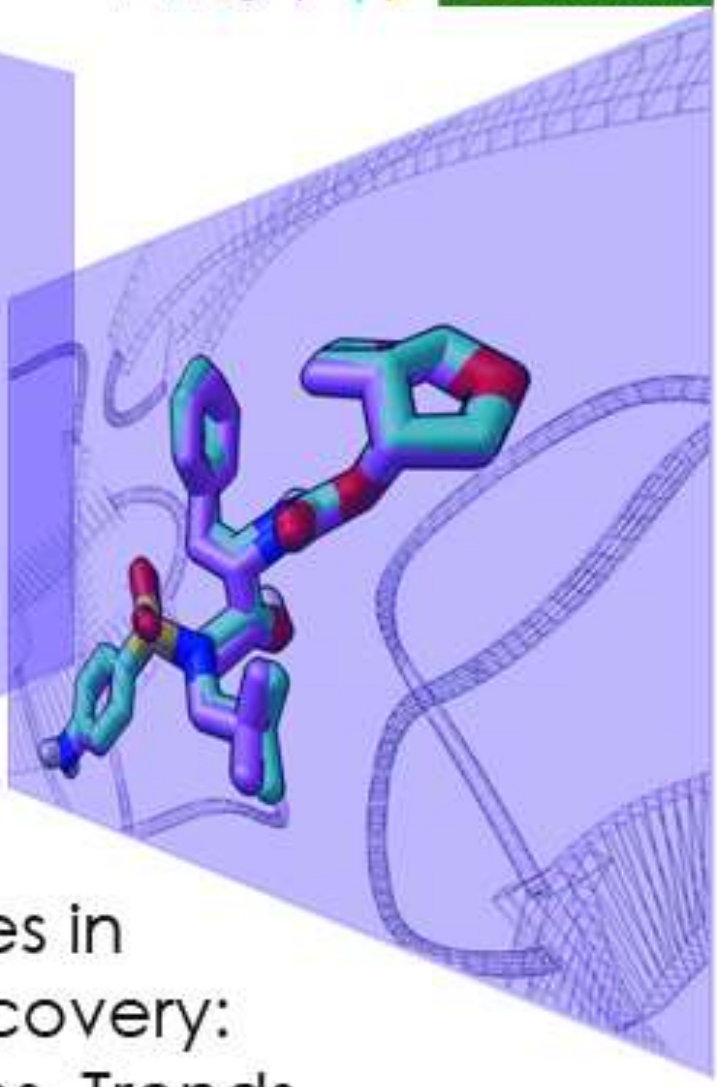
XVII NATIONAL MEETING

UNIVERSIDAD CEU SAN PABLO

MADRID 2-4 OCTOBER 2013

Sociedad Española de Química Terapéutica

SEQT



Advances in Drug Discovery: Successes, Trends and Future Challenges

CEU Universidad San Pablo

SEQT

Dear Colleagues,

The Organising Committee welcomes you to the XVIIth National Meeting on "Advances in Drug Discovery: Successes, Trends and Future Challenges" in Madrid, on October 2-4, 2013. The Meeting has been organised jointly by the Spanish Society of Medicinal Chemistry (Sociedad Española de Química Terapéutica, SEQT) and the University CEU San Pablo, continuing a long tradition established by biennial meetings in different cities and research and academic institutions throughout Spain.

About 130 scientists from 12 nations will meet at one of the centers of University CEU San Pablo, located in c/Tutor nº 35 (Madrid). This newly renovated building, placed in the heart of Madrid, offers the best facilities to support speakers, exhibitors and participants. About 13 expert speakers, 16 oral communicators and 56 poster presenters will discuss the latest advances in drug discovery: successes, trends and future challenges, to treat severe diseases in many different therapeutic areas. The Meeting will cover different topics related to Medicinal Chemistry as the design of drugs, new emerging targets, molecular recognition, chemical biology approaches in drug discovery, epigenetics in medicinal chemistry, predictive tools, diagnostic agents, or labelled ligands employed as pharmacological tools.

The XVIIth National Meeting has been organized with the support of Companies within the field of Medicinal Chemistry: Almirall, Janssen, Glaxo, LifeChemicals, Agilent, Lilly, Brucker, Galchimia and Esteve together with Fundación Universitaria San Pablo CEU. The Organizing committee and SEQT want to acknowledge their active support to this event.

Madrid is the capital of Spain and October is the ideal time to enjoy the natural and cultural beauties of the city. The dynamic atmosphere of Madrid is enhanced by picturesque streets, lively squares, beautiful boulevards, impressive monuments, spacious parks, interesting restaurants and an active cultural and night life. We believe that participation in the **XVIIth National Meeting on "Advances in Drug Discovery: Successes, Trends and Future Challenges"** in Madrid will be for you an unforgettable scientific and personal experience.

We look forward to your active participation!

The organising committee

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SCIENTIFIC PROGRAM

SCIENTIFIC PROGRAM

Wednesday, October 2nd

13:30 **Registration**

15:30 **Opening**: Coral Barbas, Vicechancellor of Research, Universidad CEU San Pablo
Beatriz de Pascual-Teresa, Organizing Committee, Universidad CEU San Pablo
Antoni Torrens, President SEQT

Chairperson: Antoni Torrens/Javier Rojo

16:00 **Inaugural Lecture**: *"Antibody-Based Therapeutics for Cancer Therapy"*
Peter Senter**,** Seattle Genetics, USA

17:00 **IS1**: "*Synthetic biology and smart therapeutic systems"*
Guillermo de la Cueva Méndez (BIONAD- Centro Andaluz de Nanomedicina y Biotecnología, Málaga, Spain)

17:30 **IS2**: *"Glycofullerenes for Biological Applications"*
Nazario Martín León (Universidad Complutense de Madrid, Spain)

18:00 **SEQT Awards**

18:30 **OC1:** "*A new strategy to gain selectivity within the gelatinases subfamily*".
Benjamin Fabre. (Universidad CEU San Pablo, Madrid, Spain) Ramón Madroñero SEQT Award

20:00 **Welcome Cocktail**

Thursday, October 3rd

Morning Session

Chairperson: Mª José Camarasa/Mª Luz López

09:00 **IS3**: *"3D Pharmacophore Models: Efficient Tools for Hit Identification, Lead Optimization, and Ligand Profiling"*
Thierry Langer (Prestwick Chemical, Illkirch, France)

09:30 **IS4**: "*Integrating Intramolecular Hydrogen Bonding (IMHB) considerations in Drug Discovery using lipophilicity as a Tool"*
Giulia Caron (University of Turin, Italy)

10:00 **IS5**: *"Nucleic acids in G-quadruplex conformations as innovative targets against cancer"*
Stefano Alcaro (Universitá "Magna Graecia", Catanzaro, Italy)

10:30 **Coffee Break**

11:00 **IS6:** *"ORL-1 Antagonists: Lead Optimization and PET Tracer Development"*
Angeles Martínez Grau (Lilly, S.A., Madrid, Spain)

11:30 **IS7**: *"The asymmetric architecture of G protein-coupled receptor heteromers with G proteins explains signalling cross-talk*
Peter McCormick (Universidad de Barcelona)

12:00 **Oral Communications** (**OC2-OC5**)
OC2: "*Synthesis and Pharmacological Evaluation of Acetaminophen Analogues with Antinociceptive Properties"*.
Pilar Goya (Instituto de Química Médica, CSIC, Madrid, Spain)

OC3: *"Applications of click chemistry in sphingolipid research"*
Antonio Delgado (IQAC-CSIC, Barcelona, Spain)

OC4: *"Computational chemistry for identifying differences among choline kinases active sites"*
Lucía Serrán Aguilera (Universidad de Granada, Spain)

OC5: *"Impact of fluorination on proteolytic stability of peptides: a case study using α-chymotrypsin and pepsin"*
Jeremie Mortier (Free University of Berlin, Germany)

13:00 **Lunch**

Afternoon Session

Chairperson: Pilar Goya/Julio Álvarez Builla

15:00 **IS8**: *"Amino Acid-Derived β–Lactams: Synthesis, Biological Activity and Beyond"*
Rosario González Muñiz (Instituto de Química Médica, CSIC, Madrid, Spain)

15:30: **OC6**: *"New non-metathetic processes catalysed by ruthenium complexes for the synthesis of heterocycles"*
Javier Pérez-Castells (Universidad CEU San Pablo, Madrid, Spain)

15:45 **Poster Session and Coffee**

16:45 **Oral Communications** (**OC7-OC8**)
OC7: *"Merging of pineal neurochemicals. pharmacological profiles and neurogenic potential"*
Mario de la Fuente Revenga (Instituto de Química Médica, CSIC, Madrid, Spain)

OC8: *"Drugging the von hippel lindau E3 ubiquitin ligase: potential drugs and useful chemical tools"*
Carles Galdeano (University of Dundee, Dundee, UK)

19:30 **Cultural evening**

Friday, October 4th

Morning Session

Chairperson: Jordi Gracia/Javier Fernández Gadea

09:00 **IS9**: *"Next-Generation Inhibitors for Kinases, Histone-Deacetylases, and Histone Methyltransferases: The discovery of Slowness"*
Gerhard Müller (Mercachem, The Netherlands)

09:30 **IS10**: *"Exploring the kinome with kinase broad screening"*
Gary Tresadern (Janssen R&D, Beerse, Belgium)

10:00 **IS11**: *"Antibiotic Treatment of Tuberculosis: Old Problems, New Solutions"*
Mónica Cacho Izquierdo (GlaxoSmithKline, Madrid, Spain)

10:30 **Coffee Break**

11:00 **Oral Communications** (**OC9-OC12**)
OC9: *"Design of mycobacterium tuberculosis shikimate kinase inhibitors inspired by the enzymatic movements for catalytic turnover"*
Verónica Prado (CIQUS, Universidad de Santiago de Compostela, Spain)

OC10: *"From hit to lead in search of new inhibitors of the enzyme isoprenylcysteine carboxyl methyltransferase (ICMT)"*
Silvia Ortega-Gutiérrez (Universidad Complutense, Madrid, Spain)

OC11: *"Design, synthesis and biological evaluation of cyclic acylguanidines as bace-1 inhibitors: effect of ring size on activity and selectivity versus BACE-2"*
Myriam Ciordia (Janssen Pharmaceutical Research & Development, Toledo, Spain)

OC12: *"-Omics tools for studying changes in metabolic pathways of new drugs"*
Isidre Masana (Agilent Technologies)

12:00 **SEQT Meeting**

13:00 **Lunch**

Afternoon Session

Chairperson: Concepción González Bello/Antonio Pineda

15:00 **IS12**: *"ETP-CNIO Academic Early Drug Discovery: PIM Kinase Inhibitors"*
Joaquín Pastor (CNIO- Centro Nacional de Investigaciones Oncológicas, Madrid, Spain)

15:30 **OC13**: *"Different levels of apoptosis caused by enantiomers in breast cancer cells"*
Joaquín M. Campos (Universidad de Granada, Spain)

15:45 **Poster Session and Coffee**

16:45 **Oral Communications** (**OC14-OC16**)
OC14: *"Tumor vascular disrupting agents: identification and optimization of novel hits"*
Mª Dolores Canela (Instituto de Química Médica, CSIC, Madrid, Spain)

OC15: "*3-Substituted indole derivatives as antimitotic agents"*
Raquel Álvarez Lozano (Universidad de Salamanca, Salamanca, Spain)

OC16: *"Chemical biology for drug discovery: two case studies in drug repurposing"*
Jordi Quintana (Parc Científic de Barcelona, Spain)

17:30 **Concluding Remarks. Poster and Oral Communication Awards**

21:00 **Gala Dinner**

ABSTRACTS

INAUGURAL LECTURE

Peter SENTER
(Seattle Genetics, EEUU)

Dr. Peter Senter. PhD in Chemistry from the University of Illinois followed by postdoctoral research at the Max-Planck Institute in Göttingen, Germany. He joined Seattle Genetics in 1998 and has served as Vice President, Chemistry since September 2002. Dr. Senter is responsible for research programs covering antibody-drug conjugates, anticancer prodrugs, and protein engineering. Before joining Seattle Genetics, he held positions at Cytokine Networks, Bristol Myers Squibb Pharmaceutical Research Institute, and the Dana Farber Cancer Institute. Dr. Senter is an Affiliate Professor in Bioengineering at the University of Washington and is the Senior Editor of Bioconjugate Chemistry, an ACS journal. He has authored more than 130 scientific publications and holds more than 40 patents. He belongs to several Scientific Advisory Boards for PharmSelex (2008-2010), PharmaIn (2009-present), OBodies (2012-present), Permeon (2012-present), Imaginab (2013-present), and belongs to the Editorial Advisory Board of Molecular Pharmaceutics (ACS) and MedChemComm (Royal Society of Chemistry).

Research Areas

- Drug targeting and drug delivery
- Protein chemistry and biology Drug development (approved drugs: ADCETRIS and Etopofos)

ANTIBODY-BASED THERAPEUTICS FOR CANCER THERAPY

P. D. Senter

Seattle Genetics, 21823 30th Dr SE, Bothell, WA USA

Monoclonal antibodies (mAbs) have played a major role in cancer medicine, with active drugs such as trastuzumab (Herceptin), cetuximab (Erbitux), bevacizumab (Avastin) and rituximab (Rituxan) in a wide range of therapeutic applications. The mechanism of activity of these agents involves cell signaling, effector functions through interactions with Fc receptor positive cells, and complement fixation. In order to improve activity, attention has turned towards enhancing mAb ADCC activity by selecting stronger Fc receptor binders. This has been accomplished using engineered cell lines that generate mAbs with optimized Fc regions designed for enhanced receptor binding (Xencor technology), or by changing the carbohydrate structures on the heavy chains of mAbs (Glycart and Biowa technologies). We have discovered an alternative approach involving the identification of biochemical inhibitors of the enzymes fucosyl transferase and GDP-d-mannose dehydratase (GMD). The inhibitors are fucose analogues, and can be added to cells that not only produce mAbs, but other proteins in which fucosylation is important for activity. Several applications of this technology will be discussed, both in vitro and in vivo (1).

mAb activity can also be enhanced by appending highly potent cytotoxic drugs to them. While this area has been investigated for many years, it has only been recently that mAb-drug conjugates have demonstrated the potential for playing a convincing role in cancer chemotherapy. The field has advanced significantly, with new insights gained into the roles that antigen target, normal tissue expression, drug potency, drug mechanism, linker stability, and mechanism of drug release play in generating active antibody drug conjugates (ADC) with acceptable safety profiles. ADCETRIS (Brentuximab vedotin, SGN-35) is an example an ADC that has been designed with these parameters in mind (2). In August 2011, ADCETRIS was approved by the US Food and Drug Administration for use in relapsed or refractory Hodgkin lymphoma and relapsed or refractory systemic anaplastic large cell lymphoma, two diseases with significant unmet medical needs. An overview of how this drug was developed and how we are extending the technology will be provided.

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PLENARY LECTURES

Guillermo DE LA CUEVA MÉNDEZ
(BIONAD- Centro Andaluz de Nanomedicina y Biotecnología, Málaga, Spain)

Dr. Guillermo de la Cueva Méndez is a Biochemist and Molecular Biologist from Madrid. During his PhD he studied the regulation and mode of action of toxin Kid and antitoxin Kis, two proteins that improve the survival of antibiotic resistance factor R1 in bacterial hosts. He then did a postdoct at the Wellcome/CRC Institute in Cambridge, UK, where he showed that toxin Kid induces apoptosis in human cells, and that this effect is neutralized by antitoxin Kis. This results led him to propose the possibility of constructing synthetic systems capable of achieving selective cell killing by controlling Kid/Kis ratios precisely and differentially in human cells, depending on their origin, differentiation state, environmental stimuli or genetic integrity. To explore the biotechnological and therapeutic potential of this proposal Guillermo joined the MRC Cancer Cell Unit in Cambridge, as Principal Investigator. Using Synthetic Biology, his group has produced smart therapeutic systems using Kid and Kis, capable of inducing apoptosis in cells exposed to specific oncogenic insults and, simultaneously, protecting other cells from collateral damage. Currently, Guillermo has moved to the Andalusian Centre for Nanomedicine and Biotechnolgy (BIONAND), where he is Head of the Therapeutic Nanosystems Area.

SYNTHETIC BIOLOGY AND SMART THERAPEUTIC SYSTEMS

G. de la Cueva-Méndez[1,2*], B. Pimentel[1], M. Preston[1], A. Turnbull[1], A. Arnáiz-Vivas[1], C. Bermejo-Rogríguez[1], I. Dionne[1]

[1] MRC Cancer Cell Unit. Hutchison/MRC Research Centre. Hills Road, Cambridge CB2 0XZ, United Kingdom.
[2]Andalusian Centre for Nanomedicine and Biotechnology (BIONAND). Parque Tecnológico de Andalucía. C/Severo Ochoa, 35. 29590 Campanillas. Málaga. Spain.

Our understanding of the molecular and cellular basis of cancer has improved considerably over the past few decades. However, this has not been paralleled by proportional increases in long-term survival rates of patients affected by the disease, which remains the second leading cause of death in Europe and North America.

Pharmacological treatment of cancer requires potent inducers of cell death that are also very selective. Classical chemotherapeutic agents are very effective at killing cells but display poor selectivity, leading to excessive collateral damage and limiting their efficacy and clinical application. Molecularly targeted drugs are more selective than classical chemotherapeutic agents. However, most of these compounds do not induce significant cancer cell killing.

Innovative approaches, combining killing efficiency of classical chemotherapeutic agents and target selectivity of molecularly targeted drugs are required to treat cancer effectively. our laboratory has produced synthetic systems that distinguish cancerous cells from normal cells. These systems exploit cancer biomarkers to promote the suicide of tumor cells whilst conferring active protection to normal cells, and therefore avoid off-target toxicity. The rationale behind these systems, their design and current performance, as well as their full potential and challenges ahead will be presented and discussed.

Nazario MARTÍN
(UNIVERSIDAD COMPLUTENSE DE MADRID, Madrid, Spain)

Dr. Nazario Martín Nazario Martín is full professor at the University Complutense of Madrid and vice-director of the Institute for Advanced Studies in Nanoscience of Madrid (IMDEA-Nanoscience). Recently he has been appointed as Dr. h.c. by La Havana University. He has published over 450 papers and co-edited six books related with carbon nanostructures. He has given over 290 lectures in scientific meetings and research institutions, and supervised 28 theses. He has served as a member of the Editorial Board of well-known international journals. He is a member of the Royal Academy of Doctors of Spain as well as a fellow of The Royal Society of Chemistry. In 2006-2012 he has been the President of the Spanish Royal Society of Chemistry. He has received the "Dupont Prize of Science" in 2007, and the "Gold Medal and Research Award" in 2012, the highest distinction given by the Spanish Royal Society of Chemistry. He has been appointed with the Spanish national "Jaime I Award for basic research" 2012, and the recipient of the "Alexander von Humboldt Award" and "Richard E. Smalley Research Award" in 2013. He is the last chemist distinguished with the "EuCheMS Lecture Award" in 2012. Last year 2012 he has received the "Advanced Grant" of the European Research Council.

GLYCOFULLERENES FOR BIOLOGICAL APPLICATIONS

N. Martín León*

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[b]IMDEA-Nanociencia, Facultad de Ciencias, Ciudad Universitaria de Cantoblanco, Módulo C-IX, 3[a] planta, 28049, Madrid

The carbohydrate-protein interaction is a key issue in a variety of biological processes. Actually, this molecular recognition is usually one of the selective steps triggering different biological processes affording biological functions. This interaction is characterized by a high selectivity, strong dependence of some specific metal cations and a remarkable low affinity.
The way in which mother Nature solves this low affinity is by means of using the *so-called* multivalent interactions by using many copies of receptor (lectines) and a multivalent presentation of the ligand (sugar). Thus, the development of the required tools for a better understanding of the biological processes involving carbohydrates are based in the design and preparation of multivalent carbohydrates systems.
We have recently shown that fullerenes are appealing suitable spherical molecular scaffolds for the construction of globular structures decorated at will on the periphery in the search for new biologically active spherical supermolecules (1).

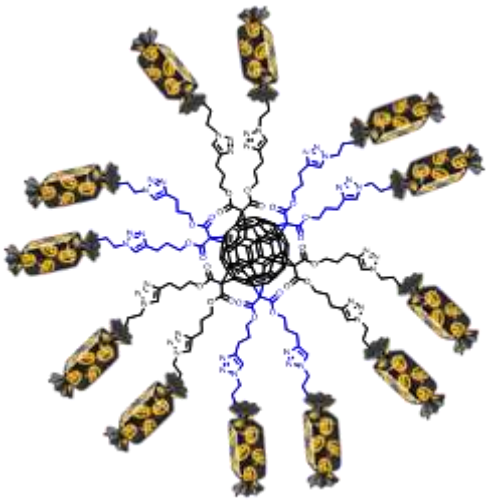

Figure. Fullerene C_{60} hexa-adduct decorated con sugar units at the periphery

Furthermore, we have recently reported an efficient method for preparing amphiphilic fullerenes by using "click chemistry". The aggregation study of these molecules reveals that the obtained morphology depends on the concentration of the studied samples. This type of aggregation in wires and micelles could find further applications in the design of functional materials as well as in biological sciences (2).

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Thierry LANGER
(PRESTWICK CHEMICAL, Illkirch, France)

Dr. Thierry Langer, Pharm MS, PhD, CEO and President, joined Prestwick Chemical in 2008. Previously he was Professor at Leopold-Franzens-University of Innsbruck, Austria, where he began his career after completing a post doctoral fellowship at the Université Louis Pasteur (Strasbourg, France) in 1992. Prof. Langer holds an M.S. degree in Pharmaceutical Sciences (1988) and a Ph.D. (Medicinal Chemistry, 1991) from the University of Vienna, Austria. In 2003, together with Prof. Stuppner and Dr. Wolber he founded the spin-off company Inte:Ligand GmbH, in which he also was CEO prior to joining Prestwick Chemical. His research interests have been focused on computer-assisted medicinal chemistry, including pharmacophore-based methods as well as 3D-QSAR molecular modeling techniques. Since 1993 he established at Innsbruck University the Computer Aided Molecular Design Group, was appointed Associate Professor of Pharmaceutical Chemistry in 1997, and served as Head of the Institute of Pharmaceutical Chemistry and of the Pharmaceutical Chemistry Department in 1998 and 1999. Prof. Langer is a well known scientist in the field of computer aided molecular design: His scientific work has culminated in more than 170 original articles and invited reviews in peer reviewed journals, several book chapters, one edited book, and more than 200 presentations at scientific meetings.

3D PHARMACOPHORE MODELS: EFFICIENT TOOLS FOR HIT IDENTIFICATION, LEAD OPTIMIZATION AND LIGAND PROFILING

T. Langer

Prestwick Chemical SAS, 220 Blvd Gonthier d'Andernach, 67000 Strasbourg-Illkirch, France

Pharmacophore-based virtual screening and activity profiling has become one of the most popular in silico techniques for supporting medicinal chemists in their hit finding, hit expansion, hit to lead, and lead optimization programs. (1)

At Inte:Ligand GmbH, the program LigandScout (2) was developed as a software containing rapid and efficient tools for automatic interpretation of ligand-protein interactions and subsequent transformation of this information into 3D chemical feature-based pharmacophore models. Additionally, algorithms were developed for ligand-based pharmacophore modeling in the absence of a target structure, as well as for accurate virtual screening. As an extension of this approach, parallel pharmacophore-based screening has been introduced as an innovative in silico method to predict the potential biological activities of compounds by screening them with a multitude of pharmacophore models.

In the presentation, Prof. Langer will give an overview of this technology and will present the results of several success stories: Examples range from proof of concept studies employing a set of antiviral compounds that were submitted to in silico activity profiling using a subset (3) of the Inte:Ligand Pharmacophore Database (4) to in silico fragment-based discovery of novel enzyme inhibitors. Additionally, several medicinal chemistry application examples yielding clinical candidates will be highlighted.

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(4) The Inte:Ligand Pharmacophore Database is available from Inte:Ligand GmbH, Vienna, Austria (http://www.inteligand.com)

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Giulia CARON
(UNIVERSITY OF TURIN, Turin, Italy)

Dr. Giulia Caron studied at the University of Torino (Italy) where she received a B.Sc. in Pharmaceutical Chemistry and Technology in 1992, and a B.Sc. in Pharmacy in 1994. Then she moved to the University of Lausanne (CH) where she was awarded a Ph.D. in Pharmaceutical Sciences in 1997 under the supervision of prof. B. Testa. She is Assistant Professor at the Molecular Biotechnology and Health Sciences Dept. of the University of Torino where teaches pharmaceutical analysis and chemometrics also with the support of blended learning technologies. Her primary scientific activity was experimental lipophilicity, then moved to the computational prediction of ADME properties by QSAR strategies. Molecular descriptors to predict intramolecular hydrogen bonding and their influence on biodistribution are also focused in her studies. The design of cell penetrating peptides is one of her more recent field of interest. She is coauthor of more than 60 papers and 2 software products.

IS4

INTEGRATING INTRAMOLECULAR HYDROGEN BONDING (IMHB) CONSIDERATIONS IN DRUG DISCOVERY USING LIPOPHILICITY AS A TOOL

G. Caron[1,*], G. Ermondi[1]

[1]Molecular Biotechnology and Health Sciences Dept., University of Torino, via Quarello 15, 10135 Torino, Italy

The optimisation of molecular properties of a compound is fundamental in the drug discovery process, mainly due to their influence on absorption and distribution *in vivo*.
The propensity of a compound to form intramolecular hydrogen bonds (IMHB) is an emerging molecular property in medicinal chemistry since IMHB can strongly influence the fate of drugs both from a pharmacodynamic and a pharmacokinetic point of view.
This communication demonstrates that $\Delta\log P_{oct\text{-}tol}$ ($\log P_{oct} - \log P_{tol}$) is a molecular descriptor that distinguishes compounds with high propensity to form IMHB (1). New experimental and computational tools used to determine and understand $\Delta\log P_{oct\text{-}tol}$ are also highlighted, e.g. the Block Relevance analysis (2).

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Stefano ALCARO
(UNIVERSITÁ "MAGNA GRAECIA", Catanzaro, Italy)

Dr. Stefano Alcaro received in 1990 the degree with full marks in Medicinal Chemistry and Technology at the University of Rome "La Sapienza", discussing an experimental thesis in computational chemistry.

