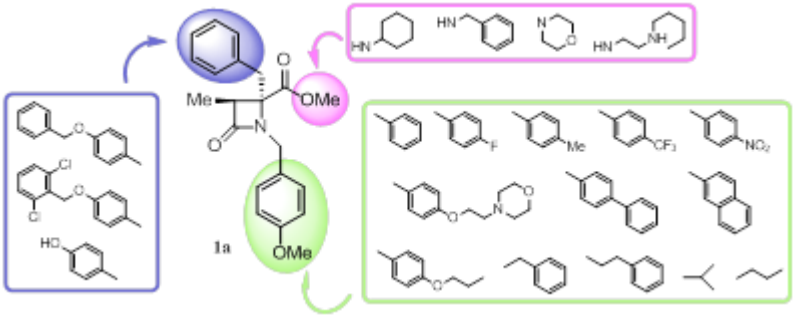
The *in vitro* cytotoxicity assays of several enantiopure (3*S*,4*S*)- and (3*R*,4*R*)-1,3,4,4-tetrasubstituted β–lactams derived from amino acids have shown that the (3*S*,4*S*)-4-benzyl-1-p-methoxybenzyl-3-methyl-4-methoxycarbonyl derivative **1a**, obtained from Phe[1], displays significant activity, which is comparable to that of the anticancer drug Doxorubicin against HT29 cell lines.

In view of this result, we decided to carry out various structural modifications on **1a** to improve its cytotoxic activity. These modifications include a) replacement of the *p*-methoxybenzyl group at position 1 by different substituents; b) replacement of the Phe side-chain at position 4 by other aromatic side-chains (Tyr and O-benzyl Tyr), and c) formation of 4-carboxamides from selected compounds resulting from modifications a) and b). Both solid-phase[2] and solution methodologies were used for the preparation of these new β–lactam derivatives.



This communication will describe the synthesis and the *in vitro* cytotoxicity of these new modified analogues of **1a**.

Acknowledgements: P. P.-F. thanks Faes Farma S.A. for the award within the XV Call of the SEQT awards for young scientists

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NEW KINESIN SPINDLE PROTEIN INHIBITORS AS POTENTIAL ANTIPROLIFERATIVE AGENTS: SYNTHESIS AND BIOLOGICAL EVALUATION

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Kinesin spindle protein (KSP, also known as Eg5 or kinesin-5) is a motor protein that participates in mitosis by crosslinking antiparallel microtubules emerging from the duplicated centrosomes. This results in sliding of microtubules in opposite directions, which is essential for the establishment of a bipolar spindle[1]. The requirement of KSP early in mitosis, together with its non-redundant function, makes it a promising target for antiproliferative chemotherapy, as an alternative to the classical antimitotics (antitubulin agents), which alter the normal microtubule dynamics but often cause toxicity as result of tubulin being required for a variety of other physiological functions[2].

As part of our research on KSP inhibitors, we have designed a series of analogues of monastrol, the first reported KSP inhibitor[3] (Fig. 1). In this work, we have introduced modifications on the 3-hydroxyphenyl ring, which has been reported as important for binding[4]. Thus, mono- and bicyclic systems are replacing such moiety, in order to gain insight about the structural requirements for potent KPS inhibition. The synthesis and biological evaluation of these compounds will be reported in this communication.

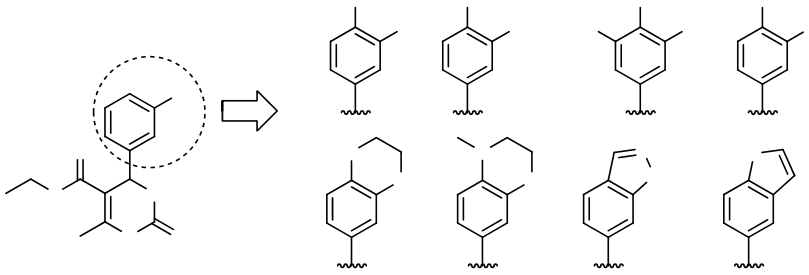

Fig. 1 Structure of the compounds synthesized as analogues of monastrol.

Acknowledgements: We thank Universidad de Salamanca for financial support.

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SYNTHESIS AND BIOLOGICAL EVALUATION OF NOVEL HETEROCYCLIC ANALOGUES OF PHENSTATIN

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Microtubules are built up by polymerization of αβ-tubulin dimers, being the target for antimitotic drugs. The search for new microtubule binding agents continue to be an active field, in order to develop new antimitotic drugs that overcome the drawbacks of the existing ones (neurotoxicity, resistance)[1]. Phenstatins inhibit tubulin polymerization by binding at the colchicine site, thus leading to cell cycle arrest and ultimately to cell death. Structure-activity relationships for phenstatins are not fully established, for they only partially match those of the closely related combretastatins[2].

As part of our research on tubulin inhibitors we have designed a new family of phenstatin analogues that bear a heterobicyclic system as replacement for the B ring (Fig. 1). The A ring, a common moiety for many of the tubulin binding agents that has been proven important for high potency, has been conserved. This strategy will allow us to determine the influence of several factors in biological activity: type and position of heteroatoms, ring size and flexibility of the system, providing that some of them are partially hydrogenated. The carbonyl bridge linking systems A and B will be also modified. In this communication we will present the synthetic route to these compounds, as well as the results of their biological evaluation.

