SIMCC 2015

SPANISH-ITALIAN MEDICINAL CHEMISTRY CONGRESS

BARCELONA, SPAIN - JULY 12-15

BARCELONA BIOMEDICAL RESEARCH PARK (PRBB)

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On behalf of the Organising Committee and of the Sociedad Española de Química Terapéutica and the Divisione di Chimica Farmaceutica della Società Chimica Italiana, it is a pleasure to invite you to participate in the "Spanish-Italian Medicinal Chemistry Congress" (SIMCC-2015) that will take place in the very attractive setting of the Barcelona Biomedical Research Park (PRBB), on July 12-15, 2015. Both societies are members of EFMC, the main organisation for the European Medicinal Chemistry community.

In the frame of the close relationship between both Societies, this Congress will give the opportunity to delegates to strengthen scientific ties and develop common interests and joint collaborative efforts. Of course, we hope these goals will involve not only Spanish or Italian participants but also scientists from other countries active in different areas of the Medicinal Chemistry field.

We are working to set up an attractive scientific program that offers a broad outlook into the Medicinal Chemistry world. This is particularly important for young people attending the Congress, in order to expand their scope about the future trends of their professional career. Thus, in addition to updates on choice topics and future trends in Medicinal Chemistry, there will be keynote lectures, invited speaker presentations, short oral communications and poster exhibits on drug discovery advances in selected therapeutic areas. The program will also cover recent progress in lead identification and optimization, drug design and profiling technologies, as well as highlight current challenges in the interface between chemistry and biology.

We look forward to seeing you in Barcelona.

Yours faithfully,

Ángel Messeguer
Congress Co-Chairman

Gabriele Costantino
Congress Co-Chairman

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SCIENTIFIC PROGRAMME

SUNDAY, 12 JULY 2015

Barcelona Biomedical Research Park (PRBB) Auditorium

15:00 **REGISTRATION**

17:00 **OPENING CEREMONY**
Chairs: Antoni Torrens and **Girolamo Cirrincione**

17:30 **SOCIETIES AWARDS**

17:45 **SEQT Award Communication**

Synthesis and biological evaluation of novel chalcones as antimitotics and vascular disrupting agent
Oskia Bueno, *Instituto de Química Médica (CSIC). Madrid, Spain*

18:00 **DCF Award Communications**

New challenges in drug discovery: targeting protein-protein interactions
Francesca Morreale, *Università di Messina, Italy*

Design and synthesis of novel heterocyclic compounds as receptor or protein/protein interaction modulators
Elisabetta Barresi, *Università di Pisa, Italy*

18:30 **PLENARY LECTURE**

Characterization of a new peptide exhibiting dopamine effects by targeting brain angiotensin converting enzyme
Jean Martínez, *University of Montpellier, France*

19:15 **WELCOME COCKTAIL**

MONDAY, 13 JULY 2015

Barcelona Biomedical Research Park (PRBB) Auditorium

SESSION 1

Chairs: Angel Messeguer and **Paolo Caliceti**

09:00 PLENARY LECTURE

Medicinal Chemistry, Quo Vadis? – A personal view backwards on successful drug discoveries within the changing climate of Pharmaceutical R&D
Helmut Buschmann, *Research, Development & Consulting, Aachen, Germany*

09:45 **New indole tubulin assembly inhibitors with stable arrest of mitotic progression, enhanced stimulation of natural killer cell cytotoxic activity and repression of Hedgehog-dependent cancer**
Giuseppe La Regina, *Sapienza Università di Roma, Italy*

10:05 **Challenges in the design of sigma-1 receptor antagonists for the treatment of pain**
Carmen Almansa, Esteve, *Parc Cientific Barcelona, Spain*

10:25 **A green synthesis of vidarabine 5'-monophosphate *via* a one-pot multi-enzymatic reaction catalyzed by immobilized biocatalysts**
Daniela Ubiali, *Università degli Studi di Pavia, Italy*

10:45 COFFEE BREAK

SESSION 2

Chairs: José María Cid and **Francesco Peri**

11:15 KEYNOTE LECTURE

Privileged structures in Medicinal Chemistry: under-exploited opportunities for target families
Gerhard Müller, *Mercachem, Nijmegen, The Netherlands*

11:45 **New inhibitors of angiogenesis with antitumor activity in vivo**
Nagore I. Marín Ramos, *Complutense University of Madrid, Spain*

12:05 **Synthesis and biological activity of a new series of nortopsentin analogues**
Barbara Parrino, *Università degli Studi di Palermo, Italy*

12:25 **Targeting triple negative breast cancer through CB_2 cannabinoid receptor activation: from synthesis to in vivo studies**
Nadine Jagerovic, *Instituto de Química Médica - CSIC, Madrid, Spain*

12:45 **A new application of click chemistry in Drug Discovery: the identification of store-operated calcium entry modulators**
Tracey Pirali, *Università del Piemonte Orientale, Novara, Italy*

13:05 **LUNCH**

14:15 **POSTERS**

SESSION 3

Chairs: Ersilia De Lorenzi and **Jordi Quintana**

15:00 **PLENARY LECTURE**
DNA-Directed strategies for multiplexed diagnostic platforms
Pilar Marco, *Institute for Advanced Chemistry of Catalonia - CSIC, Barcelona, Spain*

15:45 **KEYNOTE LECTURE**
Analytical challenges in development of supramolecular multifunctional drug delivery systems
Paolo Caliceti, *University of Padova, Italy*

16:15 **COFFEE BREAK**

16:30 **KEYNOTE LECTURE**
Analytical approaches in Alzheimer's disease Drug Discovery
Vincenza Andrisano, *University of Bologna, Italy*

17:00 **Novel purinone derivatives as JAK inhibitors for the inhaled treatment of respiratory diseases**
Jordi Bach, *Almirall S. A., Barcelona, Spain*

17:20 **Vinyl sulfones covalently react with serum albumin. A potential selectivity issue for the development of irreversible cysteine protease inhibitors**
Luca Regazzoni, *Universitá degli Studi di Milano, Italy*

17:40 **KEYNOTE LECTURE**
Metabolomics: a tool for drug discovery and personalized medicine
Coral Barbas, *University San Pablo-CEU, Madrid, Spain*

TUESDAY, 14 JULY 2015

Barcelona Biomedical Research Park (PRBB) Auditorium

SESSION 4

Chairs: David Andreu and **Vincenza Andrisano**

09:00 **PLENARY LECTURE**

The importance of understanding attrition to drive lead optimization strategies towards the selection of higher quality clinical candidates
Stefano Fontana, *DMPK at Aptuit, Verona, Italy*

09:45 **Discovery of new antibiotics targeting shikimate kinase: from the reaction mechanism to the structure-based design of inhibitors**
Veronica Prado, *CIQUS, Universidad de Santiago de Compostela, Spain*

10:05 **Ion pairing to enhance antibiotic drug efficacy. A new approach to fight problematic infections?**
Stefano Giovagnoli, *University of Perugia, Italy*

10:25 **Assembling new chemical boxes as an open source of starting points for drug discovery against three kinetoplastid parasites causing Neglected Tropical Diseases**
Pilar Manzano, *GSK Kinetoplastids, Tres Cantos, Spain*

10:45 **COFFEE BREAK**

SESSION 5

Chairs: Coral Barbas and **Antimo Gioiello**

11:15 **KEYNOTE LECTURE**

Toll-like Receptor 4 modulation by small organic molecules: new therapeutic opportunities
Francesco Peri, *University of Milano, Italy*

11:45 **Novel positive allosteric modulators of the metabotropic Glutamate Receptor 5 as potential antipsychotic agents**
José Bartolomé Nebreda, *Neuroscience Medicinal Chemistry, Janssen Research and Development, Toledo, Spain*

12:05 **Combining flow chemistry with multicomponent Povarov reaction: stereoselective synthesis and characterization of tricyclic tetrahydroquinolines**
Bruno Cerra, *University of Perugia, Italy*

12:25 **A drug-like photoswitchable GPCR allosteric ligand with light-dependent control of animal motility**
Amadeu Llebaria, *Institute for Advanced Chemistry of Catalonia - CSIC, Barcelona, Spain*

12:45 **In vivo pharmacokinetic study and CNS distribution of the sigma1 receptor agonist (R)-RC-33, a promising neuroprotective agent**
Annamaria Marra, *University of Pavia, Italy*

13:05 **LUNCH**

14:15 **POSTERS**

SESSION 6

Chairs: Pilar Goya and **Gerhard Müller**

15:00 **PLENARY LECTURE**
Pd, Cr and Ag in C-H activation: reactivity and selectivity control in the synthesis of biaryls
Igor Larrosa, *University of Manchester, United Kingdom*

15:45 **KEYNOTE LECTURE**
Thermodynamics and kinetics of drug-target binding through molecular simulations
Andrea Cavalli, *University of Bologna and Italian Institute of Technology, Italy*

16:15 **COFFEE BREAK**

16:30 **KEYNOTE LECTURE**
Controlling DNA recognition with external inputs
Eugenio Vázquez, *University of Santiago de Compostela, Spain*

SESSION 7

Chairs: Angel Messeguer and **Gabriele Costantino**

17:00 **ROUND TABLE. The Medicinal Chemist: future career avenues**
Speakers:
- Sandro Cosconati, *Univesity of Napoli, Italy*
- Albert Palomer, *Abac Therapeutics, Esplugues de Llobregat, Spain*
- Ferran Sanz, *Universitat Pompeu Fabra, Barcelona, Spain*
- Mario Varasi, *IEO-European Institute of Oncology, Milano, Italy*

18:00 **SEQT General Meeting**

21:00 **GALA DINNER**

WEDNESDAY, 15 JULY 2015

Barcelona Biomedical Research Park (PRBB) Auditorium

SESSION 8

Chairs: Antonio Pineda-Lucena and **Andrea Cavalli**

09:00 **PLENARY LECTURE**

Polyglutamates as versatile Drug Delivery carriers
María Jesús Vicent, *Centro de Investigación Príncipe Felipe, Valencia, Spain*

09:45 **Combining simple chemistry and in silico approaches for the rational design of carnosine analogues as bioavailable and effective carbonyl quenchers**
Giulio Vistoli, *University of Milan, Italy*

10:05 **Development of palladium-labile prodrugs for bioorthogonally-activated chemotherapy**
Belén Rubio-Ruiz, *University of Edinburgh, United Kingdom*

10:25 **Development of novel in vivo active chemical chaperones as a potential anti-ALS (amyotrophic lateral sclerosis) drugs**
Arie Gruzman, *Bar-Ilan University, Ramat Gan Israel*

10:45 **COFFEE BREAK**

SESSION 9

Chairs: Gabriele Costantino and **Ana Castro**

11:15 **KEYNOTE LECTURE**

Small molecules useful for intervention against emerging viruses: triazolopyrimidines and chikungunya virus as an example
María-Jesús Pérez-Pérez, *Instituto de Química Médica-CSIC, Madrid, Spain*

11:45 **Discovery of the first potent and systemically active Inhibitors of acid ceramidase**
Daniela Pizzirani's, *Drug Discovery and Development, Istituto Italiano di Tecnologia, Genova, Italy*

12:05 **Small molecule inhibition of the KRAS-PDEδ interaction impairs oncogenic K-RAS signaling**
Gemma Triola, *Institute for Advanced Chemistry of Catalonia - CSIC, Barcelona, Spain*

12:25 **Identification and biological characterization of novel inhibitors of KDM1A**
Paola Vianello, *IEO - European Institute of Oncology, Milano, Italy*

12:45 **Design and synthesis of dual inhibitors targeting MMP2 and CK2: a new approach to antitumor compounds**
Miryam Pastor, *Universidad CEU San Pablo, Madrid, Spain*

13:05 **PLENARY LECTURE**

A Medicinal Chemist journey in the Drug Discovery eco-system
Roberto Pellicciari, *TES Pharma, Perugia, Italy*

13:50 **POSTERS PRIZES AND FINAL REMARKS**

PLENARY LECTURES

Characterization of a new peptide exhibiting dopamine effects by targetting brain angiotensin converting enzyme

Jean Martinez

Institut des Biomolécules Max Mousseron UMR 5247, Université de Montpellier, CNRS, ENSCM, Faculté de Pharmacie, 15 Av. C. Flahault, BP 14491, 34093 Montpellier, France

Angiotensin-Converting Enzyme (ACE) is a well-known enzyme involved in the regulation of blood pressure. There is increasing evidence that brain ACE is a major actor in neurodegenerative pathologies through its peptidase activity that results in the production of angiotensin derivatives. We have discovered and characterized a new nonapeptide named "Acein", which, when injected into the brain of rodents, exhibits dopamine-like effects. This peptide is able to bind to a single class of binding sites with a high affinity in the nanomolar range, specifically localized in the Striatum and Substantia Nigra of rodent brains. Cross-linking experiments showed that the peptide was able to bind a glycosylated protein of about 160 Kda. Using a photoactivable derivative, the striatal protein target of JMV3042 was characterized by a photoaffinity UV cross-linking approach combined with subsequent affinity purification of the ligand covalently bound to its receptor. We succeeded to purify a ~151 kDa protein that was identified by MS/MS as Angiotensin Converting Enzyme (ACE). The activity of JMV3042 on ACE bound membranes in the brain is promising for further investigation of the peptide-dopaminergic system interactions and associated pathologies. This new peptide and its target opens the way to the study of an original dopamine release and regulation pathway, and on the pathologies associated with the deregulation of dopamine balance in the Central Nervous System. Further investigations should focus on the neuroprotective capacity of the action of this peptide and its analogues on brain membrane bound ACE.

PL 02

Medicinal Chemistry, Quo Vadis? - A personal view backwards on successful drug discoveries within the changing climate of Pharmaceutical R&D

Helmut Buschmann
Research, Development & Consulting GmbH (RD&C)

Today's innovative drug discovery is costly and time consuming, with very few novel therapeutics making it to the market place. The enterprise of drug discovery and development is fundamentally shifting in the last decade. Dramatic and irreversible changes are reshaping the roles of the pharmaceutical, biotechnology and academic areas and consequently also the role medicinal chemistry.

Over the past two decades, scientific and business needs have driven the pharmaceutical industry to more closely align drug discovery and drug development efforts. The general view is that the process whereby future drugs are discovered and developed will be fundamentally different to how these activities were performed in the past.

Traditional medicinal chemistry approaches adopted during the 1970s and 1980s were focused primarily on analoguing of endogenous ligands and industry leads, moving from in vivo models to a single target selective drug for a single mechanism. Following the lock and key model proposed by Ehrlich more than a century ago, over the previous decades, drug discovery efforts have focused on identifying single selective drugs that target a single mechanism; that is, identifying ligands ('keys') that fit into specific targets ('locks'). Achieving target specificity of active compounds has, for at least three decades, been considered a hallmark of drug discovery efforts, as a consequence of an increasingly sophisticated molecular approach to discovery, primarily focusing on isolated targets and specific binding assays.

A personal view backwards based on successful drug discoveries will be presented within the changing climate of pharmaceutical research and development strategies and technologies.

DNA-Directed Strategies for multiplexed diagnostic platforms

M. Pilar Marco

Nanobiotechnology for Diagnostics (Nb4D) group, IQAC-CSIC, Barcelona, Spain CIBER de Bioingeniería, Biomateriales y Nanomedicina (CIBER-BBN), Barcelona, Spain pilar.marco@cid.csic.es

Microarray technologies combined with optical detection has been crucial to reach the actual status of knowledge in genomics and proteomics. *Protein microarrays* are key tools for investigation of protein expression and drug development, allowing the analysis of the interactions of important pharmacological targets with proteins. Moreover, the advances in genomics and proteomics, point to a future in which clinical diagnostic will be based on molecular signatures characteristic of the health/disease status of the individuals, for which simultaneous detection of multiple biomarkers will be required. In this respect, *protein microarrays* represent a big challenge in diagnostics. However, protein microarray technology is not as straightforward as DNA technology due to the molecular variability and complex nature of proteins (different hydrophobicities, acidic or basic characters, functionality, etc.). Unlike nucleic acids, which are relatively homogeneous in terms of structural and electrostatic properties, proteins can be extremely diverse regarding chemical structure and biological properties. Preventing protein denaturation and maintaining structural conformations are key issues in microarray technology (see reference [1] for a recent review on immobilization strategies). An alternative to circumvent some of the limitations of the protein microarray technology consists on the use of oligonucleotide probes with well-known sequences and their subsequent hybridization with their complementary oligonucleotides previously immobilized on the surface. This strategy, known as DNA-Directed Immobilization (DDI), has been used to spatially assemble mixtures of molecular components, such as nanoparticles, proteins and polypeptides[2]. It not only provides greater immobilization efficiency than conventional adsorption techniques, but also allows reversible immobilization of biomolecules allowing development of reusable microarrays and biosensor chips. In combination with antibodies, DDI also provide a useful strategy to construct *immunochemical microarrays*, expanding the number of substances that can be analyzed considering the wide variety of selectivities provided by the antibodies and their exceptional features as natural bioreceptors[3]. In this communication we will present the work performed in our group in the direction to develop microarray optical diagnostic platforms. Thus, DDI strategies have been used to develop fluorescence site-encoded DNA addressable hapten-microarray platforms[4]. The same strategy has been used for the development of multiplexed SPR immunosensors. In the same direction, noble metal nanostructured surfaces for LSPR sensing can also be prepared using the self-organizing capabilities of the DNA hybridization. This strategy has been used for site-directed immobilization of distinct gold nanoparticles on glass substrates and their subsequent use for sensing.

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PL 04

The importance of understanding attrition to drive lead optimization strategies towards the selection of higher quality clinical candidates

Simone Braggio, Stefano Fontana

Drug Design & Discovery Aptuit Verona Via A. Fleming, 4 37135 Verona Italy

Drug discovery is a high-risk endeavour. The number of approvals for new drugs has fallen steadily in recent years, despite increasing R&D expenditure. Cost effective and innovative approaches to drug discovery and development have therefore become particularly important to ensure shareholder value. Improvements to the lead generation and optimization process aimed to improve the quality of the candidates selected for development are key initiatives for companies aiming to avoid expensive compound failures in the latter stages of the drug discovery process. Lead optimization aims at enhancing the most promising compounds to improve effectiveness, diminish toxicity, or increase absorption. While the approaches taken may vary, the central theme is the same: make it better, faster, and more efficient.