At the same University he reached the Ph.D. in Pharmacology, continuing his research activity in the field of interactions between bio-molecules by innovative molecular modeling tools.

In 1994 Stefano Alcaro was postdoct at the Dept. of Chemistry of Columbia University (NYC, USA) where he joined the computational team of Prof. Clark W. Still. In 1996 he moved to his inborn place as researcher at the new University "Magna Græcia" of Catanzaro, creating the Computational Chemistry lab (CClab).

From 2002 to 2011 he was associate professor of Medicinal Chemistry in the Faculty of Pharmacy and the head of the CClab of the same academic institution. From June 2011 he is full professor at the same University.

His scientific interests are related to the development of new molecular modeling tools useful in the drug discovery process and to the application of them and other methods, mainly in the design of anticancer and antiviral agents. He is co-author of more than 110 peer-reviewed manuscripts and numerous communications to national and international meetings.

NUCLEIC ACIDS IN G-QUADRUPLEX CONFORMATIONS AS INNOVATIVE TARGETS AGAINST CANCER

Stefano Alcaro

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G-quadruplex (G4) structures are non-canonical nucleic acid conformations occurring in guanine-rich sequences connected *via* Hoogsteen's type hydrogen bonds (1) among four guanines and stabilized by monovalent cations (Figure 1).

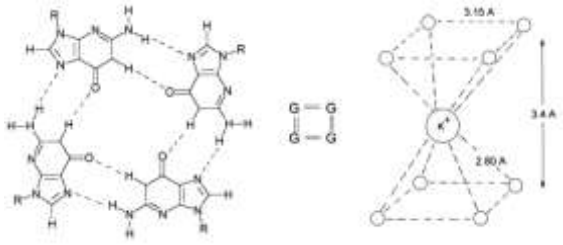

Fig. 1: Hoogsteen's type H bonds in G4 core forming typical G-quartets and main distances with respect to the K^+ stabilizing cation.

Some relevant key locations at genome level, such as telomeric ends or oncogenic promoters, are involved in G4 folding. The rationale of stabilizing the G4 conformation represents a novel approach for the drug design of innovative antineoplastic agents. The Protein Data Bank (PDB) includes several X-ray and NMR determined models of G4 structures in some case complexed with stabilizing ligands. These PDB entries are ideal starting points for rational drug discovery campaigns.

Recently we have started *in silico* studies with the characterization of PDB models of human telomeric sequence *d*[$AG_3(T_2AG_3)_3$] (h-TELO) by conformational studies, docking simulations (2) and, more recently, virtual screening experiments (3) carried out by means of combined ligand/structure based approaches. The issue of the flexibility and conformational polymorphism of the G4 targets (4) has been explicitly considered since the molecular recognition of stabilizing ligands can be strongly influenced.

In this communication some *in silico* experiences carried out in our laboratory are presented highlighting especially the successful identification of new G4 binders.

This research work is supported by the Italian Ministry of Education FIRB_IDEAS for the years 2009-2014 (code RBID082ATK_002), PRIN 2009 (code 2009MFRKZ8_002).

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Peter McCormick
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Dr. Peter McCormick performed his graduate work in the lab of Arthur E. Johnson. He developed a novel in vitro approach that incorporated a labeled lysyl-tRNA into a growing nascent polypeptide. This approach led to a seminal understanding that the pore through which all membrane proteins pass, plays an active role in membrane protein biogenesis. This work was followed by a study that was the first demonstration that transmembrane regions begin to form within the tunnel of the ribosome.

For his post-doctoral work he received a fellowship to work in the lab of Juan Bonifacino at the National Institutes of Health (USA). They answered a long standing question in the field of immunology about what path the MHC II molecules take to reach the antigen containing compartments. Next, he accepted a position in the group of Giovanna Tosato at the National Cancer Institute (USA), where he began to work on GPCRs.

In 2009, he obtained a Ramon y Cajal fellowship from the Spanish government. His current and long term goals are to continue studying how dimerization of GPCRs occurs, how it is regulated, and how it impacts cellular function.

THE ASYMMETRIC ARCHITECTURE OF G PROTEIN-COUPLED RECEPTOR HETEROMERS WITH G PROTEINS EXPLAINS SIGNALLING CROSS-TALK

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Despite the importance of G-protein-coupled receptors (GPCR) as targets for therapy, there has been a sharp drop in the number of successful drugs targeting these receptors. A possible complication in targeting these receptors is that they can form what are called heteromers, or the combination of two different GPCRs into a larger macromolecular structure. It has been proposed that one of the advantages to forming GPCR oligomers is to provide a platform for communication and asymmetry across different receptors. However, the dynamics and structural characteristics underlying heteromers are not known, and thus designing compounds to target heteromers as proven difficult. Using single particle tracking experiments, biophysics and molecular modeling we provide experimental evidence to support a model of Adenosine A_1-A_{2A} receptor complexes that can explain how heteromers can be inherently asymmetric, and what interfaces are responsible for the intermolecular communication. Our hope is that these data lay the groundwork for future strategies on targeting GPCR heteromers.

Rosario González Muñiz
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Dr. Rosario González-Muñiz studied chemistry at the Madrid Autónoma University (UAM, BSc 1982) and did her Ph.D. at the Medicinal Chemistry Institute (IQM-CSIC, 1987). Post-doctoral research (1988-1990) was carried out in the group of Bernard P. Roques at the René Descartes University (Paris V). In 1989 she became a tenured scientist at the IQM-CSIC, where presently is a scientific researcher. She was also the Deputy Director of the Institute (Oct. 2005-May 2011), and a member of the executive board of the Spanish Society of Medicinal Chemistry (2003-2011). During the PhD thesis and the postdoctoral period she worked on bioactive peptides, moving shortly after to the peptidomimetic's field. Her main contributions have been related to the generation of new peptide secondary structure mimetics and the search of chiral small-molecules to modulate peptide GPCRs and protein-protein interactions of interest in different therapeutic areas. Her current research interests are mostly focused in the design and synthesis of new chemical entities as modulators of different ion channels and closely associated proteins. .

AMINO ACID-DERIVED B–LACTAMS: SYNTHESIS, BIOLOGICAL ACTIVITY AND BEYOND

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The 2-azetidinone (β–lactam) ring has been recognized as the fundamental pharmacophore group for a wide range of bioactive compounds (antibiotics, inhibitors of proteases, antitumor agents, etc.), as well as useful intermediates for the synthesis of a variety of high-added value products (1-3).

The work of our group in the field of β–lactams began some years ago, when we discovered by serendipity that the N-haloacetyl derivatives of amino acids could easily be transformed into innovative 4,4-disubstituted 2-azetidinones (4). From there, we have developed enantioselective routes for their preparation, based on the memory of chirality phenomenon, and on the use of chiral auxiliaries or enantiopure 2-haloalcanoyl derivatives (5). Some of these β–lactams, which can be prepared both in solution and on solid-phase, displayed significant antitumor activity, constituted the starting point for a new family of non-covalent HCMV inhibitors with antiviral potential, and have served as modulators of TRP channels. In addition, as reaction intermediates, they have been transformed into different conformationally restricted amino acids (α–alkyl Asp and Asn, α–alkylazetidine-containing derivatives, quaternary α,α–2-azepane derivatives, and $\beta^{2,3,3}$–amino acids bearing a 2-piperidinone ring). Some of these non-proteinogenic amino acids are effective scaffolds to constrain peptide conformations, being able to induce the adoption of specific secondary structures and, therefore, of interest in the generation of peptidomimetics.

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Gerhard MÜLLER
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Dr. Gerhard Müller is Senior Vice President Medicinal Chemistry at Mercachem. He obtained his PhD in Organic Chemistry by the Technical University of Munich in 1994 together with Prof. Horst Kessler. The first 10 years of his professional career he spent in the pharmaceutical industry working for Glaxo in Verona, Italy, for Bayer AG in Leverkusen, Germany, and for Organon in the Netherlands, where he was section head Medicinal Chemistry.In 2003 he changed into the Biotech industry working as Chief Scientific Officer for Axxima Pharmaceuticals in Munich, then became VP Drug Discovery at GPC Biotech. Gerhard is specialized in a wide range of target classes from numerous disease areas. Over the last 10 years he specialized in kinase as well as epigenetic enzyme inhibitor research establishing novel design paradigms, proven by numerous publications.

NEXT-GENERATION INHIBITORS FOR KINASES, HISTONE-DEACETYLASES, AND HISTONE METHYLTRANSFERASES: THE DISCOVERY OF SLOWNESS

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In medicinal chemistry, a paradigm shift has occurred over the last decade in that more emphasis is laid on the improvement of ADME-related properties and off-target effects early in the drug discovery process, rather than pursuing a mere IC_{50}-hunting campaign. Despite these advances, lead finding and optimization programs frequently struggle with achieving high cellular and in-vivo efficacy for a given compound series, despite excellent biochemical activity and good physicochemical properties of the frontrunner candidates. To improve the correlation between e.g. a biochemical and the cellular and in-vivo efficacy, it is advantageous to consider the lifetime of the ligand-target complex by measuring and optimizing the residence time of compound-target complexes.

In this presentation we emphasize the relevance of binding kinetic attributes of inhibitors against kinases, as well as a variety of epigenetic targets, such as histone-deacetylases (HDACs) and histone-lysinemethyltransferases (HKMTs), respectively.

In the area of protein kinases, the prospective engineering of a binding kinetic signature into inhibitors is exemplified, that exhibit a slow koff by applying “deep-pocket-directed” scaffolds.

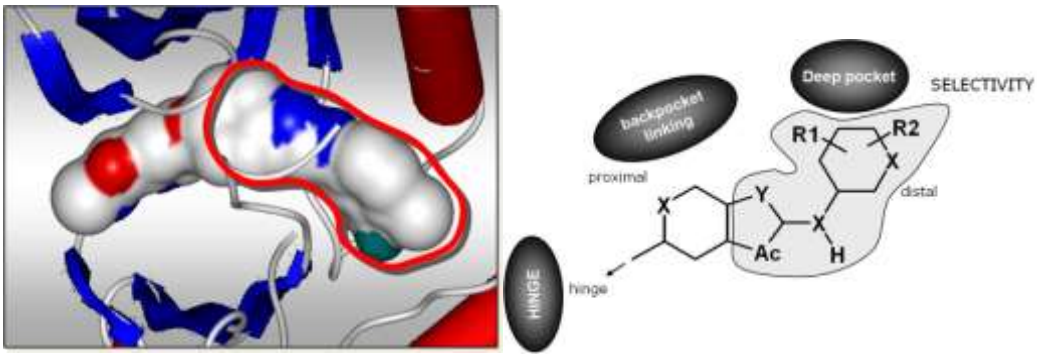

Figure 1: A type II kinase inhibitor accommodated by a conformationally rearranged kinase target (left); the pharmacophoric features derived from comparative structure studies of type II inhibitors (right)

We will demonstrate that a thorough understanding of the precise pharmacophoric requirements on the target’s binding site is essential to pre-engineer the desired slow off-rate into new, thus literature-unprecedented scaffolds that qualify as privileged structures for the target family of kinases. The details of the so-called “retro-design” approach for type II kinase inhibitors (see figure 1) will be exemplified by hit-to-lead and lead optimization campaign that yielded novel and highly efficacious CDK-8 inhibitors (1).

In addition to kinases, novel design principles to next-generation HDAC and HKMT inhibitors with pre-designed slow off-rates will be introduced that exploit the conformational flexibility that is associated with transient binding sites and product release channels.

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Gary TRESADERN
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Dr. Gary Tresadern currently heads the Molecular Informatics (MI) group at Janssen R&D in Belgium . Gary joined Janssen in 2005 as a computational chemist and molecular modeller and has contributed to multiple discovery projects reaching clinical evaluation. He has a particular interest in hit and lead generation, GPCRs, Kinases, and virtual screening. He graduated with a PhD in computational chemistry from the University of Manchester in 2003 and worked in the UK for Tripos Discovery Research from 2002 to 2005. He is co-author of 24 scientific articles, 2 book-chapters and co-inventor on 13 published patent applications.

EXPLORING THE KINOME WITH KINASE BROAD SCREENING

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Kinase broad screening is an established tool in pharmaceutical kinase drug discovery. Here we describe recent efforts at Janssen in this area. Kinase broad screening is one of the main initiatives within our cross therapeutic area kinase platform. Details and comparison of different assays will be discussed. The resulting kinase broad screening data feeds many initiatives, from immediate hits for kinase projects, selective molecules for target validation and engagement, through to cheminformatics approaches for data mining and analysis.

Mónica CACHO IZQUIERDO
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Dr. Mónica Cacho Izquierdo. BSc in Chemistry from the "Universidad Complutense de Madrid" (1996).
PhD in Chemistry in 2002 from the "Universidad San Pablo CEU". PhD Thesis: Synthesis and biological activity of new antitumor inhibitors of the cell cycle.
Short stay at the Institute for Chemical Research, Bayer A.G. (Wuppertal, Germany) to develop the project Synthesis of a new series of Factor Xa inhibitors (June-September 1999).
Postdoctoral research at the University of Nottingham (2002-2003) working in Prof. Stephen Clark group in the Stereoselective synthesis of the cyclic ether core of (+)-laurenyne.
Decem. 2003-Sep. 2004, Professor in Department of Chemistry at Universidad San Pablo CEU. She also participated in the project Design and synthesis of agonists/antagonists of adrenomedullin.
Oct. 2004-Jan. 2005, Research Position in Department of Medicinal Chemistry, Noscira S.A. (Zeltia), focused on developing innovative treatments for neurodegenerative diseases.
Feb. 2005-May 2005, Research Position at the "Institute of Medicinal Chemistry (CSIC)" financed by the Spanish Ministry of Education ("Programa Juan de la Cierva") working in Prof. Camarasa group in the identification of novel inhibitors of the viral enzyme Reverse Transcriptase (RT).
In May 2005, she joined the Tres Cantos Medicines Development Center, GlaxoSmithKline as a Senior Scientist. She has been working in the Drug Discovery Chemistry department participating in projects in the area of Tuberculosis. She has been promoted to Principal Scientist in 2012.
Coauthor of 15 publications and coinventor of 5 patent applications.

ANTIBIOTIC TREATMENT OF TUBERCULOSIS: OLD PROBLEMS, NEW SOLUTIONS

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Tuberculosis (TB) represents an enduring, deadly infectious disease for all of mankind. Nearly two billion people are currently infected with TB with a staggering 13.7 million active cases worldwide (1). The World Health Organization (WHO) estimates that nearly 1.5 million people die from TB each year with the overwhelming majority of these from developing portions of the world (2).

In recent years, the significance of the disease has increased dramatically as TB is also the major cause of death among patients co-infected with HIV. In addition, new drug-resistant strains of TB have emerged which are exceedingly difficult to treat successfully. Every year, nearly half a million new cases of multidrug resistant tuberculosis (MDR-TB) are estimated to occur (3). Extremely drug resistant TB (XDR-TB) is fatal in a large proportion of cases (4).

For those suffering from drug sensitive (DS) tuberculosis, the standard treatment involves an oral, four-drug combination (isoniazid, rifampin, pyrazinamide, and ethambutol) given daily over a period of 6 to 9 months. Treatment options for patients with drug resistance forms of TB are even more limited, with much longer treatment periods (12-18 months or longer) requiring second-line, injectable agents which can only be administered in hospital/clinic settings. There is urgent need for more effective and tolerable treatment of drug susceptible and drug resistant disease and latent TB infection.

One way to speed up the TB drug discovery process is to search for antimicrobial activity among compounds already known to posses some of the properties needed for a useful antitubercular, such as whole cell activity, oral absorption and safety profile.

Taking advantage of broad expertise of GSK in the antibacterial drug discovery, compounds already made or investigated at GSK for any type of antimicrobial activity, and not known to posses class-related cytotoxicity, have been requested and tested for inhibition of *M. tuberculosis* growth.

In this context, we present in this talk the story of Pleuromutilins and *Mycobacterium tuberculosis* DNA Gyrase Inhibitors (MGI's), novel families of promising compounds with potential for the treatment of TB disease, emerging from GSK's Antibacterium collection (7).

Acknowledgements: We would like to thank all the members of Pleuromutilins and BTI programs (GSK AB DPU) for their spirit of collaboration and all the hard work done. We would also like to thank the Global Alliance for TB for its support and funding.

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Joaquín PASTOR
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Dr. Joaquín Pastorr obtained his Ph.D. in Chemistry by the University of Alcalá de Henares working in the development of intercalating agents based on azolium salts. His Ph.D. thesis was carried out in the "Heterobetaines Group" directed by Prof. J. Alvarez-Builla. After his Ph.D. he moved for a postdoctoral stage (1994-1998) to the chemistry group directed by Prof. K.C. Nicolaou at The Scripps Research Institute (la Jolla CA, US), where he did research in the Solid Phase Synthesis of natural products and the development of novel solid supported reagents. As a highlight, he was coauthor of the SPS of the anti-mitotic agents epotholines published in Nature in 1997. He came back to Spain with a research contract to the UAH, but accepted an offer to join Janssen (J&J PRD) as head of the newly created High-throughput Medicinal Chemistry group. During his stay at Janssen Dr. Pastor held several positions as Team Leader in different projects and therapeutic areas such as CNS, metabolic disorders and oncology, collaborating in the progression of compounds to NME stage. Finally, he was also appointed as head of the Hit Generation Group for J&J PRD Europe. After 10 years of service at Janssen, in 2008 he joined the CNIO as Director of the Medicinal Chemistry Department of the Experimental Therapeutics Programme, where he played a leadership role in several drug discovery projects for novel oncology targets, which have delivered candidates already licensed to pharma companies. In March 2011 he was appointed to his current position of Director of the Programme leading the drug discovery activities of the CNIO. He is author and co-author of more than 70 scientific publications and patents in the field of drug discovery.

ETP-CNIO ACADEMIC EARLY DRUG DISCOVERY: PIM KINASE INHIBITORS

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PIM kinases are a family of serine/theronine kinases that includes three highly homologous members (PIM1, PIM2 and PIM3), which do not require posttranslational modifications to induce kinase activity; their activity is manly regulated by their expression. PIM kinases have been found to be over expressed in many hematological malignancies and solid tumors. Moreover, up-regulation of PIMs correlates with poor prognosis in multiple cancer types and promoted resistance to chemo and targeted therapies. PIM expression is mediated by the JAK/STAT and NF-□B pathways, and regulates several cellular processes such as cell cycle progression, apoptosis, metabolism, homing and migration (1). The presentation will cover aspects such as *in vivo* target validation and the generation, synthesis and optimization of a chemical series of highly potent PIM inhibitors with with low nanomolar activity against PIMs and exquisite selectivity within a panel of 456 kinases (Kinomescan^{TM}). Lead compounds have optimized *in vitro* ADME properties and a good pharmacokinetic profile. They show PIM associated biomarker down regulation *in vivo* and displayed significant antitumor efficacy in mouse models of human cancer.

The PI3K/AKT pathway is commonly activated in human cancer. Several inhibitors have been progressed to clinical trials, but the efficacy of these drugs is compromised by the stimulation of compensatory signaling pathways, which provide back-up mechanisms that allow cancer cells to escape to targeted therapies. One of them is that driven by PIM kinases, which produce parallel oncogenic signals to AKT and mTOR and share several downstream molecular targets (2,3). PIM mediates resistance to AKT and PI3K/mTOR inhibition (4,5) and, therefore, co-targeting both pathways would improve the efficacy of current PI3K/AKT/mTOR inhibitors in anticancer therapy. In the second part of the presentation, we will show the design, synthesis and characterization of dual and triple inhibitors of PIM/PI3K and PIM/PI3K/mTOR. After exploration of the initial hit, we have selected two representative orally available lead candidates for further development. They show excellent kinase selectivity profile against a panel of 456 kinases (Kinomescan^{TM}). Both compounds have been profiled for their antiproliferative behavior using a diverse panel of tumor cell lines. We have identified leukemia, lymphoma, colon and NSCLC lines, which exhibit a strong sensitivity to dual and triple inhibition. Mechanistically, cells respond to dual and triple inhibitors with cell cycle arrest and marked apoptosis in AML and NSCLC cell lines, and strong down regulation of AKT, 4EBP-1 and BAD phosphorylation.

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ORAL COMMUNICATIONS

A NEW STRATEGY TO GAIN SELECTIVITY WITHIN THE GELATINASES SUBFAMILY

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Matrix metalloproteinases (MMPs) are zinc endopeptidases that degrade the extracellular matrix (ECM) and are related to several pathological conditions.(1) Among the MMP family, MMP-2 and MMP-9 constitute the gelatinase subfamily and share highly homologous catalytic domains. MMP-2 is a validated target for cancer therapy whereas MMP-9 is considered as an anti-target.(2) Compounds that selectively inhibit MMP-2 over MMP-9 are thus sought for cancer therapy.

Our research group focuses on the selective inhibition of MMP-2, being particularly interested in reaching selectivity over MMP-9. Using a click-chemistry approach, we recently discovered two families of clicked MMP-2 hydroxamic acid inhibitors (Fig.1a). (3,4) In particular compound **1** displayed an interesting profile with an IC_{50} against MMP-2 in the

nM range, a MMP-2/MMP-1,-8,-14 selectivity profile (MMP-1,-8 and -14 are considered as anti-targets for cancer therapy (2)), a good MMP-2/MMP-9 selectivity and a water solubility in the mg/mL range.

In order to rationalize the MMP-2/MMP-9 selectivity of this promising compound, we carried out 100-ns long molecular dynamics (MD) of both MMP-2 and MMP-9, alone or in complex with **1**. These studies point out the higher flexibility of the Ω-loop (that delimitates the bottom part of S1'pocket, refer to Fig.1b) of MMP-2 as the possible origin of the selectivity. To verify this hypothesis we designed and synthesized a new series of hydroxamic derivatives **2** (Fig.1c), that present bulkier groups in the part of the inhibitor located at the bottom section of S1' pocket. The high selectivity of **2b** supports our hypothesis and suggests a new strategy to selectively inhibit MMP-2 over MMP-9.

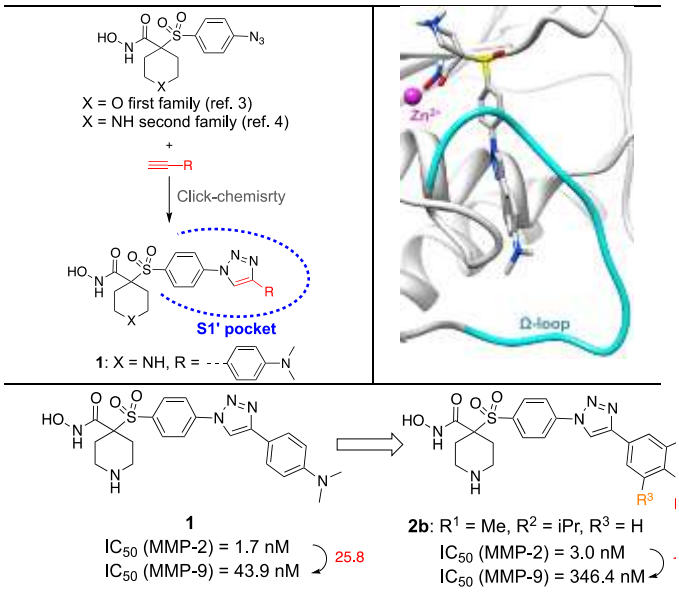

Fig 1. a) The click approach: various R groups are introduced to screen S1' pocket, known as the selectivity pocket. b) Proposed binding mode of 1 in MMP-2 (MD snapshot). The flexible part of Ω-loop that partially delimitates S1'pocket is depicted in cyan. c) Design of new hydroxamate inhibitors with improved MMP-2/MMP-9 selectivity.

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OC2

SYNTHESIS AND PHARMACOLOGICAL EVALUATION OF ACETAMINOPHEN ANALOGUES WITH ANTINOCICEPTIVE PROPERTIES

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Although acetaminophen also known as paracetamol, was found to be an effective analgesic more than a century ago, its mechanism of action is complex and the subject of continuous research mainly due to the extensive metabolism of acetaminophen in animals and humans.
Despite the fact that it has been on the market for decades, acetaminophen can be considered a "standalone" drug of which no effective analogues are on the market.
We report the synthesis and biological evaluation of new analogues of acetaminophen with important analgesic properties. The mechanism of nociception is not exerted through direct interaction with cannabinoid receptors, nor by inhibiting COX. One of them behaves as novel selective TRPA1 channel antagonist, which may be responsible for its analgesic properties. *In vivo* experiments, showed an analgesic efficacy significantly higher than acetaminophen and similar to morphine. Further studies are being carried out in order to gain a deep insight into the mechanism of these interesting derivatives.

APPLICATIONS OF CLICK CHEMISTRY IN SPHINGOLIPID RESEARCH

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Over the last years, click chemistry has become a versatile strategy with multiple applications in medicinal chemistry and related areas (1). However, examples in the lipid arena and, particularly, in sphingolipid research are still scarce (2,3).

In this communication we intend to present some examples of our current research addressed at the applications of click chemistry to the study of sphingolipids, ranging from structural aspects to cell metabolism and modulation. To this end, we have synthesized a series of azide labelled sphingolipids (N_3SL) amenable to orthogonal reactions with suitable tags by means of click chemistry protocols (Fig.1).

Several examples will be used to illustrate the applications of our N_3SL in biophysical studies using fluorescence membrane lipids, cell trafficking and localization studies, quantitative cell metabolism, and also as active site directed probes.

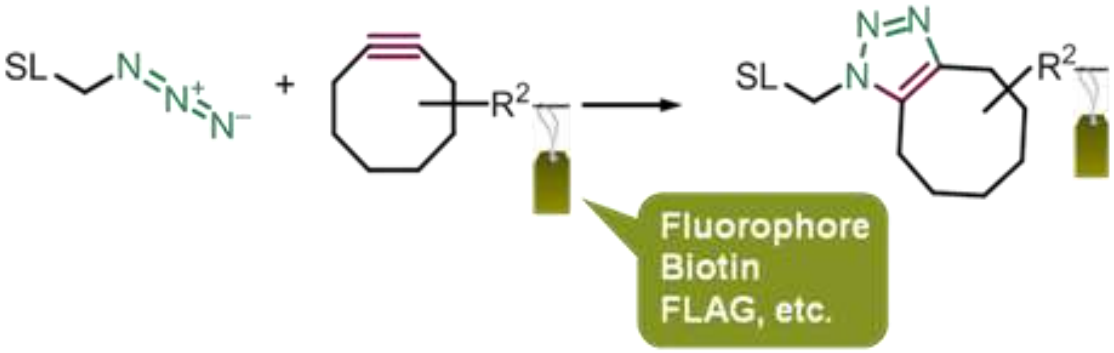

Fig. 1: Use of click chemistry lo tag N_3-labelled sphingolipids.