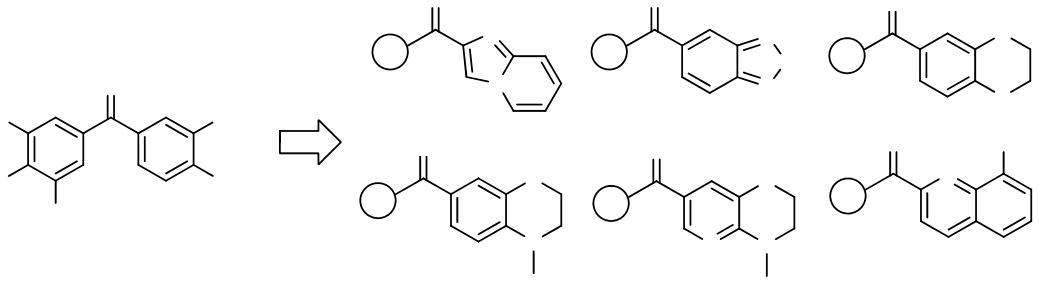

Fig.1. Heterocyclic compounds designed as analogues of phenstatin.

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Synthesis and Biological Evaluation of New Fluorinated Thiazole Coumpounds Useful for Cancer Treatment

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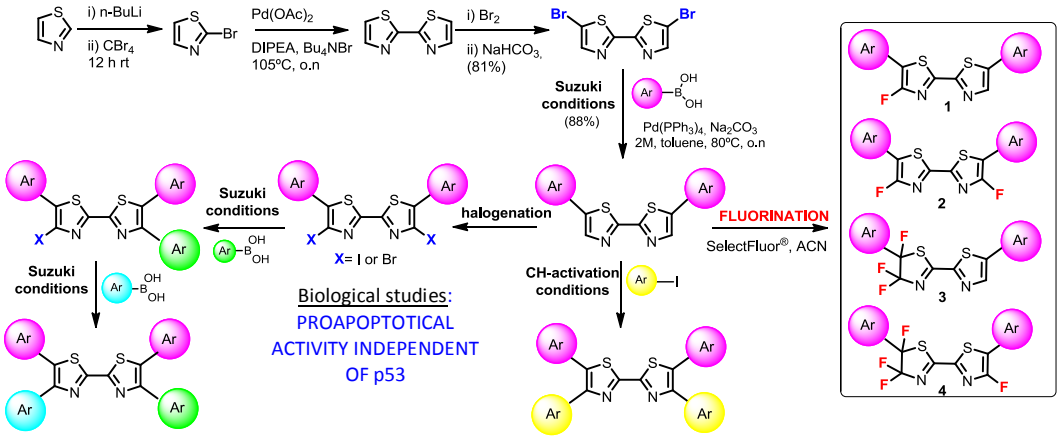
Nowadays, despite the research efforts invested in the past, there is still a need to find effective antitumoral agents, and in particular therapies which act independently of p53 would be of great interest. The presence of thiazole rings is common in many natural bioactive compounds, especially with antitumoral properties. Particularly, polyarylated bisthiazoles (**A**) constitute a new target, as they display potent and selective cytotoxic activities and can be related to a new class of small bioactive molecules.[1]

Our group has developed a new family of polyfluorinated thiazoline compounds substituted by a variety of diversely functionalized aromatic rings which rapidly induce apoptosis in cancer cells, independently of the mutational status of p53.[2] In order to establish an efficient synthetic approach to such library of thiazoline derivatives, a practical method, based on palladium (0) catalyzed cross-coupling reactions, has been developed. In this way, starting from bisthiazole though a sequence involving halogenations, Suzuki couplings and CH-activation arylations, followed by a novel electrophilic fluorination process, the final targets are conveniently prepared.



Remarkably, the structural simplicity of the hits, the availability of the starting materials and the reduced number of synthetic steps make the preparation of these compounds fast and affordable, a key issue for developing the ongoing SAR studies together with toxicological and *in vivo* experimentation.[3] Currently, we are focused on the elucidation of the mechanism of action of thiazolines-induced apoptosis.[4] The identification and characterization of their still unidentified molecular target is essential to provide new targets for cancer therapy as well as for drug development.

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[4] In Collaboration with Prof. Handa (Tokyo Institute of Technology)

Tricyclic Benzothienopyridinols: a Novel Chemotype within Antimalarial 4(*1H*)-pyridones

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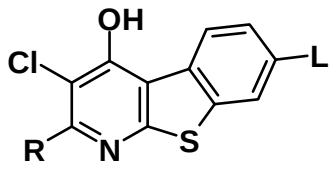
DDW Tres Cantos, Drug Discovery Chemistry Department

4(*1H)*-pyridones are selective inhibitors of Plasmodium mitochondrial function by blocking the electron transport chain at the cytochrome b level. Benzothienopyridinol derivatives belong to a novel family of tricyclic 4(*1H*)-pyridone derivatives that have displayed high *in vitro* antimalarial activity in both *Pf* Cytbc1 and whole cell *P. falciparum* and similar *in vivo* efficacy to the candidate. Some compounds bearing different aromatic lipophilic chain have improved the *in vitro* potency, maintaining *in vivo* efficacy in the standard *P. yoelii* murine model, in spite of their poor oral bioavailability. The tricyclic conjugated nature of the main scaffold allows us to prepare stable alkaline salts which have shown lightly better oral exposure than the neutral version. Additionally, the synthesis of derivatives bearing a hydroxymethyl group could allow the preparation of phosphate prodrugs as like strategy to improve oral bioavailability.

It is noteworthy that, resistant mutants to Pyridones generated in the laboratory are hypersensitive to Bezothienopyridinols. This effect seems to indicate that, although they are potent inhibitors of the electron transport chain at the cytochrome b level, their binding mode within the active site can be different to other pyridones.

The general structure of the Antimalarial Benzothienopyridinols is depicted in Fig 1.