How companies direct their early phases of lead optimization may spell the difference between success or failure; nevertheless although all the investments, strategies and discussions, the fact remains that R&D productivity has not improved over the ensuing years.

The purpose of this talk is to direct our attention to the processes we follow in drug discovery, and explore the implications of a focus on quality. The aim is to stimulate thinking about how such a focus could present opportunities to improve the overall productivity of pharmaceutical research, to ultimately change how we approach drug discovery.

Quality by design in lead optimization: a new strategy to address productivity in drug discovery.
Curr Opin Pharmacol. 2011 Oct;11(5):515-20
Rossi T1, Braggio S.

Probing the links between in vitro potency, ADMET and physicochemical parameters.
Nat Rev Drug Discov. 2011 Mar;10(3):197-208
Gleeson MP1, Hersey A, Montanari D, Overington J.

Drug efficiency: a new concept to guide lead optimization programs towards the selection of better clinical candidates.
Expert Opin Drug Discov. 2010 Jul;5(7):609-18.
Braggio S1, Montanari D, Rossi T, Ratti E.

Pd, Cr and Ag in C-H activation: reactivity and selectivity control in the synthesis of biaryls

Igor Larrosa
University of Manchester School of Chemistry, Oxford Road, Manchester, M13 9PL Igor.Larrosa@manchester.ac.uk

The development of greener and more efficient synthetic methodologies is essential for organic chemistry to reach its full potential in its application to many applied and fundamental scientific problems. Biaryls are structural motifs predominant in numerous pharmaceuticals, agrochemicals, chiral catalysts, liquid crystal displays, and even molecular switches and motors. The most common methodology for their synthesis involves the traditional cross-coupling between an organometallic compound, Ar-M, and a haloarene, Ar-X. In the last few years, two promising alternatives to these cross-couplings *have emerged: direct C–H arylation*, where a readily available Ar-H is coupled with Ar-X, and *oxidative double C–H activation*, where two different Ar-H are cross-coupled. These approaches use non-prefuntionalized starting materials, thus eliminating several synthetic steps and consequent chemical waste associated to traditional cross-couplings. However, several challenges have to be resolved before this new approaches can be widely applied: 1) the development of mild reaction conditions with a broad scope, 2) the control of the regioselectivity of C–H activation and, in the case of oxidative couplings, 3) the control of the selectivity of homo- versus cross-coupling, and 4) the development of conditions that can be safely used in industry.

In this talk I will present some of our group's approaches towards addressing these challenges. In particular, we will discuss bimetallic synergistic systems utilizing Pd/Ag, Pd/Cr and Au/Ag based methodologies that allow enhanced selectivity control and reactivity.

For some representative publications, see:

1. Carlos Arroniz, J. Gabriel Denis, Alan Ironmonger, Gerasimos Rassias, Igor Larrosa **An Organic Cation as a Silver(I) Analogue for the Arylation of sp^2 and sp^3 C–H Bonds with Iodoarenes** *Chem. Sci.* **2014**, *5*, 3509-3514.
2. Junfei Luo, Sara Preciado, Igor Larrosa **Overriding ortho-para Selectivity Via a Traceless Directing Group Relay Strategy: the meta-Selective Arylation of Phenols** *J. Am. Chem. Soc.* **2014**, *136*, 4109-12.
3. Paolo Ricci, Katrina Krämer, Xacobe C. Cambeiro, Igor Larrosa **Arene-Metal π-Complexation as a Traceless Reactivity Enhancer for C–H Arylation** *J. Am. Chem. Soc.* **2013**, 135, 13258-61.
4. Xacobe C. Cambeiro, Tanya C. Boorman, Pengfei Lu, Igor Larrosa **Redox-Controlled Selectivity of C-H Activation in Oxidative Cross-Coupling of Arenes** *Angew. Chem. Int. Ed.* **2013**, *52*, 1781-4. (*Hot Paper*)
5. Josep Cornella, Marika Righi, Igor Larrosa **Carboxylic acids as traceless directing groups for formal** meta-**selective direct arylation** *Angew. Chem. Int. Ed.* **2011**, *50*, 9429-32.
6. Pengfei Lu, Tanya C. Boorman, Alexandra M. Z. Slawin, Igor Larrosa **Gold(I)-mediated C-H activation of arenes** *J. Am. Chem. Soc.* **2010**, *132*, 5580-1.
7. Nathalie Lebrasseur, Igor Larrosa **Room temperature and phosphine free palladium catalyzed direct C-2 arylation of indoles** *J. Am. Chem. Soc.* **2008**, *130*, 2926-7.

PL 06

Polyglutamates as versatile Drug Delivery carriers

María Jesús Vicent

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Polyglutamates are highly biocompatible, biodegradable and multifunctional polymers, which have been effectively used as building blocks in polymer drug conjugates and polymeric micelles for various medical applications ranging from cancer[1] to ischemic processes.[2] Moreover, it is expected its approval by regulatory agencies after approval of PGA-paclitaxel conjugate, Opaxio™ for the treatment of various cancers alone or in combination.[3] In addition, polypeptides are envisaged to achieve a major impact on a number of different relevant areas within biomedicine and biotechnology.[4] Acquired knowledge and the increasing interest on amino acids, peptides and proteins is establishing a large panel of these biopolymers whose physical, chemical and biological properties are ruled by their controlled sequences and composition. The development of new and more defined architectures with higher *M*w (to enhance passive targeting by the EPR effect), predictable structure and conformation, lower heterogeneity, higher drug loading capacity and greater possibility for multivalency are main research lines in polymer therapeutics in particular, and in nanomedicine in general.[1,5] Control on polymer chain length and stereochemistry have been one of the major challenges in synthetic approaches over the past years.

Recently in our group, we have demonstrated how to overcome those limitations with precise controlled reactions followed by an adequate characterization yielding to well-defined polypeptidic architectures by NCA polymerization techniques.[6] Furthermore, a number of architectures based on PGA, including stars, grafts, and hybrid diblock copolymers have been designed. In addition, a variety of functionalities such as alkyne, azides, reactive disulphides, protected amines… can be easily introduced by "post-polymerization modification" reactions yielding a set of orthogonal reactive attachment sides[6] suitable for further bioconjugations. In addition, this strategy has been efficiently applied in the synthesis of star-based polypeptide architectures with capacity of self-assembling to yield supramolecular architectures with interesting properties.[7] Those constructs have been used as drug delivery systems/ imaging probes as single agents and in combination therapy with application in cancer[8] and in neurodegenerative disorders.[9]

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A Medicinal Chemist journey in the Drug Discovery eco-system

Roberto Pellicciari

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The role of the medicinal chemist has changed through the years, both in Industry and Academia, in order to adjust to the constantly changing and rapidly evolving drug discovery scenario. While, however, the Industrial medicinal chemist is normally in a team as part of an interdisciplinary matrix system, their Academic counterparts generally operate within highly focussed research groups specialising on one research area that tend to operate independently from each other, thus lacking the cross-discipline integration that is vital for innovation in the drug discovery and development process. In order to overcome this problem, my group has in the past collaborated with both big Pharma and Biotech companies to also provide this collaborative environment in an Academic group. More recently, we have decided to explore a third way with the creation of an innovative start-up, TES Pharma, aimed at converting in-depth Academic innovation into novel approaches against key targets in metabolic diseases, aging and oncology. In this presentation I will give a few examples as illustrative points. Amongst which, I will highlight a project that led to the discovery of Obeticholic Acid (OCA), a first-in-class FXR agonist in the final clinical stage for primary biliary cirrhosis (PBC) and which has recently has received "breakthrough therapy designation" from the U.S. Food and Drug Administration (FDA) for the treatment of patients with nonalcoholic steatohepatitis (NASH) with liver fibrosis.

KEYNOTE LECTURES

Privileged structures in Medicinal Chemistry: under-exploited opportunities for target families

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The majority of today's therapeutically relevant protein targets cluster into multi-member gene families which exhibit structural and functional commonalities in their ligand or substrate recognition principles across the families. A detailed understanding of the family-wide commonalities in molecular recognition features offers an unprecedented opportunity to enhance medicinal chemistry productivity in that novel privileged structures that are complementary to those family-wide shared features can be generated.

In this context, we have focused our interest on the multi-member target class of G protein-coupled receptors by application of latest cheminformatics approaches that guided the search into new chemical space while maintaining close proximity to bioactive compound space. Details on the European Lead Factory-embedded scaffold selection and validation will be given, highlighting the novelty aspect of the underlying chemotypes.

In addition, novel privileged structures for protein kinases and histone deacetylases will be introduced that allow for pre-engineering of long residence time into the final inhibitor candidates. In the case of kinases, the design focuses on disrupting the hydrophobic spine, a key element that is highly conserved throughout the entire target family and as such qualifies to inform the design process of privileged structures. In the area of histone deacetylases, the existence of a transient product release channel serves as blueprint for the generation of HDAC-directed privileged scaffolds that exhibit front-loaded binding kinetic signatures.

The advantages of pre-engineering binding kinetic features into final inhibitor candidates at the stage of the privileged scaffold are manifold, since increased cellular and in-vivo efficacy, a better therapeutic index by applying lower doses, and increased selectivity can be achieved.

KL 02

Analytical challenges in development of supramolecular multifunctional drug delivery systems

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Over the past years, multifunctional nanotechnology has emerged as an advanced approach to create nanopharmaceuticals with optimized therapeutic performance. As a result, last generation delivery systems capable of complex functions, which enable sequential overcoming of multiple biobarriers following a certain time/site determined "logic" of events, have been developed. These nanocarriers can provide long drug circulation, high tolerability, and site specific delivery; factors that result in better patient outcomes. Cancer represents the field of medicine application to which multifunctional nanotechnology made the most prominent contributions. Nevertheless, other fields of therapeutic applications have been benefited of this novel formulation approach.

The development of novel performing nanopharmaceuticals that sequentially behave responding to specific environmental conditions, requires the use of a variety of chemical functions chemically or physically assembled into smart nano-objects. Targeting agents, cell penetrating enhancers, physicochemical modifiers, stealthing materials are combined with an inert platform acting as anchoring structure for the other moieties. Natural and synthetic polymers, as well as inorganic nanoparticles, are landmark materials for production of smart nanomedicines. Multivalent, amphiphilic and stimuli responsive polymers have been exploited to bestow drug bioconjugates and self-assembling colloidal systems with peculiar physicochemical and biopharmaceutical properties.

The pharmaceutical performance of nanocarriers depends on the physicochemical, biopharmaceutical and biological properties of the single functional and structural components as well as on their molar content in the supramolecular system. Therefore, a rational scientifically based design of the multifunctional nanocarriers must be performed in order to avoid random try-and-fail ineffective approaches. Actually, there is an unmet need of in silico platforms that can be properly applied to the design of multifunctional supramolecular systems.

Analytical approaches in Alzheimer's disease Drug Discovery

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The research efforts in drug discovery field are based on the knowledge of the molecular aspects of the disease and on the development of new techniques necessary to investigate the biological systems at molecular level. The selection of new leads is therefore a challenging task and involve various essential steps, the first being the identification/validation of new targets, then the selection of molecules able to bind to the target(s), and finally the study of the effects of hitting the target at molecular, cellular and whole animal level. In the case of Alzheimer's disease (AD), the most common form of dementia in adults, acetylcholinesterase (AChE) has been the first target for the development of new drugs since the discovery of the cholinergic deficit in the central nervous system. However, basic research showed that cognitive impairment could be due not only to a cholinergic deficit but also to a cascade of biochemical events leading to the accumulation in the brain of proteins such as ß-amyloid (Ab) and hyper-phosphorylated tau protein. Important targets are amyloid fibrillogenesis, beta-secretase (BACE1), one of the enzymes which cleave APP (amyloid precursor protein) and GSK3b, a tau protein phosphorylating kinase. On the other hand, other non cholinergic role of AChE in the AD has been discovered: some evidences suggest that AChE peripheral binding site may play a key role in the development of senile plaques, accelerating Ab deposition. Once the disease targets have been selected, the determination of the activity of the new compounds must be carried out quickly and in a way that allows the verification of the design hypothesis. Drug activity is in fact mediated by different types of interactions with specific biological targets and the esteem of these interactions may elucidate the mechanism of action. To this aim, in a first instance, high throughput screening methods (HTS) of a large number of compounds for the selection of few lead compounds are required. Secondly, specific methods, which elucidate the selected compound mechanism of action, must be employed, before the ultimate and most advanced tools, transgenic animal models of the disease, can be used to study the effects of single compounds on the disease phenotype.

Here we report the development of purposely designed integrated methodologies to define the multifunctional activity profile for new AD drug discovery. With regard to the assessment of the activity of chemical libraries, the affinity chromatography on HPLC immobilized-enzyme column (or immobilized enzyme reactors, IMER) is one of most promising methodologies for HTS applications. Human recombinant AChE and BACE1 monolithic micro-IMERs (immobilized enzyme reactor) have been developed for on-line automated HT HPLC inhibition studies (IC_{50} and mechanism of inhibition); secondly, fluorometric, circular dichroism, mass spectrometry, AFM methods were optimised for monitoring the inhibition of AChE-induced Aβ fibril formation and the inhibition of spontaneous Aβ aggregation, elucidating at which intermediate level of the Aβ aggregation cascade the inhibitors halt the process (monomer, soluble oligomers, protofibrils, fibrils). Finally, mass spectrometry combined with UHPLC was applied to investigate the mechanism of action of GSK3β inhibitors. By the application of these integrated approaches, new leads as the prototype of new classes of multifunctional compounds for AD treatment were discovered.

KL 04

Metabolomics: a tool for drug discovery and personalised medicine

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Metabolomics is the study of small molecules (< 1500 Da) involved in a biological process. The typical metabolomics workflow consists of developing as many signals as possible from the biological samples to be studied; applying a differential analysis between at least two groups; identifying the signals, translated into compounds, responsible for differences and interpreting the changes through the metabolic pathways that have been altered. This workflow is an untargeted approach where there are no *a priori* hypothesis about metabolic alterations and therefore a real discovery phase. Complementary, target analysis of metabolites can confirm a preliminary hypothesis when existing.

Studying the effects of drugs on the metabolome constitutes a huge part of the metabolomics discipline. Whether the approach is associated with drug discovery (altered pathways due to the disease that provide future targets and information into the mechanism of action or resistance, etc.) or pharmacometabolomics (studying the outcome of treatment), our group has participated in different studies applying different separation techniques coupled to mass spectrometry:

-Ketamine mechanism of action and resistance in neurological diseases [1].

-Metabolomic evaluation of mitomycin C and rapamycin in a personalised treatment of pancreatic cancer [2]

-Leishmania: pathways responsible for parasite survival, mechanism of action and resistance of some common drugs, drug repositioning [3].

Examples of those studies will be presented in order to show different tools and capabilities of metabolomics in this context.

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Toll-like Receptor 4 modulation by small organic molecules: new therapeutic opportunities

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Toll-like receptor 4 (TLR4) detects minute amount of Pathogen Associated Molecular Patterns, PAMPs, namely bacterial endotoxin (lipopolysaccharide and oligosaccharide, LPS and LOS and their bioactive part, the lipid A) and activate the immune and inflammatory responses to pathogen infections. However, deregulated or excessively potent TLR4 activation and signaling generates serious syndromes such as septic shock and sepsis. TLR4 stimulation by endogenous factors also called Danger Associated Molecular Patterns, (DAMPs), has recently been associated to a wide array of diseases ranging from autoimmune, inflammatory and circulatory diseases, to some types of diabetes and tumors. Small molecules active in modulating TLR4 activity are promising lead compounds for developing specific therapeutics against infectious and inflammatory pathologies. We synthesized glycolipids and other cationic and anionic amphiphiles that are active in modulating the TLR4-mediated inflammatory and innate immunity responses to bacterial endotoxins (lipopolysaccharide, LPS).[1] Cationic glycolipids derived from D-glucose[2] and anionic lipid A mimetics (Figure)[3] inhibit endotoxin-induced cytokine production in innate immunity cells. These molecules are active in vivo in contrasting septic shock and DAMP-dependent syndromes, such as neuropathic pain, caused by microglial TLR4 activation.[4] Some promising preliminary results in neuroinflammation and Amyotrophic Lateral Sclerosis (ALS) animal models will be presented. The mechanism of action of synthetic compounds and nanoparticles active on TLR4 will be discussed in detail. The activity of some these molecules in inhibiting LPS-dependent TLR4 activation is due to specific targeting of CD14 that chaperones endotoxin association to TLR4.[5] New perspectives in the modulation of TLR4 signal with synthetic lipid A-like compounds and natural compounds (mainly plant metabolites) will be presented.

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KL 06

Thermodynamics and kinetics of drug-target binding through molecular simulations

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Drug-target recognition and binding is a complex physicochemical process that represents the first event at the basis of the therapeutic action of drugs. Indeed drugs, navigating into the cell, recognize their biological counterparts, loosely interact with them, and finally establish tight binding interactions with targets. This complex process is regulated by thermodynamic and kinetic parameters, which can eventually account for drug potency, and in vivo drug efficacy.

Molecular simulation is emerging as a powerful tool for investigating protein-ligand binding. In particular, molecular dynamics (MD) simulation is emerging as a fundamental tool for drug discovery, and MD-related approaches, including enhanced sampling methods, are playing an increasing pivotal role in computational drug design. While extensive MD simulations in the microsecond to the millisecond timescale are nowadays able to simulate protein-ligand binding "spontaneously", enhanced sampling methods, including metadynamics, steered-MD, umbrella sampling, etc., can improve the sampling of that part of free energy that can be relevant for the biological process under investigation.

In this talk, I will be presenting the use of extensive molecular dynamics simulations to investigate spontaneously protein-ligand binding. Subsequent free energy calculations will be presented to identify the minimum free energy path from the bulk of the solvent into the protein-binding pocket, and to determine thermodynamic and kinetic parameters associated to drug-target recognition and binding. Then, I will focus on applications of enhanced sampling methods carried out to accelerate ligand binding and unbinding and to estimate kinetics and thermodynamics, in simulation timescale more compatible with the requirements of speed and accuracy of the pharmaceutical research. All these simulations will be discussed in the framework of drug design and discovery, highlighting the role of these approaches in real-life drug discovery endeavors.