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OC4

COMPUTATIONAL CHEMISTRY FOR IDENTIFYING DIFFERENCES AMONG CHOLINE KINASES ACTIVE SITES

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Choline Kinase plays an important role in cellular membrane phospholipid generation in eukaryotic cells and has been validated as a therapeutic target in cancer[1] and malaria[2]. In order to identify differences among active sites of human and *Plasmodium falciparum* choline kinases, crystal structures of the previously mentioned species were submitted to Molecular Dynamics simulations and SiteMap calculations. No significant differences in ligand recognition or physicochemical properties in the ATP and choline binding sites were found, leading to the hypothesis that the same ligands may show similar inhibitory activities among species. This observation is important to retrieve in the design of new inhibitory compounds with a multitarget profile. To validate the above statement, several compounds were synthesized to carry out tryptophan fluorescence experiments in order to determine the dissociation constants (Kd) against purified human and *Plasmodium falciparum* choline kinases. The results showed similar Kds for each enzyme and agreed with our initial hypothesis inferred from the computational studies.

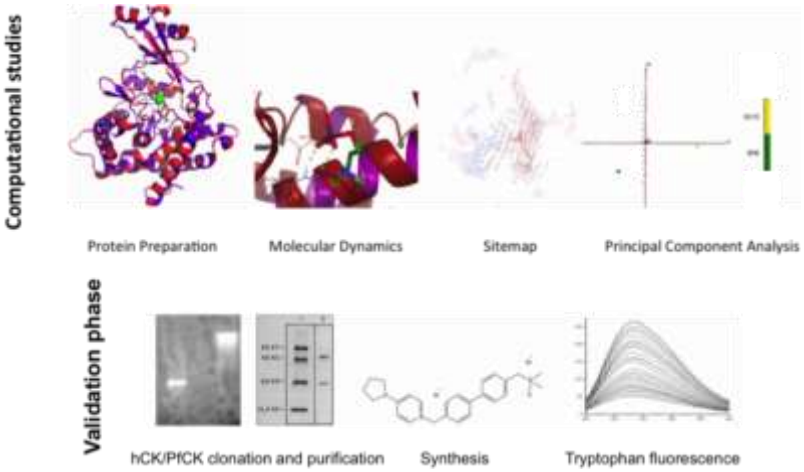

Fig. 1: Computational studies and validation steps for the physicochemical characterization of human and *Plasmodium falciparum* choline kinases active sites.

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OC5

IMPACT OF FLUORINATION ON PROTEOLYTIC STABILITY OF PEPTIDES: A CASE STUDY USING A-CHYMOTRYPSIN AND PEPSIN

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Protease stability is a key factor in the development of peptide-based drugs. A major approach to increase the bioavailability of pharmacologically active peptides is the integration of non-natural amino acids. Due to the unique properties of fluorine, fluorinated organic molecules have been proven useful in the development of therapeutically active molecules. (1, 2)

Our study presents data on the ability of fluorinated amino acids to affect proteolytic stability when incorporated into peptide sequences that are based on ideal protease substrates. Different model peptides containing fluorinated amino acids or ethylglycine in P2, P1'and P2' positions were designed according to the specificities of the serine protease α-chymotrypsin and aspartic protease pepsin (Fig 1). The proteolytic stability of the peptides toward these enzymes was determined by an analytical RP-HPLC assay and compared to the control sequence. Molecular modeling was used to support the interpretation of the structure-stability relationship based on the analysis of potential ligand-enzyme interactions. Surprisingly, an increase in proteolytic stability was observed only in very few cases. After a preliminary study with blood plasma, (3) this systematic study shows that proteolytic stabilities of fluorinated peptides is not predictable but rather a complex phenomenon that depends on the enzyme, the position of substitution relative to the cleavage site and the fluorine content.

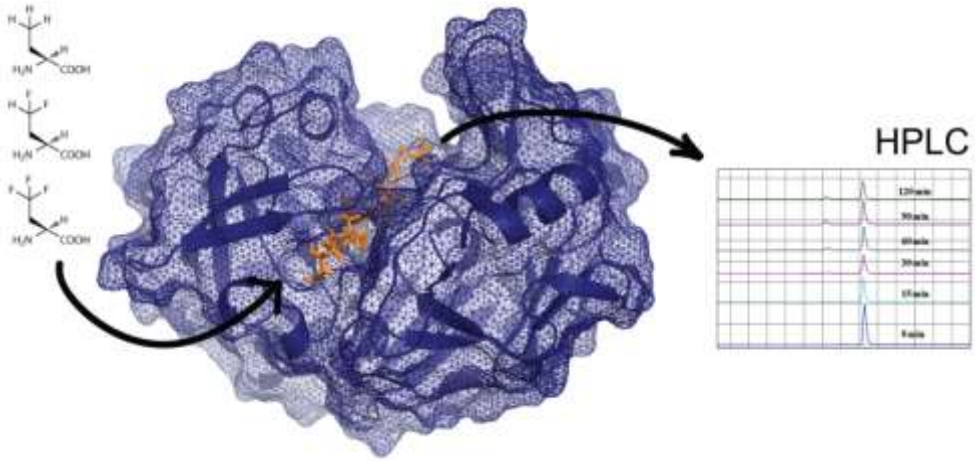

Fig. 1: Impact of fluorination on proteolytic stability of peptides: a case study with α-chymotrypsin and pepsin

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OC6

NEW NON-METATHETIC PROCESSES CATALYSED BY RUTHENIUM COMPLEXES FOR THE SYNTHESIS OF HETEROCYCLES

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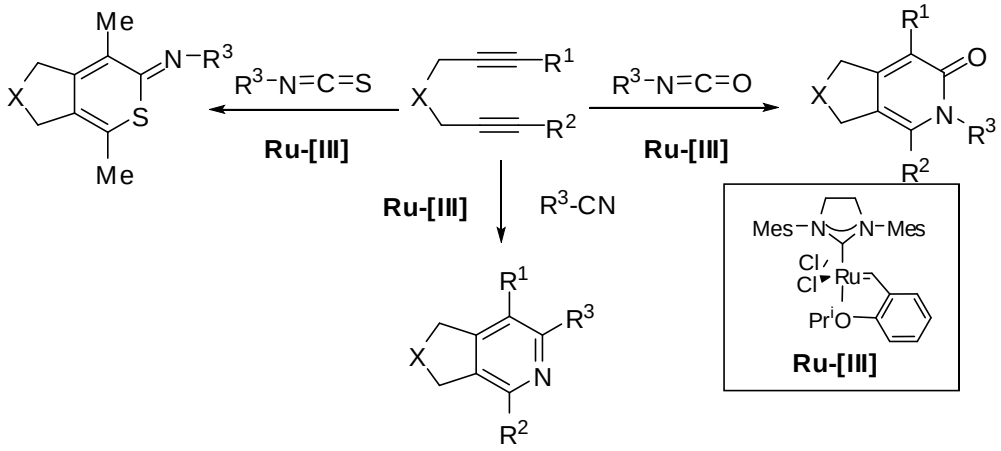
Non-metathetic transformations(1) include oxidations, hydrosilylations of alkynes, hydrogenation of olefins, cyclopropanations, cycloaddition reactions and olefin isomerizations. These are side reactions observed frequently when developing metathesis reactions catalysed by ruthenium alkylidene catalysts(2) that when optimized, may be useful transformations.

Metal catalyzed [2+2+2] cycloadditions are elegant, atom-efficient and group tolerant reactions catalyzed by complexes of more than 17 metals.(3) When this reaction involves a nitrile or an isocyanate it gives access to valuable heterocycles like pyridines or 2-pyridones, which are useful for the production of biologically active products.(4) Pyridines and pyridones display a variety of interesting biological properties including anticancer, antiviral, and antibacterial activity.

Herein we present the ability ruthenium species coming from the thermal modification of Grubbs' 2nd generation catalyst to promote cyclotrimerization reactions in good yields and selectivity. No metathesis reaction has been observed. (5)



ACKNOWLEDGEMENTS:

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OC7

MERGING OF PINEAL NEUROCHEMICALS. PHARMACOLOGICAL PROFILES AND NEUROGENIC POTENTIAL

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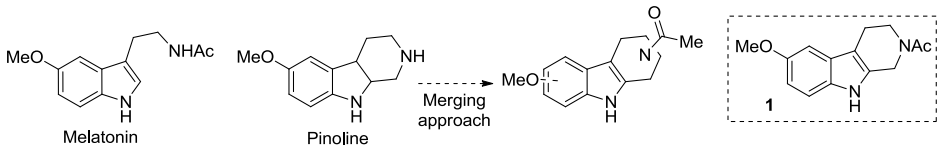
The neurohormone melatonin and the natural occurring β-carboline pinoline are produced in the pineal gland, and both have been found in various human tissues. Melatonin has been widely investigated; especially its effects mediated via MT_1 and MT_2 GPCRs and antioxidant properties. Its role supporting mitochondrial function and its likely additional actions mediated via nuclear receptors are gaining weight in the recent years, opening a vast field of study beyond MT_1 and MT_2 receptors (1).

Pinoline is a much potent antioxidant than melatonin found in similar concentrations to melatonin in different tissues, but its pharmacology remains much less studied. Melatonin's chronobiological role as circadiam rhythm regulator is well known while the main role of pinoline in the organism remains unknown. It has been speculated that it may play an important role in sleep pattern and the dreaming process. Besides its antioxidant properties, pinoline also inhibits monoamine oxidase (MAO) and serotonin transporter (SERT). Studies conducted with [H^3]pinoline have revealed high specific binding to nuclei of cells in the cerebral cortex and adrenal gland, suggesting a plausible effect at this organelle level (2).

In order to gain more knowledge about these intriguing molecules we have based our approach in the systematic study of their pharmacological profiles (5-HTR, SERT, MAO, MT_1/MT_2, ORAC, AChE). Furthermore we have extended our efforts to the synthesis and evaluation of additional structures resulting from the merging of both pineal neurochemicals. So far, we have been able to determine that pinoline is a rather potent (EC_{50} = 33 nM) full agonist at the 5-HT_{2C} which is in agreement with the role this receptor subtype displays in the sleeping homeostasis (3). Moreover we have been able to identify compound **1**, as a potent *in vitro* neurogenic compound (Tuj+) able to promote neural maturity (MAP+) to a higher extent than melatonin. Being totally devoid of any affinity for the neurogenic 5-HTRs and unlikely to have any for MT_1/MT_2 receptors we are currently conducting studies to establish whether nuclear receptors could be implied in its neurogenic effect.



Acknowledgments: MINECO (SAF2012-31035), Fundación Mutua Madrileña Automovilística (AP103952012), CSIC (PIE-201280E074), and PhD Brian L. Roth (NIMH PDSP).

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OC8

DRUGGING THE VON HIPPEL LINDAU E3 UBIQUITIN LIGASE: POTENTIAL DRUGS AND USEFUL CHEMICAL TOOLS

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Protein ubiquitination occurs through a cascade of enzymatic reactions, involving an E1 ubiquitin activating enzyme, an E2 ubiquitin conjugating enzyme and an E3 ubiquitin ligase. E3 ubiquitin ligases confer substrate specificity to protein ubiquitination pathways, and are thus attractive drug targets (1). However, to date, efforts to target E3 ligases using small molecules have been rewarded with limited success, resulting in this protein family being considered as “undruggable”.

In 2012 we published the first example of ligands targeting the von Hippel-Lindau protein (VHL) (2,3,4), the substrate recognition subunit of an E3 ligase, an important target in cancer, chronic anemia, and ischemia (5). Using a rational design and a molecular recognition strategy we have very recently generated nanomolar inhibitors of the VHL:Hif-1α interaction, the natural substrate of VHL. These new inhibitors have been caractherized by Isothermal Titration calorimetry (ITC) and Fluorescence Polarization (FP). We confirmed the ligand binding site and elucidated binding modes using both protein X-ray crystallography and Protein NMR spectroscopy.

These small molecule inhibitors of the VHL:HIF-1α interface showed huge potential to be developed into cell-penetrant chemical probes: on one hand, cell-based assays using chemoproteomics targeting the native VHL E3 ligase complex are underway, on the other hand, these ligands are perfect starting points for the development of novel drug-like PROTACS (PROteolysis TArgeting Chimeric moleculeS) for the recruitment of target proteins to the proteasome for degradation.

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OC9

DESIGN OF MYCOBACTERIUM TUBERCULOSIS SHIKIMATE KINASE INHIBITORS INSPIRED BY THE ENZYMATIC MOVEMENTS FOR CATALYTIC TURNOVER

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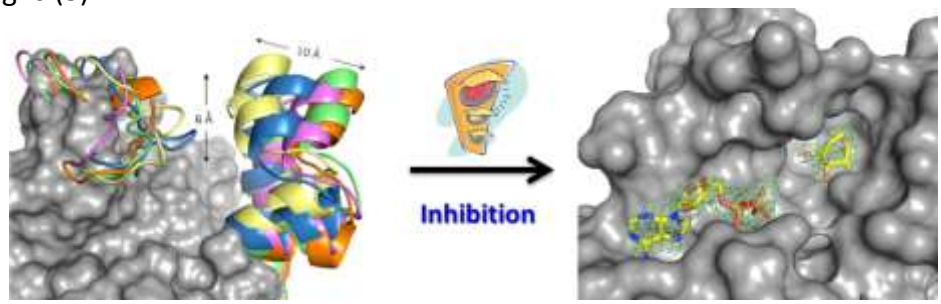
The essential nature of the shikimic acid pathway in certain microorganisms, combined with its absence in mammals, makes it an attractive target for the development of new antimicrobials (1). The important features of the shikimic acid pathway have not gone unnoticed by the scientific community and in recent years a great deal of effort has been focused on the development of inhibitors of the enzymes involved in this route. Shikimate kinase (SK, EC 2.7.1.71, *aroK* gene) is an essential enzyme in *Mycobacterium tuberculosis*, *Helicobacter pylori*, *Acinetobacter baylyi*, *Haemophilus influenzae*, *Francisella novicida* and *Pseudomonas aeruginosa*. It would be therefore an attractive target for the development of new drugs against several important bacterial diseases. This enzyme catalyzes the conversion of shikimic acid into shikimate 3-phosphate. The key interactions of the substrate and product binding and the enzyme movements that are essential for catalytic turnover of the *M. tuberculosis* shikimate kinase enzyme have been investigated by structural and computational studies (2). Inspired by the essential enzyme movements, several substrate analogs were designed and assayed. These studies reveal that the fixation of the diaxial conformation of the C4 and C5 hydroxyl groups recognized by the enzyme or the replacement of the C3 hydroxyl group in the natural substrate by an amino group is a promising strategy for inhibition because it causes a dramatic reduction of the flexibility of the LID and shikimic acid binding domains. Molecular dynamics simulation studies showed that the product is expelled from the active site by three arginines (Arg117, Arg136 and Arg58). This finding represents a previously unknown key role of these conserved residues. These studies highlight the key role of the shikimic acid binding domain in the catalysis and provide guidance for future inhibitor designs (3).



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(3) Financial support from the Spanish Ministry of Economy and Competitiveness (SAF2010-15076) and the Xunta de Galicia (10PXIB2200122PR and GRC2010/12) is gratefully acknowledged. VP and BB thank the Spanish Ministry of Economy and Competitiveness and the Spanish Ministry of Education for their FPI and FPU fellowships, respectively.

OC10

FROM HIT TO LEAD IN SEARCH OF NEW INHIBITORS OF THE ENZYME ISOPRENYLCYSTEINE CARBOXYL METHYLTRANSFERASE (ICMT)

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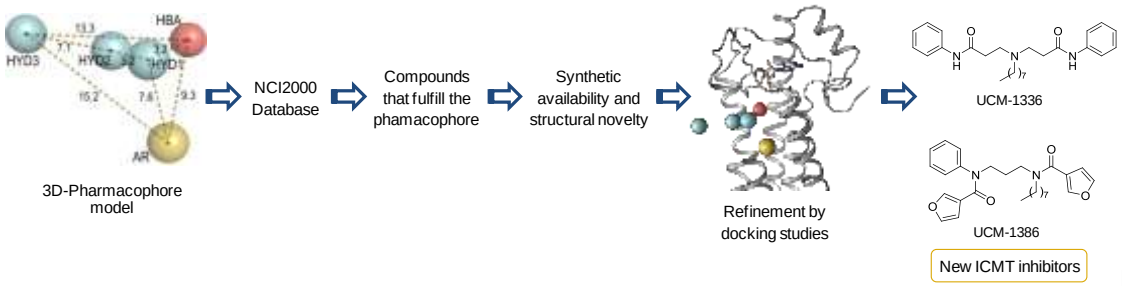
Activating mutations in Ras proteins have been found in almost 30% of all cancers, including 50% of colon and up to 90% of pancreatic tumors. In absence of its post-translational modifications, Ras losses its ability to induce tumor transformation. Therefore, the blockade of the enzymes involved in these modifications represents an attractive strategy to inhibit Ras activity. The enzyme isoprenylcysteine carboxyl methyltransferase (ICMT), which catalyzes the last step of the sequence of post-translational modifications of Ras, is receiving an increasing attention as a new therapeutic target in oncology (1). Up to date, very few structurally distinct inhibitors have been disclosed and only one molecule (cysmethynil) has been characterized as an ICMT inhibitor not only in vitro but also in cellular systems (2). These findings provide a compelling rationale for the development of ICMT inhibitors as another approach to anticancer drug development.

To address the design of new ICMT inhibitors we have built a 3D pharmacophore model that has been used as a query in the NCI database. This model has been further refined using homology models based on the recently described crystal structure of a prokaryotic ICMT ortholog (3). This approach has allowed us to identify some hits with good ICMT inhibitory activities (UCM-1336 and UCM-1386, which inhibit the 93% and 92% of the control ICMT activity at 50 μM, respectively) that also show good pharmacokinetic properties (4). All these results and the ongoing research aimed at the cellular validation of the compounds will be presented.



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OC11

DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION OF CYCLIC ACYLGUANIDINES AS BACE-1 INHIBITORS: EFFECT OF RING SIZE ON ACTIVITY AND SELECTIVITY VERSUS BACE-2

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Alzheimer's disease (AD) is the most prevalent type of dementia, comprising an estimated 70% of all dementia cases, and affecting some 25 million people worldwide. The neuropathological hallmarks of the disease include extracellular amyloid β (Aβ) deposition in the form of plaques in brain parenchyma and intracellular neurofibrillary tangles (NFT). (1)

BACE-1 cleavage of □-amiloyd precursor protein (β-APP) is the rate-limiting step of Aβ peptide generation. BACE-1 has been claimed as the most promising therapeutic target for the treatment of AD. However, after more than 15 years of research BACE-1 has proven to be an exceptionally difficult target, where identifying small-molecule inhibitors which combine good pharmacological and pharmacokinetic properties remains a challenge. (2)

Current small-molecule inhibitors present some shortcomings, among which is combining low inhibitory activity with good selectivity over other aspartyl proteases such as BACE-2, pepsin, renin or cathepsin D.

In this work, we present our initial efforts towards the rational design, synthesis and biological evaluation of series of cyclic acylguanidines as potential BACE-1 inhibitors with improved selectivity over BACE-2 (Figure 1). Preliminary results on the influence of ring size in BACE-1 activity will be reported.

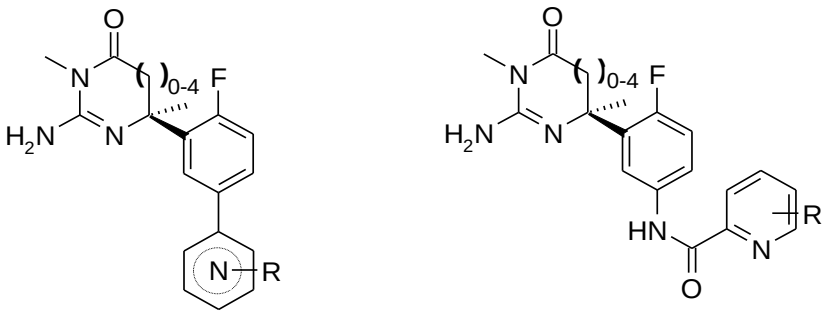

Fig. 1: General structure of the cyclic acylguanidines

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OC12

-OMICS TOOLS FOR STUDYING CHANGES IN METABOLIC PATHWAYS OF NEW DRUGS

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The complexity of biology continues to challenge researchers' ability to find answers through omics approaches (1). While genomics, transcriptomics, proteomics, and metabolomics are in wide use in both industry and academia, these experiments—performed alone—often lack the statistical power to uncover meaningful correlations, due to the high levels of noise than omics experiments typically generate. Multi-omics and metabolic pathways drived approaches helps to reduce this noise, allowing researchers to better understand the interaction of a drug with the organism. Three examples of it are:

- Pandey, A. and his Hopkins University group work done recently combining transcriptomic data with proteomic profiling to uncover the mechanism of action of sulforaphane (SFN), a potential chemopreventive for breast cancer, and to identify potential pharmacodynamics biomarkers (2).
- Rajagopalan, S. team work: "A Comprehensive Analysis of Rapamycin-treated Cells Using Agilent's Multi-omics Approach" shows the integration of genomics, transcriptomics, proteomics, and metabolomics studies on rapamycin-stimulated versus untreated HEK293 cells (3).
- Hartung, T. is developing integrated testing and multi-omics approaches to identify biomarkers for toxicity, that can be used for in vitro, cell-based tests. Not only will these tests allow high-throughput screening of compounds for toxic effects, they are expected to provide more accurate assessment of toxicity in humans than current animal-based approaches (4).

The base of multi-omics studies is the use of pathways analysis tools to:

- Evaluate which pathways are differentially expressed, when are compared several sample populations differing between them, by some drug treatment, or disease status, or patient phenotype (to classify patients according their different responses to some drug treatment/ disease/….), among other possibilities. It lets to confirm the expected new drug organism interaction behaviour, and also helps to reveal unexpected drug secondary effects, toxicity problems,…
- Reduce biological noise, founding pathways & nodes showing similar behaviours among the several –omics experiments, corroborating the experimental conclusions by different –omics.
- Improve the quality of the results in proteomics & metabolomics, by pathway driven experiments; according some previous hypothesis about the expected relevant pathways involved on the study. In genomics, it reduces microarrays cost/experiment using specific targeted microarrays, customized trough web by customer using Agilent "Bases-Jet Printing Technology" (same than HP Inkjet printers).

One good strategy for multi-omics studies, is to begin with NON targeted (hypothesis free) transcriptomics & metabolomics experiments, followed at the end, by targeted proteomics experiments (typically by LC/QqQ MRM data acquisition); focused on those proteins involved on the statistically relevant pathways found previously, by the differential statistical analysis of genomics AND metabolomics untargeted data sets.

The interest of researchers in metabolomics is increasing quickly. By one side, the metabolome could be considered as the "final" expression of the processes involved on a biological system (and highly influenced by endogenous & exogenous factors); by the other, during last years have improved a lot the developments in high accurate mass spectrometry (like LC/QTOF) and software tools for metabolomics. One of these new tools is the ability to create automatically a metabolites database, with all those metabolites involved in the set of user selected pathways. This tool lets to interrogate, by statistical analysis, high accurate mass data for new biomarkers DISCOVERY, but in TARGET metabolomics mode, improving the quality of the results. This is a powerful tool, for researchers having previous hypothesis about which could be the relevant pathways involved in an experiment (pathway driven experiments).

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OC13

DIFFERENT LEVELS OF APOPTOSIS CAUSED BY ENANTIOMERS IN BREAST CANCER CELLS

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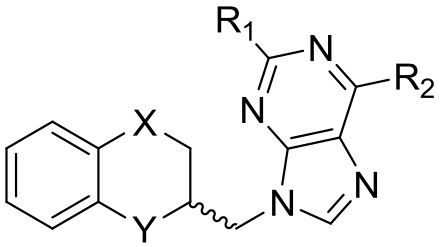
Breast cancer is the most common malignancy amongst women in the United States, and the second leading cause of cancer-related death in women (1). The issue of chiral drug is now a major theme in the design, discovery and development of new drugs (2). It has been shown for many pharmaceuticals that only one enantiomer contains the desired activity, and the synthesis of such drug molecules in their optically pure form is becoming increasingly important. Racemic compounds **1**-**6** were selected to the enantiospecific synthesis because of their notable anticancer activity against the human breast cancer cell line MCF-7 (3,4). Mitsunobu reaction was carried out between (*R*)- and (*S*)-3,4-dihydro-2*H*-1,5-benzoxathiepin-3-ol and purines under microwave irradiation. A contraction into a six-membered ring takes place with concomitant inversion at the stereocentre with excellent enatiomeric excesses giving rise to the homochiral 9-(2,3-dihydro-1,4-benzoxathiin-3-ylmethyl)-9*H*-purines. The anti-tumour activity of all enantiomers is reported against the caspase-3-deficient MCF-7 and the wild type SKBR-3 human breast cancer cells. The most active homochiral compound displays an IC_{50} of 1.85 μM and induces inhibition of the translation initiation factor eIF2α. All homochiral compounds included in this study show different apoptotic effects between both enantiomers with levels up to 99%. We have analyzed caspase-mediated apoptotic pathways on enantiomers and racemates. We have found a homochiral derivative that activates the canonical intrinsic caspase-8/caspase-3 apoptotic pathway on the MCF-7 cells, and a racemic compound that induces caspase-2 activation. Moreover, we demonstrate the involvement of caspase activation during cell death induced by these compounds in SKBR-3 cells.



1 X= S, Y= O, R_1 = H; R_2 = Cl
2 X= S, Y= O, R_1 = H; R_2 = Br
3 X= S, Y= O, R_1 = R_2 = Cl
4 X= O, Y= S, R_1 = H; R_2 = Cl
5 X= O, Y= S, R_1 = H; R_2 = Br
6 X= O, Y= S, R_1 = R_2 = Cl

Fig. 1: Racemic compounds with anti-tumour activity against the breast cancer cell line MCF-7.