R = CH_3, CH_2OH
L = lipophilic moiety

Fig 1. General formula of Antimalarial Benzothienopyridinols

COMPUTATIONAL APPROACHES FOR THE DISCOVERY OF PDE7 INHIBITORS ACTIVE ON CNS DISEASES

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Phosphodiesterases (PDEs) have been emerged, in the last years, as an important family of druggable targets with a central role in signal transduction by regulating intracellular levels of cyclic AMP and GMP[1]. Specifically, PDE7 is one of the eleven isoforms expressed abundantly in different brain areas, which suggests its potential involvement in different CNS diseases interfering with both the inflammatory and neurotransmitter cascades[2]. Therefore, the identification of selective inhibitors targeted against PDE7 enzyme has become an attractive area of research[3].

By using different computational approaches we were able to discover a new chemical family of PDE7 inhibitors. The enzymatic assays confirm this result and we have also evidence that these compounds reduce the inflammatory activation of primary cultures of astrocytes and microglia, in response to lipopolysaccharide.

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Synthesis and biological evaluation *in vitro* and *in vivo* of 1-alkoxy-2-aminoimidazoline derivates as possible prodrugs for the treatment of sleeping sickness

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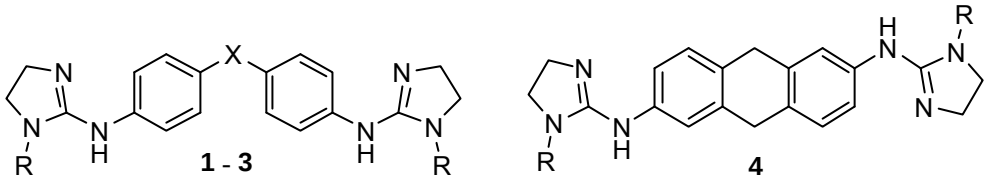
Human African trypanosomiasis (HAT), also known as sleeping sickness, is a deadly tropical disease caused by parasites of the genus *Trypanosoma*. Two subspecies (*T. brucei rhodesiense* and *T. brucei gambiense*) provoke acute and chronic forms of the disease, respectively[1]. There is a lack of safe and efficient drugs to fight the late-stage disease (neurologic phase) meaning that new treatments are urgently needed. A requirement for drugs targeting late-stage HAT is the capacity to cross the blood–brain barrier (BBB) to reach the parasites within the CNS[2]. To improve the BBB permeability of trypanocidal lead compounds active in the first stage HAT in mice[3], we have synthesized new 1-alkoxy-2-aminoimidazoline derivates (**1b-f**–**4b-f**) as possible prodrugs of the lead compounds. *In vitro* assay of these derivates showed a decrease in activity against *Trypanosoma brucei rhodesiense* (*T. b. rhodesiense*) > 100 times when compared with the lead compounds. In a murine model of acute sleeping sickness (hemolymphatic stage), the *N*-hydroxylated derivates were able to increase the mean time of relapse of the parasitemia. Moreover, compound **1e** was curative at doses of 20 mg/kg (ip) in this model. These data suggest that these compounds are acting as prodrugs of the lead compounds **I-IV**. Studies of the antitrypanosomal potential of these prodrugs in the late-stage disease are ongoing.



X= NHCO (**1**); NHCONH (**2**); CH_2CH_2 (**3**)
R = H (**a**); OMe (**b**); OEt (**c**); OTHP (**d**); OH (**e**); OBn (**f**)

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LECTIN MIMETICS BASED ON A TRIETHYLBENZENE SCAFFOLD SUBSTITUTED WITH NON-AROMATIC AND FLUOROPHENYL RINGS AS INHIBITORS OF HIV INFECTION

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The envelope glycoprotein gp120 of Human Immunodeficiency Virus (HIV) is of particular importance during viral fusion and entry, as it serves as the first point of contact with the host cell. This envelope glycoprotein is the main target for neutralizing antibodies that appear during natural infection. The HIV gp120 protein is heavily glycosylated, so that approximately 50% of its molecular weight is due to a dense carbohydrate (glycan) shield that partially hides the viral envelope antigens.[1] Therefore, agents that interact with the glycans on the viral envelope may disrupt the efficient interaction between gp120 and its (co)receptors.

Some lectins, proteins that bind carbohydrates, showed a potent inhibitory activity against HIV. It has been proposed that the binding of these lectins with the glycans of gp120 affect the interaction with the (co)receptors in the host cell surface.[2] Moreover, appearance of glycosylation site mutations in response to lectin pressure might trigger an efficient immune response directed against the mutated virus, since the previously hidden immunogenic epitopes of gp120 become exposed upon glycan deletions and, therefore, the host immune system may produce neutralizing antibodies against them.[3] However, due to their high molecular weight and protein nature, lectins may be endowed with unfavourable pharmacokinetic properties that prevent their development as suitable drugs.

Our research efforts are focused on the discovery of synthetic small molecules, “lectin mimetics”, able to act through a mechanism similar to that of the natural lectins. With this aim, compounds containing a triethylbenzene scaffold substituted with polyphenolic moieties as carbohydrate-binding ligands have been previously prepared in our group. Some of them showed inhibitory activity against HIV. To complete our structure–activity relationship studies here we describe structural modifications on our lead compound by replacing the polyphenolic moieties by non-aromatic entities or mono-, di- and trifluorophenyl rings. Details about the synthesis and anti-HIV activity of these compounds will be presented.

Acknowledgments: This work has been supported by the project SAF2009-13914-C02-01 of MICINN (Spain), the CSIC-Intramural Programme (PIF08-022) and “The Centers of Excellence” of the K.U.Leuven (EF-05/15 and PF-10/18).