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Controlling DNA recognition with external inputs

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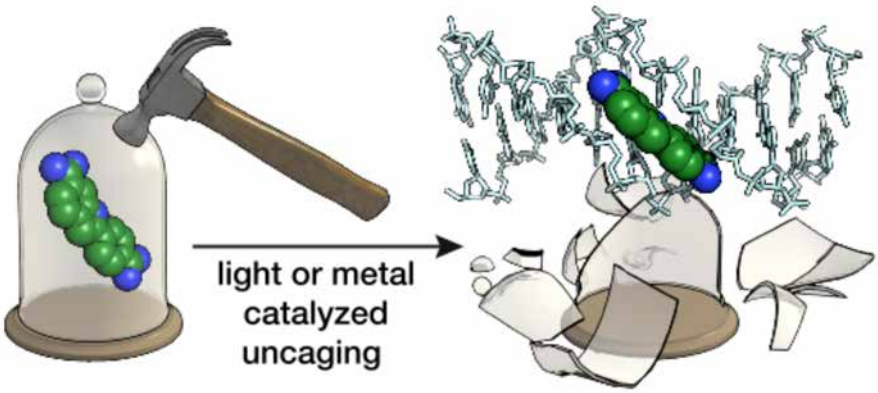
A major research goal in chemical biology is the efficient and selective targeting of double stranded DNA with small molecules. Towards this goal, a wide range of synthetic DNA binders, from small molecules to larger peptides, have been developed over the years.[1] However, in addition to the search for better sequence selectivity, there is also an increased focus in the external control of the DNA binding of these molecules, so that they could be activated at will, when and where required. This has been usually approached through the synthesis of bioactive compounds masked with photosensitive protecting groups—caged compounds—so that irradiation with UV light releases the parent molecules, thus triggering a biological response. In this context, we will describe our latest developments in light-activated DNA-binding small molecules, and peptide derivatives, which rely in the use of *o*-nitrobenzyl or bisbipyridyl ruthenium complexes as UV-photolabile groups.[2]

On the other hand, the possibility of releasing bioactive molecules by using a catalytic process is particularly appealing, particularly by reactions that rely on the use of transition metal catalysts. However, despite the extensive use of organometallic catalysis in synthetic chemistry, metal-based catalytic reactions have been, until recently, largely overlooked in biological settings.[3,4] We will show that simple alloc-protecting groups might prevent DNA binding of small molecules, which can be restored using metal-π-allyl chemistry for catalytic uncaging both in vitro and in cell culture.[5]



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KL 08

Small molecules useful for intervention against emerging viruses: triazolopyrimidines and chikungunya virus as an example

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Despite the progress made in increasing our antiviral armamentarium against world spread viral diseases such as AIDS or hepatitis B and C, there are still a number of very relevant viral infections that lack any treatment. In addition globalization, favoring exchange of goods and people all over the world, and demographic trends, including urbanization, has a profound effect on the dissemination of viral diseases.[1] Current outbreaks of viral diseases that quickly raise social concern like chikungunya fever in the Caribbean coasts, Ebola virus in West Africa or avian influenza in Asia are well known examples of how these diseases represent an increasing medical and social challenge.

Small molecules continue to be a key source to provide tools that help to better study the replicative cycle of these viruses. Additionally, and through the corresponding medicinal chemistry programs, novel chemical entities can be obtained for the therapeutic treatment for these viral diseases.

Among the above mentioned viral emerging diseases, we have been working in the search of small molecules able to interfere with Chikungunya virus (CHIKV). CHIKV, traditionally circumscribed to certain areas of Africa and Asia, has jumped to the Americas and several cases have also been reported in the Mediterranean countries. CHIKV causes painful arthritis-like symptoms that last for moths to years; in some cases, neurological complications have also been described.[2] So far, no antiviral treatment is available for these patients that receive just symptomatic alleviation.

Recently, we have identified small molecules able to inhibit CHIKV replication.[3] Optimization of the initial hit and extensive SAR exploration has allowed the improvement of the antiviral potency of the hit. Moreover, in a joint effort with European virology, biochemistry and molecular biology groups, we have identified a viral enzyme as the putative target of our compounds opening the way for therapeutic intervention.

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ORAL COMMUNICATIONS

Synthesis and biological evaluation of novel chalcones as antimitotics and vascular disrupting agent

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Among the current approaches for innovative anticancer therapies, Vascular Disrupting Agents (VDAs) constitute a particularly promising therapy complementary to the existing ones, and no resistance mechanisms have so far been described.1 In particular compounds that bind the α/β tubulin heterodimers at the colchicine binding site have shown a dual mechanism of action: antimitotic and VDAs. The most representative example is combretastatine A4 (CA-4).2 However current candidates in clinical trials suffer from several drawbacks such as low chemical stability and poor solubility.

Inspired by the structure of natural products that bind the cochicine-binding site in tubulin, we have addressed the synthesis of chalcone-based compounds. We were glad to find that these compounds showed antiproliferative activity against tumor and endothelial cells in the low nanomolar range. Competition assays in cell culture as well as fluorescent assays confirmed that these compounds bind the colchicine-binding site in tubulin with Kd values in the order of 10-7 M-1. In addition the crystal structure of our prototype compound in complex with α/β tubulin confirmed the E-configuration of the chalcone double bond as the bioactive structure. In order to perform "in vivo" studies, a prodrug strategy was applied that improved the solubility 2000-fold compared to the parent compound. Testing of the prodrug in mice inoculated with melanoma cells B16F10 that express luciferase have shown a significant antitumoral effect as followed by imaging techniques and by weighting of the tumor. Thus we may conclude we have in hand a very promising family of antitumoral compounds targeting the colchicine-binding site in tubulin.

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New indole tubulin assembly inhibitors with stable arrest of mitotic progression, enhanced stimulation of natural killer cell cytotoxic activity and repression of Hedgehog-dependent cancer

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Arylthioindoles and aroylindoles are potent inhibitors of tubulin polymerization and cancer cell growth by binding to the colchicine site on beta-tubulin [1]. Several derivatives were more potent than colchicine, combretastatin-A4, vinblastine and paclitaxel and have potential as novel therapeutic agents to treat cancer.

Molecular modelling studies led us to design new 2-phenylindole derivatives as potential anticancer agents bearing the 3,4,5-trimethoxyphenyl moiety with a sulphur, ketone or methylene bridging group at position 3 of the indole and halogen or methoxy substituent(s) at positions 4-7. New derivatives inhibited tubulin assembly and growth of a large panel of cancer cells at low micro- and nanomolar concentrations, respectively. Among these, 6-methoxy-3-((3,4,5-trimethoxyphenyl) thio)-1*H*-indole and 6,7-dichloro-3-((3,4,5-trimethoxyphenyl)thio)-1*H*-indole strongly inhibited the growth of the P-glycoprotein-overexpressing multidrug resistant cell lines NCI/ADR-RES and Messa/Dx5. At 10 nM, the same derivatives stimulated the cytotoxic activity of natural killer cells, while at 20-50 nM arrested >80% of HeLa cells in the G2/M phase. Finally, 6,7-dichloro-3-((3,4,5-trimethoxyphenyl)thio)-1*H*-indole and 2-(1*H*-imidazol-1-yl)-3-((3,4,5-trimethoxyphenyl)thio)-1*H*-indole showed strong inhibition of the Hedgehog signalling pathway, blocking the growth of NIH3T3 Shh-Light II cells with IC50's of 72 and 38 nM, respectively.

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Challenges in the design of sigma-1 receptor antagonists for the treatment of pain

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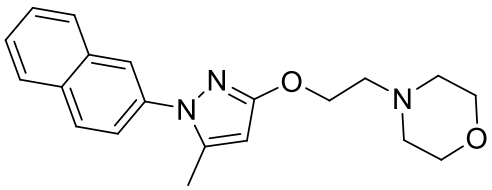
The σ receptor (σR) is a molecular chaperone which modulates the function of different receptors and ion channels when they become challenged by disease-related stress or mutations. The σR presents at least two different subtypes, σ_1R and σ_2R, which show distinct functions and are related to different potential therapeutic indications. Studies in animal models support a role for σ_1R antagonists in the treatment of pain states where hypersensitivity develops as hyperalgesia and allodynia, two common symptoms encountered in neuropathic pain and other chronic pain conditions. The recent progression of the σ_1R antagonist E-52862 (**1**), up to clinical trials for the treatment of different pain states will reveal how this relevant preclinical evidence translates into man.

We report here a summary of our recent work towards the identification of σ_1R antagonists for the treatment of pain, which is challenged by the nature of the target itself. Thus, in spite of the simplicity of the σ_1R pharmacophore, which consists of a positive ionisable nitrogen atom and an aromatic group at a certain distance, the design of therapeutically useful σ_1R ligands, *ie* selective and drug-like, is complicated by the high lipophilicity of the σ_1R binding site and the overlap of the σ_1R pharmacophore with that of a number of other receptor systems, including the hERG channel. In addition, due to the intracellular localization of the receptor and the lack of truthful, mechanistic-based functional assays with adequate throughput to drive comprehensive medicinal chemistry programs, the identification of σ_1R antagonists relies on *in vivo* testing.



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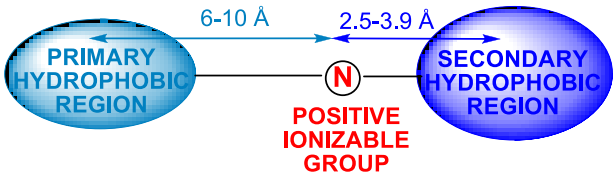

σ_1 Receptor pharmacophore

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A green synthesis of vidarabine 5'-monophosphate *via* a one-pot multi-enzymatic reaction catalyzed by immobilized biocatalysts

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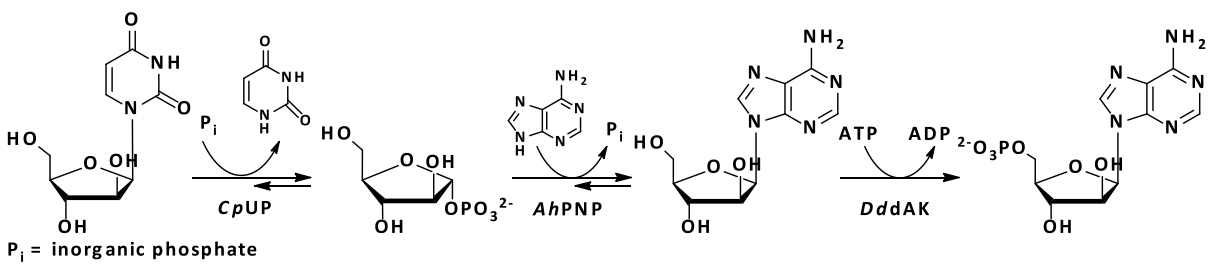
#This work is dedicated to the memory of Prof. Jure Piškur

In nature, enzyme cascades can be found in many metabolic pathways. The idea of using multi-enzymatic systems to mimic these processes is gaining interest for production of chemical compounds. A type of multi-enzymatic application is the use of multiple enzymes for shifting reaction equilibria. This strategy relies on removing intermediates, inhibitory products or by-products, *via* a second enzymatic reaction. In the context of a multi-enzymatic system, a one-pot process uses more than one enzyme in a single reactor.[1]

We here describe a three-step sequential enzymatic reaction for the one-pot synthesis of vidarabine 5'-monophosphate (araA-MP), an antiviral drug, using arabinosyluracil (araU), adenine (Ade) and adenosine triphosphate (ATP) as precursors. To this aim, three immobilized biocatalysts involved in the biosynthesis of nucleosides and nucleotides were used: uridine phosphorylase from *Clostridium perfringens* (*Cp*UP),[2] a purine nucleoside phosphorylase from *Aeromonas hydrophila* (*Ah*PNP),[2] and deoxyadenosine kinase from *Dictyostelium discoideum* (*Dd*dAK).[3] Specifically, *Cp*UP catalyzes the phosphorolysis of araU thus generating uracil and α-D-arabinose-1-phosphate. *Ah*PNP catalyzes the coupling between this latter compound and Ade to form araA (vidarabine). This nucleoside becomes the substrate of *Dd*dAK which produces the 5'-mononucleotide counterpart (araA-MP) using ATP as the phosphate *donor* (Scheme 1). Reaction conditions (*i.e. medium*, temperature, immobilization carriers) and biocatalyst stability have been balanced and optimized to achieve the highest productivity. Vidarabine 5'-monophosphate was obtained in 95.5% conversion. Optimization of the purification step is in progress.

Scheme 1 One-pot multi-enzymatic synthesis of vidarabine 5'-monophosphate (araA-MP)



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New inhibitors of angiogenesis with antitumor activity in vivo

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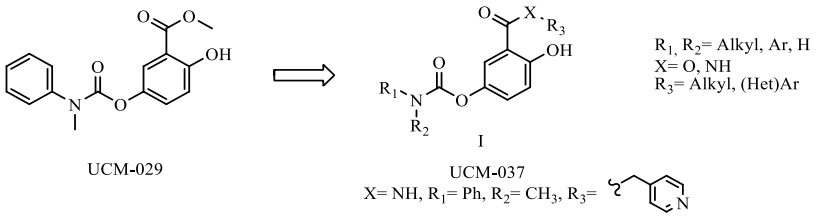
Angiogenesis is essential for the sustainable growth and proliferation of solid tumors. Thus, the development of new compounds that induce a sustained inhibition of the pro-angiogenic signaling generated by tumor hypoxia remains as an important unmet need.[1] It is considered that the most effective way to stop this process would be through the simultaneous inhibition of various signaling pathways to counteract the homeostatic mechanisms of the cell.[2] With this aim, we have designed and synthesized a new series of compounds that block simultaneously the cellular mechanisms of adaptation to hypoxia and the induction of pro-angiogenic growth factors as a potential effective way to block tumor growth and metastatic processes in the long term.

Initially, we identified compound UCM-029 as a hit able to inhibit the cellular migration induced by fibroblast growth factor (FGF). In order to increase its potency as well as to improve the ADME properties, we designed and synthesized new compounds of series I, among which we selected UCM-037 as a lead due to its promising properties in vitro.[3] This compound inhibits the pro-angiogenic signaling under hypoxic conditions in breast cancer cells, decreasing from 40% to 100% the levels of pro-angiogenic factors FGF, vascular endothelial growth factor (VEGF) and nitric oxide (NO). Besides, it affects the MEK/ERK and Akt signaling pathways, impairs cellular migration and significantly changes the transcription of genes related with angiogenesis. Finally, it also decreases the total levels of iNOS as well as those of the phosphorylated FGF and VEGF receptors. The observed effects are mainly mediated by HIF-1α, since when its expression is knocked-down the effects of UCM-037 mostly disappear and the compound does not affect the levels of upstream HIF-1α anf HIF-2α proteins. Furthermore, administration of UCM-037 in a xenograft model of breast cancer reduced tumor growth from 46% to 55% in 38% of the treated animals without causing any toxic side effect. Importantly, in the responding tumors, a significant reduction in the number of blood vessels and VEGF mRNA levels was observed, further supporting the mechanism of action of the compound. These findings provide a rationale for the development of new antiangiogenic compounds that could eventually lead to new drugs suitable for the treatment of some types of tumors alone or in combination with other agents.



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Synthesis and biological activity of a new series of nortopsentin analogues

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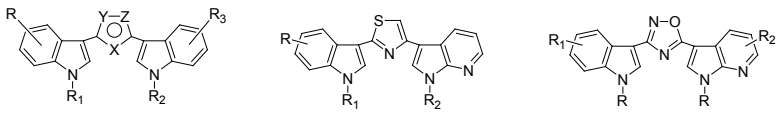
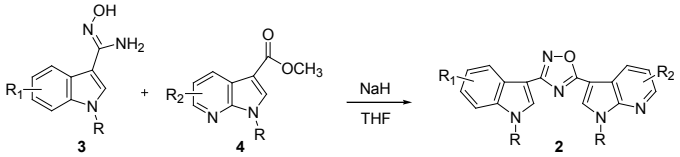
Marine organisms constitute a very important source of biologically active natural products. In particular, bis-indole alkaloids, characterized by two indole units bound to a spacer through their 3 position, have emerged as an important structural class because of their wide variety of biological activities such as anti-inflammatory, antimicrobial, antiviral and antitumor.

Nortopsentins A-C, having a characteristic 2,4-bis(3'-indolyl)imidazole skeleton, showed *in vitro* cytotoxicity against P388 cells (IC_{50} 0.34-0.90 μg/ml).[1] Due to the considerable biological activities shown by nortopsentins and their several analogues, we reported the synthesis and antitumor activity of different bis-indolyl-5-membered heterocycles, in which the central imidazole ring of nortopsentin was replaced by thiophene, pyrazole, isoxazole, and furan rings. All these series showed antitumor activity from micromolar to sub-micromolar level.[2] Many other analogues such as 2,4-bis(3'-indolyl)-thiazoles and 3,5-bis(2-indolyl)pyridines showed strong inhibitory activity against a wide range of human tumor cell lines. Moreover, the structural manipulation of the natural nortopsentins, was extended to one or both indole units leading to 3-[(2-indolyl)-5-phenyl]pyridine and phenylthiazolyl-7-azaindole derivatives. Both series of compounds showed good antiproliferative activity and remarkably inhibited the activity of the cyclin-dependent kinase 1 (CDK1).[3]

More recently, 3-[2-(1*H*-indol-3-yl)-1,3-thiazol-4-yl)-1*H*-7-azaindoles **1** were synthesized and compounds of this series were tested against the NCI full panel of human cancer cell lines and STO and MesoII cells, derived from human diffuse malignant peritoneal mesothelioma (DMPM). The most active compounds, that also act as CDK1 inhibitors, consistently reduced DMPM cell proliferation and induced a caspase-dependent apoptotic response.[4]



Considering the significant biological activity showed by nortopsentin analogues and in particular those with 7 aza-substitution, we synthesized new analogues **2** in which the central imidazole ring was replaced by [1,2,4]oxadiazole ring and the 7 aza-substitution was maintained, in order to study how these structural modifications influence biological activity. The synthesis was performed reacting two different key intermediates, *N'*-hydroxy-1*H*-indole-3-carboxamide **3** and methyl 1*H*-pyrrolo[2,3-*b*]pyridine-3-carboxylate **4**, in the presence of sodium hydride and tetrahydrofuran under reflux. All compounds were preliminarily tested on HCT116 and MCF7 cell lines. In particular, two of them showed GI_{50} values at sub-micromolar concentrations. Further studies on the possible mode of action will be discussed.