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OC14

TUMOR VASCULAR DISRUPTING AGENTS: IDENTIFICATION AND OPTIMIZATION OF NOVEL HITS

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Resistance mechanisms emerging from current antineoplastic treatment demand novel approaches in the design of anticancer drugs. Vascular Disrupting Agents (VDAs) constitute an innovative anticancer therapy with an increasing interest owing to their original mechanism of action, complementary to other existing therapies.[1] VDAs act directly and selectively over the tumoral endothelium, inducing crucial morphological and functional changes. As a result, blood flow inside the tumour is quickly and dramatically decreased which unleashes the final necrosis due to the specific tumour hypoxia.[2] Although there are a few VDA candidates in clinical trials, they present serious drawbacks, such as low chemical stability and limited solubility. Therefore, the development of new VDAs with better pharmaceutical profile is critical.

Our approach for the design of new VDAs structurally different from those in the clinic has relied on a ligand 3-D shape similarity virtual screening (VS) approach.[3] The "query" has been the 3-D structure of two VDAs acting at the colchicine-binding site in tubulin: colchicine itself and TN-16. As shown in Figure 1 both compounds explore a close but distinct area in tubulin.

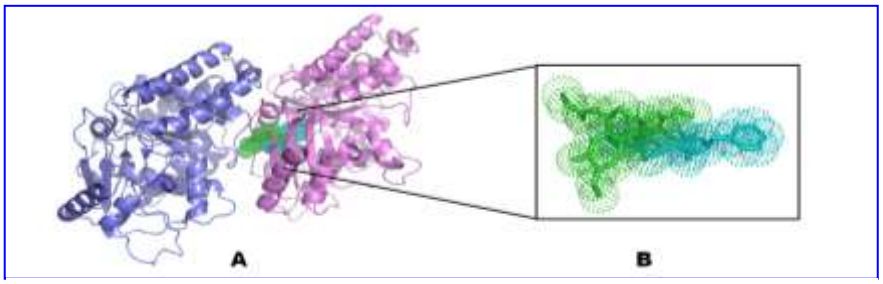

Fig. 1: (A) Colchicine Binding Site in α,β-tubulin dimer. (B) Superposition of the ligands used in the virtual screening: colchicine (green) and TN-16 (cyan)

The VS campaign afforded a list of potential hits that were purchased or synthesized. Fortunately, once tested, one of the hits showed a very significant antiproliferative activity, both in tumoral cells and endothelial cells. Therefore, we have undertaken the synthesis of structural analogues. Interestingly, several of these new analogues have shown antiproliferative activity in the sub-μM range. Moreover, we have demonstrated that they produce vascular disruption by inhibiting the established endothelial tubular network. Finally, we have confirmed that the mechanism of action of these compounds is through a direct binding to the colchicine site in tubulin. In conclusion, our approach has led to a new family of promising antiproliferative compounds with VDA properties.

Acknowledgements: M.D.C.. thanks the FSE and the JAE-Predoc programme for a predoctoral fellowship. This work has been supported by a grant of the Spanish CICYT (SAF2012-39760-C02)).

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3-SUBSTITUTED INDOLE DERIVATIVES AS ANTIMITOTIC AGENTS

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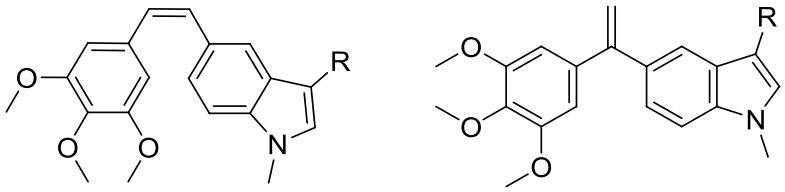
Antimitotic agents prevent right mitotic spindle formation, which leads to cell cycle arrest and, subsequently, to cell death. They act by interfering tubulin polymerization- depolymerization dynamics. Indole - Combretastatins and Isocombretastatins bind to αβ-tubulin dimers at the colchicine site and they have shown to be amongst the more potent tubulin polymerization inhibitors (1). One of the main drawbacks that these compounds present as potential drugs is their low water solubility, which means that either more water soluble ligands or prodrugs need to be prepared.



In this sense, we have designed, synthesized and evaluated new indole derivatives of combretastatins and isocombretastatins that incorporate different polar groups to the molecule. The solubilizing groups or anchors to incorporate them, have been introduced at position-3 of indole nucleus (2).

Some of the synthesized analogues have improved water solubility, while others have resulted in more potent analogues which could be formulated as prodrugs.

Tubulin polymerization inhibition and cytotoxic assays have pointed out different SAR patterns for combretastatins and isocombretastatins.

The disposition of the aromatic rings and possible interactions with the protein have been studied by molecular docking experiments.

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OC16

CHEMICAL BIOLOGY FOR DRUG DISCOVERY: TWO CASE STUDIES IN DRUG REPURPOSING

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The Spanish initiative in Chemical Biology (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.

Two case studies describing how the Chembiobank workflow, structure and applications may be applied to drug repurposing will be shown: a) how to discover possible new mechanisms of action and therapeutic applications for known drugs from the Prestwick Chemical Library, and how this knowledge may be transferred to a biotech or a pharmaceutical company; and b) how to discover and validate the chemical biology profile of approved orphan drugs, and how to repurpose drugs for rare diseases.

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). An update of the current status of this project and its implications and benefits for the medicinal chemistry community in Europe will be described.

POSTERS

EVALUATION OF ANTICANCER ACTIVITY OF TWO NEW BIURET DERIVATIVES AGAINST T47D AND K562 CELL LINES IN PRESENCE OF NATURAL KILLER CELLS

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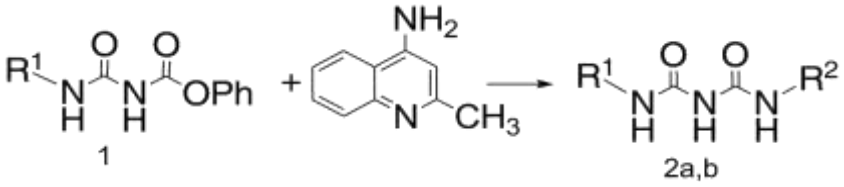
Background and Aims: Different approaches such as synthesis of potent compound, finding natural product with fewer side effects or using antiproliferative effects of natural killer (NK) cells are considered for better treatment of cancers. In this study, the use of NK cells along with two most potent derivatives (2a,b) of several biurets which have been reported as cytotoxic agents against T47D breast cancer cell lines in our previous study[1], was considered to assess the possibility of enhancing their cytotoxicity.

Methods: Biurets 2a,b were prepared from the reaction of allophanates with 4-aminoquinaldine[1] (Scheme 1). To assess cytotoxicity, different number of NK cells, which extracted from whole blood, were added to various concentrations of the tested biurets and cancer cells (T47D and K562) in 96 well plates. Cytotoxicity was measured by lactate dehydrogenase (LDH) assay kit[2]. Results were compared to those wells that contain biurets or NK cells alone with cancer cells. Analysis of covariance was used to analyze the results.

Result: For 1-(3-phenylpropyl)-5-(quinaldin-4-yl) biuret (2a), presence of NK cells (50000 in each well) led to a significant increase (average of 12%) in the cytotoxic activity on T47D ($p<0.0001$). Cytotoxic effect of this compound against K562 cancer cells in the presence of the same number of natural NK cells, showed an average decrease of about 7% ($p=0.0001$). 1-(2-phenylethyl)-5-(quinaldin-4-yl) biuret (2b) showed significantly increased cytotoxicity (10%) on T47D cell line ($p=0.0002$).However, presence of NK cells resulted in 7-17% decrease of cytotoxicity on K562 which was significant at number of NK cells of 80 ($p=0.0203$).

Conclusion: Results show that NK cells could enhance the cytotoxic action of the tested biurets on T47D cell. However, it seems that presence of NK cells might lead to a decrease in cytotoxicity of these biurets against the K562 cell line.



Compound 2a: R^1=3-phenyl propyl, R^2=4-aminoquinaldinyl

Compound 2b: R^1=2-phenethyl, R^2=4-aminoquinaldinyl

Scheme 1. Synthesis of biurets 2a,b

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P2

NEW ANTITUMORAL ANALOGUES OF PHENSTATINS

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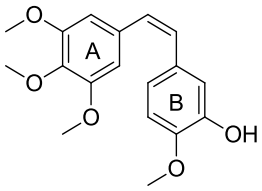
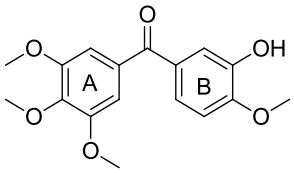
Phenstatins(1) are benzophenone derivates with cytotoxic activity derived from their effect on microtubule dynamics and mitosis. Phenstatins were synthetized taking as model Combretastatins(2), natural products with two rings (A and B) bonded by a *cis*-ethylene bridge. *Cis* disposition is required for Combretastatins activity and their transformation into *trans* isomers produce a lack of activity. Phenstatins maintain the activity of Combretastatins without the problem associated to the decrease of activity by isomerization.

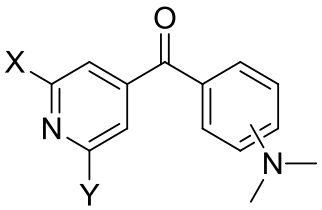


Combretastatin A-4
IC_{50} IPT: 3-4uM



Phenstattins (Benzophenone)
IC_{50} IPT: 1uM

The main problem of Combretastatin and Phenstatins is their low aqueous solubility. In order to increase their solubility, we have synthesized several families of pyridine analogues of Phenstatins:



X: OMe, SMe
Y: OMe, SMe

Their trimethoxyphenyl ring has been replaced by a pyridine ring with two different substituents: methoxy and thiomethoxy groups. Furthermore, the effect of the position of a dimethylamino group in B ring has been tested. The solubility of these compounds has been measured and it is observed that the introduction of the dimethylamino group and the pyridine ring leads to an increase in solubility of the compounds.

The obtained compounds have been evaluated on tumoral cells lines: HL-60 (leukemia), HT-29 (colon cancer) and Hela (cervical cancer). None of them have cytotoxic activity at concentrations lower than 10^{-6} M. The tubulin polymerization inhibitory activities have been assayed but none of them have shown an increase in the potency with respect to model compounds.

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DEVELOPMENT OF NEW FtsZ INHIBITORS AS 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. In this sense, FtsZ "a widely conserved tubulin-like GTPase" has been proposed as an attractive target for antibacterial drug discovery due to its essential role in bacterial cell division.(1) From a structural point of view, two main cavities have been identified for ligand binding in FtsZ (i) the GTP-binding pocket and (ii) the long cleft between the nucleotide-binding and the C-terminal domain, also known as the PC190723 binding site (Figure 1).(2) In the last decade, compounds that specifically target FtsZ and inhibit its function in bacterial division have been identified.(3) Among them, PC190723 is the most promising FtsZ inhibitor discovered so far due to its potent activity both in vitro and in vivo against *S. aureus*.(4) However, it is inactive against a range of other Gram-positive and no antibacterial effect was observed for Gram-negative strains. Therefore, the development of new inhibitors of FtsZ able to act as broad spectrum antibacterials still needs to be addressed and will be the focus of the present work.

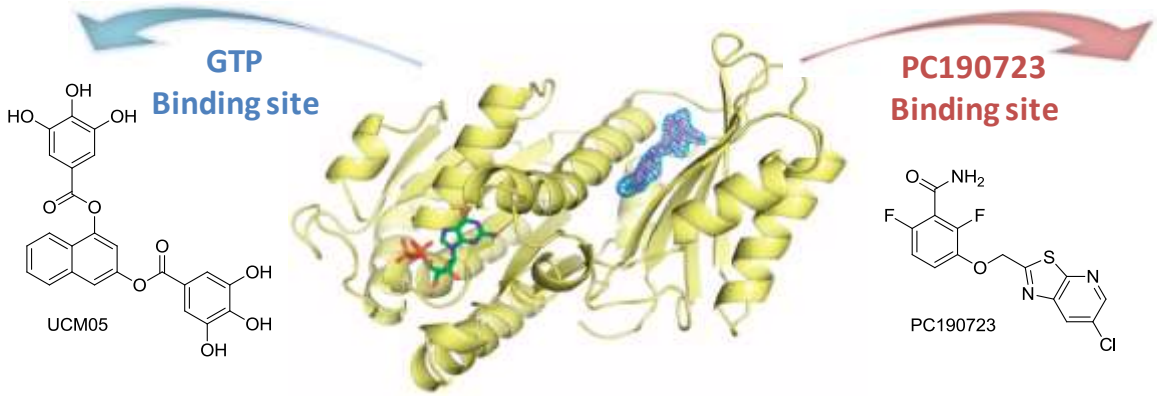

Figure 1. Crystal structure (2.0 Å) of *S. aureus* FtsZ-GDP in complex with PC190723

Here, we will present our latest results on the discovery of FtsZ inhibitors targeting both binding sites. For the GTP-binding pocket, we identified hit UCM05 from the screening of our in-house library, and a hit to lead process had led to new derivatives with improved FtsZ-affinity and antibacterial activity.(5) Regarding the PC-binding site, fluorescent derivatives of PC190723 have been synthesized to obtain a valuable tool to set up a fluorescent assay for the assessment of the affinity of new compounds for this binding site.

Acknowledgments: This work has been supported by grants from the Spanish Ministerio de Economía y Competitividad (MINECO, SAF2010-22198) and Comunidad de Madrid (SAL-2010/BMD2353). The authors thank MINECO for a predoctoral grant to M.A.

P4

A HELICAL PEPTIDE LIBRARY FOR MODULATION OF PROTEIN-PROTEIN INTERACTIONS

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The modulation of protein-protein interactions (PPIs) plays pivotal roles in key biological processes like immune response, and cell signaling and growth. Therefore, PPIs are currently considered very interesting targets for the discovery of new drugs (1). When the structure of the targeted proteins is unknown, a valid approach to identify new modulators is the evaluation of diverse compounds, including peptides, peptidomimetics and small-molecules.

In this context, α-helix secondary structures are extensively involved in the intercommunication among proteins and are crucial for many PPIs. Quite frequently, the key contacts between these helical motifs and the complementary helix-binding sites are mediated by hydrophobic residues (2-3).

As a simple approach to discover initial hits for PPI modulation, we have designed and synthesized a collection of 81 linear helical peptides (13-mer) bearing hydrophobic residues at 5, 9 and 12 positions. Conformational studies by CD and NMR have confirmed the tendency of these peptides to adopt the expected helical structures. This library, which has been validated in two well-known PPIs, p53-MDM2 (2) and VEGF-VEGFR-1 (3), has led to the identification of compounds able to significantly block some TRP channels.

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NOVEL POLYCYCLIC COMPOUNDS WITH POTENT ACTIVITY AGAINST THE AMANTADINE-RESISTANT L26F AND V27A MUTANT M2 CHANNELS OF INFLUENZA A VIRUS

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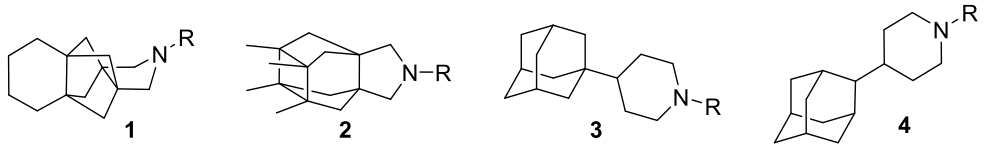
Influenza is a worldwide spread disease that affects millions of people each year and has caused non-negligible morbidity and mortality. The M2 protein of influenza A viruses forms a homotetrameric proton-selective channel that is targeted by amantadine and rimantadine, which have been widely used as anti-influenza drugs (1). However, most of the current influenza A viruses carry drug-resistant mutations alongside the drug binding site. Although a variety of mutations can lead to amantadine-resistance in vitro, only three mutants, S31N, V27A and L26F, are generally observed in transmissible viruses (2). Of note, computational and experimental studies have shown that the V27A and L26F mutants have larger drug-binding sites (3).
Recently, we have rationally designed many polycyclic amines larger than amantadine able to fill the extra space of these M2 channel mutants. Here we will present the design, synthesis and pharmacological evaluation of several families of compounds with low-micromolar or even submicromolar IC_{50} activities as L26F and V27A mutant M2 channels inhibitors (4).



a, R = H; **b**, R = $C(NH)NH_2$

Fig. 1: Selected structures of novel amantadine-analogs.

	wt M2 channel	V27A M2 channel	L26F M2 channel
Amantadine	16	>100	>100
1b	3.4	0.3	ND
2a	18	0.7	8.6
3b	2.9	4.2	ND

Table 1: Inhibitory effect of selected compounds on A/M2 *wt*, V27A and L26F proton channels functions. Isochronic (2 min) values for IC_{50} are given.[a]. ND = not determined.

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P6

DRUGS4RARE: A DRUG DISCOVERY REPURPOSING TOOL FOR RARE DISEASES

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The repurposing approach (1) is based on finding new medical indications for existing drugs that have reached the market. Using this approach, the clinical development for the new indication can be performed with less investment, potentially faster than the *de novo* development, and with a lower risk in terms of toxicity and safety.

We have developed a platform for Drug Discovery for Rare Diseases, Drugs4Rare, with three main objectives: a) to gather information on the chemical structures, published mechanisms of action, and orphan diseases, associated to chemical compounds of therapeutic interest; b) to carry out a virtual polypharmacology analysis for each chemical compound, predicting its interactions with a large number of biological targets; and c) to validate experimentally the highest affinity predicted chemico-biological interactions.

As a proof of concept, we have started the platform development analyzing 49 chemical compounds which have been approved in Europe for the treatment of certain rare diseases. After compiling information about these compounds and associated rare diseases from the Orphanet database (www.orpha.net), we analyzed the information on their chemical structures, biological targets, and mechanisms of action in public (Pubchem, ChEMBL) and private access (SciFinder, Integrity) databases. Then we used the virtual polypharmacology predictive platform developed in the Chemogenomics Laboratory at IMIM (2,3), which allowed us to generate a profiling of the 49 compounds in front of around 4500 biological targets. Analysis and prioritization of this virtual chemical biology matrix led us to the selection of an small set of compounds, for which experimental validation of new biological targets was determined.

We are currently expanding this initial "proof of concept" of our Drugs4Rare Drug Discovery Platform to additional compounds from the Orphanet database, as well as to other compounds of therapeutic interest.

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CLICKED PEG-PETIDE CONJUGATES FOR STUDYING CALMODULINE-Kv7.2 CHANNEL BINDING

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The ubiquitous calcium sensor calmodulin (CaM) is essential for Kv7 channels to reach the plasma membrane and for Ca^{2+}-dependent modulation (1,2). In the nervous system, Kv7.2 and Kv7.3 are the main components of the M-current, a voltage-dependent, non-inactivating K^+ current that play a fundamental role in controlling the activity of both peripheral and central neurons (3). In addition, some spontaneous mutations at the CaM-binding domain of Kv7.2 or Kv7.3, which interfere with CaM binding, are linked to Benign Familial Neonatal Convulsions (BFNC), dominant inherited idiopathic human epilepsy (4).

The CaM-binding domain in Kv7.2 channels is formed by two discontinuous sites (helix A and helix B), with high probability of adopting an alpha helix conformation, linked by a long loop of 141 residues. Some of us have previously demonstrated that either recombinant or synthetic peptides having helix A or B sequences are able to bind to CaM, but both sites cooperate to increase the apparent affinity for this Ca^{2+} sensor (5). Since the linker between helices A and B does not seem to play a major role in CaM binding, non-peptide spacers could serve to joint A and B fragments in a single molecule, facilitating the study of its interaction with CaM. Considering that solubility could be an issue in peptides longer than 6 residues, the linker could be selected to improve the solution of the bis-peptide conjugates in aqueous media. To this end, we have designed and synthesized poliethyleneglycol-peptides (PEG-peptides) derived from the amino acid sequence of Kv7.2 helices A and B. These conjugates were modified with azide and alkyne groups, respectively, suitable for Cu-catalyzed click chemistry. The obtained 1,4-disubstituted 1,2,3-triazolyl-PEG-peptide conjugates showed high affinity for CaM, comparable to that of the entire CaM binding domain (219 residues). This strategy could be of interest to generate biological tools for better understanding the role of CaM binding in Kv7 channels function.

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P8

ANTIMITOTIC AGENTS: SOME NEW AMINO-PYRIDINE DERIVATIVES OF ISOCOMBRETASTINS

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CA-4 is a highly potent inhibitor of tubulin polymerization and cytotoxic agent against murine lymphocytic leukemia and human ovarian and colon cancer cell lines. A large number of analogues has been published in order to overcome the low aqueous solubility of these analogs due to the hydrophobic nature of the colchicine site, chemical instability due to their isomerization to the inactive but thermodynamically more stable *trans* isomers, and insufficient cytotoxicity against cells in the tumor rim, resulting in tumor regrowth (1).
With this purpose, we have described different families of analogues (2) and more recently a new family of combretastatin isomers, which we have called isocombretastatins, which are potent inhibitors of tubulin polymerization and cytotoxic compounds (3).
In this communication we will present the results obtained by substitution of the B ring of CA-4 by an aminopyridine moiety resulting in new analogues of isocombretastatin and combretastin.

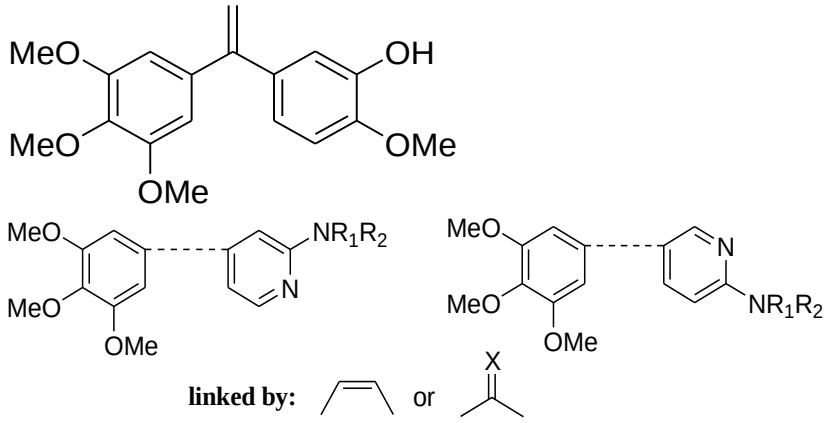

Fig. 1: Isocombretastatin, and analogues of CA-4 and isocombretastatin described in this communication

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trans-Pt(II) COMPLEXES AS COVALENT AND INTERCALATOR BINDERS: A DOUBLE DNA-TARGETING APPROACH

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Cisplatin is today the most used platinum drug in clinic, and can be used also in combination with some other drugs. However, its clinical efficiency is limited due to its low solubility and its inefficacy against resistant tumors, and also because it produces several side effects. In view of these limitations, research has been extended to new platinum analogues with the aim of overcoming these unwanted effects. Most of this research has been performed with cis geometry (1) because initial research reported a lack of activity of transplatin. However, several exceptions for the trans platinum complexes *trans*-[$PtCl_2$(L)(L')] were reported indicating that synthetic variations in the coordinating ligands could lead to new active species (2). Using aliphatic amines as spectator ligands, our research group was also able to demonstrate that active *trans* Pt(II) complexes were achievable (3). We now described a new family of non-conventional platinum metallodrugs (fragment–linker–DNA targeted ligand, Fig. 1) based on naphthalimide ligand. Thus, we hypothesized that the synergistic combination of an intercalator (DNA recognition) and a metal covalent binder (DNA fixation) would improve the selectivity and enhance the known antitumor activity of platinum complexes with *trans* geometry. Several platinum-based drugs containing aliphatic amines and the targeted ligand, naphthalimide, were synthesized. The antiproliferative activity of the complexes was evaluated and the results showed better activity versus cancer cell lines over the ligand and cisplatin. Furthermore, the distance of the polyaromatic DNA-binding group to the metal center is determinant for the cytotoxic activity of these complexes. In addition, DNA interaction assays have been carried out to study the binding mode and compare with cisplatin.

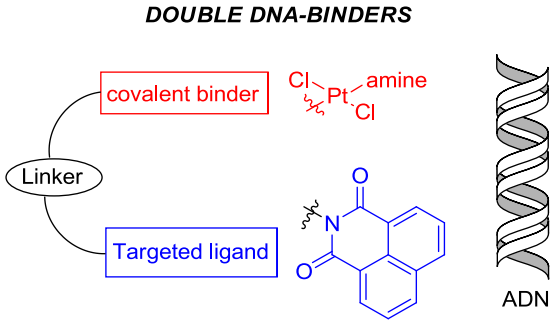

Fig. 1: Doble DNA-binders platinum complexes.

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P10

STEREOSPECIFIC ALKYLATION OF SUBSTITUTED ADENINES BY THE MITSUNOBU REACTION UNDER MICROWAVE-ASSISTED CONDITIONS

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In the search for new compounds of therapeutic interest, purine derivatives are very important for their possible interaction with many biological targets.

Our interest in the topic lies in the fact that pyrimidine and purine alkylated products seem to be attractive structures endowed with anti-proliferative properties (1,2). We wish to report herein the reactivity of the racemic and enantiomers of 3,4-dihydro-2*H*-1,5-benzoxathiepin-3-ol and several adenines, such as 6-*N*,*N*-dimethyl-, 2-chloro-6-*N*-methyl-, and 6-*N*-methyl-adenines. It reveals a complete inversion of the stereogenic centre of the secondary alcohol giving an alkylated purine linked to a homochiral six-membered ring. The results are interpreted in terms of multident nucleophile reactivity and a S_N2 transition state for the alkylation reactions. Alkylation sites have been determined by 2D NMR techniques and for three compounds have been confirmed by X-ray crystallography. The *N*-9´/*N*-3´ regioselectivity can be justified by the electronic effects of the substituents at positions 2' and 6' of purine.