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DEVELOPMENT OF A NOVEL DRUG DELIVERY SYSTEM BASED ON PROTEIN-POLYACETAL CONJUGATES.

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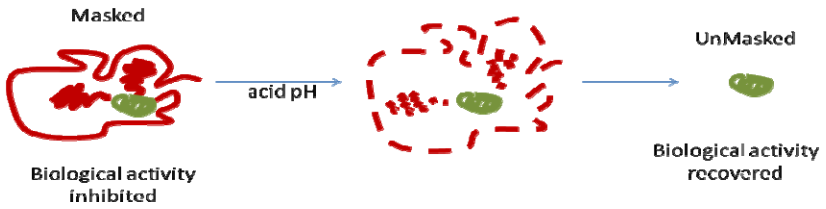
Polymer Therapeutics has been successfully applied to clinics. The clinical benefit already achieved with polymer–protein conjugates routinely used has established their potential in anticancer therapy.[1] In addition, these results have provided a firm foundation for more sophisticated constructs that deliver the newly emerging target-directed bioactive agents in addition to polymer–based drug combinations[2]. However, Polymer Therapeutics have rarely been designed to promote tissue repair[3]. In this work we explore the application of polymer-protein conjugates in this important field by making use the concept of Polymer-masking-UnMasking-Protein Therapy (PUMPT)[4,5]. Our conjugate has to be able to deliver the protein (e.g. a growth factor) to the damaged tissue and release it successfully but always preserving its biological activity.

Amino-pendant polyacetals[6] have been selected as biodegradable carriers for specific protein conjugation through bioresponsive linkers that make them appropriate for the applications we are studying. Likewise, trypsin[4,7] was chosen as model protein because it is well characterized and described in the specialized literature, providing a feasible model to design the system for a localized and controlled drug delivery.

A family of trypsin-polyacetals has been synthesized by copolymerization of 2-amino-1,3-propanodiol, PEG_{4000} diol, and triethilenglycoldivinil ether and subsequent trypsin conjugation. The protein was loaded by linking the amino group of a lysine in the protein to a previously succinoylated polymer main chain. The chemical structure of the conjugate as well as its masking-unmasking behavior in solution have been analyzed and will be discussed in this communication.



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DESIGN, SYNTHESIS AND ANTIPROLIFERATIVE ACTIVITY OF NOVEL SELENOCARBAMATES

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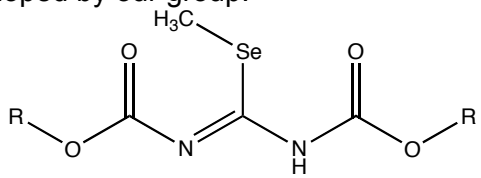
Prostate cancer continues to be the most common malignancy among men an the third leading cause of cancer-related mortality in the Unted States and the eighth cause in Spain.

There is an increasing evidence that cancer can be prevented not only by avoiding exposure to recognized risk factors but also by favoring the intake of protective factors and by modification of defense and DNA repair mechanisms of the host organism [1].

Selenium (Se) is an essential dietary component of fundamental importance to human health. More than 200 studies support the anticarcinogenesis effects of Se and several mechanisms have been suggested. The major ones are reduction of DNA damage, oxidative stress, inhibition of cell cycle and angiogenesis and induction of apoptosis [2].

The synthesis of a serie of twelve new selenocarbamates with the structure 1 and their evaluation for antitumoral activity *in vitro* against prostate cancer cell line (PC3) have been developed by our group.



R = aliphatic, aromatic

1

Values of GI_{50}, TGI and LD_{50} in PC-3 have been determined. The GI_{50} values for seven of the compounds were below 1 μm, lower than some standard chemotherapeutic drugs used as references.

Acknowledgements: B. Romano acknowledges the Association of Friends of the University of Navarra for a PhD Grant and the project funding by Ministerio de Ciencia e Innovación

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INFLUENCE OF LIPOPHILICITY OF NON-SYMMETRICAL CHOK INHIBITORS AS ANTICANCER AGENTS

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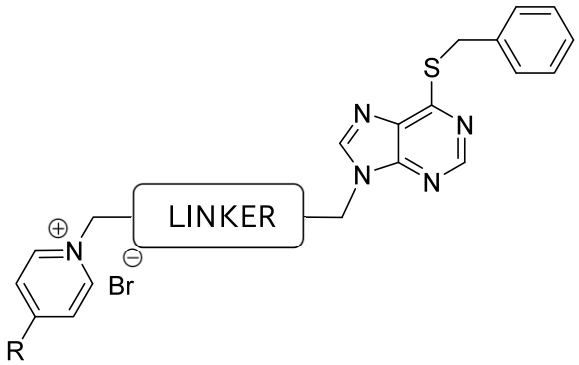
Choline (Cho) is phosphorylated by choline kinase (ChoK) to generate phosphocholine (*P*Cho), which represents the first step in the biosynthesis of a membrane phospholipid, phosphatidylcholine. Several oncogenes such as RAS or RHOA increase ChoKα activity resulting in higher intracellular levels of *P*Cho.[1] Moreover, it has been reported that hypoxia can induce ChoK expression in cancer cells.[2] These results suggest ChoK as a promising target due to its specific inhibition leads an efficient antitumoural activity in vivo. We have previously synthesized a series of non-symmetrical ChoK inhibitors using different 4-substituted pyridinium salts that could be stabilized into the Cho binding site and adenine that mimics the ATP adenine moiety connected both by an appropriated linker. We present here a series of compounds in which the polar amino group in position 6 of the adenine has been changed for a benzylthio substituent in order to increase the lipophilicity of the structures. The antiproliferative effect observed in human hepatoma HepG2 cell line for these new series is higher than the previous reported one[3] demonstrating that the increase in lipophilicity should favorably affect the passage through the cytoplasmic membrane.