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Targeting triple negative breast cancer through CB_2 cannabinoid receptor activation: from synthesis to in vivo studies

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Triple-negative breast cancer does not respond to endocrine therapy or other available targeted agents so it represents an important clinical challenge. Consequently, there is an evident need to develop new therapeutic strategies for the management of this disease. Chemotherapy with its well-known side effects is currently used as a systemic treatment for this cancer.

Within this context, new chromenopyrazolediones have been designed and synthesized as anticancer agents using the multibiological target concept involving cannabinoid antiproliferative properties and quinone cytotoxicity. These compounds showed to be fully selective CB_2 cannabinoid receptor ligands eliminating any psychotropic side effect derived from CB_1 receptor activation in the brain. They decreased cell proliferation in four different human triple negative breast cancer cell lines showing full cytotoxicity selectivity for cancerous cells versus noncancerous human mammary epithelial cells. Mechanistic studies allowed us to determine that these compounds exert antitumor effects by inducing cell apoptosis through activation of CB_2 receptors and through induction of oxidative stress. This antiproliferative effect was reproduced in vivo. Upon one month of treatment, histopathological analysis of different organs of treated mice revealed no signs of toxicity. Research on the incidence of neuropathies (sensorial and gastrointestinal, an important side effect produced by current triple negative treatments) upon chromenopyrazoledione treatment is currently being carried out.

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A new application of click chemistry in Drug Discovery: the identification of store-operated calcium entry modulators

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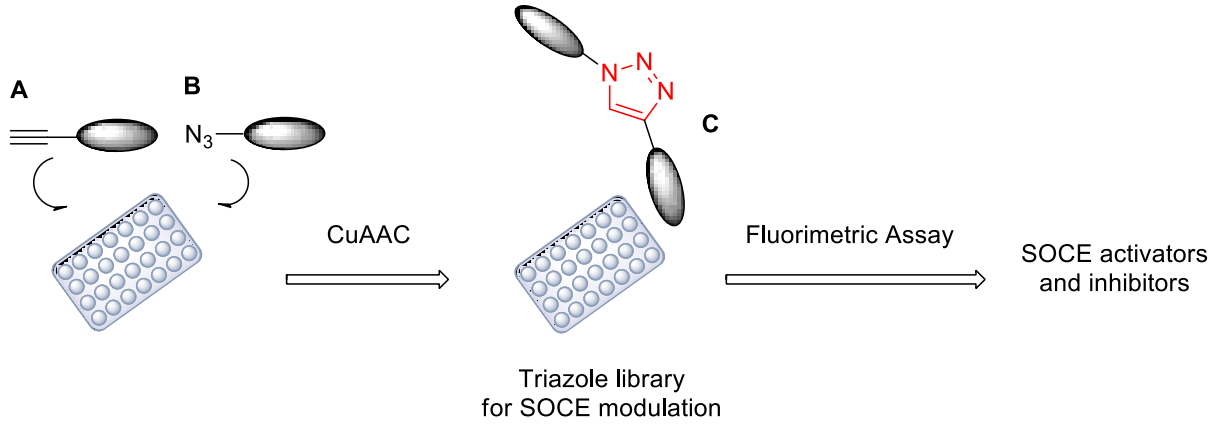
Store-operated calcium entry (SOCE) is a process by which the depletion of calcium from the endoplasmic reticulum activates calcium influx across the plasma membrane.[1] The key molecular components of SOCE machinery are Orai1 and TRPC channels whose activity is orchestrated by STIM1, the endoplasmic reticulum calcium sensor.

Potent, specific and widely available pharmacological tools are highly desirable for further analysis of the contribution of these proteins to calcium signaling and downstream cellular events, but there are no published crystal structures on which the design of modulators can be based. Thus, the field is at a stage of serendipitously identifying small molecule modulators from chemical libraries.[2]

Starting from the structure of known pyrazole derivatives (BTP, Pyr),[3] we designed a library of SOCE modulation candidates and these compounds were synthesized by the reliable CuAAC reaction.[4] Screening was performed in BV-2 cells, a cell line derived from raf/myc-immortalised murine neonatal microglia that predominantly expresses Orai channels and led to the identification of both SOCE activators and inhibitors.



The identified compounds appear suitable for modulation of SOCE, paving the way towards better understanding of calcium entry mechanisms and development of novel therapeutic strategies.

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Novel purinone derivatives as JAK inhibitors for the inhaled treatment of respiratory diseases

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Janus (JAK) kinases (JAK1, JAK2, JAK3 and TYK2) signal in multiple cytokine receptors that play a role in asthma and COPD. A pan-JAK inhibitor blocking the multiple cytokine signalling constitute a potential new therapeutic approach for the treatment of these respiratory diseases.

This presentation will focus on the design, synthesis and biological activity of a series of purinone derivatives as potent pan-JAK inhibitors. Systematic SAR efforts addressed to improve potency of starting molecules led to the identification of an advanced lead with balanced activities against JAK1-3, long lung retention and proven efficacy in the rat LPS model by intratracheal administration.

Vinyl sulfones covalently react with serum albumin. A potential selectivity issue for the development of irreversible cysteine protease inhibitors.

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ABSTRACT

Vinyl sulfones are used as warheads for the development of irreversible inhibitors of cysteine proteases (CPs)*1*. Their activity was demonstrated to be due to an alkylation of the cysteine thiol moieties in the catalytic pocket of CPs*2*. Such a mechanism was claimed to be selective since vinyl sulfones need the catalytic machinery of CPs for activation as electrophiles*1*. The lack of reactivity towards small circulating thiols (i.e. glutathione) was considered as a sufficient evidence of such a selectivity so far*1*. Nevertheless, vinyl sulfones showed some toxic effects in animal models*3*. Herein, we demonstrate that vinyl sulfones are unstable in human serum since they react covalently with serum albumin (HSA). Specifically, the Cys34 and His146 residues of HSA were identified as the most important hotspots undergoing to such modifications. Interestingly, the high reactivity of Cys34 was explained by an *in silico* model*4* showing a stabilization of the thiolate anion, which is largely superimposable with the catalytic mechanism of CPs proposed by independent authors*5*. This was apparently the first time that a reaction between vinyl sulfones and a non-thiolic group (e.g. His146) was proven, which confirm that the alkylation mechanism is non-selective. The main consequence of such a side reaction is that molecules bearing a vinyl sulfone warhead can potentially be toxic or poorly bioavailable. Therefore, the development of such compounds should be designed to include reliable early tests to evaluate their selectivity and stability *in vitro*. The use of HSA as molecular probe looks promising for the setup of new methods for such investigations.

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Discovery of new antibiotics targeting shikimate kinase: from the reaction mechanism to the structure-based design of inhibitors

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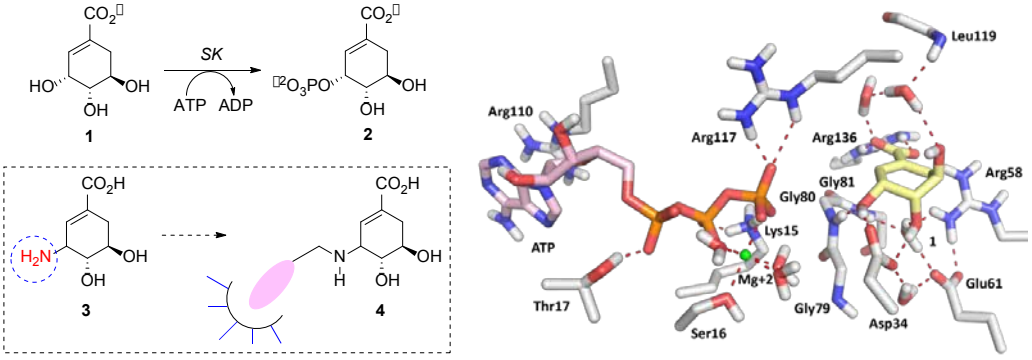
The loss of effectiveness of current antibiotics caused by the development of drug resistance has become a severe threat to public health. There is therefore great interest in the discovery of novel drugs and therapies to tackle antimicrobial resistance, in particular drugs that target other essential processes for bacterial survival. Many drugs that are highly successful in human clinical use mimic a substrate, a transition state or a product of essential enzymes. For this purpose, a detailed knowledge of the catalytic mechanism and the binding determinants of selected enzymes involved in biosynthetic pathways or processes that do not have mammalian homologs, but are essential for bacterial survival, can be valuable for the rational design of these mimetics (inhibitors) that can be used as drugs.

In our research group we are studying the possible development of new antibiotics by the selective inhibition of shikimate kinase (SK), the fifth enzyme of the biosynthesis of the aromatic amino acids.[1] It catalyzes the stereospecific phosphorylation of the C3 hydroxyl group of shikimic acid (**1**) by transferring the γ-phosphate group of ATP to the hydroxyl group to provide shikimate 3-phosphate (**2**) and ADP.[2] It is an essential enzyme in relevant pathogenic bacteria such as *Mycobacterium tuberculosis*, *Helicobacter pylori* and *Pseudomonas aeruginosa,* but do not have any counterpart in human cells. Here we report results from NMR, biochemical and Molecular Dynamics simulation studies that help to understand the catalytic mechanism of the SK enzyme. Based on these results, several competitive inhibitors of the enzyme, namely compounds **3** and **4**, were designed. Our recent results on this project will be presented.[3]



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Ion pairing to enhance antibiotic drug efficacy. A new approach to fight problematic infections?

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University of Perugia

Background The worldwide increased burden of bacterial resistance urges novel approaches to enhance control and treatment. It is estimated that only in Europe one million resistant strains related deaths will be recorded by 2025. We propose ion pairing as a tool to enhance antibiotic drug efficacy against problematic lung infections, e.g. Tuberculosis.

Methods Amikacin and kanamycin were ion paired with dehoxycholic acid (DCA) and vancomycin with ursodehoxycholic acid (UDCA) in *green* conditions at different stoichiometry ratios. The new compounds were characterized by DSC, FTIR, UV-vis spectrophotometry, molecular modeling and NMR. The minimum inhibition concentration (MIC) was measured on sensitive *Staphilococcus aureus* and clinical isolates of methicillin resistant *S. aureus* (MRSA). The inhibition capacity and activity on MSRA biofilms were also measured and morphologically investigated by fluorescence microscopy.

Results The ion pairs formed spontaneously with high yield at 1:4, 1:3 and 2:1 ratios for amikacin, kanamycin and vancomycin, respectively. Both DCA and UDCA showed high binding affinity with the respective drugs and the structures were partially resolved by combining *in silico* modeling and NMR analysis. The activity of the ion pairs was 5 to 10 fold higher than that of the parent drugs. Higher inhibition as well as enhanced efficacy in the treatment of established MSRA biofilms was observed.

Conclusions The enhanced efficacy on MRSA biofilms suggests that ion pairing may result an important platform to improve the treatment and control of problematic infectious diseases. This strategy possesses two intrinsic advantages:1) a likely faster bench-to-market pathway, 2) easy formulation as dry powder to allow inhalation.

Acknowledgements This work was supported by the grant (2014.0078.021 Ricerca Scientifica e Tecnologica) by the *Cassa di Risparmio di Perugia* Foundation.

Assembling new chemical boxes as an open source of starting points for drug discovery against three kinetoplastid parasites causing Neglected Tropical Diseases

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Kinetoplastids are a group of flagellated protozoans that include the species *Leishmania* and *Trypanosoma*, which are human pathogens with devastating health and economic effect. The most common human diseases caused by kinetoplastids are included within the list of 17 core NTDs (Neglected Tropical Diseases) declared by WHO. They are human African trypanosomiasis, which is caused by subspecies of *Trypanosoma brucei;* Chagas disease, which is caused by the infection with *Trypanosoma cruzi*; and various clinical manifestations of leishmaniasis, which are caused by more than 20 species of *Leishmania*. All NTDs have been categorized as "tool ready," yet also "tool deficient" because many of these tools (i.e. drugs and diagnostics) and implementation strategies are inadequate to achieve the desired goals. New effective, safe, and affordable drugs, preferably oral, are needed.

As part of the fight against the neglected tropical diseases, recently GlaxoSmithKline has published three anti-kinetoplastid chemical boxes of around 200 compounds each as an open source for future lead discovery or chemical biology research.[1] The GSK 1.8 million compounds diverse collection has been phenotypically screened against their causative parasites, respectively *Leishmania donovani*, *Trypanosoma cruzi* and *Trypanosma brucei*, using the state-of-the-art methodologies available in high throughput screening.

Secondary confirmatory and orthogonal assays have been applied in order to confirm anti-parasitic activity and to identify potential cytotoxicity activity. Hit compounds have been chemically-clustered and triaged for suitable physicochemical properties. As a result of this effort, three anti-kinetoplastidal boxes of approximately 200 compounds each have been assembled, which represent all the chemical and biological diversity identified and are intended to serve as an open source of starting points for further lead discovery programs, as well as to address important research questions.

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Novel positive allosteric modulators of the metabotropic Glutamate Receptor 5 as potential antipsychotic agents

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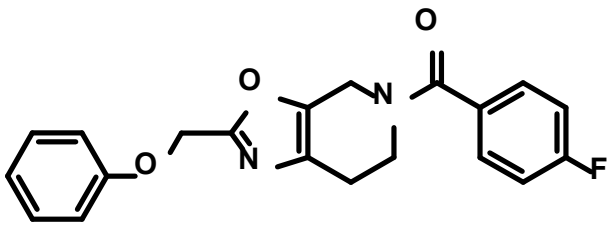
Positive allosteric modulators (PAMs) of metabotropic glutamate receptor 5 (mGlu5) represent a promising therapeutic approach for the treatment of schizophrenia in virtue of the prominent roles of mGlu5 receptor in synaptic plasticity, cognition, learning, and memory process[1,2]. Starting from a high throughput screening (HTS) campaign hit, a focused medicinal chemistry optimization effort led to the identification of a novel series of oxazolepiperidine derivatives with mGlu5 PAM activity. Structure–activity relationships (SAR) within this series, which uncovered VU0409551, a novel potent and highly selective mGlu5 PAM will be summarized. A detailed in vitro potency and selectivity profile, in vitro and in vivo DMPK properties, in vivo efficacy in animal models predictive for antipsychotic efficacy as well as a preliminary toxicological profile for VU0409551 will also be presented.



VU0409551

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Combining flow chemistry with multicomponent Povarov reaction: stereoselective synthesis and characterization of tricyclic tetrahydroquinolines

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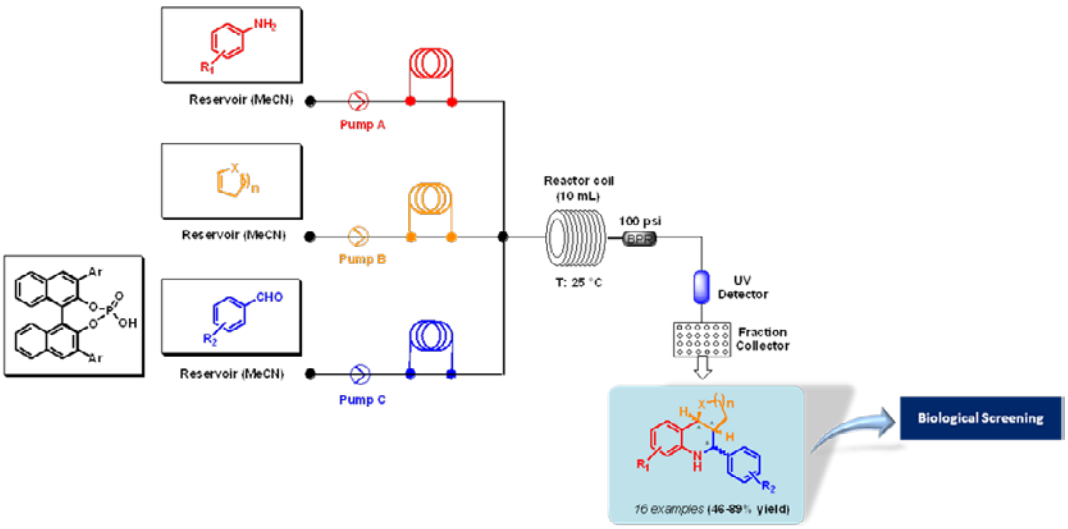
Ring-fused tricyclic tetrahydroquinolines (TC-THQs) represent an important class of compounds which appear as structural framework in many natural products, chemical tools and therapeutic agents.[1] They exhibit a wide spectrum of biological activity being able to interact with different biological targets including both nuclear and membrane receptors, enzymes and ion channels.[1] The facile construction of TC-THQs is therefore particularly sought in both synthetic and medicinal chemistry programmes.

As a continuation of our ongoing research toward the development of steroid-responsive nuclear receptor modulators[2] and innovative synthetic solutions,[3] in this communication we report the synthesis and preliminary biological evaluation of TC-THQs as an appealing class of compounds useful to explore the steroidal-targeted bio-chemical space. To this aim, for the first time, the multicomponent Povarov reaction was successfully conducted under flow conditions to rapidly build a TC-THQ library. In particular, the reaction was optimized in terms of experimental conditions, yield and productivity, costs and environmental impact, and investigated from a stereoselectivity standpoint to afford stereochemically pure products ready for biological screening.



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A drug-like photoswitchable GPCR allosteric ligand with light-dependent control of animal motility

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An azobenzene molecule (Alloswitch-1) [1] is a photoswitchable negative allosteric modulator (NAM) of metabotropic glutamate receptor 5 ($mGlu_5$) with a potency in the nanomolar range and high selectivity over other mGlu receptor subtypes, a family of GPCRs with important roles in synaptic transmission and validated as therapeutic targets in CNS diseases.

After illumination with violet light (390 nm) Alloswitch-1 isomerizes from the stable *trans*- isomer to the *cis* isomer. This structural change can be reversed with blue-green light (490 nm), or spontaneously by thermal relaxation and the cycles can repeated for extended time. The light-dependent isomerization serves to control $mGlu_5$ activity in HEK cultured cells *in vitro*. In the dark, *trans*-azobenzene fully antagonizes the orthosteric activation of $mGlu_5$ receptor defining a classical NAM profile. Upon 390nm illumination, no effects were observed, indicating different activities for the *cis* and *trans* isomers on $mGlu_5$.