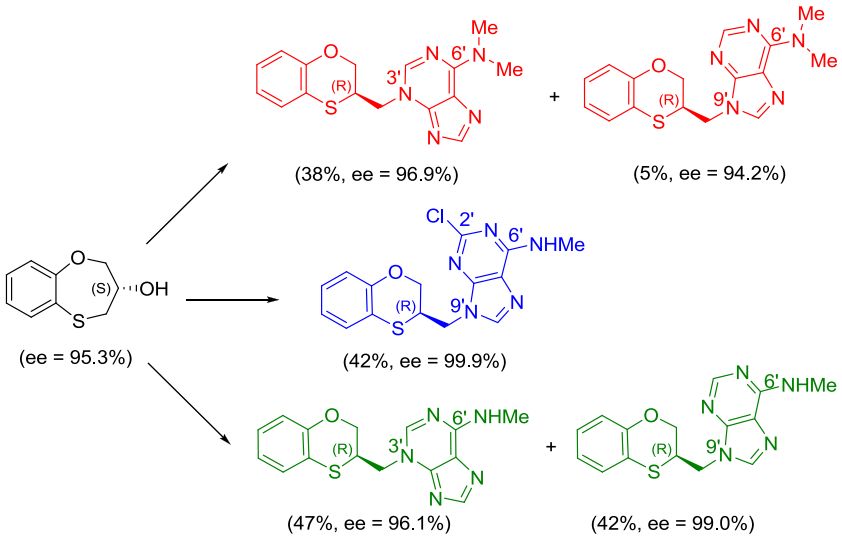

Fig. 1: Alkylated aminopurines with the 3,4-dihydro-2*H*-1,5-benzoxathiepin-3-ol moiety by the Mitsunobu reaction under microwave-assisted conditions.

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P11

INSIGHTS ABOUT THE INTERACTIONS OF AMP-ACTIVATED PROTEIN KINASE WITH PP1-R6 PHOSPHATASE COMPLEX

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The hydrolysis of ATP drives virtually all of the energy-requiring processes in living cells. A prerequisite of living cells is that the concentration of ATP needs to be maintained at sufficiently high levels to sustain essential cellular functions. In eukaryotic cells, the AMPK (AMP-activated protein kinase) cascade is one of the systems that have evolved to ensure that energy homoeostasis is maintained (1). AMPK acts as a cellular energy sensor and regulator, balancing the energy at both the cellular level and the whole body. Disturbances in energy homeostasis underlie a number of disease states such as: Type 2 diabetes, obesity and cancer, making AMPK useful target for therapeutic intervention (2).

Identification of novel protein-protein interaction is a challenge approach in the drug discovery process. In this context, protein phosphatase holoenzyme composed of PP1 and the regulatory subunit R6 have been described as a physiologically relevant complex on AMPK regulation process and specifically, R6 subunit was reported to physically interact with AMPK (3).

Based on these results, we present different approaches, by means of biomolecular and biophysical techniques, in order to identify the putative regions of interaction between AMPK and PP1-R6 complex.

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P12

EXPLORING NEW APPROACHES FOR NEURODEGENERATIVE DISEASES BY TARGETING THE DREAM PROTEIN

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Altered neuronal calcium homeostasis and early compensatory changes in transcriptional programs are common features of many neurodegenerative pathologies including Alzheimer's disease (AD), Down syndrome (DS) and Huntington's disease (HD). DREAM (Downstream Regulatory Element Antagonist Modulator), also known as calsenilin or KChIP-3 (potasium channel interacting protein-3), is a multifunctional Ca^{2+} binding protein that controls the expression level and/or the activity of several proteins related to Ca^{2+} homeostasis, neuronal excitability and neuronal survival (1). This protein is widely expressed in the brain and, depending on the cell type and physiological conditions, shows multiple subcellular localizations, in the nucleus, cytosol or cell membrane (2).

Initially, the interest in DREAM was based on its key role in the regulation of intracelular Ca^{2+} levels (1,3). An early reduction in DREAM levels is found in the pre-symptomatic phase of several neurodegenerative mouse models, including AD, DS and HD. These data support the idea that an early down regulation of the DREAM level in neurons during the pre-symptomatic phase of the AD, DS and HD might be part of its neuroprotective mechanism. These findings suggest that DREAM could be a novel and versatile target for therapeutic intervention in neurodegeneration and that molecules able to bind to DREAM and block its physiological functions could be candidates of drugs to treat neurodegenerative diseases. Up to know, low molecular weight molecules have not been described able to interact with DREAM and to modulate its action.

In this communication we report the rational design, the synthesis and the biological evaluation of novel DREAM-binding molecules.

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P13

UREA AND THIOUREA KINURENAMINE DERIVATIVES: SYNTHESIS AND BIOLOGICAL EVALUATION AS NITRIC OXIDE SYNTHASE INHIBITORS

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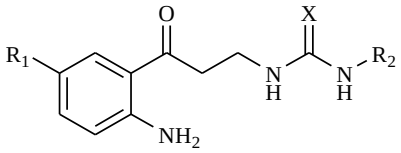
Nitric oxide (NO) is an inorganic free radical that has diverse physiological roles, including regulation of blood pressure, neurotransmission and macrophage defense systems. NO is synthesized from the enzyme catalysis of L-arginine to L-citrulline in several cell types by a family of nitric oxide synthase (NOS) isoenzymes: neuronal NOS (nNOS), endothelial NOS (eNOS) and inducible NOS (iNOS). An overproduction of NO by nNOS has been associated with strokes, migraine headaches or neurodegenerative disorders such as Parkinson (1) and Alzheimer's diseases (2). On the other hand, an overproduction of NO by iNOS causes inflammatory bowel disease, arthritis, and neuropathic pain (3). Also, inflammatory reaction in Parkinson's disease is associated with iNOS. Thus nNOS and iNOS represent a therapeutic target since inhibition of these enzymes can help in the treatment of several disorders, and a selective inhibition of one of these isoforms would be desired.

Previously, we have described a series of nNOS inhibitors with a kynurenamine (4) and 3-phenyl-4,5-dihydro-1H-pyrazole structure (5). Here we describe a new group of kynurenamine derivatives and their inhibition values of both neuronal and inducible NOS. The general structure is represented in Figure 1. These compounds have an urea or thiourea moiety with several substituents (Me, Et and Pr) and a phenyl group with electron-withdrawing (Cl), electron-donating (OMe) substituents or without any substituent. In general, the biological results indicate that the synthesized compounds are good inhibitors of both isoforms and some of them are iNOS selective.



X= O, S
R_1= H, Cl, OCH_3
R_2= Me, Et, Pr

Fig.1: General structure of new kinurenamine derivatives

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P14

BINDING MODE PROPOSAL FOR THE BINDING OF A NOVEL SERIES OF NONCHELATING MMP2 INHIBITORS OBTAINED BY CLICK CHEMISTRY

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Matrix metalloproteinases (MMPs) are Zinc-dependent proteins that play an important role in cell migration, both in healthy normal tissues and in metastatic processes.(1) These proteins are involved in the breakdown of connective tissue and, therefore, are considered as important targets in the development of inhibitors in a number of inflammatory, degenerative or malignant diseases. Although they were first thought to only take part on the metastatic processes in advanced cancers, MMPs were shown to be also implicated in primary tumour growth by liberation of stroma-associated growth factors, remodelling of the extracellular matrix near the primary tumour, and their implication in angiogenesis. Among the members of this family, MMP-2 is a validated pharmacological target in cancer as it stimulates tumour growth, angiogenesis and metastasis through its implication in the degradation of the extracelullar matrix. The great majority of MMP2 inhibitors that have been discovered to date present a zinc-binding group (ZBG),(2-4) but, as the result of their unselective interaction with Zn ions, these inhibitors are very toxic. Therefore, there is an increasing need in the development of new inhibitors that bind selectively to MMP2 without a ZBG. Here we report the binding mode proposal of a number of new molecules obtained by “click chemistry” that present MMP2 inhibition activity with no known ZBG. These ligands were docked in the active site of MMP2 and the stability of the complexes was assessed by means of molecular dynamics (MD) simulations. Moreover, we propose a rational for the different binding affinities based on the conformational study carried out with MD simulations, and the calculation of the microstate probability for the most populated conformers in water of each ligand.

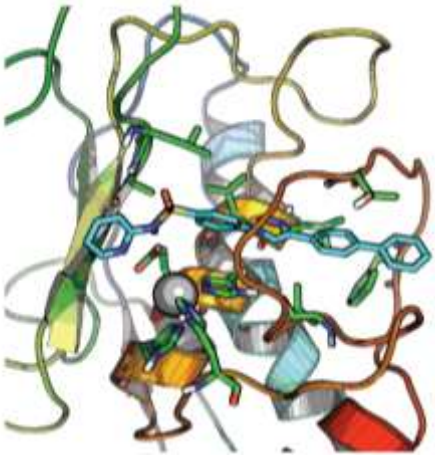

Fig. 1: Proposed binding mode of the highest affinity MMP2 inhibitor of the series.

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P15

MOLECULAR MODELLING OF THE δ-ALA-D ENZYME: ACTIVE SITE CONFIGURATION AND BINDING OF THE $(PheSe)_2$ INHIBITOR

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Diphenyl diselenide $(PheSe)_2$ is an organochalcogen molecule with antioxidant, antiulcer, neuroprotective, anti-inflammatory, anti-hyperglycemic, anti-atherosclerotic and anti-hypercholesterolemic properties (1). However $(PheSe)_2$ also displays cytotoxic effects partially associated to the inhibition of thiol-containing proteins. Among them, δ-aminolevulinic acid dehydratase (δ-ALA-D) is an essential metalloprotein in porphyrin biosynthesis, whose inhibition is involved in a set of physiological disorders (2). Thus, the rational design of derivative compounds from $(PheSe)_2$ with low affinity against δ-ALA-D requires the elucidation of the molecular mechanism for the interaction of $(PheSe)_2$ with the active site of δ-ALA-D.

Starting from the high resolution X-ray structure 1H7N of the δ-ALA-D enzyme from *Saccharomyces cerevisiae*, we built molecular models of the unbound form of the α/β barrel active site as well as in the presence of $(PheSe)_2$ using a broad array of computational tools. We focused on a large region of the very large octameric 1H7N structure centered (~50 Å) at the active site of the first enzyme unit. Then electrostatic pK_a calculations were employed to investigate the protonation state of important residues (e.g., Lys_{263}). Similarly, the coordination environment around the Zn ion in the active site was initially studied using quantum mechanical (QM) calculations. Four different configurations of the active site were then examined by means of molecular dynamics (MD) simulations using the ff99SB version of the Amber molecular mechanics (MM) force field. The structural analyses of the simulations complemented with semiempirical QM/MM and QM calculations on cluster models allowed us to determine the most likely configuration of the δ-ALA-D site. On the other hand, we derived MM parameters for representing the $(PheSe)_2$ compound and tested them carefully against QM data. Finally, the structure and dynamics of a prereactive complex between δ-ALA-D and $(PheSe)_2$ was studied using flexible-docking calculations and MD simulations.

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P16

BENZO[*h*][1,6]NAPHTHYRIDINES AS A NOVEL FAMILY OF ACETYLCHOLINESTERASE INHIBITORS

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We have recently developed a novel family of inhibitors of acetylcholinesterase (AChE) that were rationally designed to simultaneously reach both the active and peripheral sites of the enzyme, whose main structural feature was the presence of a pyrano[3,2-*c*]quinoline scaffold as the peripheral site interacting unit (1). Indeed, molecular dynamics simulations supported the ability of this scaffold to establish π-π stacking interactions with the characteristic peripheral site residue Trp286. On the basis of these results we inferred that substitution of the oxygen atom of this moiety by a nitrogen atom would increase the basicity of the quinoline nitrogen atom, thereby enabling additional cation-π interactions with Trp286 upon protonation at physiological pH, which might result in increased inhibitory potency against the catalytic activity of AChE, and more importantly against the AChE-induced aggregation of the β-amyloid peptide (Aβ), one of the main actors in the neuropathogenesis of Alzheimer's disease. Herein we report on the synthesis and *Electrophorus electricus* AChE (eeAChE), human AChE (hAChE), human butyrylcholinesterase, and Aβ aggregation inhibitory activities of a new family of "pyrido[3,2-*c*]quinoline" derivatives, properly named as benzo[*h*][1,6]naphthyridines, differently substituted at positions 1, 5, and 9. Also, the binding mode of some selected compounds to AChE has been studied by molecular dynamics simulations and kinetic studies, and by propidium displacement assays as well.

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SYNTHESIS AND SCREENING OF A SMALL LIBRARY OF QUINOLINES AND TRICYCLIC HETEROFUSED QUINOLINES AGAINST *TRYPANOSOMA BRUCEI*

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Human African tripanosomiasis, a major protozoan parasite disease caused by *Trypanosoma brucei*, is responsible for thousands of deaths per year in developing countries. Notwithstanding its mortality and morbidity, conventional treatments are far from optimal due to either poor brain penetration, and, hence, low efficiency for treating the late stage central nervous system infection, or high toxicity. In this light, there is an acute need for better trypanocidal drugs. We have recently reported on the trypanocidal activity of a series of huprines, a structural family bearing an aminoquinoline scaffold (1,2). Herein we report on the synthesis based on the Povarov multicomponent reaction of a small library of structurally different quinolines and tricyclic heterofused quinolines, namely pyrano[3,2-*c*]quinolines, pyrrolo[3,2-*c*]quinolines, azepino[3,2-*c*]quinolines, benzo[*h*][1,6]naphthyridines, and thiazolo[5,4-*c*]quinolines, and their evaluation against cultured bloodstream forms of *Trypanosoma brucei* as well as the determination of their cytotoxicity against rat myoblast L6 cells and the prediction of their brain penetration through the PAMPA-BBB assay.

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P18

SYNTHESIS AND CHEMOPREVENTIVE ACTIVITIES OF THIOHYDROXYTYROSOL DERIVATIVES ON PARENTAL AND MULTI-DRUG RESISTANT HL60 CELLS

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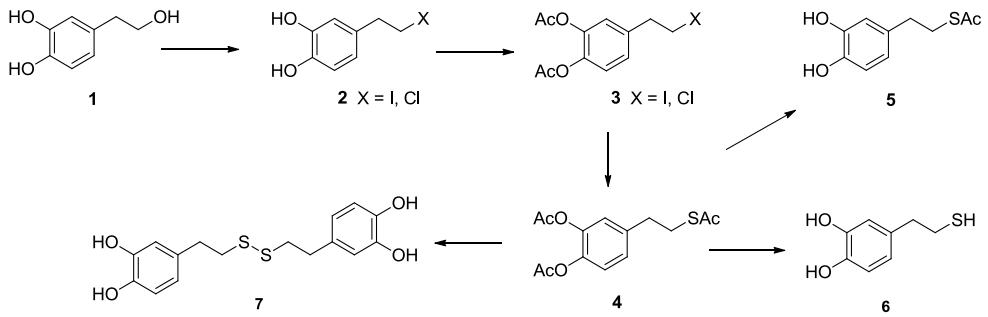
Hydroxytyrosol (HT) **1** is a potent antioxidant present in olive oil. Its biological properties have been deeply investigated (1) to explain the epidemiological data indicating an inverse correlation between olive oil consumption and the risk of some degenerative diseases, in particular coronary heart diseases and cancer (2). Regarding the cancer chemopreventive activities, it has been shown that HT is able to inhibit both initiation and promotion/progression phases of carcinogenesis by preventing the DNA damage induced by different genotoxic molecules (3) and inducing the apoptosis in different tumors cell lines. This effect was dependent upon the ability of HT to induce the accumulation of hydrogen peroxide (H_2O_2) in the cell culture medium (4).
Thio derivatives of hydroxytyrosol **5-7** containing thiol, thioacetate and disulfide functionalities were synthesized from natural HT via 3,4-dihydroxyphenethyl halides **2** (5). These compounds, containing the combination of catechol moiety and sulfur functions, were tested for the pro-apoptotic and anti-proliferative activities on both parental HL60 and multi-drug resistant HL60R cells. It was found that all synthesized compounds were more effective than HT in inducing apoptosis on HL60R cells, and that the hydroxytyrosol disulfide was the most active pro-apoptotic and anti-proliferative compound on both HL60 and HL60R cells. Different from HT, all thio derivatives of hydroxytyrosol induced apoptosis by a mechanism not involving the release of H_2O_2 in the culture medium. The data on HL60R cells suggest that these compounds could be able to reverse the resistance toward the most common drugs in cancer therapy.



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P19

NOVEL MULTIVALENT SYNTHETIC POLYPHENOLS AS MIMETICS OF NATURALLY-OCCURRING TANNINS DIRECTED AGAINST HIV-1

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Contemporary treatment of the Human Immunodeficiency Virus (HIV) infection has converted AIDS from a rapid, lethal disease into a chronic, controlled condition in an impressively short time. However, resistances to the currently available chemotherapeutic arsenal, the appearance of toxicities and the development of side effects are major hurdles in the fight against this pathogen. Thus, a significant effort in the search of novel strategies and alternative therapeutic agents directed against the virus are still required (1).

Polyphenols are a new structural class of versatile anti-HIV agents recently described (2). Among them, the family of tannins has aroused considerable interest due to their remarkable ability to inhibit HIV replication. This group of polyphenols comprises usually a common architecture based on a central distributing scaffold functionalised by several polyhydroxy aromatic moieties populating the outer of their molecular frameworks. In recent years our research efforts have been focused on the discovery of novel synthetic molecules, lately inspired on the multivalent architectures of tannins, containing new polyphenolic moieties that differ structurally of the naturally-occurring motifs.

Based on this experience, we describe herein the design, syntheses and the assessment of the anti-HIV activity of a set of tannin mimetics, containing a 2,3,4-trihydroxyphenyl subunit differently linked at the position 1 of the aromatic ring to flexible distributing scaffolds. The syntheses of these compounds have been carried out through simple, reliable transformations that allow a versatile methodology to reach amides, carbamates, ureas or triazoles as bridging linkers between the polyhydroxybenzene and the suitable scaffolds. The role of the multivalency has been studied by the achievement of mono- di-, tri- and tetrapodal series of compounds.

Preliminary SAR studies on these series of compounds emphasize the importance of the 2,3,4-trihydroxyphenyl ring, the presence of an amide as linker and the multivalent architecture of these molecules as the anti-HIV activity increases as the number of polyphenol subunits do. A novel tetrapodal compound bearing four *N*-(2,3,4-trihydroxyphenyl)amide groups exhibits a remarkable selective activity against HIV-1 in the sub-micromolar range and a safe profile. The presented data support the interest on the potential of the "*synthetic tannins*".

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P20

MOLECULAR MODELING OF NEW PDE10A INHIBITORS: DOCKING, SYNTHESIS AND BIOLOGICAL EVALUATION

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Parkinson's disease is a neurodegenerative disorder that is characterized by the progressive loss of dopaminergic neurons in the substantia nigra of brain, in which neuroinflammation has been recognized to be involved as a primary mechanism in this pathogenesis.[1]
Phosphodiesterases are a family of enzimes encoded by 21 genes and subdivided into 11 families according to their structural and functional properties.[2] These enzymes selectively degrade cyclic purine nucleotides cAMP and cGMP to their inactive 5'-monophosphate forms. PDE10A is the single member identified from the PDE10 family and it is highy expressed in the striatal medium spyny neurons.[3] Since PDE10A inhibitors can be used to raise levels of cAMPC and cGMP within cells that express the PDE10 enzyme and the levels of these nucleotides play an important role in neuroprotection and neuroinflammation, PDE10 inhibitors may be useful in treating Parkinson's and other related diseases.
In the present work, we use virtual screening to identify new hits from our in-house library. From this screening, we selected some molecules that have potential to be recognized selectively at the PDE10A pocket. On the basis of the most important interactions between the enzyme and the ligands, we designed new structural modifications to achieve new more potent and selective inhibitors. The synthesis and biological evaluation of new compounds are in progress.

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PPV-PAMAM HYBRID DENDRIMERS: SELF-ASSEMBLY AND STABILIZATION OF GOLD NANOPARTICLES

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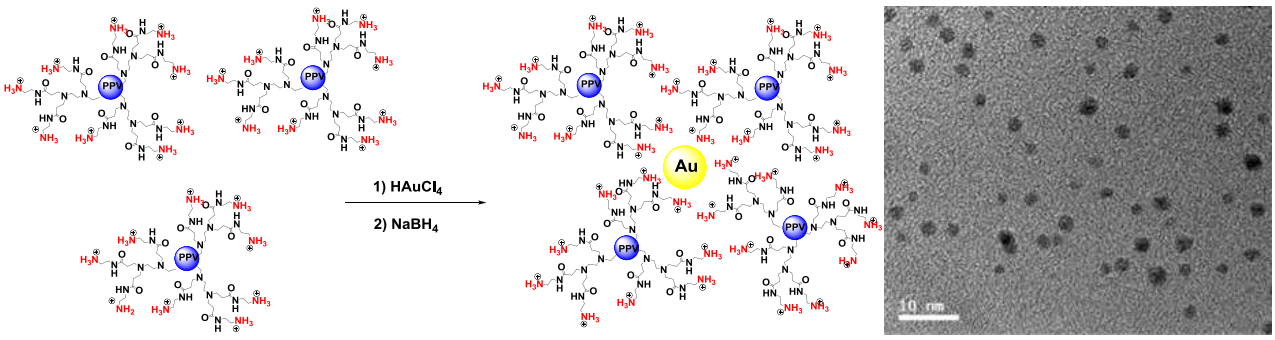
Dendrimers have emerged as a novel class of nanoparticle platform in nanobiotechnology because of their well-defined architecture and unique characteristics (1). The void area within a dendrimer, the extent of its branching, its ease of modification and preparation, and size control offers great potential within the focus of nanomedicine among other interdisciplinary fields (1). Recently, we reported the synthesis and characterization of a new polyphenylenevinylene-polyamidoamine (PPV-PAMAM) hybrid dendrimer that was able to bind and release small interfering ribonucleic acid (siRNA) and lacks any toxicity on neurons at the concentrations used to deliver siRNA. We also demonstrated that dendriplexes formed by this dendrimer and RNAs (siRNAs) could be incorporated into more than 90% of the neurons indicating that PPV-PAMAM dendrimers might be a promising non-viral gene delivery carrier (3). As far as the mechanism of gene transfer relies on supramolecular interactions, self-assembly interactions between molecules are interesting in order to understand the supramolecular organization of the dendrimer as well as their interactions with other molecules. Taking advantages of the supramolecular organization very small gold nanoparticle can be stabilized by these dendrimers. This assembly allows us to combine the previously cited biological properties with the unique properties of gold nanoparticles that make them useful as biomarkers in living whole cells.



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P22

SYNTHESIS AND BIOLOGICAL EVALUATION OF IMIDAZOLYLINDOLES AND HOMOTRYPTAMINES AS NEW POTENTIAL SSRIs

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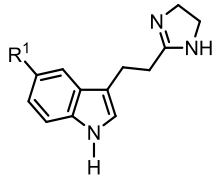
Herein we report the synthesis and evaluation of imidazolylindoles **1a** and **1b**, and homotryptamines **2a** and **2b** as potential selective serotonin uptake inhibitors (SSRIs). The in vitro studies showed that while compounds **1a**, **1b** and **2b** were able to inhibit serotonin uptake by SERT in an only micromolar range (IC_{50} 0.9-4.9 µM), a high inhibition was observed for **2a** (IC_{50} 43.5 nM), similar to that induced by the reference compound fluoxetin (IC_{50} 41.7 nM).

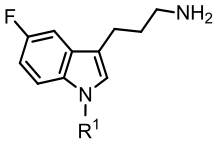
Inhibition of serotonin transporter (SERT) prevents the reuptake of serotonin (5-HT) into presynaptic terminals, regulating the 5-HT levels and potentiating the synaptic function of 5-HT (1). It forms the full or partial basis for the mechanism of action of many antidepressants (SSRIs) (2). Although many clinically useful SSRIs have been discovered, the development of compounds with higher affinity and target selectivity is still an area of interest (3). On this basis, we decided to synthetize 5-HT analogues**1a**, **1b**, **2a**, and **2b** modulated by (i) the nature of the electron-withdrawing group on the aromatic ring, (ii) substitution on the indolic nitrogen atom, (iii) insertion of an additional methylene group into the side chain, and (iv) replacing the basic amino group of the side chain by an imidazoline ring, in order to be tested as SSRIs

Compounds **1a**, **1b**, **2a** and **2b** were evaluated for their ability to inhibit the uptake of 5-HT by SERT in frontal cortex synaptosome of male rats. [^{3}H]-5HT were used as specific radiolabelled ligand for SERT. The results of the reuptake inhibition assays, determined and expressed here as SERT IC_{50} values, show that all the synthesized compounds were able to inhibit 5-HT uptake by SERT in a concentration dependent manner with IC_{50} values ranging from 43.5 nM for compound **2a** to 4.85 µM for compound **1a**.



1a: R^1 = H

1b: R^1 = F



2a: R^1 = H

2b: R^1 = 

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PLURIPOTENT PLATFORMS: A TOOL FOR DIVERSITY GENERATION

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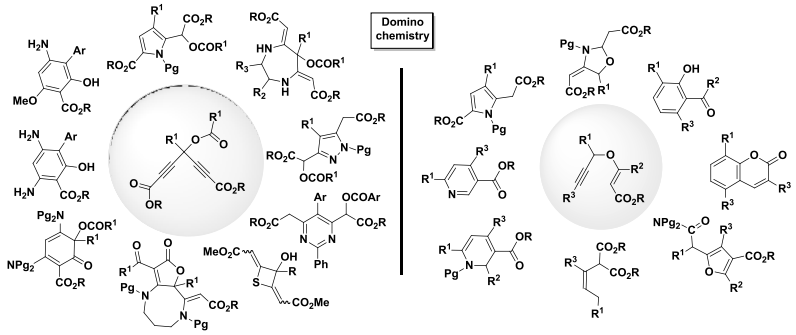
Pluripotent platforms, in analogy with the biological concept, are defined as an array of interrelated chemical functionalities supported on a minimal chemical connectivity and enable to express more than one reactivity profile (outcome). Thus, they constitute a very versatile tool for diversity oriented synthesis (DOS), and specifically in synthetic programs based on the coupling-building-pairing strategy. During the last years, we have been involved in a wide program of synthesis and methodology to develop new strategies for the fast, efficient and diversity-oriented synthesis of small molecules for mapping bioactivity (mainly antitumor activity) in the chemical space (1). In this communication we will outline the application of this concept to the synthesis of structurally diverse libraries of small molecules including heterocyclic, carbocyclic and acyclic structures.