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GSK´s INITIATIVE IN NEGLECTED DISEASES

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In the will to address obvious unmet medical needs in the field of Neglected Diseases, GSK is starting to get engaged in kinetoplastid research. A Kinetoplastid Discovery Performance Unit (DPU) was recently created in Tres Cantos (Spain) adding up to the existing Malaria and Tuberculosis entities to complete the Diseases of the Developing World R&D center activity. The Kinetoplastid DPU will function based on a collaborative model, being dedicated to the discovery of small molecules as new drugs for Chagas disease, Sleeping Sickness and Leishmaniasis. The responsibility of the DPU is to discover clinical candidates with the final goal to deliver new and better medicines for people living in endemic countries. GSK is calling for partners, willing to establish collaborations and participate in projects at every level in a win-win model, from early stage R&D to clinical phase. In the area of most tropical neglected diseases, it is important to highlight the unprecedented initiative from the company to open the Intelectual Property, releasing number of patents into the public domain through the Pool for Open Innovation, in collaboration with BioVenture Global Health. GSK is also opening its Tres Cantos facilities with the creation of the “Tres Cantos Open Lab initiative”, welcoming experts in Malaria, Tuberculosis or Kinetoplastid fields who would need the GSK resources to boost their projects, then collaborating with them in order to ultimately enable a significant impact on the global community.

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http://www.gsk.com/collaborations/tres-cantos.htm

DEVELOPMENT OF APAF-1 INHIBITORS AS THERAPEUTIC TOOLS TO DECREASE UNWANTED APOPTOSIS

Mónica Sancho[a], Rebeca Montava[a], Eliana Sirvent[a], Sandra Marchán[b], Carmen Herrero[b], Ángel Messeguer[c], Carmen Lagunas[b], Mar Orzáez[a] and Enrique Pérez-Payá[a,d].

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Previous studies from our laboratory have identified a new structural class of trimers of N-alkylglycine derivatives as inhibitors of the apoptosome, a central multiprotein complex that regulates mitochondrial-dependent apoptosis. The most active, named peptoid-1, has been structurally improved for cellular-based studies rendering the active heterocyclic compound SVT016426. This new apoptosome inhibitor binds to the CARD domain of Apaf-1 and precludes the recruitment of procaspase-9 to the apoptosome, inhibiting the intrinsic apoptotic cellular pathway. A new generation of compounds has been developed in order to study whether molecular inhibition of apoptosome is a legitimate therapeutic strategy against cellular injury. These new compounds with improved cell activity and solubility will facilitate the study of the detailed molecular mechanism of action in a series of chemical biology-based assays such as *in vitro* reconstitution of apoptosome and Apaf-1 depleted HEK293 extracts where rApaf-1 is added to induce apoptosome formation. Furthermore the anti-apoptotic activity of our inhibitors has been analyzed in cellular models that resemble pathological situations of interest such us apoptosis induced by toxic products and drugs (e.g., Doxorrubicine, Cisplatin) or hypoxic-ischemic conditions (renal ischemia).

NEW QUINUCLIDINIUN DERIVATIVES COMPOUNDS AS CHOK INHIBITORS WITH ANTIPROLIFERATIVE ACTIVITY.

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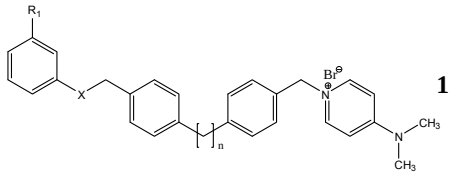
Docking Studies performed on the crystal structure of human ChoK5 show that biscationic compounds could be inserted simultaneously in both the choline and ATP binding sites. One of the cationic head can bind the specific site of union of the positive charge of choline, while the other one is situated on the ATP binding site, were the union of ATP to the enzyme is established through H-bond and not through electrostatic interactions.[1]

The crystal structure of ChoK co-crystallized with HC-3, suggest that only one of the quaternized centre is necessary for the inhibition of choline kinase.

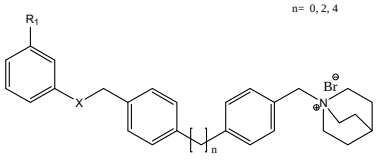
For these reasons, the main purpose of this research is the synthesis of new non-symmetrical ChoK inhibitors bearing only one cationic head, with the purpose to explore the chemical and physics characteristics of both sites of union. All of these observations led us to prepare and evaluate two novel and unusual series of non symmetrical inhibitors with different linkers. In previous works we had synthesized a series of 1-[4-(aminophenoxy)methylbenzyl]-4-(1-pirrolidinyl)pyridinium bromides and 1-[4-(hydroxiphenylamino)methylbenzyl]-4-(1-pirrolidinyl)pyridinium bromides derivatives represented by the general formula **1**, who showed a good results as inhibitors of ChoK and antiproliferative compounds.

Herein we present the synthesis and activities of new compounds, which a phenyl group as a linker, a quinuclidiniun fragment that acts as a cationic head and a aminophenol aromatic moiety that mimics adenine.

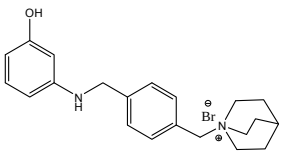


1

R_1= OH, NO_2, NH_2
X= -O-, -NH-
n= 0, 2, 4



R_1= OH, NO_2, NH_2
X= -O-, -NH-
n= 0, 2



R_1= OH, NO_2, NH_2
X= -O-, -NH-

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STRUCTURE-BASED DESIGN AND SYNTHESIS OF NOVEL ANTITUMOUR DRUGS

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Choline Kinase has been validated as a biological target in anticancer[1] therapy. Hemicholinium-3[2] was its first inhibitor but it was neither selective nor potent. Due to this fact, new potential more selective and potent antitumor agents have been developed (Table 1).