Imaging real-time intracellular calcium in individual cells after Alloswitch application in the dark showed inhibition of the intracellular calcium oscillations evoked by the orthosteric activation of $mGlu_5$, and 390 nm promoted isomerization to the *cis* form restored the receptor activity. Isomerization of the *cis*-azobenzene back to the *trans* form with 490-nm light blocked again calcium oscillations. This cycle can be repeated several times showing that $mGlu_5$ activity in living cells could be reversibly switched with Alloswitch-1 by simply setting illumination at 390 or 490 nm.

Finally, wild type *Xenopus tropicalis* tadpoles and Zebra fish show a clear behavioral effect of Alloswitch-1 at different wavelengths that affects natatorial capacity of both species. These optically controlled switches of animal motility were reversible suggesting a potential for wild type $mGlu_5$ activity control *in vivo* with photoswitchable drugs.

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In vivo pharmacokinetic study and CNS distribution of the sigma1 receptor agonist (R)-RC-33, a promising neuroprotective agent

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Sigma-1 receptor plays an important role in neuronal plasticity, a process implicated in the pathophysiology of neuropsychiatric diseases and their activation by agonists causes beneficial effects in the cells. Sigma1 receptor agonists have been proved to prevent neuronal death caused by glutamate toxicity, and to promote *in vitro* neurite sprouting and elongation.[1] In this context, the identification of new, potent, and highly selective sigma1 agonists is of significant interest, not only for better understanding of the role played by sigma1 receptors in various pathologies, but also, as a more challenging task, to develop neuroprotective agents. Therefore, sigma1 receptor agonists could represent an innovative pharmacological approach for the treatment and prevention of neurodegenerative diseases.[1]

In this scenario, we recently discovered, (*R*)-**RC-33**, a potent and metabolically stable sigma1 receptors agonist.[2-4]

In this communication we will report on i) the set up of a method suitable for gram-scale production of (*R*)-**RC-33**; we explore and compare the diverse options which can be applied to reach pure enantiomers (asymmetric synthesis, chiral chromatography and fractional crystallization); ii) the development of a simple UFLC-MS/MS method for the determination of (*R*)-**RC-33** in mouse plasma, brain and spinal cord homogenates; iii) the *in vivo* pharmacokinetic study and CNS distribution.

Overall, results evidenced an excellent distribution of (*R*)-**RC-33** in the central nervous system, thus suggesting that it could be an optimal candidate for proof-of-concept *in vivo* studies in animal model of neurodegenerative diseases.

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Combining simple chemistry and in silico approaches for the rational design of carnosine analogues as bioavailable and effective carbonyl quenchers

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Reactive carbonyl species (RCS) are endogenous electrophilic compounds able to condense with suitable nucleophilic residues yielding protein adducts, the pathogenic role of which was confirmed by several studies which also revealed the molecular mechanisms by which they can contribute to the onset and progression of several oxidative-based diseases. Similarly, in vivo studies emphasized the beneficial role of compounds able to inhibit protein carbonylation which indeed can be seen as a novel and attractive drug target. Among the possible strategies, the direct carbonyl quenching appears to be the most effective one since it involves an irreversible trapping of the reactive carbonyl species which are transformed into nontoxic and easily excreted derivatives. Among the reported quenchers, L-carnosine appears a really interesting molecule due to its well-established reactivity towards RCS such as 4-hydroxy nonenal (HNE) and acrolein combined with a satisfactory selectivity since it is unable to react with physiological carbonyls such as pyridoxal. Nonetheless, L-carnosine cannot have a therapeutic role since it is actively absorbed by hPepT1 at the intestinal level but is rapidly hydrolyzed by a specific serum carnosinase. As a general rule, the rational design of improved carnosine analogues would lead to derivatives which (1) are still recognized by intestinal transporters; (2) are reasonably stable in human serum and (3) are able to retain or possibly enhance the reactivity and selectivity towards RCS. Starting from the available natural carnosine derivatives, the communication will describe how these primary objectives can be conveniently pursued by introducing simple modifications to the carnosine structure which are suggested by in silico approaches. In detail, computational studies allowed the generation of reliable and predictive homology models for both hPepT1 and serum carnosinase as well as a deeper understanding of the structural features determining the reactivity and selectivity towards RCS. Taken collectively, these studies led to the design of promising peptidomimetics, whose quenching activity will be discussed along with the encouraging results of some preliminary in vivo studies and ADME-PK profiling.

Development of palladium-labile prodrugs for bioorthogonally-activated chemotherapy

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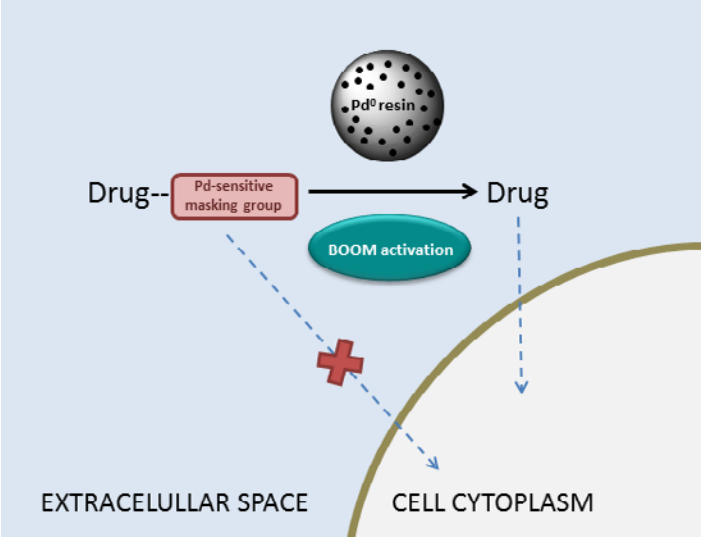
Based on the ability to interfere with and/or halt cell division at different stages, cytotoxic agents of various classes have been widely used in chemotherapeutic treatments[1]. Although highly effective, the clinical dose of most cytotoxic drugs is limited by their lack of selectivity for cancer cells that render systemic side effects. To reduce these unwanted effects while increasing the levels of drug in the disease area, a number of novel methods originated from the Chemical Biology field have emerged during the last years to explore the site-specific activation of cytotoxic drugs[2,3]. One of those novel concepts, pioneered by Dr. Unciti-Broceta's group, is based on the use of palladium to activate drug precursors by heterogeneous bioorthogonal organometallic (BOOM) catalysis[4]. Using an alkylation strategy to mask functional groups essential for the cytotoxic mode of action of a well-known-clinically-used drug, different Pd^0-sensitive prodrugs were developed. Our results show that the cytotoxic properties of drugs were successfully eliminated and selectively restored in cancer cell culture by extracellular Pd^0 catalysis.



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Development of novel in vivo active chemical chaperones as a potential anti-ALS (amyotrophic lateral sclerosis) drugs

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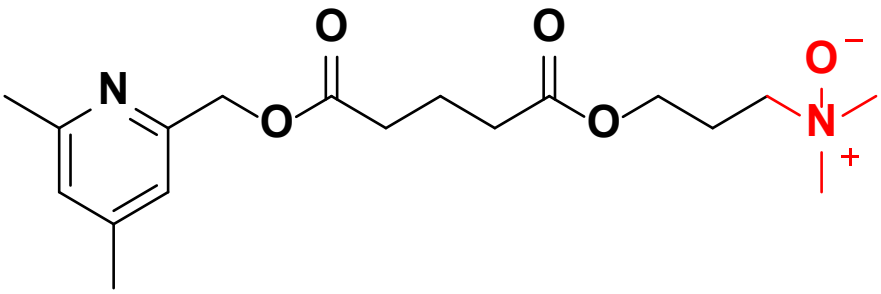
Artificial chaperones have been linked to ability to reverse the mislocalization and aggregation of proteins associated with different human diseases. The most limiting factor using of chemical chaperones as drugs is their very high active concentration (mM). We have synthesized several lipophilic derivatives of a known chemical chaperone (TMAO). One of them: 3-((5-((4,6-dimethylpyridin-2-yl)methoxy)-5-oxopentanoyl)oxy)-N,N-dimethylpropan-1-amine oxide (compound **14**) demonstrated anti-ALS effects in μM concentrations range. Compound **14** was shown to significantly decrease the formation of misfolded mutated SOD1 aggregates which cause familial ALS[1]. Furthermore, **14** prevented ER stress-induced apoptosis in primary culture of ALS mice astrocytes. Finally, the lead compound was evaluated in vivo. Compound **14** was administrated I.P. one time a day (10 mg/kg), in male and female groups. Already on the 95th day of the experiment significant difference in body weight between treated and non-treated mice was detected. In the end of the experiment (125 days) the deferens between treated and untreated group was so dramatic (in neurological functions and in the body weight) that we were able to conclude that the compound causes significant improvement of clinical symptoms in ALS mice[2]. While future characterization of compound **14** in terms of its mechanism of action is clearly needed, we believe that this specific compound, as well as other compounds sharing the same underlying design philosophy (i.e., chemical chaperone derivatives) may be useful in other pathologies resulting from protein aggregation.

Compound **14**



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Discovery of the first potent and systemically active inhibitors of acid ceramidase

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The sphingolipids are a class of bioactive lipid molecules that serve multiple regulatory functions in health and disease.[1] Central to sphingolipid metabolism, the ceramides are involved in the control of cellular senescence, inflammation, and apoptosis.[2] Additionally, these lipids are the biochemical precursors of sphingosine-1-phosphate (S1P), which mediates opposite effects, promoting cell survival and proliferation.[3] Ceramide hydrolysis by acid ceramidase (AC) stops the biological activity of these substances and influences survival and function of both normal and neoplastic cells. As key regulator in ceramide metabolism, AC may offer a novel molecular target in disorders with dysfunctional ceramide-mediated signaling, such as certain types of cancer and inflammation.

In the present communication, we report on the discovery of benzoxazolone carboxamides as the first potent and systemically active inhibitors of AC.[4] Prototype members of this class inhibit this enzyme with low nanomolar potency by covalent binding to the catalytic cysteine residue. Their metabolic stability and high *in vivo* efficacy suggest that this compounds can be used as probes to investigate the roles of ceramide in health and disease, and that this scaffold may represent a promising starting point for the development of novel therapeutic agents.

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Small molecule inhibition of the KRAS-PDEδ interaction impairs oncogenic K-RAS signaling

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K-ras, an important oncogene found mutated in several types of cancers, is posttranslationally lipidated with a farnesyl moiety at the C-terminal cysteine, what together with a polybasic sequence mediates its plasma membrane association, crucial for its activity. Several attempts to selectively target K-ras have been reported, albeit with limited success.

The δ subunit of retinal rod phosphodiesterase (PDEδ) is a prenyl-binding protein that recognizes the C-terminus of Ras proteins. Previous work indicated the essential role of PDEδ in keeping the correct localization and function of Ras proteins. Moreover, genetic PDEδ down-modulation resulted in randomized Ras distribution to all cellular membranes and suppression of Ras signaling in cancer cells.

Herein, we have investigated the inhibition of the interaction between PDEδ–prenylated Ras using small molecules as a novel strategy for cancer treatment. With this aim, an assay was established and employed to screen a 200.000 compound library. A structure-based optimization process was performed resulting in low nanomolar inhibitors. Additional investigations proved that the synthesized compounds also inhibit the PDEδ-Ras interaction in cells and suppress proliferation of human pancreatic ductal adenocarcinoma in mouse models.[1,2] This finding provides a novel strategy to impair oncogenic Ras signaling and thereby tumor growth.

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Identification and biological characterization of novel inhibitors of KDM1A

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KDM1A (LSD1), a flavin adenine dinucleotide (FAD) dependent amino oxidase which demethylates mono- and dimethylated H3K4 and H3K9, has been widely accepted as an attractive target in oncology. Its involvement in cancer has been demonstrated for neuroblastoma, prostate cancer and non small cell lung cancer, besides being overexpressed in hematological malignancies, specifically in acute myeloid leukemia.

The inhibition of this demethylase has demonstrated its efficacy *in vivo* in several haematological and solid tumors, and two irreversible inhibitors, structurally related to tranylcipromine, are currently under clinical trials: ORY-1001 (Oryzon) and GSK2879552 (GSK).

We have developed a potent novel series of tranylcypromine based irreversible KDM1A inhibitors, obtained by versatile and scalable enantioselective synthetic process, which allowed the synthesis of the optically-active trans isomers of the cyclopropane ring.

Biochemical as well as biological characterization of several compounds, including transcriptional effect on KDM1A targets and their efficacy in reducing colony formation in leukemia cells, will be reported.

ADME and pharmacokinetic properties, compatible with an oral in vivo administration, of most interesting compounds will be presented. The *in vivo* activity in a murine promyelocytic leukemia model of selected compounds showed a significant survival increase associated to evidences of target modulation, after oral administration. Moreover, in the attempt to find new reversible KDM1A inhibitors, we conducted a successful HTS campaign, screening 34000 compounds as a subset of our internal chemical collection. The effort led to the identification of 4 chemical series with initial SAR indications. 3D structure of the target with our preferred hit paved the way for an intense effort of hit expansion guided by structure-based drug design, allowing the discovery of nanomolar reversible KDM1A inhibitor.

Design and Synthesis of Dual Inhibitors Targeting MMP2 and CK2: A new approach to antitumor compounds

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In this project, we seek to design, synthesize and evaluate new dual agents based on the inhibition of two enzymes involved in the development, progression and dissemination of cancer processes such as CK2 and MMP2. CK2 is a serine/threonine kinase that constitutes an interesting target for the treatment of cancer due to its favorable cellular environment for tumor progression and maintenance.[1] On the other hand, within the MMPs family, MMP2 plays a prominent role in cancer because it stimulates tumor growth, angiogenesis and metastasis, through its involvement in the degradation of extracellular matrix.[2]

Traditional drug design strategy based on a single target has serious difficulties in developing new therapies for diseases such as cancer. An alternative approach is to design multi-target modulators, directed to different disease mechanisms. Recently, our research group has demonstrated that CK2 is a regulator of the activity of MMP2[3] and, therefore, the use of dual modulators of these two enzymes constitutes an innovative and at the same time viable strategy.

The design of these modulators is based on the previous experience of the group in the design and synthesis of inhibitors of these two targets through a fragment based process.[4] The connection, in most cases, is carried out using a click chemistry reaction, namely the cycloaddition between an alkyne and an azide catalyzed by Cu (II).

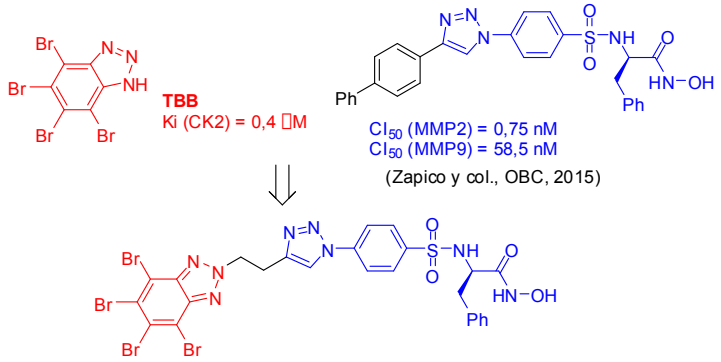

Figure 1. Structure of a dual CK2/MMP2 inhibitor

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POSTERS

Novel CF_3-containing pyrrolidines as amidine-based BACE1 inhibitors

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Alzheimer's disease (AD) is a progressive neurodegenerative disorder considered to be the most common cause of dementia. Nowadays, this devastating disease is estimated to affect 25 million people worldwide and it represents a major public health challenge of growing significance due to the continuous aging of the population.(1)

Since the discovery that BACE1 is essential for processing amyloid precursor protein (APP), this enzyme has become the prime target for the treatment of AD. However, after more than 15 years of research BACE1 has proven to be an exceptionally difficult target, where identifying small-molecule inhibitors which combine good pharmacological and pharmacokinetic properties has been a challenge.(2)

Amidine- or guanidine-containing heterocycles were recently discovered to establish an ideal hydrogen-bonding network with the catalytic dyad of the enzyme. However, control of the basicity of the amidine moiety in a BACE1 inhibitor is essential not only to achieve a suitable interaction with the catalytic aspartates but also to improve central penetration. (3) The pKa of the amidine could be modulated by the introduction of electron withdrawing groups (EWG) in the warhead. Often, fluorine or polyfluoro alkyl groups have been used, as these functional groups also have a marked effect on physicochemical properties of molecules.

Synthesis of substituted pyrrolidines has been reported previously in literature through the Michael addition of preformed imines from different α-amino esters to methyl and trifluoromethyl crotonates. We report the one-pot synthesis of a new family of quaternary fluorinated pyrrolidines starting from unprotected aromatic amino esters using as key synthetic step a tandem Michael addition/cyclization reaction as well as its further derivatization into novel amidine-based BACE1 inhibitors with tunable pKa.

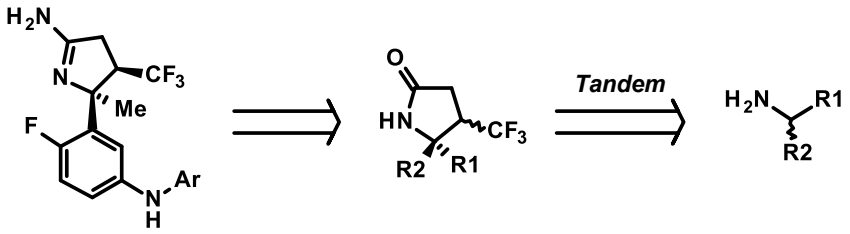

Fig. 1: General structure of the target pyrrolidines derivatized into BACE1 inhibitors

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Synthesis and antimicrobial effects of some new 1,3,4-thiadiazole derivatives

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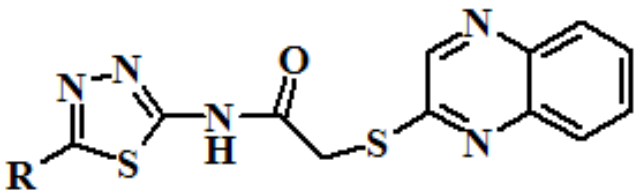
Compounds containing the thiadiazole nucleus exhibit a broad spectrum of biological activity such as antibacterial [1], antifungal [2]. Microbiological importance of thiadiazoles prompted our research group to synthesize a new series of 1,3,4-thiadiazoles derivatives and to investigate their antimicrobial activities. Various 1,3,4-thiadiazole-2-amines (1a) were acetylated by chloroacetyl chloride to obtain N-(substituted-1,3,4-thiadiazole)-2-chloro-acetamide derivatives (2a). Ring closure of ethyl glyoxylate with o-phenylenediamine under microwave irradiation gave quinoxaline-2-one (1b) which was reacted with phosphorus pentasulfide under microwave irradiation to afford quinoxalin-2-thiol (2b). In the final reaction step, the compounds (2a) and (2b) were reacted to obtain N-(substituted-1,3,4-thiadiazole)-2-[(quinoxalin-2-yl)sulfanyl]acetamide (3a) derivatives. Structures of the target compounds were confirmed by spectroscopic methods. Final compounds were tested for antibacterial and antifungal activity. Antifungal activity of the synthesized compounds were better than their antibacterial activity.