Each library member is fully accessible in a fast manner and a multigram scale!!

Fig. 1: DOS of small molecules libraries using the *pluripotent platform concept*.

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EMERGING VIRAL DISEASES: IDENTIFICATION AND EXPLORATION OF NOVEL CHIKUNGUNYA VIRUS REPLICATION INHIBITORS

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Chikungunya fever is a mosquito transmitted viral disease caused by the Chikungunya virus. It has a serious impact on human health causing fever, rashes, arthralgia and myalgia. Indeed "Chikungunya" is a Makonde word meaning "that which bends up".[1] During the past 50 years, there have been different CHIKV re-emergences in Africa and Asia. However, the outbreak in 2005-2006 in La Reunion island, a French territory in the Indian Ocean, marked a before-and-after in the epidemiology of this disease.[2] More than one third of the population of the island got infected, and 237 deaths were reported associated to the virus infection. After this, an outbreak occurred in the north-east of Italy, being the first reported case in the occidental world. The ability of the virus to adapt to a new mosquito species, *Aedes albopictus*, may have contributed to these outbreaks and is expected to favour the worldwide spread of this pathogen.

At this moment there is no drug to treat this infection and no vaccine has been approved for clinical use. Therefore there is a clear need to identify and develop new chemical entities able to selectively inhibit CHIKV replication. Our laboratories and others have been involved in a wide screening campaign devoted to this aim.[3,4] In this joint effort, and after submitting to screening a historical collection of chemically diverse compound series, we have identified a new family of low molecular weight heterocyclic compounds that afforded almost full protection against the cytopathic effect of CHIKV to Vero cells at non-toxic concentrations. A synthetic programme was set up to prepare and evaluate new structural analogues. The synthetic pathways involve three to five steps and make use of microwave-assisted synthesis for the crucial steps. As a consequence we have improved the antiviral potency of the hit and have identified the structural requirements for anti-CHIKV activity.

Acknowledgements: A.G. thanks the FSE and the JAE-Predoc programme for a predoctoral fellowship. This work has been supported by a grant of the Spanish CICYT (SAF2009-13914-C02-01).

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CHOLINE KINASE INHIBITORS AS POTENTIAL ANTILEISHMANIASIS DRUGS

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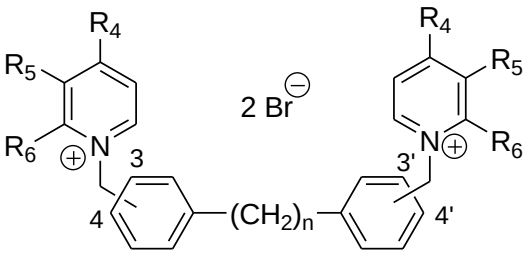
Leishmaniasis is a major and globally widespread group of parasitic diseases caused by the intracellular protozoan Leishmania. Main clinical manifestations include visceral leishmaniasis (VL) and cutaneous leishmaniasis (CL). Whereas CL has a tendency to spontaneously self-heal with resulting scars, VL is a severe form in which the parasites have migrated to the vital organs and is fatal when left untreated. An effective vaccine against leishmaniasis is not available and chemotherapy is the only way to treat all forms of disease. However current therapy is toxic, expensive and the resistance has emerged as a serious problem (1) which emphasizes the importance of the development of new drugs against leishmaniasis.

We have previously reported the design and synthesis of a new set of bis-quaternary compounds, **1** that showed very good Choline Kinase (ChoK) inhibitory activity (2) These compounds have been tested for their ability to produce sensitivity in promastigotes and intracellular amastigotes of Leishmania spp. Some of them showed IC_{50} below 1 μM, suggesting a new approach to be considered for treatment of leishmaniasis.



1

R_4 = cyclic tertiary and acyclic amino groups
$R_5 + R_6$ = 2H, $(CH=CH)_2$ or CH=CH-CCl=CH_2
Isomers: 3,3' when n = 0; 4,4' when n = 0, 2, 3, 4

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P26

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

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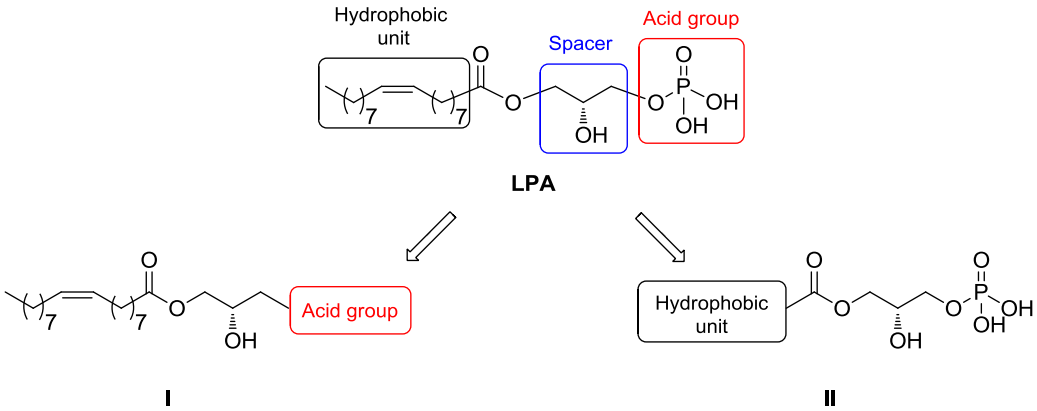
Lysophospholipids (LPs) are lipid molecules that are receiving growing attention because, in addition to their structural function in the cell membrane, they are now regarded as important regulators for diverse biological functions through activation of specific receptors. These receptors have been characterized during the last two decades as G protein-coupled receptors (GPCRs) and are divided in two main families: $LPA_{1\text{-}6}Rs$ and $S1P_{1\text{-}5}Rs$. Despite their interest, the high structural similarity over the two groups has restrained the possibility of obtaining selective and high affinity ligands and therefore the elucidation of the role of these receptors in the central nervous system (CNS).

Given the interest of the LPA signaling in the CNS (2), we started a project in our research group aimed at the development of new LPA_1R selective ligands, which was started with the design, synthesis and biological evaluation of two series of compounds based on the structure of the endogenous ligand LPA. From this initial set, four ligands were identified as promising hits with low micromolar EC_{50} values for LPA_1R. In order to improve affinity, the most relevant structural features of these hits are being combined on a new series of compounds that are currently under study.



Acknowledgements: This work has been supported by grants from the Spanish Ministerio de Economia y Competitividad (MINECO, SAF2010-22198) and Comunidad de Madrid (S2010/BMD-2353). The authors thank MINECO for a predoctoral grant to I.G-G.

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HYDROXY CHALCOGENIDE-PROMOTED MORITA-BAYLIS-HILLMAN-ALKYLATION REACTION: NEW INTERMOLECULAR APPLICATIONS WITH ALKYL HALIDES AS ELECTROPHILES

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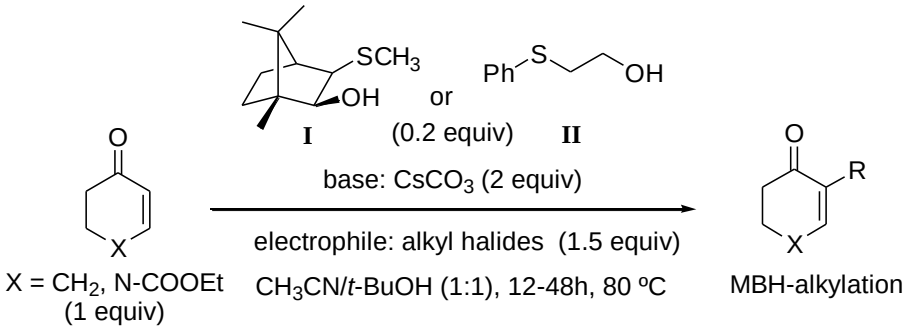
The Morita–Baylis–Hillman reaction (MBH) is an organocatalytic reaction involving construction of a carbon-carbon bond between the α-position of an activated alkene and an electrophile under the influence of a catalyst providing a densely functionalized molecule. During the past twenty years there has been an explosive growth of this reaction with respect to all the three essential components required.[1]

In the course of our studies in the MBH reaction methodology we have recently developed an unprecedented used of the enantioselective hydroxy chalcogenide **I** as catalysts to performed efficiently the alkylation version of this interesting reaction, under basic conditions, when α,β-unsaturated lactams and lactones were used as activated alkenes and allyl, alkyl halides, propargyl halides or epoxides as electrophiles.[2]

In our previous work we already indicated that the presence of the free hydroxyl group in catalyst **I** was important as no MBH adduct was observed when others no bifunctional compounds were used as catalyst.

We now present in this work recent extensions of our method to a broader catalyst scope, including a variety chiral and non chiral sulfanyl alcohols such as the hydroxyl chalcogenide **II** among others, Scheme 1.



Scheme 1. MBH alkylation reaction of cyclic enones with different electrophiles using sulfanyl alcohols as catalysts.

The procedure works efficiently for a range of cyclic enones including cycloalkenones and dihydropyridinones as activated alkenes and allyl and alkyl halides, propargyl halides as electrophiles.

ACKNOWLEDGEMENTS:
Grants from the MEC (CTQ2012-31063) and the CEU-San Pablo University (PC14/12) have supported this work.
R. Ferreira thanks Airbus Military for a pre-doctoral fellowship.

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P28

SYNTHESIS OF A NOVEL TYPE OF FLUORESCENT GFP-CHROMOPHORE ANALOGUES

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The unique properties of green fluorescent protein (GFP) exploited in novel bioimaging techniques have revolutionized many areas in life sciences and motivated numerous experimental and theoretical studies (1). Despite the enormous contribution of the GFP to biological research, only a limited number of small molecular model compounds have been reported for a GFP chromophore (2). This is probably because the GFP chromophore does not exhibit fluorescence when it is outside the barrel structure of the GFP (3).
Recently, in the course of studies focused on the synthesis of unusual indole and azaindole derivatives, we have obtained as by-products a family of highly fluorescent compounds related to GFP chromophores (4).

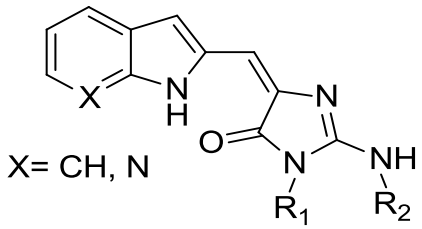

Fig. 1: Synthesized family of fluorescent GFP chromophores.

Due to the interest of this new family of GFP chromophores, we have designed a short and efficient synthesis to obtain these products. Moreover, we have studied their fluorescent properties and tested them as fluorescent probes in *PC-3* human prostate *cancer cell* lines.

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P29

NOVEL MMP-2 INHIBITORS CONTAINING NON-HYDROXAMATE ZBGS. DESIGN, SYNTHESIS AND BIOLOGICAL ACTIVITY

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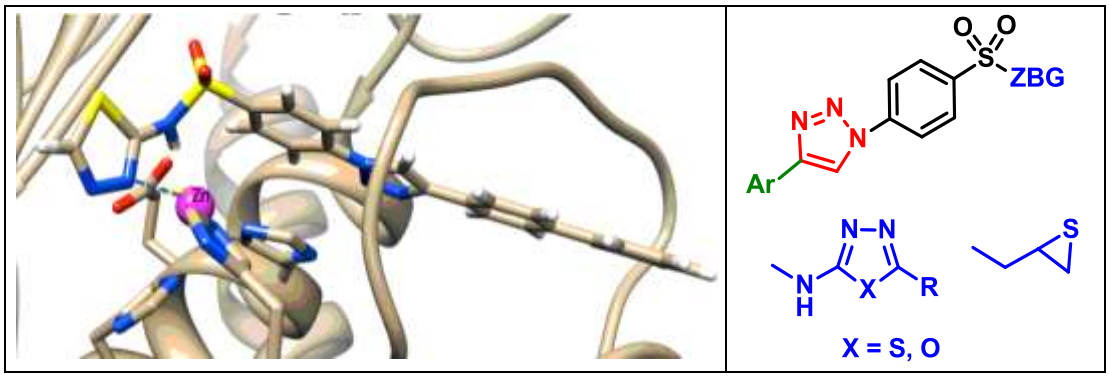
Matrix metalloproteinases (MMPs) are zinc containing enzymes that mediate the breakdown of connective tissue, degrade and remodel the extracellular matrix and are implicated in a wide variety of biological processes. Abnormal levels of MMPs are related to cancer and many other diseases, thus, they are potential targets for therapeutic inhibitors.[1]

The general structure of an effective MMP inhibitor (MMPi) includes a zinc binding group (ZBG) capable to bind the catalytic zinc(II) ion of these proteinases, and a peptidomimetic backbone. Although hydroxamic acid derivatives were found to be one of the most powerful ZBG, the use of this scaffold in drugs led to extreme undesirable effects, due to the poor bioavailability of such function and especially to its high transition-metal binding ability.[2]

We describe herein our efforts aimed at finding alternative non-hydroxamate ZBGs. Based on the previous work on fragment-based drug design of MMPi's developed in our group,[3] we synthesized a novel family of this class of inhibitors by employing oxadiazoles, thiadiazoles and thiiranes as ZBGs. These structures bear an azide moiety and were linked to the alkyne-containing fragment by a Cu-catalyzed click chemistry approach to obtain the final triazoles (**Figure 1**).

The activities against MMP-2 were evaluated by colorimetric assays, showing promising results in some of the synthesized inhibitors. Docking and NMR experiments (Water-LOGSY and STD competition) have been used to reveal the potential interactions that govern the recognition and binding to MMP-2.



a) **b)**

Figure 1. a) Snapshot extracted from a MD simulation (MMP-2). b) General structure of the designed MMPi's and the corresponding ZBGs (in blue).

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P30

DEVELOPMENT OF NEW REVERSIBLE INHIBITORS OF THE ENZYME MONOACYLGLYCEROL LIPASE (MAGL)

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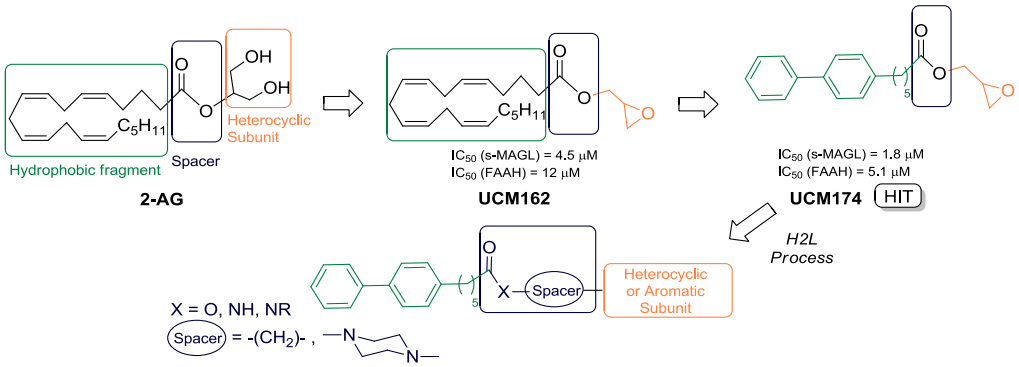
The activation of the cannabinoid receptors CB_1 and CB_2 by exogenous and endogenous ligands regulates many physiological events such as memory, locomotion and immune system function. Accordingly, they represent important targets for the development of new therapeutic strategies for the treatment of neurodegeneration, excitotoxicity, pain and inflammation. It is known that the use of direct agonists of the CB_1 receptor produces psychotropic effects; instead, the increase of the levels of the endogenous ligands 2-arachidonoylglycerol (2-AG) and anandamide (AEA), would avoid these undesirable effects. Due to the key role of 2-AG in the central nervous system, we have started a project aimed at the development of compounds able to increase the levels of 2-AG by blocking its hydrolysis via the inhibition of the MAGL, the main enzyme responsible for its inactivation. Up to date, only a few potent and selective irreversible MAGL inhibitors have been described (1). However, after repeated administration, they produce CB_1 desensitization, fact that eventually impairs their antinociceptive properties. Accordingly, our objective is to develop new potent and selective compounds that inhibit MAGL activity but in a reversible manner, in order to avoid these undesirable effects.

Based on the structure of the endogenous substrate 2-AG, and after an initial structure-activity relationship exploration, we identified the compound UCM174 as a reversible inhibitor of MAGL, although with moderate potency and selectivity (2). Herein, we present the *hit to lead* process in terms of potency and selectivity vs other hydrolases, together with the *in vivo* activity of the selected lead in a mouse model of multiple sclerosis.



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TOWARDS THE 'IN SILICO' PREDICTION OF THE BIOACTIVE CONFORMATION OF SMALL MOLECULES

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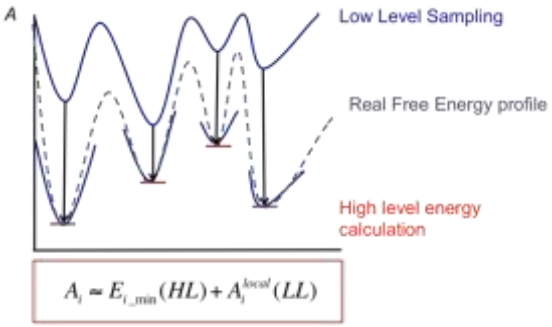
To properly describe the conformational energy surface of small molecules, we have recently reported (1) a new strategy that relies on the predominant-state approximation in order to explore the conformational preferences of molecules. This approach partitions the conformational space into distinct conformational wells, which are located by using low-level (LL) methods for sampling the conformational minima, whereas high-level (HL) techniques are subsequently used for calibrating their relative stability. In the first implementation, the LL sampling was performed with the semiemprirical RM1 Hamiltonian (solvent effects included through the RM1 version of the MST continumm solvation model) and the HL refinement was performed by combining geometry optimizations of the minima at the B3LYP level, and single point MP2 computations using Dunning's augmented basis set, with the local curvature of the well sampled at the semiempirical level.

The use of the RM1 method for the LL sampling avoids the need of parametrizing the ligand, though it implies a significant cost in terms of computational cost. Hence one might argue whether the use of classical force field might be a more efficient option, even in spite of the effort required for ligand parametrization. In this work we discuss preliminary results obtained by using the AMBER-GAFF force field in conjunction with explicit solvent for the LL sampling, addressing the parametrization step through standard tools. We apply our methodology to a set of biogenic amines whose conformational preferences has been the focus of several theoretical and experimental studies (2-3).



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NEUROPROTECTIVE EFFECT OF GRAMINE AND ITS DERIVATES

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In the search of new drugs for the treatment of neurodegenerative diseases acting by original pharmacological properties, we have found that the natural alkaloid gramine (Poaceae) and its derivatives have been described to feature an interesting biological and therapeutic profiles (1), with properties such as the ability to regulate $[Ca^{2+}]_c$, analgesic and anti-inflammatory effects, antihistaminergic, antagonism of 5-HT_6 receptors, etc. Based on the properties ascribed to gramine, we decided to deep into its effect and those from its derivates on different models of neurodegeneration. A first model is the mitochondrial dysfunction and the oxidative stress, which are key processes in the pathogenesis of neurodegenerative diseases such as Alzheimer's (AD) (2). Thus, gramine showed protection against an oxidative stress model, defined by the cocktail of rotenone and oligomycin A on SH-SY5Y cells. Moreover, recent discoveries emphasize that maintenance of the activity of protein phosphatase 2A (PP2A) as a new drug target in AD, since this enzyme is largely responsible for the dephosphorylation of *tau* protein (3). Gramine and its derivatives synthesized in our laboratory have shown neuroprotective properties in in vitro models related to *tau* hyperphosphorylation and glutamate- induced Ca^{2+} overload, toxic stimuli antagonized by the phosphatase activity of PP2A. All of these findings allow us to consider the gramine and its derivates as a potential new family of compounds with indication for the treatment of neurodegenerative diseases and stroke.

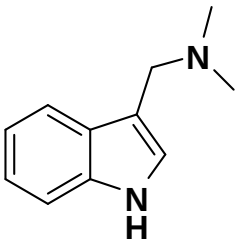

Fig. 1: Chemical structure of gramine.

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POLYPHENOL PEPTIDES DERIVED FROM L-AMINOACIDS: SYNTHESIS AND BIOLOGICAL EVALUATION

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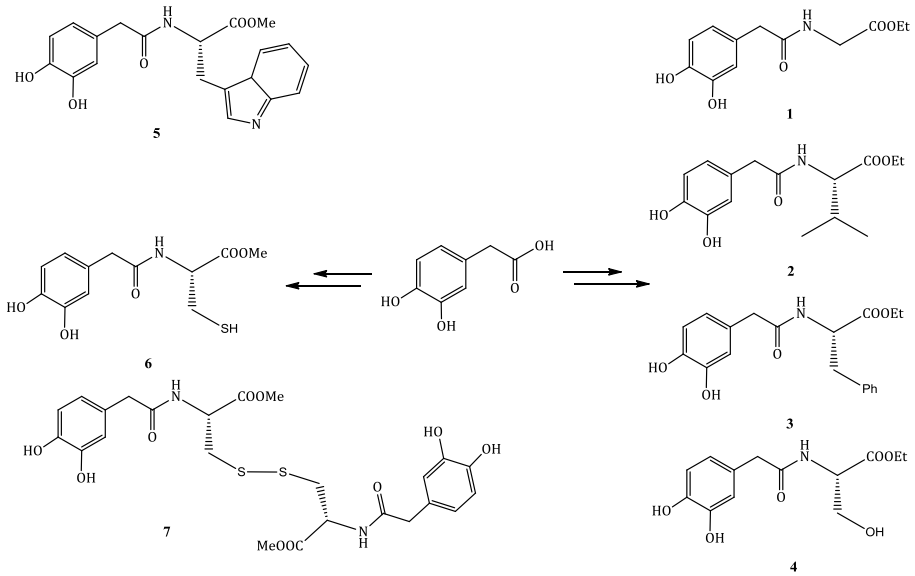
Polyphenols comprise a heterogeneous family of natural compounds found in a great variety of food of vegetal origin, like fruits, wine, tea, cocoa, or very specially, virgin olive oil. Such compounds have been found to be potent antioxidant agents, and also to exert beneficial effects against cardiovascular and degenerative diseases, like cancer, Parkinson, or Alzheimer disorders, among others (1), (2).

Most of these compounds are quite polar molecules, and therefore, a chemical modification can afford a modulation of their biological activities, together with an improved biodisponibility in biological systems.

Herein we report the preparation of peptides **1**-**7** derived from 3,4-dihydroxyphenyl acetic acid and a series of L-aminoacids (glycine, phenylanaline, valine, serine tryptophan, cysteine and cysteine), using a peptide coupling as the key synthetic step. The final compounds have been evaluated as antioxidants (anti-radicals, anti-H_2O_2) and as antiproliferative agents against cancer cervical cells (HeLa, CaSki, ViBo).



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DYNAMIC FINGERPRINT OF IMATINIB SENSITIVE KINASES

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Protein kinases constitute one of the largest and most functionally diverse protein families, involved in
most cellular pathways. They are of crucial importance for signal transduction, and their deregulation is
related to numerous human diseases, including cancer.
Among kinases, c-Abl and c-Src are of particular interest for cancer research. The BCR-Abl fusion protein, is the initial cause of chronic myeloid leukemia disease (CML) (1). CML is treated with the powerful anti-cancer drug Imatinib, which acts as ATP-competitor.
Imatinib, however, is not so selective and targets numerous kinases (2), beside c-Abl and c-Src, like c-KIT, PDGFR, Syk, Lck and many others, showing towards some of them a lack of activity.
For instance it effectively inhibits BCR-Abl (IC_{50}=0.2 uM) but not Src (3), no matter their high sequence homology (47%), neither that the bound structure of Src with Imatinib is very similar to that of Abl with Imatinib.
In the same way, the drug inhibits c-KIT (IC_{50}=0.41 uM) but not Syk(3), although they share the same sequence identity of 40% with both Abl and Src. Striking is the case of Lck, quite effectively inhibited by Imatinib (IC_{50}=9 uM) (3) , despite the high identity with Src kinase (70%).
Recently, in our work concerning the two kinases c-Abl and c-Src, we have shown that the difference in the Imatinib activity can be attributed to a larger flexibility of c-Abl (4). Now in this follow up work we have extended the analysis to the other cited kinases, analyzing their flexibility profiles.
Performing classical molecular dynamic simulations, followed by RMSF analysis, we have identified regions with a higher flexibility common to all the kinases sensitive to Imatinib (c-Abl, c-KIT and also Lck) and a more rigid profile in the same regions for those not responding to the drug (c-Src and Syk).
From these preliminary results we could recognize flexibility as a possible hallmark of all the studied kinases sensitive to Imatinib, probably due to its paramount importance for active site accessibility.
Detecting the features responsible for the observed differences is essential for the development of new effective anticancer drugs but also useful to identify possible hallmarks of the variegate kinase superfamily.

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MECHANISTIC BASIS OF THE INHIBITION OF TYPE II DEHYDROQUINASE ENZYME BY (2*S*)- AND (2*R*)-2-BENZYL-3-DEHYDROQUINIC ACIDS

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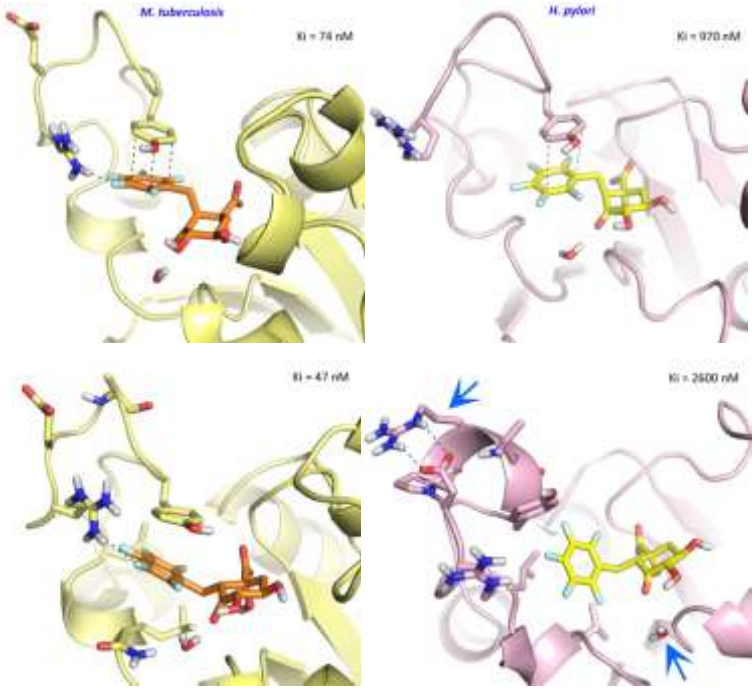
Our research group is studying the possible development of new antibiotics by inhibition of the type II dehydroquinase (DHQ2), an essential enzyme in *Mycobacterium tuberculosis*, the causative agent of tuberculosis, and *Helicobacter pylori*, the causative agent of gastric and duodenal ulcers, which has also been classified as a type I carcinogen. In this communication, we report structural and computational studies that show how 2-benzyl-3-dehydroquinic acids (1) inactivate DHQ2 by causing a significant conformational change (2) in the flexible loop of the enzyme (3). These changes depend on the substitution of the aromatic moiety of the inhibitors. This loop closes over the active site after substrate binding and its flexibility is essential for the function of the enzyme. These differences have also been investigated by molecular dynamics simulations in an effort to understand the significant inhibition potency differences observed between some of these compounds and also to obtain more information about the possible movements of the loop (4).