Its design has been carried out in three steps:

1st) Theoretical docking studies[3]: They direct synthesis towards a more successful way by ruling out the possibility of losing money and time with compounds that are unlikely active. It has provided clues to group together a biphenyl-type spacer, a cationic head and a tertiary amine.

2nd) Synthesis: Firstly, spacer has been prepared. After that, cationic head has been inserted by nucleofilic substitution and finally, synthesis has been completed by tertiary amine placement.

3rd) Biological Evaluation: Their biological activity is being determined in the HepG2 tumour cell line.

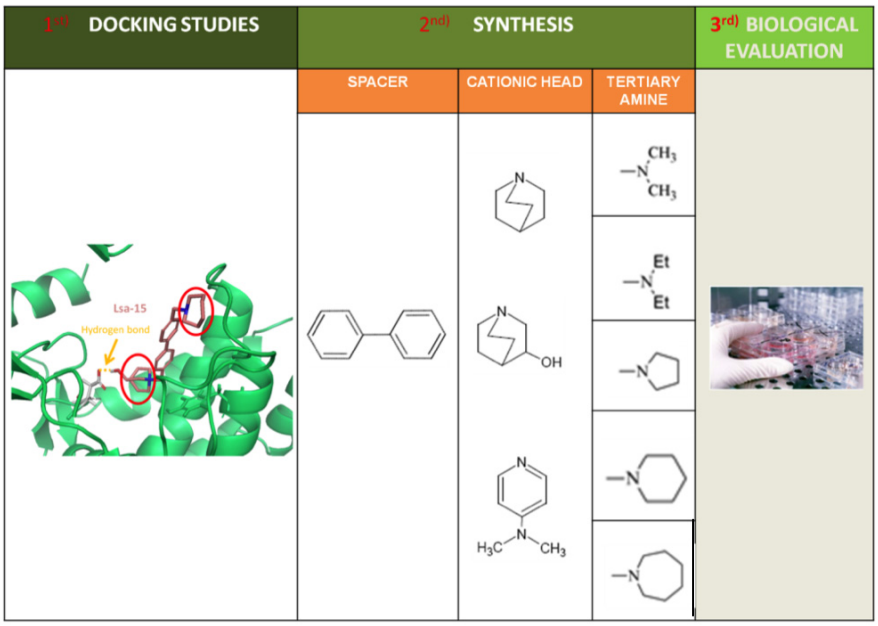

Table 1

Acknowledgements: Ministerio de Educación (FPU) and CTS-130 Research Group.

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A PRODRUG APPROACH FOR IMPROVING ANTI-TUBERCULOSIS ACTIVITY OF POTENT *MYCOBACTERIUM TUBERCULOSIS* TYPE II DEHYDROQUINASE INHIBITORS

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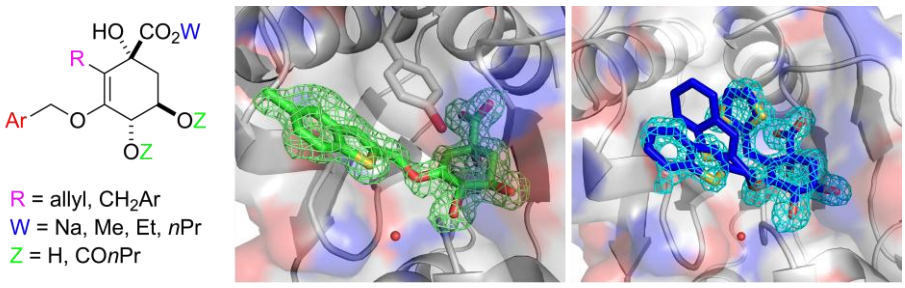
Today, there is a wide range of antibiotics for the treatment of bacterial infections. For approximately four decades (1940s to 1970s) the pharmaceutical industry provided a steady flow of new antibiotics. However, despite this enormous initial development progress in this area has been limited. Hence, there is big concern in developing new antibiotics with novel mode of action.[1]

Our research group is studying the potential of the aromatic amino acids biosynthesis as a new therapeutic target, particularly for the development of new anti-TB agents.[2] In this communication, the design and the synthesis of high-affinity reversible competitive inhibitors of *Mycobacterium tuberculosis* type II dehydroquinase (DHQ2), an essential enzyme in *Mycobacterium tuberculosis* bacteria, is presented.[3] The inhibitors reported here are mimics of the enol intermediate and the effect of substitution on C2 was studied. The crystal structures of DHQ2 in complex with three of the reported inhibitors show that an aromatic substituent on C2 prevents the closure of the active site by impeding the hydrogen-bonding interaction of Arg108 with the essential Tyr24 of the flexible loop, the residue that initiates catalysis. Chemical modifications of the reported acids to improve internalization into *Mycobacterium tuberculosis* through an ester prodrug approach will be also presented.



Acknowledgements: Financial support from the Xunta de Galicia (10PXIB2200122PR and GRC2010/12) and the Spanish Ministry of Science and Innovation (SAF2010-15076) is acknowledged. LT thanks the Spanish Ministry of Science and Innovation for a FPU fellowship.