R: -H, -CF_3, -CH_3, -C_2H_5, -SCH_3, -SC_2H_5

Figure: Structure of the target compounds

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Synthesis of Novel 4-Substituted-Piperazine-1-Carbodithioic Esters and Investigation of Their Inhibitory Activity against Cholinesterase Enzymes

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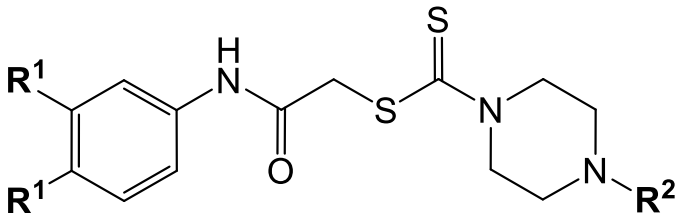
Carbamate derivatives inhibit the cholinesterase enzyme via carbamoylation of the esteratic site. Thus, decrease of enzyme level in brain may be prevented [1]. Dithiocarbamate derivatives can act as cholinesterase inhibitors because of their structural similarity to carbamates. Hence, in the present work some novel 4-substituted-piperazine-1-carbodithioic esters were synthesized and their potency against cholinesterase enzymes were investigated. Structures of the final compounds were elucidated by ^{1}H-NMR, IR, MS spectroscopic methods and elemental analyses. In the series, some the compounds showed significant inhibitory potency.



R^1: -Cl, -F, $-OCH_3$ **R^2:** $-CH_3$, $-C_6H_5$, $-CH_2C_6H_5$

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Photoisomerizable phenylazopyridines enable the control of metabotropic glutamate receptor subtype 5 activity with light

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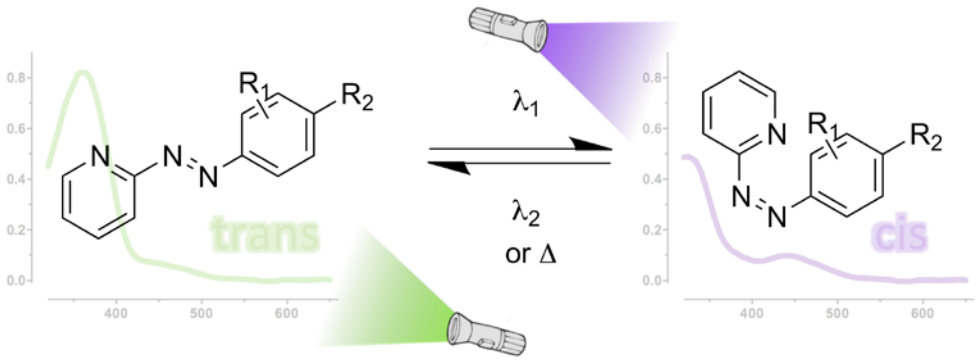
Metabotropic glutamate receptors (mGluRs), are class C GPCRs widely distributed through the CNS and considered pharmacological targets for neurologic disorders. For example, blocking group I mGluRs or activating group II and group III induces antinociceptive and neuroprotective effects[1].

Recently, our group published alloswitch-1 as the first GPCR photoswitchable allosteric modulator with activity in-vivo[2]. Alloswitch-1 is a structurally substituted phenylazopyridine and selectively performed NAM activity in $mGlu_5$ in the *trans*-isomer, whether in the *cis* disposition it was inactive. This behaviour was easily controlled by illumination with 380 nm and 500 nm of wavelength light and was consistent and reversible in cultured cells and native cultures. Moreover, it allowed the possibility to control the natatorial motility native *Xenopus Tropicalis* with light.

We next design an synthesise new related phenylazopyiridnes not only to study the structure - pharmacological activity relationship, but also the efficiency of photoisomerisation form *trans* to *cis* isomers and the rate of thermal relaxing from *cis* to *trans* isomers. Thus, we obtained many compounds with a very similar potency to alloswitch-1, but other with an enhanced potency from 4- to 10-fold shift or with improved photoswitching properties. Moreover, we obtained new active compounds, which required wider wavelength for photoisomerizating, what is less harmful for in-vivo tissues, and we also proved that these phenylazopyridines are more stable in physiologic conditions than common azobenzenes.

Overall, we proved that optopharmacology might be more advantageous than optogenetics, because of the possibility to perform assays with native tissues or in-vivo with no genetic modification and even with a better stability in physiologic conditions.



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Derivatives of aziridine-2-carboxamide and their biological evaluation

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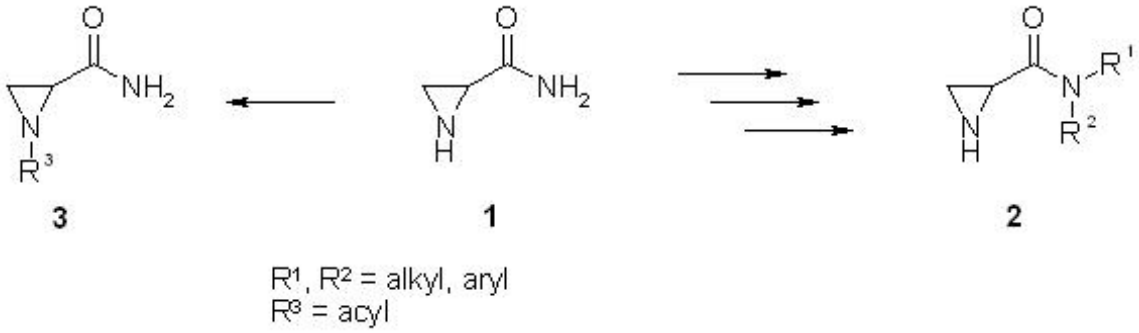
Leakadine (aziridine-2-carboxamide, **1**) is anti-cancer drug which was developed in 1970s in Latvian Institute of Organic Synthesis. [1, 2]

Here we report a synthesis of novel aziridine-2-carboxamide derivatives **2** and **3**.



Synthesized series of compounds **2** and **3** have exhibited high biological activity on several cell lines. The highest cytotoxicity was obtained on the cell line HT-1080 (human lung fibrosarcoma) with best hit of IC_{50} = 11 μg/ml.

The obtained results of structure – activity relationship will allow to design and synthesize more active compounds and will help to obtain new anti-cancer agents.

Acknowledgements:

InnovaBalt
Strengthening the research and innovative capacities of the Latvian Institute of Organic Synthesis

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Chemical modification of an aldolase enzyme Involved in bacterial virulence

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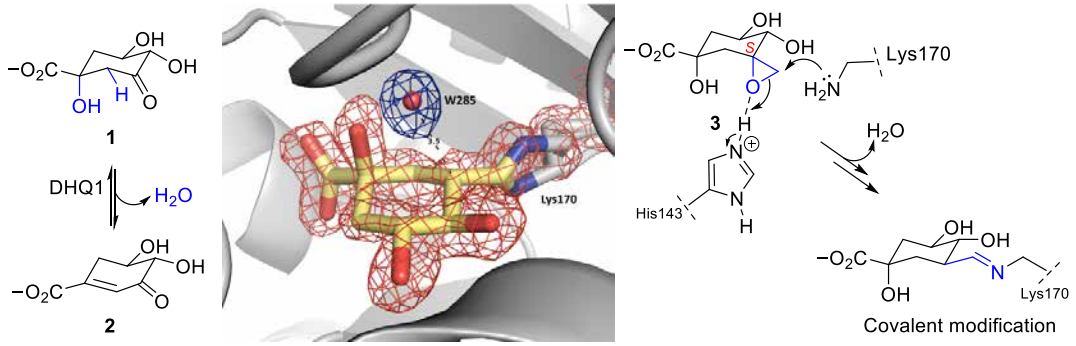
Type I Dehydroquinase (DHQ1), the third enzyme of the shikimic acid pathway, is a class I aldolase enzyme that catalyzes the reversible dehydration of 3-dehydroquinic acid (**1**) to form 3-dehydroshikimic acid (**2**) by multi-step mechanism that involves the formation of Schiff base species. It is present in plants and several bacterial sources, such as *Escherichia coli, Salmonella typhi and Staphylococcus aureus,* but do not have any counterpart in human cells. It has been suggested that DHQ1 may act as a virulence factor *in vivo* as the deletion of the *aroD* gene, which encodes DHQ1, has been proven to afford satisfactory live oral vaccines. This fact has identified DHQ1 as a promising target in the search of new anti-virulence agents to combat widespread antibiotic resistance, which has become one of the most important public health issues of the early 21st century.

We report here the synthesis of several mimetics of the natural substrate that were designed as irreversible inhibitors of the DHQ1 enzyme and to study the binding requirements of the linkage to the enzyme.[1,2] The crystal structure of DHQ1 from *S. typhi* (*St*-DHQ1) covalently modified by epoxide **3**, which was solved at 1.4 Å, revealed that the modified ligand **3** is covalently attached to the essential Lys170 by forming a stable Schiff base after several chemical modifications.[2] Biochemical and Molecular Dynamics simulation studies suggest that the resulting *St*-DHQ1/**3** adduct is obtained by activation of His143 followed by nucleophilic ring opening of the epoxide and subsequent dehydration and isomerization reactions. These studies highlight the huge importance of the conformation of the C3 carbon of the ligand for covalent linkage to this type of aldolase I enzyme, revealed the key role played by the essential His143 as a Lewis acid in this process and show the need of a neatly closed active site for catalysis.[3]



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4-Cyclyl pyrazolo[3,4-d]pyrimidines as sigma-1 receptor antagonists for the treatment of pain

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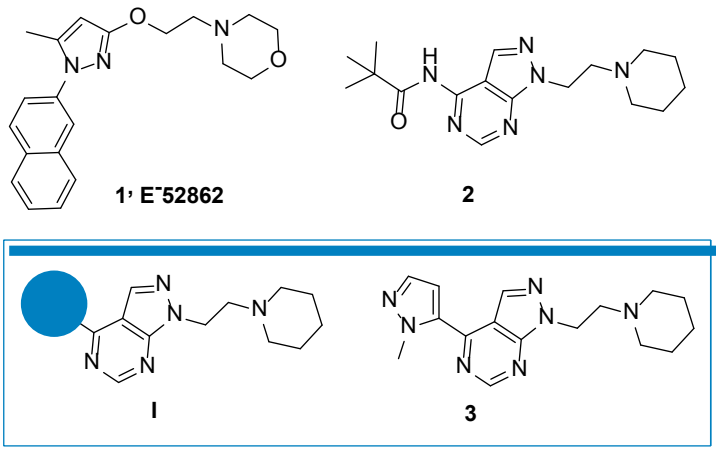
The σR receptor (σR) is a unique molecular chaperone, which modulates the function of different proteins, specially under disease-related stress or mutation conditions. The σR presents at least two different subtypes, σ_1R and σ_2R, which show distinct functions and are related to different potential therapeutic indications. Increasing preclinical evidence support a role for the σ_1R in opioid analgesia modulation and in pain control when central sensitization occurs.[1] Several σ_1R antagonists have shown antinociceptive properties in pain models of different aetiologies and the recent progression of E-52862 (**1**),[2] up to clinical trials for the treatment of different pain conditions will reveal how this relevant preclinical evidence translates into man.

We report here the synthesis and SAR study of a series of 4-cyclyl pyrazolo[3,4-d]pyrimidines (**I**)[3] that were derived from the previously reported 4-acylamino derivatives, represented by **2**.[4] Compounds were prepared using a straightforward two step metal catalyzed process starting from commercially available 1H-pyrazolo[3,4-d]pyrimidine building blocks. Introduction of both aliphatic and aromatic cyclic substituents led to highly active σ_1R ligands, which in general exhibited also affinity for the σ_2R. Phenyl or pyrazole groups were preferred by the σ_1R and only certain substituents in the orto position, combined with adequate Log*P* and dipole moments avoided hERG inhibition. The 4-(2-methylpyrazol-3-yl) derivative, **3**, was the more selective σ_1R ligand, showing as well potent antinociceptive properties in several pain models in mice, indicative of its antagonistic behaviour.



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Pyrazino[1,2-a]indoles as sigma-1 receptor antagonists for the treatment of pain

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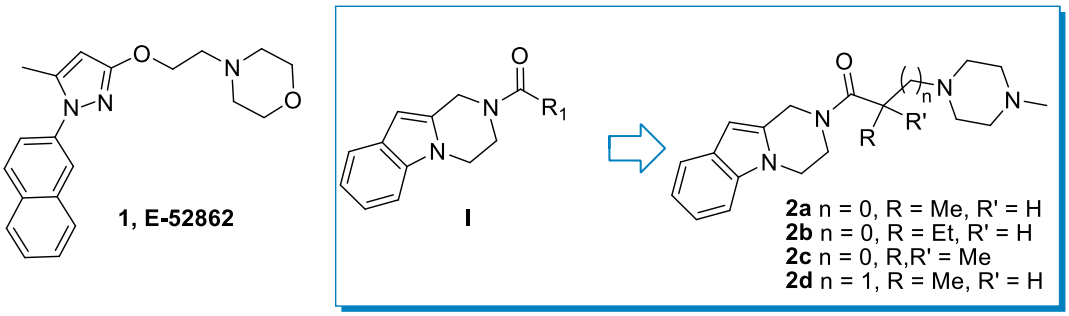
Drug Discovery and Preclinical Development, ESTEVE, Baldiri Reixach, 4-8, E-08028 Barcelona, Spain.

The sigma-1 receptor (σ_1R) is an intracellular chaperone protein, expressed in CNS regions important for pain control. Although it does not have homology to opioid receptors, it modulates opioid analgesia and studies in rodents show its clear role in pain control when central sensitization occurs.[1] The recent progression of E-52862 (**1**),[2] a selective σ_1R antagonist, up to clinical trials for the treatment of different pain conditions will reveal how this relevant preclinical evidence translates into man.

We report here the synthesis and SAR study of a series of pyrazino[1,2-a]indoles (**I**)[3] that were prepared using a two-step procedure starting from commercially available 1,2,3,4-tetrahydropyrazino[1,2-a] indoles. This scaffold was seen as one of the hydrophobic regions required by the σ_1R, while the positive ionizable group also necessary for having σ_1R affinity was introduced in the R_1 substituent. Several groups containing a basic nitrogen atom at different distances of the amide group and with different substitution patterns were explored. Many of them provided good affinity for the σ_1R but much more challenging was obtaining compounds with selectivity versus the σ_2 receptor, an isoform with a distinct pharmacological role. In order to have compounds with reduced lipophilicity, a well known factor to affect promiscuity and in vitro ADMET properties, the introduction of additional polar atoms in R_1 was explored. It was shown that a methylated nitrogen atom was tolerated, as long as alkyl groups were present in the vicinity of the amide group. Compounds **2a-d** were identified as some of the most interesting derivatives in the series, showing good affinity for the σ_1R, good selectivity versus the σ_2 and other receptors and potent antinociceptive properties in several pain models in mice, which indicates that they act as σ_1R antagonists.



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Acknowledgements: This research was supported by CDTI (PROJECT IDI-20110577).

Discovery of novel indenylethanamines as potent and selective SIGMA-1 ligands

Mesquida, Neus (1); Alcalde, Ermitas (1); Paloma, Laura (1); Almansa, Carmen (2); Diaz, José Luis (2); Dordal, Albert (2)

Universitat de Barcelona (1); ESTEVE (2)

The sigma-1 receptor (σ_1R), which modulates the function of multiple ion channels and receptors, is a ligand-regulated molecular chaperone protein widely distributed in the central nervous system (CNS) and peripheral tissues. In particular, preclinical evidence strongly supports a role for σ_1R antagonists in the treatment of pain of different origins, including neuropathic pain, and as opioid adjuvant therapy.[1]

Our research on indenylsulfonamides **1** was originally part of a project aimed at the finding of (*Z*)-stilbenes with biological effects on the CNS.[2] Continuing this research, 5-HT_6 serotonin receptor ligands were converted into σ_1R ligands by moving the position of the *N*-arylsulfonyl moiety, transforming **1**-type indenes to their isomeric counterparts **2**.

Herein, we describe the development of compounds **2** to (7-arylinden-3-yl)ethanamines **3** and (7-heteroarylinden-3-yl)ethanamines **4**, that act as σ_1R ligands with potency in the nanomolar range and show good σ_1R/σ_2R selectivity ratios. The indenylethanamines **3** and **4** were prepared following different two-step synthetic procedures from commercial bromoindanones.[3]

Acknowledgements: This research was supported by ESTEVE, Barcelona (Spain), through projects FBG2010-305952 and FBG2011-306458 *Fundació Bosch i Gimpera-Universitat de Barcelona*, Spain. Thanks are also due to the AGAUR (*Generalitat de Catalunya*) *Grup de Recerca Consolidat* 2014SGR1655.