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OXAZOLE DERIVATES: NEW AGENTS DISPLAYING MULTIFUNCTIONAL ACTIVITIES FOR A POTENTIAL TREATMENT OF ALZHEIMER'S DISEASE.

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The complex etiology of Alzheimer's disease (AD) prompts scientists to develop multifunctional compounds to combat causes and symptoms of such neurodegeneration (1). A physiological function affected in AD is cholinergic neurotransmission, which seems to be closely related to pathological amyloid β-peptide A*β*. Currently, the cholinergic strategy remains the most effective therapeutic approach for the symptomatic treatment of AD. Neuroprotection is one of the major challenges of modern medicine as a potential way to combat or slow down the progression of neurodegenerative conditions such as AD (2). From an in-house library of compounds, five different structures related to complex oxazoles (3) were selected to be tested in neuroprotective and cholinergic assays (4). Four compounds inhibit at submicromolar concentration acetylcholinesterase (AChE), AChE-induced and self-induced A*β* aggregation, and they behave as selective inhibitors of AChE of human origin. They could also penetrate the central nervous system (CNS), according to an in vitro blood-brain barrier model. Free radical capture and/or promotion of antioxidant protein biosynthesis are mechanisms that can be implicated in their neuroprotective actions (5). Due to their excellent pharmacological properties, three derivates have been selected to develop new series of multifunctional compounds that are currently in progress.

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SYNTHESIS OF BENZOTHIAZEPINE ANALOGUES AND EVALUATION OF THEIR MITOCHONDRIAL Na^+/Ca^{2+} EXCHANGER BLOCKING EFFECTS

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The calcium flux through plasma membrane and among intracellular compartments play a critical role in different functions of excitable cells, such as neuritogenesis, synaptogenesis, synaptic transmission, plasticity, or cell survival. However, in neurodegenerative disorders, the cell calcium regulatory machinery is altered, leading to synaptic dysfunction, reduced plasticity, and neurodegeneration. In this scenario, oxidative stress disrupts energy metabolism and causes aberrant aggregation of certain proteins (Amyloid beta, alpha-synuclein, huntingtin, etc) that negatively affect calcium homeostasis by different mechanisms.
In this situation, mitochondria play a pivotal role in neurodegenerative diseases. The modulation of the mitochondrial Na^+/Ca^{2+} exchanger (mNCX) could be a target for regulating Ca^{2+} homeostasis and become neuroprotective. It is known that the benzothiazepine CGP37157 blocks mNCX. Since mitochondrial Ca^{2+} overload results in cell death, we hypothesize that the modulation of the mNCX would intimately affect to the onset of neurodegenerative processes.
Therefore, we decided to synthesize analogues of benzothiazepines to improve this blocking activity as well as to increase the selectivity towards this exchanger. All synthesized molecules were subjected to different models of cell death, including oxidative stress by the toxic cocktail Rotenone/Oligomycin A in SH-SY5Y cells, or glutamate-induced calcium overload in hippocampal slices; in those compounds showing a better neuroprotective profile was analyzed in permeabilized HeLa cells transfected with aequorin protein genetically directed to the mitochondrial matrix (AEQ-Mt-mut). With these data and those obtained from the mitochondrial exchanger blockade, we selected ITH12575 to determine their IC_{50} and compare with the lead compound CGP37157.
The presented results allow us to get new ligands for the mNCX with a neuroprotective profile, which in the future may be used in the treatment of neurodegenerative diseases.

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CONVERGENT SYNTHESIS OF GLYCODENDROPEPTIDES BY "CLICK CHEMISTRY" APPROACHES

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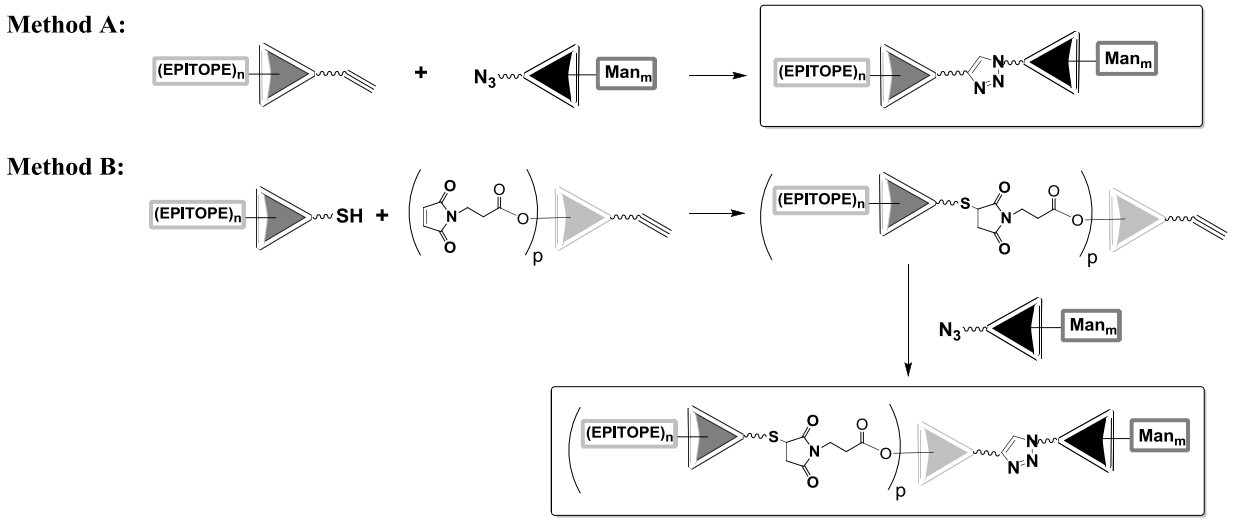
In 1997, Pieters and Koning simultaneously described the biological effects of peptide and protein mannosylation,[1] which included the facilitated uptake of such peptides and proteins through mannose receptors at the cell surface. The carbohydrates induced a receptor-dependent internalization resulting in strong T-cell stimulation. This discovery opened a way to novel vaccine strategies, particularly for those cases (cancer, HIV, etc.) where conventional vaccines had not been successful enough.

Herein, we present two versatile, complementary synthetic strategies converging on glycodendropeptides (GDPs) displaying up to 9 and 16 mannose and peptide copies, respectively. Both approaches (Scheme) rely on Cu(I)-catalyzed azide-alkyne cycloaddition (CuAAC)[2], also known as "click chemistry", for both the synthesis of glycodendron units and their subsequent conjugation to SPPS-made peptides or MAPs. This versatile and straightforward strategy for the preparation of bifunctionalized systems allows a total control on the chemical structure and provides the means to modulate easily the valency.



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CHROMENOPYRAZOLE AS VERSATILE CANNABINOID SCAFFOLD: MOLECULAR MODELLING, SYNTHESIS AND BIOLOGICAL ACTIVITY

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G-protein-coupled cannabinoid receptors, CB_1 and CB_2, have emerged as promising therapeutic targets. Classical cannabinoids showed potent activity *in vivo*, but most of them are psychoactive. The unwanted psychoactive effects of cannabinoid receptor agonists have limited their medical development. Consequently, it is important to discover new non-psychoactive cannabinoids that do $_1$not cross the blood-brain barrier and/or act on peripherally located cannabinoid receptors. In this context, we recently described a series of chromenopyrazoles as non-psychoactive and selective CB_1 cannabinoid agonists with peripheral antinociceptive properties (1). The current study aimed to target the CB_2 type receptors mainly located outside the brain. For this purpose and following with the chromenopyrazole scaffold, structural features required for CB_2 affinity and selectivity were explored by molecular modeling using the CB_1 and CB_2 receptors models described by some of us. Based on these studies, new series of chromenopyrazoles were synthesized and characterized, and pharmacologically characterized at cannabinoid CB_1 and CB_2 receptors. Affinity of new compounds was evaluated measuring their ability to displace [^{3}H]CP55940 from human cannabinoid CB_1 and CB_2 receptors transfected into HEK293 EBNA cells. Functional activity of the compounds with the highest CB_2 affinity profiles was tested in CB_2-mediated functional assays using BV2 cultured cells (a mouse microglia cell line). Chromenopyrazoles were found to constitute a versatile scaffold for obtaining potent cannabinoid receptor ligands with selectivity at either CB_1 or CB_2 receptor types.

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SYNTHESIS, MOLECULAR MODELLING, *IN VITRO* BIOLOGICAL PROFILING, AND *IN VIVO* EFFICACY STUDIES OF A NOVEL FAMILY OF HUPRINE-BASED MULTI-TARGET ANTI-ALZHEIMER COMPOUNDS

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Alzheimer's disease (AD) is among the top ten leading causes of death in developed countries, but the only one among them than cannot be cured, prevented or slowed down. It is increasingly believed that AD is not a straightforward process but a complex network of interconnected protein targets, with redundant and compensatory signalling pathways that render ineffective the modulation of a single target of the pathological network. In this light, compounds with the ability to simultaneously hit multiple targets of AD network are emerging as a realistic option for efficiently tackling the neurodegenerative process of AD.

We report herein on a new family of huprine-based hybrid compounds that exhibit a very interesting multi-target *in vitro* biological profile, encompassing very potent BACE-1 and acetylcholinesterase inhibitory activities and significant β-amyloid (Aβ) anti-aggregating properties. The Aβ anti-aggregating activity observed *in vitro* has been confirmed *in vivo* in intact *Escherichia coli* cells overexpressing Aβ42. Similarly, using *E. coli* cells that overexpress tau protein, we have found that these compounds are also able to significantly inhibit tau aggregation. Additional *in vivo* studies in mice have shown that one selected compound (AVCRI175P1) is able to protect 2-month-old C57bl6 male mice against the synaptic failure induced by acute treatment with Aβ oligomers, as evidenced by the induction of long-term potentiation and by the prevention of the loss of synaptic proteins. Very interestingly, intraperitoneal administration of AVCRI175P1 for 4 weeks to transgenic APP/PS1 mice resulted in reduction of cortex and hippocampus Aβ aggregates, reduction of astrogliosis, restoration of synaptic plasticity, reduction of tau phosphorylation, and prevention of the loss of cognitive abilities. Thus, AVCRI175P1 emerges as a very promising multi-target anti-Alzheimer drug candidate with potential to efficiently modify the natural course of the disease (1).

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SYNTHESIS AND *IN VITRO* EVALUATION OF IMIDAZOISOINDOL DERIVATIVES AGAINST *STRONGYLOIDES VENEZUELENSIS* FEMALE LARVAE

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Strongyloidosis is usually a chronic human parasitic disease of the gut, which can remain undetected for decades affecting to 50-100 million people in Africa, Southeast Asia, Latin America, Southeastern of the United States and Southern Europe (1). Treatment is based the use of albendazole, thiabendazole and ivermectin but there are reports of treatment failures or relapses after antiparasitic therapy (2). In previous studies several imidazoisoindoles demonstrate good antiparasitic activity (3-4). The purpose of this study was to assess the *in vitro* activity of 29 imidazoisoindoles against L3 and females of *Strongyloides venezuelensis*. Compounds were tested in triplicate in cultures with 200 decontaminated third stage larvae (L3) or 100 female during a week at final concentrations of 0.1, 0.5, 1, 5, 10 and 20 µg/mL. Ivermectine was used as control (5). The nematocidal activity was measured, as percentage of mortality after microscopic observation of mobility and IC_{50} was determined. Imidazoisoindoles were obtained in a two steps procedure from benzalphthalides the corresponding yielding of 70-90%. Four compounds reduced mobility of L3 more than 90 % at 7th day of the culture at a IC_{50} of 0.3 µM. Further studies will be carry out in order establish the mechanism of action.

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CYTOTOXIC ACTIVITY AND INHIBITION OF TUBULIN POLYMERIZATION OF DIMETHYLAMINO-ISOCOMBRETASTINS

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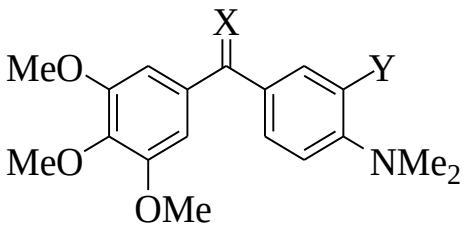
A large number of modifications of CA-4 B-ring have been described in the literature, including amino substituents replacing the 3-OH or 4-OMe substituents. Recently described isocombretastatins, 1,2-diarylethene analogues of combretastatins, have been also investigated, but the number of modifications described to the present is shorter (1,2).

Isocombretastatins display a higher inhibition of tubulin polymerization (ITP) "in vitro" than related (equally substituted at rings A and B) combretastatins, but slightly lower cytotoxicity (3,4). In this communication we will present the results of our research on isocombretastatins carrying the usual 3,4,5-trimethoxy substitution pattern in the A-ring and 3-substituted-4-dimethylaminophenyl as B-ring. Substituents at position 3 of B-ring have been introduced in order to increase the solubility of these molecules that already have the dimethylamino group to this purpose.
Initial promising results of cytotoxicity, ITP activities will be present, as well as docking studies.



X: O, CH_2, NOH

Y: NO_2, NH_2, CHO, CH=NOH, CN

Fig. 1: Isocombretastatins described in this communication

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PYRIDINE DERIVATIVES AS KSP LIGANDS: POTENTIAL ANTIMITOTIC AGENTS

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Antimitotic drugs in clinical use are limited to compounds targeting tubulin. Such drugs display severe side-effects such as neurotoxicity, arising from the fact that tubulin performs other physiological functions in the cell apart from mitosis (1). Therefore, there is a great need for new antimitotic agents devoid of such side-effects.

KSP (kinesin spindle protein, also known as Eg5) is a motor protein that promotes the formation of a bipolar mitotic spindle by crosslinking antiparallel microtubules and sliding them in opposite directions (2). Its only role in mitosis makes it an attractive target for new antimitotic agents.

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 replaced the 3-hydroxyphenyl ring, which has been reported as important for binding (4), by nitrogenated rings. Thus, pyridine systems, bound through different ring positions, are replacing such moiety, in order to gain insight about the structural requirements for potent KPS inhibition. Additionally, a 2,6-dihydroxy-4-pyridyl moiety allows the presence of hydroxy groups at positions equivalent to that in monastrol. The synthesis and biological evaluation of these compounds will be reported in this communication, including the formation of an unexpected compound.

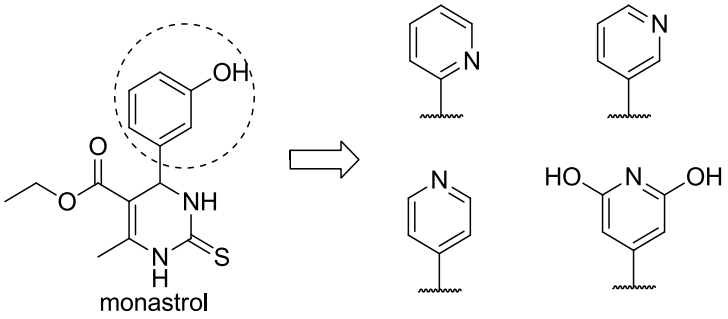

Fig. 1: Structure of the compounds designed as analogues of monastrol.

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CONFORMATIONAL STUDY OF THE HUMAN INSULIN SIGNAL PEPTIDE

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The human insulin consists of two polypeptide chains (A and B chains), linked together by disulfide bonds. It is however first synthesized by the pancreatic β-cells as a single polypeptide called ***preproinsulin***. Preproinsulin contains a 24-residue signal peptide which directs the polypeptide chain to the rough endoplasmic reticulum (RER). Preproinsulin is converted into proinsulin by signal peptidases, which remove its signal peptide from its N-terminus. Finally, proinsulin is converted into the bioactive hormone insulin by removal of its connecting peptide (C-chain).

Recent studies about Type I *diabetes mellitus* suggest that a possible cause is a mutation somewhere in the process of synthesis of insulin (1), (2). This mutation could be located in the signal peptide, preventing preproinsulin from reaching RER. The genbank store several point mutations in the signal peptide, four of these single nucleotide polymorphisms (SNP) are silent mutation. Almost no preproinsulin exists in the cell, because the removal of the signal peptide is not a separate step, so the conformation of the signal peptide is not in databases. For this reason the main objective of this work is to propose a possible tertiary structure for this signal peptide and to study the effect that changes in the position of the silent mutations has over the stability of the structure.

Starting from the primary structure of the signal peptide, we have searched in databases of *Homo Sapiens* proteins for identical amino acid sequences. We have selected the 25 peptides of highest percentage of amino acid sequence identity, downloading their structures from the Protein Data Bank.

In order to decide which angles are the most thermodynamically favourable, all the values of PSI and PHI angles have been represented in the Ramachandran plot of that specific amino acid (3). Sometimes, there are two possibilities of angles, the corresponding to α-helix or β-sheet, so two different structures have been proposed.

We have performed a geometry optimization of these structures with the programme HyperChem in order to minimize their energy.

We have also analyzed the effect of changes in the position of silent mutations on the stability of the signal peptide structure.

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SYNTHESIS OF SOME ANTICANCER COMPOUNDS VIA CHEMICAL COMBINATION OF 5-FLUORORACIL, 4-AMINOQUINALDINE AND SOME NITROHETEROCYCLES

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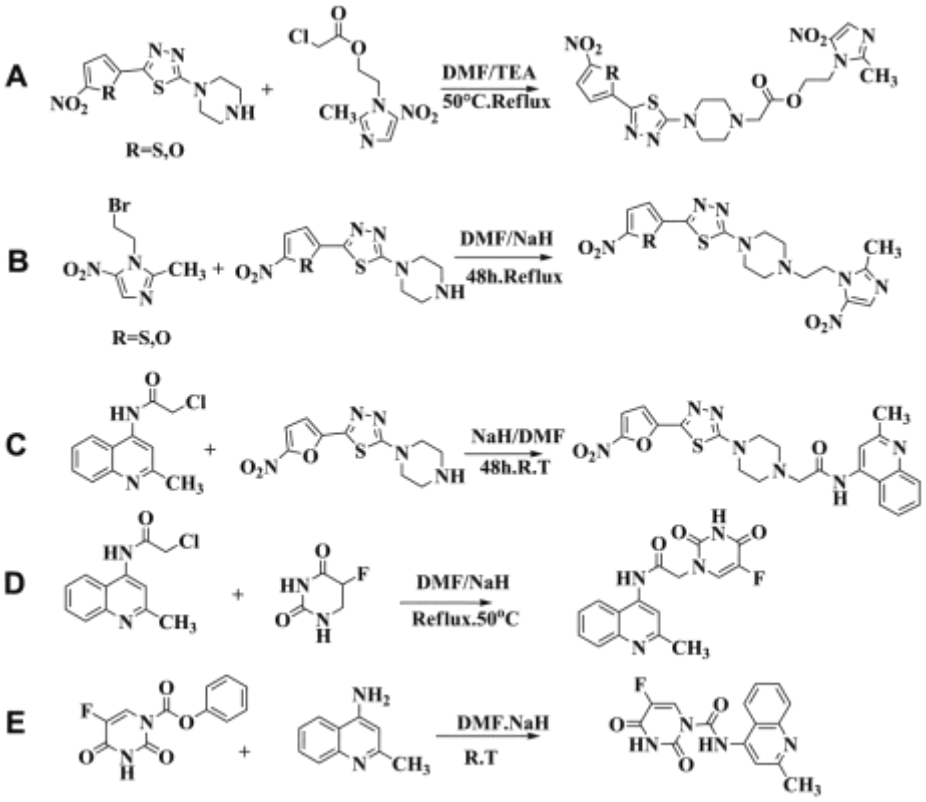
In recent years different approaches such as synthesis of potent compounds or association of two anticancer drugs in order to increase of their potency besides of less side effects have been considered. In this project, synthesis of some new compounds resulted from chemical combination of two drugs such as 5-fU and metronidazole was studied. Metronidazole is an nitroimidazole which is used as antimicrobial agent as same as its anticancer effect. 5-Fu is also prescribed for the treatment of gastrointestinal cancer. 4-AminoQuinaldine recently showed anti cancer effect in some cancer cell lines[1]. All of new compounds were prepared by nucleophilic attack of amine moieties of nitrofuran thiadiazole piperazine to mesylate or bromide leaving groups of metronidazole and chloride of 4-aminoquinaldine chloroacetyl chloride(schemes A, B and C) or by reaction of 5-FU with 4-aminoquinaldine chloroacetyl chloride and vice versa (Schemes D, E). These new synthesized compounds' anti cancer activity will be studied in the future.



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ANTIMALARIAL ACTIVITY AGAINST *P. FALCIPARUM* OF NOVEL ASYMMETRICAL BISCATIONIC CHOLINE KINASE INHIBITORS

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Malaria is a major health problem, with more than 40% of the world's population at risk, and is responsible for 200–300 million clinical cases yearly and nearly a million deaths. Choline kinase (CK) is the first enzyme in the Kennedy pathway for the biosynthesis of phosphatidylcholine, the most essential phospholipid in the malaria-causing parasite *P. falciparum* (1). The proliferation of this parasite within erythrocytes is concomitant with a massive increase of phosphatidylcholine biosynthesis. It has been demonstrated that the inhibition of *Pf*CK disrupts the Kennedy pathway which results in parasite death (2, 3). Here, we present a new series of asymmetrical bispyridinium compounds that has been evaluated as antimalarial agents against *P. falciparum* in infected erythrocytes. These molecules were firstly designed as human CK inhibitors and inhibit this enzyme very efficiently. The results show a very potent *in vitro* activity against *P. falciparum* with IC_{50} values in the very low nanomolar range, being compound **BR-25** the most active one (IC_{50}= 0.002µM). At present, biochemical studies are being performed in order to elucidate if the observed *in vitro* antimalarial activity is due to the inhibition of *Pf*CK.

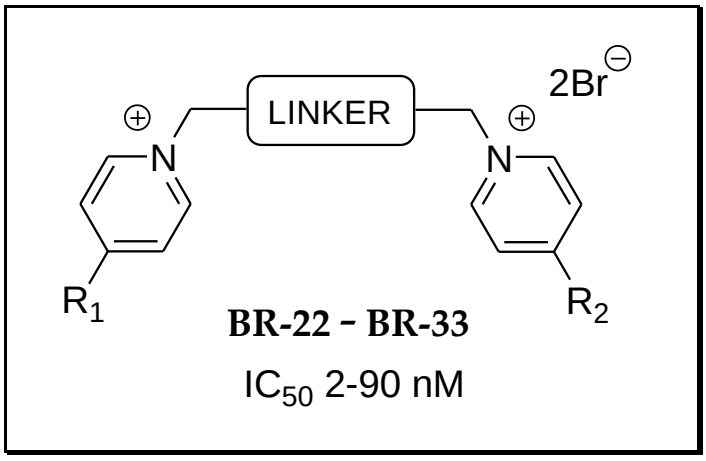

Figure 1. General structure of asymmetrical bispyridinium compounds.

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INHIBITION OF PROTEIN-PROTEIN INTERACTIONS IN TRYPANOTHIONE REDUCTASE (TryR) OF *LEISHMANIA INFANTUM*: AN INNOVATIVE STRATEGY

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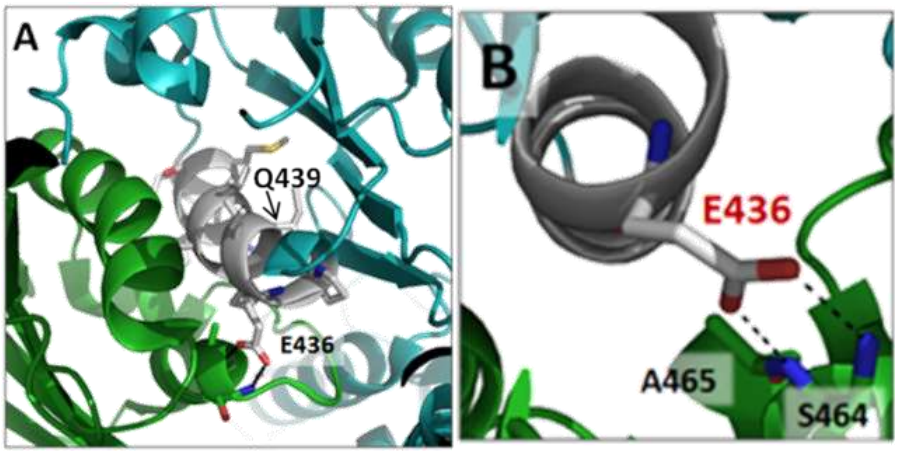
Leishmaniasis is a neglected tropical disease caused by *leishmania* parasites. According to the World Health Organization, there are 12 million people infected by this disease and further 350 million people are at risk of contracting this illness in more than 88 different countries (1). Historically, this illness was limited to underdeveloped countries but in recent years, due to the coinfection with HIV, this disease has extended to developed countries. However, only a limited and outdated drug arsenal for its treatment is available. Trypanothione Reductase (TryR) is a validated and attractive target in the search for drugs in leishmaniasis treatment. This enzyme is exclusive (does not exist in mammals) and is essential for parasites survival (2). Based on the fact that the biologically functional form of TryR of *Leishmania infantum* (Li-TryR) is a homodimer (3), we have devised a yet unexplored alternative strategy that attempts to interfere with the dimerization process of the enzyme. Molecular modeling studies and site-directed mutagenesis showed Glu436 and Gln439, contained within a α-helix in the dimerization domain of the enzyme, as two key residues (hot spots) (Figure). From a small library of peptides derived from this interfacial α-helix, linear peptide TRL14 significantly inhibits, both the activity and the dimerization of the enzyme in enzymatic assays (4).