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3-TRIFLUOROMETHYL-2-CARBONYLQUINOXALINE 1,4-DI-*N*-OXIDE DERIVATIVES AS ANTI-*TRYPANOSOMA CRUZI* AGENTS

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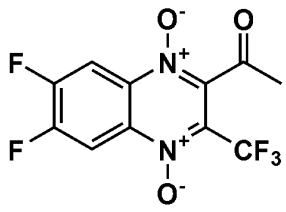
Chagas disease, caused by *trypanosoma cruzi,* remains the most relevant illness produced by protozoa in the American continent. It affects aproximately 10 million people and 25 million are at risk[1].

The drugs currently used in the treatment of Chagas disease are two nitroaromatic heterocycles, Nifurtimox (Nfx) and Benznidazole (Bnz), introduced empirically in the 1960's. Both possess important limitations that underscore the urgent need to develop new effective, safe and cost-effective therapeutic alternatives[2].

Based on the demonstrated anti-infective capability of the quinoxaline 1,4-di-*N*-oxide system against a large number of microorganism, our group evaluated a selected group of derivatives. This study allowed us to identify excellent *in vitro* anti-*T. cruzi* agents and to state the correct requirements for obtaining optimal *in vitro* anti-*T. cruzi* activity. Derivatives with electron-withdrawing substituents in the 2-, 3-, 6-, and 7-positions were the most active compounds[3].



PGI = 100 %
ID_{50} = 390 µM
SI = 27.2

Taking into account their mammal cytotoxicity, some trifluoromethylquinoxaline 1,4-di-*N*-oxide derivatives have been proposed as candidates for further clinical studies. Consequently, mutagenicity and *in vivo* analyses were performed with the most promising derivatives and very interesting results were obtained. In addition, with regard to the mechanism of action studies, it was demonstrated that mitochondrial dehydrogenases are involved in the anti-*T. cruzi* activity of the most active derivatives[3].

Acknowlegments: This work has been carried out with the financial support of Fondo de Investigaciones Sanitarias (PI080817) and PIUNA project from the Universidad de Navarra. Enrique Torres is indebted to the Asociación de Amigos of Universidad de Navarra for a grant.

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SEARCH OF NOVEL ANTIANGIOGENIC AGENTS: APPROACH TO THE DESIGN AND SYNTHESIS OF 2-OXOPIPERAZINE-BASED PAR1 ANTAGONISTS[1]

Ángel M. Valdivielso[a], M. Teresa García-López[a], Marta Gutiérrez-Rodríguez[a], Ioannis Pappos[b], Nikos E. Tsopanoglou[b] and Rosario Herranz[a].

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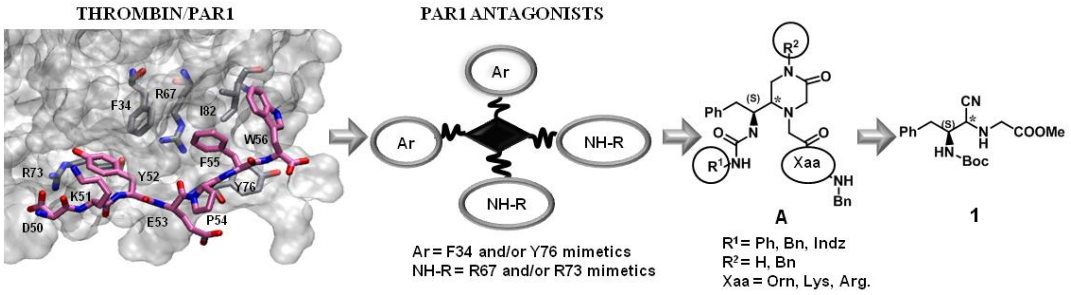
In addition to the key role of thrombin in blood coagulation, this multifunctional serine protease activates platelets and regulates the behavior of other cells through protease activated G-protein coupled receptors (PARs). PAR1 is the principal thrombin-activated receptor involved in platelet aggregation and in endothelial and tumor cell proliferation[2]. In these cells, PAR1 activation is involved in angiogenesis. Several studies have showed that the first thrombin/PAR1 interaction takes place between the thrombin exosite I and the hirudin-like domain of PAR1 ($K^{55}YEPF^{51}$), and that this interaction is essential for high affinity binding. Hydrophobic residues F34, I82, L65 and Y76 and basic residues R67 and R73 of the exosite I of thrombin are crucial for this binding (Figure). Taking into account these studies, we proposed the search of new PAR1 antagonists as potential anti-angiogenic agents based on the design of peptidomimetics that block the thrombin/PAR1 interaction at the exosite I. In this communication, we report the synthesis and biological evaluation of a library of 2-oxopiperazine-derived ureas **A** prepared by a diversity-oriented synthesis[3] using the cyanomethyleneamino pseudopeptides **1** as key diversity generation intermediates[4].



Acknowledgments: This work was supported by CICYT (SAF2006-01205 and SAF2009-09323). Á. M. Valdivielso holds a FPU fellowship from the Spanish Ministry of Education.

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NEW SEROTONIN 5-HT$_{1A}$ RECEPTOR AGONISTS WITH ANALGESIC EFFECT IN AN IN VIVO MODEL OF PAIN

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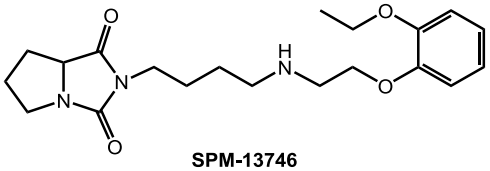
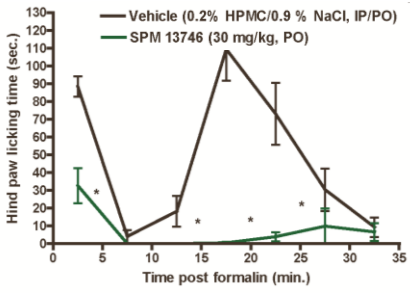
G protein-coupled receptors (GPCRs) account for more than the 50% of the druggable genome, being among the most heavily investigated drug targets.[1] The 5-HT$_{1A}$ receptor (5-HT$_{1A}$R) has been the most extensively studied within the serotonin GPCR family. Besides the well-established therapeutic areas of its agonists and partial agonists in the treatment of anxiety, depression and psychotic disorders, other interesting non-psychiatric perspectives have emerged for 5-HT$_{1A}$R agents in recent years, mostly related to neuroprotection, cognitive impairment, Parkinson's disease or pain treatment.[2] In particular, selective agonist F-13640 has shown remarkable analgesic properties, and today several studies strongly support the interest of 5-HT$_{1A}$R activation in the search for pain treatment strategies.