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Synthesis, antimicrobial, and anti-inflammatory activity, of novel S-substituted and *N*-substituted 5-(1-adamantyl)-1,2,4-triazole-3-thiols

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Introduction

The incorporation of an adamantyl moiety into several molecules results in compounds with relatively high lipophilicity, which in turn can modify the biological availability of these molecules. In almost all cases, an adamantyl-bearing compound will be more lipophilic than the des-adamantyl analog. Beyond increasing the partition coefficient, the adamantyl group positively modulates the therapeutic index of many experimental compounds through a variety of mechanisms. In addition, several 1,2,4-triazoles and their *N*-Mannich bases were reported to possess potent antimicrobial and anti-inflammatory activities. As a continuation of our interest in the chemical and pharmacological properties of adamantane derivatives we now report herein on the synthesis and antimicrobial and anti-inflammatory activity of a new series of *S*-substituted and *N*-substituted-5-(1-adamantyl)-1,2,4-triazole-3-thiol derivatives. The reaction of 5-(1-adamantyl)-4-phenyl-1,2,4-triazoline-3-thione (compound 5) with formaldehyde and 1-substituted piperazines yielded the corresponding *N*-Mannich bases 6a–f. The reaction of 5-(1-adamantyl)-4-methyl-1,2,4-triazoline-3-thione 8 with various 2-aminoethyl chloride yielded separable mixtures of the *S*-(2-aminoethyl) 9a–d and the *N*-(2-aminoethyl) 10a–d derivatives. The reaction of compound 5 with 1-bromo-2-methoxyethane, various aryl methyl halides, and ethyl bromoacetate solely yielded the *S*-substituted products 11, 12a–d, and 13. The new compounds were tested for activity against a panel of Gram-positive and Gram-negative bacteria and the pathogenic fungus *Candida albicans*. Compounds 6b, 6c, 6d, 6e, 6f, 10b, 10c, 10d, 12c, 12d, 12e, 13, and 14 displayed potent antibacterial activity. Meanwhile, compounds 13 and 14 produced good dose-dependent anti-inflammatory activity against carrageenan-induced paw edema in rats.

SAR of indole isocombretastatin analogues

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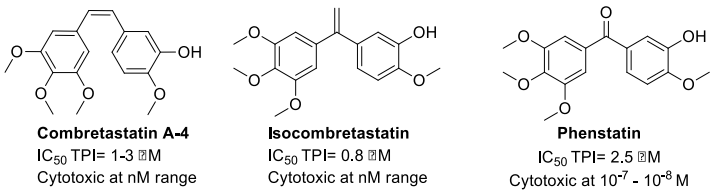
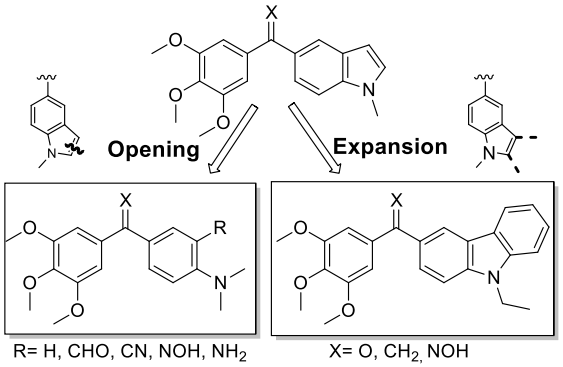
Antimitotic agents prevent right mitotic spindle formation, which leads to cell cycle arrest and, subsequently, to cell death. They act by interfering tubulin polymerization-depolymerization dynamics[1]. While Combretastatin A-4[2] is the reference ligand of tubulin inhibitors that bind to colchicine site, phenstatins and isocombretastatins are related families that have shown promising activity profiles and avoid *Z/E* isomerization, one of the main drawbacks showed by these potential drugs.[3]



While the trimethoxyphenyl ring is almost essential to keep cytotoxicity, the guaiacol ring can be substituted by different aromatic moieties being *N*-methyl indole derivatives among the most potent analogues.[4]

In this work we have explored the effect on the activity when the indole ring is replaced by two structurally related aromatic rings: a carbazole moiety (an expanded indole) or a dimethylaminophenyl ring (formally, a seco-indole).



Acknowledgments:-. Financial support came from Junta de Castilla y León (SA147U13), University of Salamanca (Plan Propio de Apoyo a la Investigación 2013-Ref. KAC8), Spanish AECID (PCI-Mediterráneo A1/037364/11) and the EU (Structural Funds)

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Synthesis and evaluation of new pyrazole-based carboxamides as modulators of the TRPV1 channel

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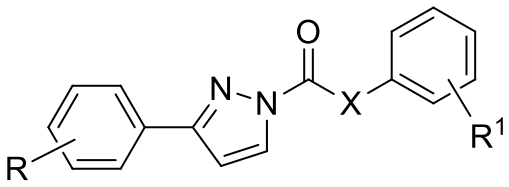
Since the cloning of the vanilloid receptor protein (TRPV1) by Caterina et al.,[1] this ligand gated nonselective cation channel has attracted growing interest due to its function associated with nociception signaling pathway. TRPV1 is activated by vanilloid ligands such as capsaicin and resiniferatoxin, noxious heat (> 42 °C), protons (extracellular pH < 6), and modulated by a variety of endogenous ligands including cannabinoid anandamide and arachidonic acid metabolites. TRPV1 activation enhances Ca^{2+} permeability that leads to an increase in intracellular Ca^{2+}, resulting in excitation of primary sensory neurons and the central perception of pain. Activation is followed by the desensitization making the C-fiber sensory neurons unresponsive to TRPV1 agonists and other inflammatory mediators. In addition, capsazepine, the first reported antagonist of TRPV1, blocks the capsaicin-induced uptake of Ca^{2+} in cell-based assays. In vivo, capsazepine demonstrates species-dependent efficacies in various models of inflammatory hyperalgesia and chronic pain. [2] Therefore, both desensitization by an agonist and the direct blockade by an antagonist of TRPV1 might have therapeutic utility in the management of acute and chronic nociceptive pain. [3] Recently, a variety of TRPV1 ligands bearing a heterocyclic linker has been reported to show very interesting both *in vitro* and *in vivo* biological profiles. Based on these findings, we designed a series of pyrazole-based carboxamide analogues (Figure 1), assuming that the rigid heterocyclic linkage can induce more appropriate conformation for TRPV1 binding.



$X = CH_2, CH(CH_3), NHCH_2$

Figure 1

In this communication, we describe the synthesis and activities of a series of these ligands, which were screened by a cell-based assay utilizing the Ca^{2+} permeability of the TRPV1 channel.

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A versatile dihydroceramide desaturase substrate to monitor enzyme activity

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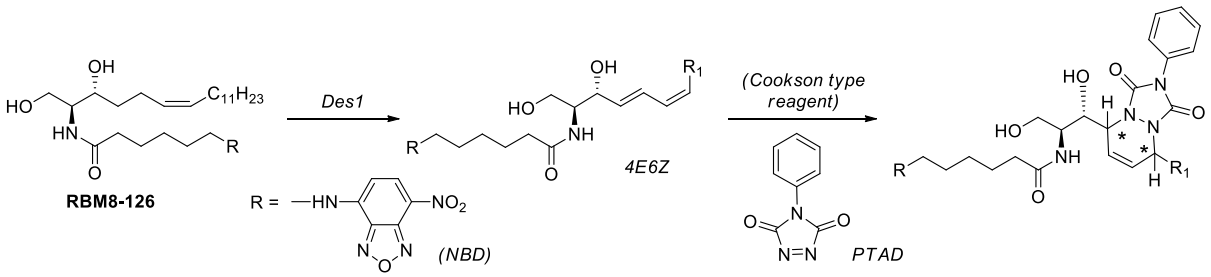
Over the past decade, sphingolipids have emerged as a new class of modulators of various cell functions. Ceramide, which is the central molecule in the biosynthesis of sphingolipids and glycosphingolipids, is involved in the regulation of different cellular events, including cell senescence, differentiation, and apoptosis.[1] In the *de novo pathway*, ceramide is biosynthesized from L-serine in four steps, the last one being the Δ^4-desaturation of dihydroceramide to ceramide by the action of dihydroceramide desaturase (*Des1*).

The discovery of new Des1 inhibitors with improved potency and selectivity would be greatly accelerated with the help of high-throughput screening (HTS) assays. Toward this end, the design of an efficient probe to quantify the enzyme activity is capital. In this Communication, we wish to report that (*Z*)Δ^6ceramide RBM8126 is a suitable Des1 substrate and that the resulting diene product can be efficiently trapped in a DielsAlder reaction with PTAD, a Cooksontype reagent [2] (Scheme 1). In addition, the unanticipated observation that the isomeric (*E*)Δ^6ceramide is a poorer Des1 substrate provides valuable information about the conformational requirements of the enzyme active site. Current efforts addressed at the adaptation of this process for the development of a HTS assay by anchoring the substrate to a solid support and the use of a fluorescent Cooksontype reagent will be presented.



Scheme 1. Desaturation of **RBM8126** by Des1 and DielsAlder reaction of the resulting diene with a Cookson type reagent

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On the irreversibility of sphingosine 1-phospahte lyase inhibition by 2-vinyldihydrosphingosine 1-phosphate stereoisomers

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Sphingosine-1-phosphate (S1P) is involved in many fundamental biological processes like proliferation, mitogenesis, inflammation, migration, angiogenesis and protection from apoptosis. Sphingosine-1-phosphate lyase (S1PL) catalyzes the ultimate step in sphingolipid's breakdown, converting phosphorylated long chain bases, like S1P, into ethanolamine phosphate and a fatty aldehyde. (1).

Our main objective is to demonstrate that chemical modulation of S1PL activity could provide the basis for future pharmacological interventions to improve functionality of damaged nervous system by increasing the S1P intracellular levels. Towards this end, we undertook the enantioselective synthesis of the four stereoisomers of the mechanism-based inhibitor 2vinylsphinganine-1-phosphate (2VS1P, Figure 1), which is reported in the literature as a mixture of isomers.(2).

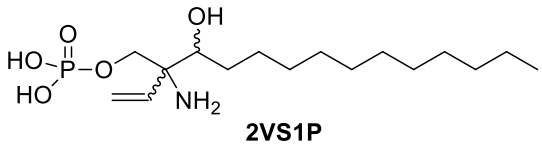

Figure.1. Mixture of isomers of 2-vinylsphinganine-1-phosphate

Based on mechanistic considerations, it is conceivable that the presence of the vinyl group gives rise to a highly reactive electrophilic intermediate able to form an irreversible covalent bond by Michael-type reaction with a nucleophilic residue close to the enzyme active site. This new bond formation would account for the irreversible inhibition expected for this family of compounds (Figure 2). This mechanistic aspect was not described in the above work and will be presented in this Communication, as well as the stereoselectivity of the enzyme inhibition by the four **2VS1P** stereoisomers.

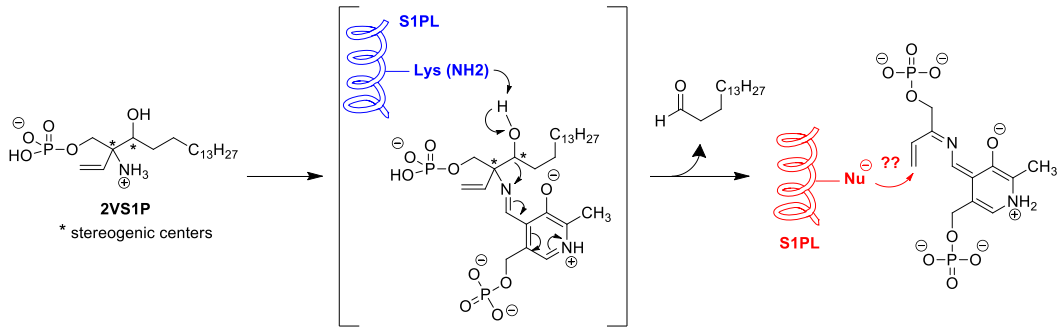

Figure. 2. Postulated irreversible inhibition of S1PL mechanism by 2VS1P.

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Design and synthesis of new sphingosine-1-phosphate lyase (S1PL) inhibitors

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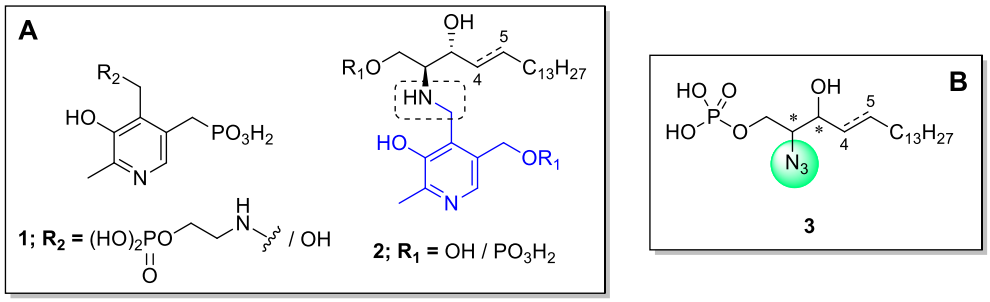
Accumulating evidence indicates that sphingosine-1-phosphate (S1P) modulates many noxious processes that follow CNS injury. Thus, the discovery of novel neuroprotective therapies may arise from interventions on S1P metabolism.[1] S1PL is a PLP-dependent enzyme that irreversibly cleaves S1P into 2-*trans*-hexadecenal and phosphoethanolamine (EAP).[2] Despite the capital role of S1PL in controlling the intracellular levels of S1P, the number of available pharmacological tools to modulate its activity is scarce.[3]major therapeutic treatments have relied on agents requiring injection delivery. In September 2010, fingolimod/FTY720 (Gilenya, Novartis

Herein we describe the synthesis and evaluation of a new series of mechanism-based S1PL inhibitors. Two families of compounds were designed taking into account two different strategies: First, a series of non-hydrolizable analogues of the S1P/EAP-PLP intermediate aldimines was considered. Additionally, the presence/absence of the phosphate groups in both the sphingoid and the cofactor moieties were evaluated in terms of biological activity (Scheme 1, A).

In a second approach, a series of azido-S1P derivatives were envisaged as suitable nonreactive, potential S1P competitive inhibitors (compounds 3, Scheme 1, B). Since the C2-amino group is replaced by an azide group, aldimine formation with the cofactor (PLP) is precluded and, therefore, analogs 3 should not be degraded after reaching the enzyme active site. In this sense, the stereoselective synthesis of azidosphingosine-1-phosphate ($SoPN_3$) and 4,5-dehydroazidosphingosine-1-phosphate ($SaPN_3$) stereoisomers is reported in this work.



Scheme 1. General structure of new S1PL inhibitors

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PPV dendrimers as antibacterial agents

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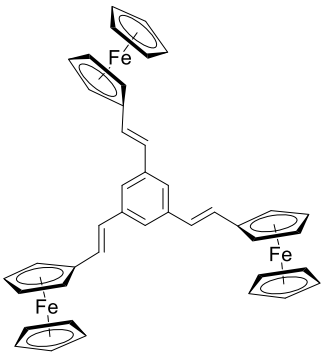
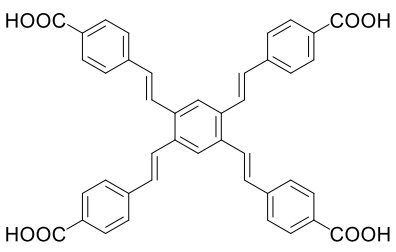
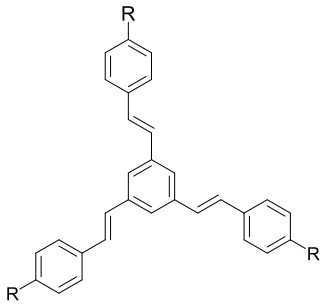
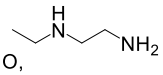
The common use of antibiotics, even for viral infection where they have no effect, causes a rise in the resistance among disease-cause microorganism. Therefore, there is a significant global need for specific new antibacterials that may stablish a relationship between activity and structure and shed light on the mechanisms of action. The design of well defined structures will help in this goal and dendrimers appear as low polydisperse and highly branched polymers. Antibiotic polyvalent dendrimers has been described bearing β-lactams (1), ferrocene, quaternary ammonium, boron complexes, carbohydrates, carboxylic acids, and peptides at their peripheries (2). But dendrimers have also very flexible arms and, consequently, many geometries and conformations difficult the correlation between structure and activity. Low generation of flat and rigid dendrimers as poly(phenylenevinylenes) (PPVs) distribute the active group in specific positions and geometrics. Here we present different PPVs decorated with ferrocene, amines, quaternary ammonium, or carboxylic acids, in order to evaluate their antibacterial activity against *escherichia coli* (Gram-negative) and *enterococcus faecalis* (Gram-positive) as well as their cytotoxicity with COS-1 y Vero cell lines. The compound were prepared with high yields through Pd-free strategy (3) and the results show that either geometry, charge or basicity are significant for bactericidal properties without compromising the cytotoxicity.

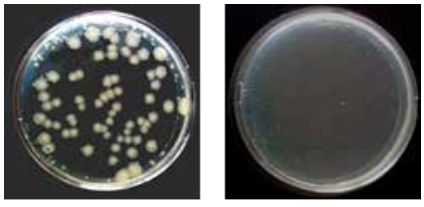






R = H, NMe_2, COOH, OC_6H_{12}, N^+Me_3, CHO,





control 10μM

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Synthesis of fluorescent pentaene-sphingolipid probes

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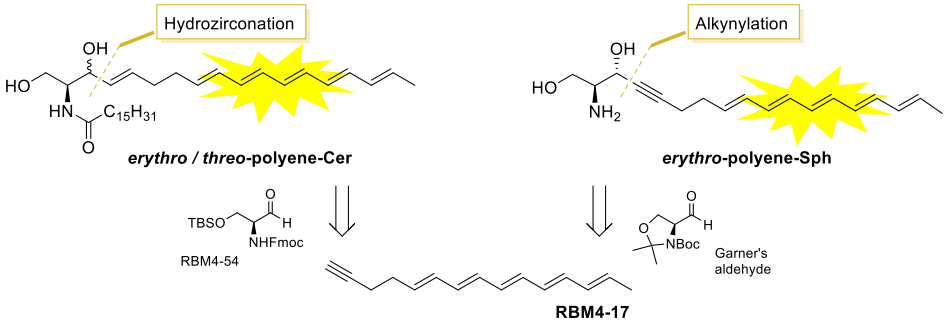
One of the ultimate goals in biomedicine is to understand the relationship between structure, function, and dynamics of biomolecules in the living cells. A wide array of biophysical tools has been designed to gain more insight into metabolism, trafficking and interaction of the sphingolipids (SLs). These techniques require the use of high molar concentrations of fluorescence lipid analogues, labelled with bulky chromophores, such as BODIPY or NBD, resulting in altered biophysical properties of the cell membrane[1].