We herein report the initial steps in the optimization process of prototype TRL14. In order to increase the chemical stability of prototype, Met residues were replaced by Nle. With the aim of studying the minimum length required for activity, a series of peptides truncated at the C-terminal end were prepared and evaluated. To stabilize the α-helical conformation, a second series of conformationally restricted peptides that incorporate a covalent amide bond between the side-chains of two residues of the sequence at different positions were investigated. The target peptides have been tested in both enzymatic and dimerization assays. Our results support that targeting the dimerization interface of Li-TryR by rationally designed peptides is a feasible goal.

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NOVEL PYRROLO[1,2-a]QUINOXALINE DERIVATIVES AS INHIBITORS OF PROTEIN TYROSINE PHOSPHATASE-1B

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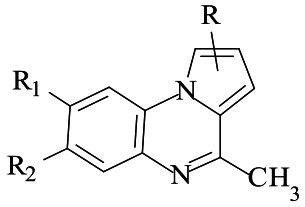
In this communication we report the preparation of novel compounds **1**, **2** and **3** which were obtained from nitroanilines.

Compounds **1**, **2** and **3** were tested as inhibitors of protein tyrosine phosphatase 1B (PTP-1B), which catalyzes protein tyrosine dephosphorylation. PTP-1B play essential roles in intracellular signal transduction by regulating the cellular level of tyrosine phosphorilation to control cell growth and differentiation, metabolism, cell migration, gene transcription, ion channel activity, the immune response, cell apoptosis and bone development (1, 2). Also, PTP-1B is involved in many human diseases, including cancer, diabetes and obesity. Small molecule inhibitors have been reported to show IC_{50} from low to high nanomolar (3).



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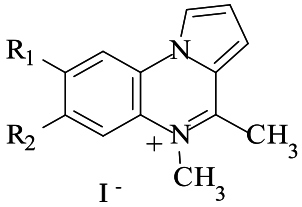

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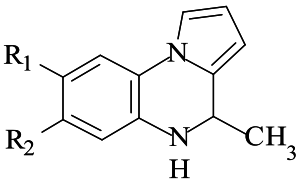

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P49

NEW MORE POLAR SYMMETRICAL CHOLINE KINASE INHIBITORS: STUDY OF SETTING UP A NEW SCAFFOLD FOR THE CANCER THERAPY

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Research into the anti-tumor properties of biscationic compounds has received significant attention over the last few years. In the challenge to improve modern cancer chemotherapy, the search of new drugs with higher therapeutic index and lower capacity to induce resistance is an active field of investigation in medicinal chemistry. As part of our drug research program in searching modified biscationic compounds that show strong growth inhibitory activities against a two cancer cell lines (1-4), we were interested in more polar biscationic compounds derivatives, which should constitute an important class of new compounds for their potential pharmaceutical applications.

A novel family of 1,2-bis(4(substituted)methyl)phenyloxy)ethane salts containing a pair of oxygen atoms in the spacer of the linker of the framework of the biscationic compounds, like hypothetical hydrogen bond acceptors with the enzyme choline kinase, were synthesized and evaluated for inhibitors of choline kinase and theirs antiproliferative activity.

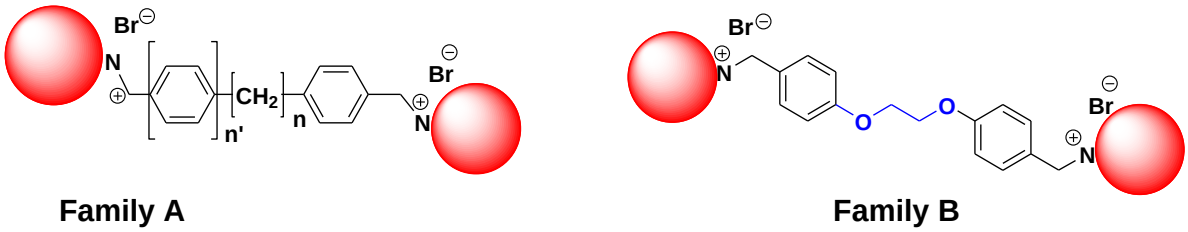

Fig. 1: General structures of symmetrical biscationic inhibitors of choline kinase Family A was previously published [1-3]. Family B are described in this work.

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P50

CRTh2 ANTAGONISTS WITH LONG RECEPTOR RESIDENCE TIMES

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The measurement of ligand binding potency is fundamental to Medicinal Chemistry (1). However, simple potency data gives no indication of the underlying kinetics of the binding and unbinding processes. Binding events can occur over seconds, minutes, hours or days, and these differences can give rise to both desirable and undesirable consequences (Fig 1) (2,3). The medicinal chemistry community is now embracing the phenomenon of residence time not only by unraveling the possible consequences of fast and slow kinetics, but by taking advantage of this extra dimension of ligand-target binding.

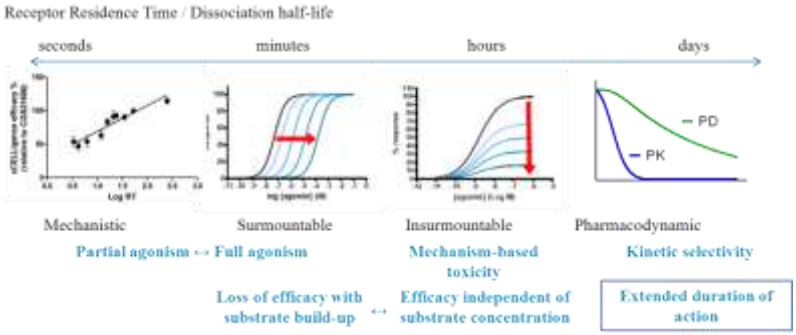

Fig 1. Possible consequences of short or long receptor

This poster outlines some of the results of our efforts to develop potent, orally bioavailable CRTh2 antagonists (Fig 2). In addition, we sought compounds with long receptor residence time to prolong the pharmacodynamic effect (4). In vitro and in vivo assays to assess compound residence time are also presented.

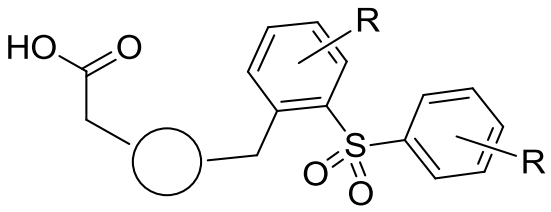

Fig 2. CRTh2 antagonist general structure. Dissociation half-lives from 0.1 - 23 h

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BIS-HUPRINES AS A NEW FAMILY OF TRYPANOCIDAL AGENTS

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The high toxicity and poor brain penetration of currently available treatments for human African trypanosomiasis, one of the major protozoan parasite diseases in developing countries, makes it necessary and urgent the development of more efficacious and safer drugs.
We have recently shown that huprines exhibit a potent activity against cultured bloodstream forms of *Trypanosoma brucei* and a few of them are also active against a chloroquine-resistant strain of *Plasmodium falciparum*, exhibiting IC_{50} values in the submicromolar to low micromolar range and brain permeability as well (1,2). However, the selectivity of these anti-protozoan activities over rat myoblast L6 cells was not completely satisfactory.
We report herein on the synthesis and evaluation of the trypanocidal activity and selectivity over L6 cells of a new series of homodimers of enantiopure (+)-(7*R*,11*R*)-huprine Y (Figure 1).

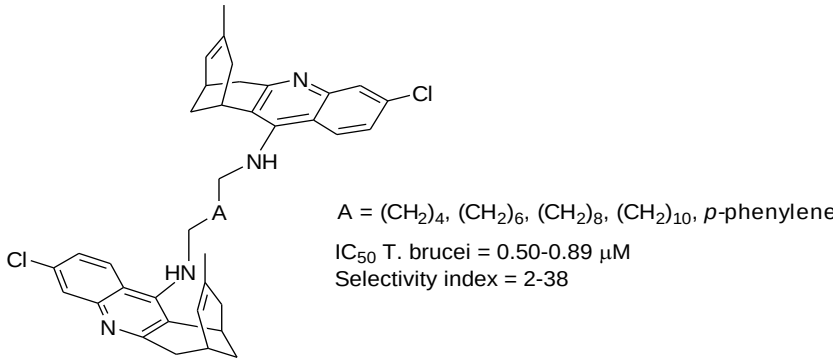

Fig. 1. Chemical structure and trypanocidal activity and selectivity of *bis*-(+)-huprine Y homodimers.

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SYNTHESIS AND BIOLOGICAL ACTIVITY OF A NEW GENERATION OF 4-AMINOSTYRYLQUINOLINE COMPOUNDS AGAINST LEISHMANIA

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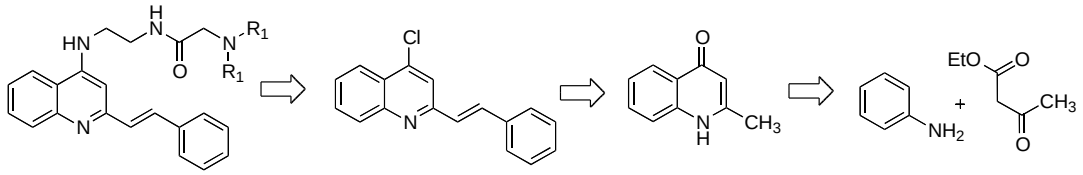
Leishmaniases are a complex group of protozoal diseases due to infestation by parasites of the genus *Leishmania*. Several species are responsible for multiple clinical forms, ranging from the cutaneous variant to the visceral leishmaniasis that can be lethal if not treated. Such maladies belong to the family of neglected tropical diseases that affect the less developed countries, for which no ideal therapeutic tools are available, since classical therapies are expensive, toxic, and require parenteral administration (1). In this scenario, we have embarked on a new drug discovery project devoted to the identification of novel small molecules for the treatment of leishmanial diseases. With this goal in mind, we noticed that several 2-substituted quinolines and styrylquinolines have been identified as effective against cutaneous and visceral leishmaniases (2). On the other hand, several anti-leishmanial compounds have amino or polyamino side chains that are considered critical for their biological activity (3). Taking into account the good pharmacokinetic properties and the high degree of drug-likeness of quinoline derivatives, we considered the combination of both fragments as potentially leading to a new privileged structure to combat protozoan diseases (4). Thus, a new library of 4-aminostyryquinoline derivatives was generated. The final products were achieved via the synthetic route outlined in Scheme 1, starting with the preparation of 2-methylquinolin-4-one from aniline and ethyl acetoacetate. This intermediate was transformed into 4-chlorostyrylquinoline in two steps, and this was followed by a nucleophilic aromatic substitution with the suitable amino chains. A preliminary *in vitro* evaluation of the anti-leishmanial activity of all the synthetized compounds revealed a good efficacy. Furthermore, styrylquinoline derivatives, thanks their native fluorescence, may be employed as fluorescent probes to better understand the leishmanicidal mechanism of action and validate the application of our molecules for diagnostic studies.



Scheme 1: Retrosynthetic route employed to obtain the 4-aminostyrylquinoline compounds

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NOVEL ANALOGUES OF MEMANTINE WITH POTENT ACTIVITY AS GLUTAMATE N-METHYL-D-ASPARTATE RECEPTOR ANTAGONISTS

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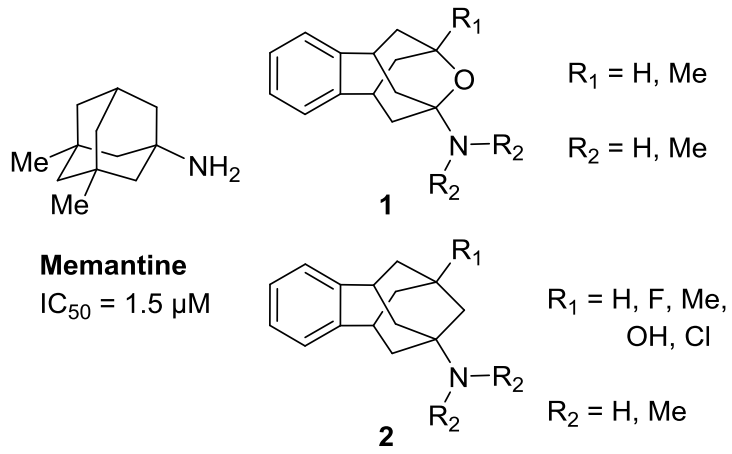
Alzheimer's disease (AD) is a neurodegenerative disorder that is increasingly becoming more problematic for the elderly. Unfortunately, current treatments neither prevent nor reduce the progression of the disease, so there is an urgent need of new drugs against this impairment (1). *N*-Methyl-D-aspartate (NMDA) receptor antagonists act by protecting neurons from excessive pathological calcium influx, which leads to neuronal damage and finally to neuronal cell death. So far, memantine is the only NMDA receptor antagonist that has been approved for the treatment of AD (Figure 1) (2).

In the last few years our research group has synthesized and carried out the pharmacological evaluation of a large variety of new memantine analogues. In this communication we will present the synthesis and pharmacological results of several novel derivatives with low micromolar IC_{50} as NMDA receptor antagonists. Some of them displayed IC_{50} values very similar to that of memantine (3). Worthy of note, all the novel compounds were synthesized using high-yield, very short synthetic sequences from commercially available starting materials.



Compound	R_1	R_2	IC_{50} (μM)
1a	Me	Me	3.9 ± 0.4
1b	H	Me	3.8 ± 0.4
2a	Me	Me	11.8 ± 0.4
2b	F	H	1.8 ± 0.3

Fig. 1: Structures of memantine, that of polycyclic scaffolds 1 and 2 and selected values of IC_{50} as NMDA receptor antagonists.

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P54

DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION OF CYCLIC ACYLGUANIDINES AS BACE-1 INHIBITORS: EFFECT OF RING SIZE ON ACTIVITY AND SELECTIVITY VERSUS BACE-2

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Alzheimer's disease (AD) is the most prevalent type of dementia, comprising an estimated 70% of all dementia cases, and affecting some 25 million people worldwide. The neuropathological hallmarks of the disease include extracellular amyloid β (Aβ) deposition in the form of plaques in brain parenchyma and intracellular neurofibrillary tangles (NFT). (1)

BACE-1 cleavage of β-amiloyd precursor protein (β-APP) is the rate-limiting step of Aβ peptide generation. BACE-1 has been claimed as the most promising therapeutic target for the treatment of AD. However, after more than 15 years of research BACE-1 has proven to be an exceptionally difficult target, where identifying small-molecule inhibitors which combine good pharmacological and pharmacokinetic properties remains a challenge. (2)

Current small-molecule inhibitors present some shortcomings, among which is combining low inhibitory activity with good selectivity over other aspartyl proteases such as BACE-2, pepsin, renin or cathepsin D.

In this work, we present our initial efforts towards the rational design, synthesis and biological evaluation of series of cyclic acylguanidines as potential BACE-1 inhibitors with improved selectivity over BACE-2 (Figure 1). Preliminary results on the influence of ring size in BACE-1 activity will be reported.

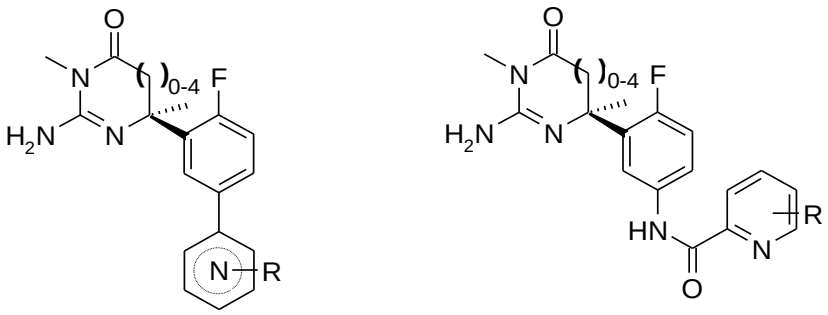

Fig. 1: General structure of the cyclic acylguanidines

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SYNTHESIS OF ENANTIOPURE CYCLIC 3-SUBSTITUTED BENZOFUSED-SULFINAMIDES AND SULFONAMIDES BY S_Hi AT THE SULFUR ATOM

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The sulfonamide group is one of the most important pharmacophores.[1] Likewise, cyclic sulfonamides[2] are useful heterocycles for medicinal chemistry.[3] A number of benzofused sultams have recently surfaced that display potent activity including anti-inflammatory (e.g., Ampiroxicam and S-2474),[4] antiepileptic agents[5] (e.g., sulthiame) and anti-HIV activity,[2] to name a few.

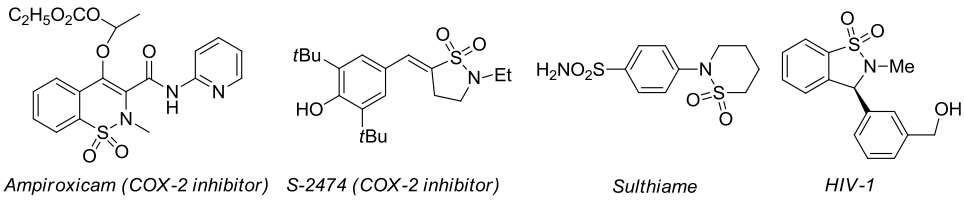
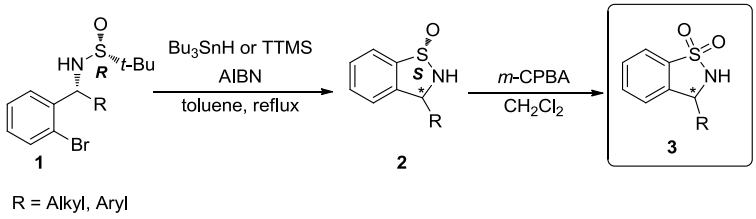


Fig. 1: Biologycally active sultams

In this communication, we report the synthesis of benzo-fused cyclic enantiomerically pure sulfinamides **2** differently substituted and functionalized mediated by an intramolecular homolytic substitution process at the sulfur atom starting from *orto*-bromobenzylsulfinamides **1.** When they were refluxed in toluene in the presence of AIBN and Bu_3SnH or TTMS, they evolved into sulfinamides **2** in good yield and complete stereocontrol.



Reaction of compound **2** with m-CPBA afforded chiral 3-substituted benzosultams differently substituted and functionalized in excellent yield and maintaining the enantioselectivity.

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TURNING THE BIOLOGICAL DISCOVERIES OF TODAY INTO THE MEDICINE OF TOMORROW

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The Drug Discovery Unit (DDU) is a fully operational, fully integrated drug discovery group working across multiple diseases based within a UK university. Our multi-disciplinary teams work closely together in an integrated management structure, operating more like a biotech company than a traditional academic group. Our initial remit was to develop new, more effective treatments for some of the world's most neglected diseases. Over the last seven years, we have extended, and continue to extend both our remit and our partnerships across the academic, industrial and charitable sectors worldwide. Today, we are focusing on tackling unmet medical need through small molecule drug discovery; collaborating with partners in Dundee and beyond to identify drug candidates, lead and hit compounds, potential new drug targets and innovative tools and approaches across a wide range of debilitating and deadly diseases, including more mainstream conditions such as cancer and diabetes.[1]

Our two areas of activity are diseases of the developing world and innovative targets and pathways. Within the diseases of the developing world area we are targeting five major diseases: human african trypanosomiasis (HAT),[2,3] Chagas disease, visceral leishmaniasis,[4] malaria and tuberculosis. Within the innovative targets and pathways area we are collaborating with world-class, life sciences researchers to develop new approaches to developed world diseases, including cancer, diabetes, and Alzheimer's diseases. Our goal is to; partially validate innovative drug targets, investigate disease pathways and identify lead compounds to develop our understanding of the underpinning biology and establish proof of concept in pre-clinical models of disease.

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N-ISOQUINOLIN-5-YL-1-NAPHTHALENESULFONAMIDE: A NEW AGENT WITH IN VIVO ACTIVITY AGAINST TRYPANOSOMA CRUZI

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Chagas disease, also known as American trypanosomiasis, is a potentially life-threatening illness caused by the protozoan parasite *Trypanosoma cruzi*. It is a widespread parasitic disease considered within the most relevant group of neglected tropical diseases. The World Health Organization has estimated about 8 million people to be infected with *Trypanosoma cruzi* worldwide, mostly in Latin America. Currently, two nitroheterocycle drugs, nifurtimox and benznidazol, are used to treat this disease. However, their use is problematic as both can cause side effects and have limited efficacy (1).

In a previous work we demonstrated the *in vitro* activity against *T. cruzi* epimastigotes of sulfonamides **1-5** (Fig. 1) (2). Herein our initial work has been extended to *in vitro* intracellular *T.cruzi* amastigotes and antichagasic *in vivo* studies as well as towards a new series of sulfonamide derivatives.

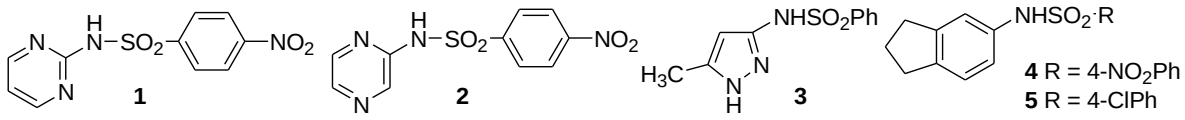

Fig. 1

Among the tested compounds, *N*-isoquinolin-5-yl-1-naphthalenesulfonamide **6** (Fig. 2) showed remarkable *in vitro* efficacy without citotoxicity against J774 macrophages and NCTC929 fibroblasts and *in vivo* activity in a murine model of acute Chagas disease (72± 20% reduction of parasitemia in BALB/c mice). Therefore sulfonamide **6** emerges as a promising lead compound for the development of an effective therapeutic treatment against *T. cruzi*.

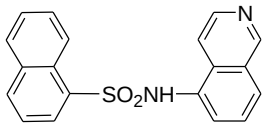

Fig. 2

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β-ANTITUBULIN STUDIES OF LEISHMANICIDAL SULFONAMIDES BY CONFOCAL MYCROSCOPY

M. E. González-Rosende[1,*], M. A. Dea-Ayuela[1,2], C. Galiana-Roselló[1,3], P. Bilbao-Ramos[2], F. Bolás-Fernández[2], E. García-España[3]

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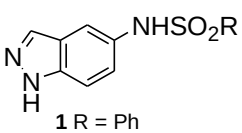
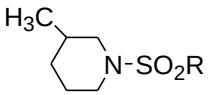
Microtubules are vital for cell shape, form, motility, growth differentiation and survival/infectivity of Kinetoplastid parasites. In this context, research has shown the antitubulin activity of sulfonamide-containing compounds (1).

In this communication, we present immunofluorescence studies with confocal microscopy, using specific antibodies against β-tubulin, in order to evaluate the effect of leishmanicidal sulfonamides **1** and **2,** on the tubulin distribution profile in *L. infantum* promastigotes.



1 R = Ph



2 R = Naphtyl

Confocal microscopic analysis clearly showed the effects of sulfonamides **1** and **2** on *L. infantum* promastigotes after *in vitro* incubation for 24 and 48 hours, in comparison to untreated controls. Whereas in untreated promastigotes, the anti-tubulin antibody is homogenously bound alongside cell cytoskelet, this pattern was visibly altered after 24h incubation with compound **2**. This disorganizing effect was even more dramatic after 48h, involving also the DNA, the kinetoplast DNA (kDNA) was missing and the nuclei appear to be undergoing apoptosis. Immunofluorescence images of *Leishmania* promastigotes treated with sulfonamide **1** showed a marked disorganizing effect on microtubules after 48h contact. However, in contrast to compound **2**, no evident changes on nuclear and kinetoplastid DNA were evidenced. Therefore, cytoskelet β-tubulin appears to be exclusively targeted by compound **1**, whereas neither nuclear nor kinetoplastid DNA seem to be altered for this compound. In conclusion we demonstrate the antinuclear and/or anti-tubulin effects on *L. infantum* promastigotes of compounds **1** and **2** by confocal microscopy analysis. Thus, our previous findings on the mode of action of leishmanicidal sulfonamides (2) have been extended by using alternative molecular tools and, at the same time, opening new windows towards the precise characterization of the mechanism of action of this class of compounds.

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SYNTHESIS AND IN VITRO EVALUATION OF LONG CHAIN DIAMINES AND AMINOALCOHOLS AGAINST T. CRUZI EPIMASTIGOTES

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Chagas' disease is a major public health problem in the Americas. Ten million people are infected in endemic areas of America and countries with Latin American immigration as United States of America, Europe, Japan and Australia (1). The drugs currently used against *T. cruzi* are the benznidazole and the nifurtimox but cause problems and toxicity affectivity (2). New drugs with fewer side-effects and increased trypanocidal activity are needed. Aliphatic aminoalcohols and diamines have shown good antiprotozoal activity (3-4).

The purpose of this study was to evaluate the *in vitro* activity of certain aminoalcohols and diamines with different chain length against two strains of *T. cruzi* that were isolated from Colombian patients: MG (MHOM/CO/04/MG) and JEM (MHOM/CO/05/JEM). Compounds cytotoxicity on mouse macrophage cells were also tested (5). Twenty five compounds were tested against epimastigotes of *T. cruzi* in final concentrations ranging 0.1, 0.5, 1, 5, 10 and 20 µg/mL. The trypanocidal activity was measured as percent of mortality after microscopic observation with vital trypan blue dye and IC_{50} was determined (6). Three diamines and 3 aminoalcohols were more effective than nifurtimox against MG strain with IC_{50} between 2.84 and 50.5 µM. With respect to the strain JEM the most effective compounds were one aminoalcohol and two diamine with IC_{50} between 2.84 and 50.5 µM. The most active compound was 4.5 times more potent than nifurtimox and 4.9 than benznidazol with low macrophage toxicity (6), selectivity index parasite/macrophage of 20. Compounds with higher chain length were more potent against epimastigotes of *T. cruzi* and could be tested *in vivo*.

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