In the present work we report the synthesis of new compounds based on structural modifications of previously described 5-HT$_{1A}$R ligands.[3] These second-generation 5-HT$_{1A}$R agonists were assessed for binding affinity, selectivity, and functional activity at the receptor. Selected candidates were also evaluated for their *in vivo* analgesic properties. Interestingly, compound SPM-13746 has been characterized as a high-affinity and potent 5-HT$_{1A}$R agonist, and exhibited analgesic effect in the formalin test as a model of pain in mice. The intraperitoneal and oral analgesic effects of SPM-13746 were virtually identical to higher doses of gabapentin, a drug clinically used for neuropathic pain treatment. Notably, SPM-13746 also revealed a good pharmacokinetic profile in metabolic and distribution studies. The results herein reported suggest the interest of further pharmacological development of compound SPM-13746 and related drugs.[4]



SPM-13746
K_i = 2.3 nM
EC_{50} = 19 nM



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DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION OF PEPTIDOMIMETIC UREAS ANALOGUES OF THE THROMBIN RECEPTOR PAR1 ANTAGONIST RWJ-58259

Pilar Ventosa-Andrés[a], M. Teresa García-López[a], Ioannis Pappos[b], Nikos E. Tsopanoglou[b], Rosario Herranz[a]

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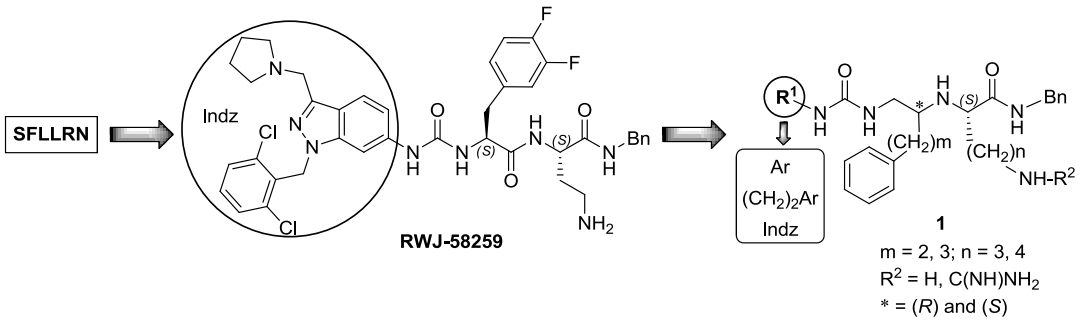
Thrombin regulates multiple cellular responses, such as platelet aggregation and tumor cell proliferation and angiogenesis. These cellular effects of thrombin are mediated by the activation of the protease-activated receptor PAR1.[1] This activation unveils at the N-terminal exodomain of PAR1 the tethered activation ligand SFLLRN. First potent PAR1 antagonists were SFLLRN-based peptidomimetic ureas, represented by RWJ-58259 (Figure), which was the first antagonist that provided *in vivo* proof of the clinical utility of PAR1 antagonists in cardiovascular and tumoral diseases.[2] Taking into account these precedents and recent structural studies on the thrombin/PAR1 interaction,[3] a small and directed library of RWJ-58259 analogue ureas of general formula **1** has been design, synthesized and screened as PAR1 antagonists.



Acknowledgements: This work was supported by the Spanish Ministry of Science and Innovation grants SAF2006-01205 and SAF2009-09323. P. V.-A. holds a FPI fellowship from the Spanish Ministry of Science and Innovation.

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DISCOVERY OF ALLOSTERIC MODULATION SITES ON GLYCOGEN SYNTHASE KINASE 3

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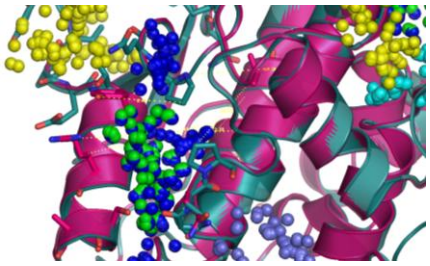
Glycogen synthase kinase 3 (GSK-3) is an important drug target for human severe unmet diseases such as diabetes type II, bipolar disorders, chronic inflammatory diseases and neurodegenerative pathologies. Discovery and/or design of allosteric kinase modulators are gaining importance in this field not only for the increased selectivity of this kind of compounds and the potential to overcome resistances, but also for the subtle modulation of the target. This last point is of utmost importance for the GSK-3 inhibition as therapeutic approach. GSK-3 activity is completely necessary for life and only the aberrant overactivity found in the pathologies should be inhibited with its inhibitors treatment.

We performed here a search for the druggable sites on the enzyme using fpocket[1] algorithm with the aim to provide allosteric potential binding sites on it and new clues for further drug discoveries. Moreover our results allow us to determine the binding site of VP0.7, a small molecule discovered in our laboratory that may be the first allosteric modulator of GSK-3.



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