In the present study, we synthesize novel intrinsically-fluorescent sphingosine (Sph) and ceramide (Cer) probes labelled with five conjugated double bonds, which faithfully mimic the natural counterparts[2] and are suitable for fluorescence detection[3]. Interestingly, the pentaene moiety has been introduced in the sphingoid base backbond for the first time, providing new tools to study the distribution of this type of SLs.

Herein, we describe the synthesis of the new polyene alkyne RBM4-17, as a versatile scaffold for our fluorescent target SLs analogues. Silver perchlorate[4] catalysed hydrozirconation of RBM4-17 with the previously unreported aldehyde RBM4-54, followed by one-pot deprotection and *N*acylation, afforded *threo*-polyene-Cer (Scheme 1). By changing the hydrozirconation catalyst to $ZnCl_2$,[5] access to the natural *erythro* configuration is also possible. Furthermore, an alkyne containing *erythro*-polyene-Sph probe is also accessible by alkynylation of Garner's aldehyde[6]17 and 18, respectively. The erythro-isomer 17 is formed by the addition to 10 of lithium pentadecyne 16 in THF/HMPT at -78\u00ba, whereas the corresponding threo-isomer 18 is produced in the presence of ZnBr2 in Et2O. Deprotection of the acetal moiety afforded 1,3-diols 19 and 20. These diols were selectively reduced with Red-Al to the (E with RBM4-17, followed by deprotection under mild acidic conditions (Scheme 1).



Scheme 1: Retrosynthesis of polyene-SLs analogues

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Design, synthesis and biological activity of new K-RAS inhibitors

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Introduction

K-Ras is considered to be the most commonly mutated oncogene in human cancers. In general, cancers with Ras mutations are aggressive with poor prognosis and no good response to standard treatment. Now there are not yet available K-Ras effective inhibitors and it is widely believed that the K-Ras protein can be undruggable.[1]

Ras protein plays an important role in several pathways involved in normal cell growth.[2] The three isoforms H-Ras, N-Ras and K-Ras were identified for their oncogenic activation in several human tumors.[3]

Objectives

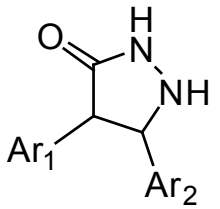
The main objectives of this work are the design of new K-Ras inhibitors and the development of new methodologies for the synthetic preparation of these disubstituted pyrazolidin-3-ones.

Methodology

Classical methodologies of organic synthesis were applied for the preparation of a series of compounds.

Results and Conclusions

A series of pyrazolidin-3-ones possessing different substituents has been prepared by a new synthetic route. The K-Ras inhibition of the synthesized compounds has been carried out on four different colon cancer cell lines (HCT K-RasSL, RKO KRasSL, COLO 320 K-RasSL and SNU-C1 K-RasSL) in three different concentrations. The activities of the prepared compounds were compared to the Lilly Lead compound. The anti-angiogenesis activity was also evaluated. The results of this synthesis and those of the biological activity will appear and be discussed in the communication.



Ar = Aromatic system

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Design, synthesis and anti-inflammatory activities of new methylsulfones

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Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) are a heterogeneous class including several structures widely used to alleviate inflammatory and pains associated with different pathological conditions. The NSAIDs are among the most widely prescribed drugs in the world. Several studies have indicated that the COX inhibitors may prevent colon cancer,[1] and its mechanism of action is not understood. However, recent literature has indicated that apoptosis and angiogenesis are involved in this chemopreventive action. *Ibuprofen* inhibits cell proliferation *in vitro* in human cell lines (HT-29) and also potentiates the antitumor activity of other agents such as *5-fluorouracile*.[2] *Sulindac* and *celecoxib* inhibit the growth of adenomatous polyps of different patients. The development of safe and effective anti-inflammatory and antitumor agents is complicated.[3]

Objectives

In this work, we designed a series of new compounds from classical NSAIDs, the acetic or propionic acid group was changed to methysulfone, a more lipophilic group with acidic properties. Molecular modelling has been used to study the influence of substituents.

Results and Discussion

Several compounds have been prepared and new routes for the synthesis were established. Compounds **2**, **3**, **4** and **7** showed significant anti-inflammatory response in comparison with control. The anti-inflammatory activities of these compounds were comparable to that of classical drugs (NSAIDs). The results revealed that the arylmethylsulfone scaffold can be determined as a new pharmacophore.

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Design, synthesis, cell growth inhibition and antitumor screening of new 6,9-disubstituted purines by inhibition of CDK4/6

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Keywords: CDKs, purines-disubstituted, cross-coupling, cell proliferation.

Introduction

DNA biosynthesis involves the formation of the nucleic acid bases followed by the addition of sugar and phosphorylation, leading to the formation of the nucleoside and nucleotide respectively.[1] In this biosynthetic process several enzymes take place such as polymerases and topoisomerases. The activity of several purines considered analogues of purine bases (6-mercaptopurine, 6-thioguanine) against cancer diseases such as leukemia and sarcoma lends support to the view that purines-derivatives might be expected to be useful against divers neoplastic diseases and as considered as being worthy of the broadest range of modification.[2] Recently, purine-derivatives have been involved in the inhibition of cyclin-dependent kinases (CDKs), which set the cell signaling pathways in motion.

Objectives

We are interested in the compounds that interfere with key enzymes of the phase I of cell cycle such as CDK4/6 by allosteric mechanism.[3] General CDKs act during different phases of the cell cycle.

Method/Design

The functionalization of the 6- and 9- positions of the purine nucleus, introduction of pyrazine at C-6 and arylation of *N*-9 position were among the structural modifications carried out. Organic Chemistry classical methods are used for the preparation of these substituted purines. Also cross-coupling optimized conditions have been useful for the *N*-arylation of purines. Finally the antitumor activity of the synthesized compounds was evaluated using MTT assay.[4] Some compounds have demonstrated great inhibition of the growth of tumor cells. Molecular Modeling studies were applied for the design and selection of structures to synthesize.

Results and Conclusions

Several compounds have been prepared and the design, synthesis and the biological activities should be discussed in the presentation of this research work.

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Synthesis and biological evaluation against nitric oxide synthase of new 4,5-dihydro-*1H*-pyrazole-1-carboxamide or carbothioamide derivatives

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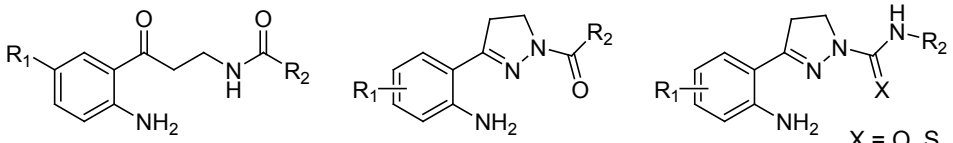
Nitric Oxide (NO) is a significant mediator in cellular signaling pathways.[1] NO is synthesized from the catalysis of *L*-arginine to *L*-citrulline by three isoenzymes of nitric oxide synthase (NOS): neuronal NOS (nNOS) which is required for normal neuronal signaling, endothelial NOS (eNOS) which regulates blood pressure and flow, and inducible NOS (iNOS) involved in immune system activation.[2] In general the cell is protected from NO's toxic effect; however, overstimulation of NO by the individual NOS isoforms, such as nNOS and/or iNOS, plays an important role in many disorders including septic shock, stroke, neurodegenerative disorders (Parkinson, Alzheimer), pain (migraine, chronic tension-type headache, visceral and neuropathic pain), arthritis, diabetes, and ischemia-reperfusion injury.[3] Therefore, the use of substances with inhibitory properties toward the different NOS isoforms has great therapeutic potential for the treatment of the above-mentioned diseases. Consequently, over the last twenty years the design and synthesis of NOS inhibitors has received much attention.



Previously we have published nNOS and iNOS inhibitors structurally related to the main brain metabolite of melatonine, the *N*-acetyl-5-methoxy-kynurenamine **1** ($R_1 = OCH_3$, $R_2 = CH_3$)[4] and we have synthesized several families of compounds, including the 4,5-dihydro-1*H*-pyrazoles **2**.[5,6]

These molecules behave like rigid analogues of the kynurenamines **1**. Basing on these precedents, and looking for new NOS inhibitors with structural relation to L-Arg, in this research project, we have synthesized a new family of derivatives, represented by the general structure **3**, where the acyl group in *N*-1 of the heterocyclic ring has been substituted for a carboxamide or carbothioamide residue, in order to find new compounds that could produce inhibition by competition with the natural substrate of NOS.

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Benzoxazinones and correlated scaffolds linked to benzylamino moieties through a butylene spacer. New sigma receptor ligands

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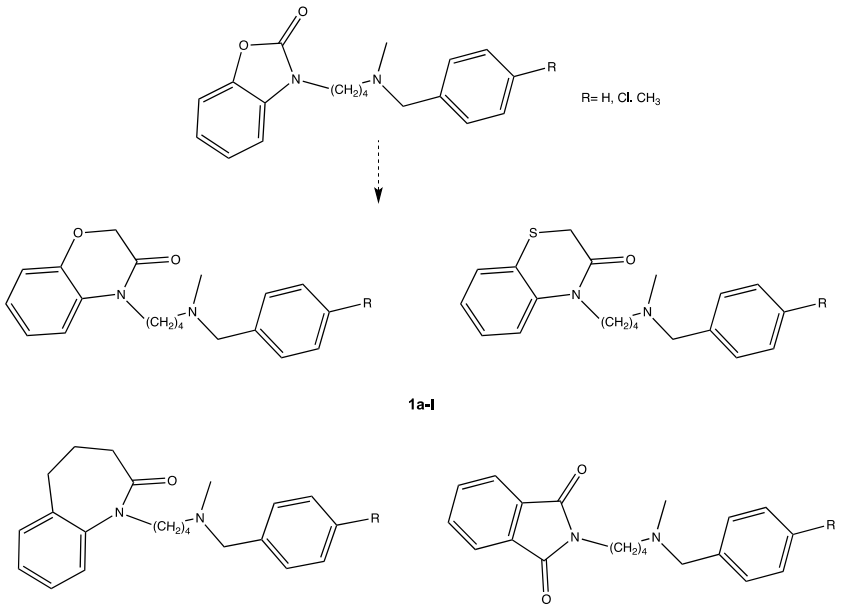
On the basis of some substituted benzoxazolones derivatives previously synthesized by us [1] and gifted with high activity toward s_1 receptor subtype (best $Kis_1 = 2.6$ nM), we have synthesized some new compounds replacing the benzoxazolone moiety with other scaffolds such as benzoxazinone, benzothiazinone, benzoazepinone, indolin-1,3-dione, (**1a-l**) in order to establish the influence of the modified moiety on s_1 receptor affinity of the corresponding compounds and their selectivity over s_2 receptor subtype.



Except for the indolin-2,3-dione series, all the tested compounds showed similar values of s_1 receptor affinities (Kis_1 range: 5.0-30 nM) and moderate selectivity (best Kis_2/Kis_1 ratio of 28. To the other hand, the indolin-2,3-dione series showed a very low σ_1 affinity (844 and >3000 nM), but a good s_2 affinity (best $Ki\sigma_2 = 42$ nM) and high selectivity versus σ_2 receptor subtype ($Ki\sigma_1/Ki\sigma_2$ ratio of >72). The additional carbonyl group, present on the indoline-2,3-dione scaffold, seems to lead the affinity and selectivity against the s_2 receptor subtype, while the minimal modification in the benzoxazinone moiety drive to compounds gifted with similar affinity/selectivity against σ receptor subtype.

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Rational design and synthesis of thioridazine analogues as enhancers of the antituberculosis therapy

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Tuberculosis (TB), brought by *M. tuberculosis* (Mtb) is one of deadliest diseases that the human kind have faced throughout the centuries, and the toll of deaths caused by this disease is still remarkable. In the last decades with the emergence of resistant strains, the world scenario became unbearable.[1] Two main hurdles characterize TB infection: drug-resistance and the presence of persistent strains, that extend the duration of the therapy and make it more difficult to eradicate. Although the hundreds of compounds disclosed, with good in vitro activity against drug-resistant and drug-susceptible Mtb, a different idea is needed to face the disease. Efflux pumps are known to be important means to modulate antibiotic resistance for many bacteria, and for Mtb in particular. Moreover efflux system are part of the innate mechanism of antibiotics resistance and their contribute is crucial in conferring high-level of resistance.[2] It has been demonstrated that with the inhibition of efflux pumps we are able to restore the activity of different antibiotics toward which the bacteria had become resistant. Therefore, inhibition of efflux pumps may help in preventing the raise of resistances, in containing its spread, and in re-establish obsolete therapeutic options. Thioridazine (TZ), an old neuroleptic, by virtue of its capability to affect efflux system of macrophage, acts as inhibitors of bacteria efflux pumps but it can also cure resistant TB when administered in combination with other antitubercular agents on compassionate basis.[3][4] However, TZ, normally administered for schizophrenia, shows general toxicity at the anti-TB therapeutic doses. We report a series of TZ analogues as inhibitors of the mycobacterial efflux pumps. We were pleased to notice that one derivative has lower cytotoxicity than TZ and it was able to enhance up to 64 times the activity of RIF in the combination assay.

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Novel epigenetic enzyme inhibitors for EHMT1/2

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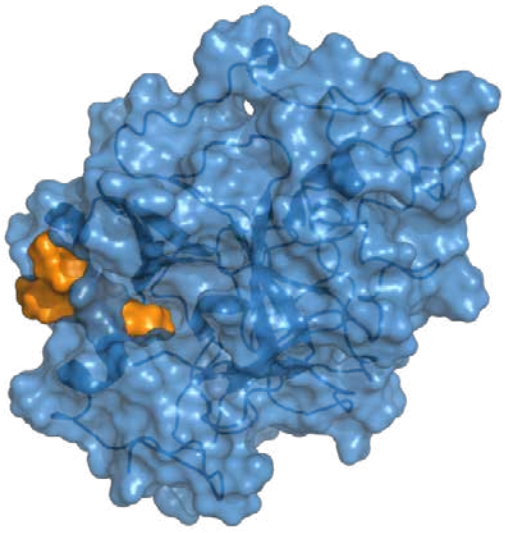
Despite the fact that all cells in an organism contain the same genetic code, the specific local and temporal expression of genes is regulated by posttranslational modifications on the DNA itself, and on the N-terminal tails of histone proteins that constitute the nucleosomes both, in normal cellular phenotypes but also in the development of human disease states.

Within the target landscape, the functional constituents of the epigenetic control machinery can be categorized into enzymes that covalently modify the DNA or, more predominantly, the N-termini of histone proteins by adding (epi-writers) or removing (epi-erasers) posttranslational marks to or from selected amino acid side chains. In addition to the enzymes, a broad range of receptor domains exists that recognize (epi-readers) the respective modification state of the affected side chain residues in a specific manner. The spectrum of posttranslational modifications ranges from conjugation of entire proteins over phosphate- or acetyl-groups to very small alterations such as adding or removing a single methyl-group to e.g. a lysine or arginine residue, thus controlling gene expression in a tight and accurate way.

HKMTs, histone-lysine(K)-methyl-transferases belong to the protein family of epi-writers and currently counts 96 family members. As HKMTs are still a relatively new target class, only a few inhibitors are currently known. These inhibitors either address the cofactor S-adenosyl-L-methionine (SAM) site or the substrate (histone) site. Since HKMT inhibitors are believed to become highly relevant e.g. as personalized cancer therapeutics, new strategies towards novel HKMT inhibitors are urgently needed.



Here we report on the concept of designing novel EHMT1/2-directed scaffolds that qualify as core structures addressing the histone binding site, and as such interfere in a protein-protein interaction.

REZI20-037-1 docked in EHMT1 (IC50=70nM)

CDK8 inhibitors with long residence time emerging from a retro-design approach: *new opportunities for cancer treatment*

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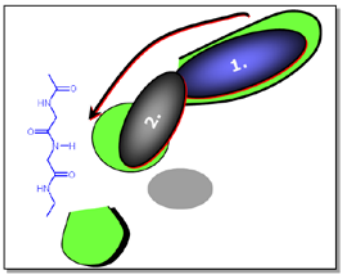
Frequent up-regulation of CDK8 has recently been described for colon cancer, gastric cancer, and melanoma, rendering CDK8 as an attractive target for the development of selective and efficacious inhibitors. This notion is strongly supported by the observation that inhibition of CDK8 by RNA-interference results in profound inhibition of in vivo tumor growth.

Based on the recent findings that in contrast to all other CDK family members, CDK8 is amenable to a type II inhibition mode, we set out to design selective CDK8 inhibitors pursuing a privileged structure-based target family-centric library approach. The employed privileged structures are tailor-made for disrupting the hydrophobic R spine within the N-terminal lobe of a kinase, thereby leading to an induced-fit mechanism of derived inhibitors that will exhibit a pre-engineered binding kinetic signature. This "Retro-Design" approach allows keeping the molecular complexity of emerging inhibitors at a minimum level since the seed scaffold is targeted towards the deep pocket of the conformationally rearranged binding site of the enzyme.

Here we report on the discovery and optimization of a new class of CDK8 inhibitors based on an IP-free scaffold. Frontrunner compounds exhibit excellent biochemical inhibition data on CDK8 and a high cellular efficacy in a variety of mechanism-of-action models as well as phenotypic models such as inhibition of anchorage-independent cell growth. The most advanced compound, MC085 show superior selectivity over a huge panel of kinases when compared to market approved drugs such as Sorafenib or Lapatinib. This selectivity is attributed to the distinct inhibition mechanism which is corroborated by detailed binding kinetic studies which reveal residence times for MC085 in the range of several hours.



Retro-Design Approach: B2F (back-to-front)
- Sets out with ***scaffolds*** rather than leads
- Disrupt hydrophobic spine
- Long residence time on target; slow k_{off}
- Option for exploration of novel IP space
- Option for increased selectivity

Compound	CDK8/CycC Biochem IC_{50} [nM]	HCT-116 IC_{50} [nM]	DLD-1 IC_{50} [nM]
MC-085	16	470	760
MC-094	5	31	34
MC-095	3	51	61
MC-096	5	8	11
MC-097	3	43	43

Figure1: Schematic presentation of the "Retro-Design" approach (left), biochemical and cellular activity for the frontrunners (right).

New sulfonamides with amido substituents targeting tubulin and bromodomains

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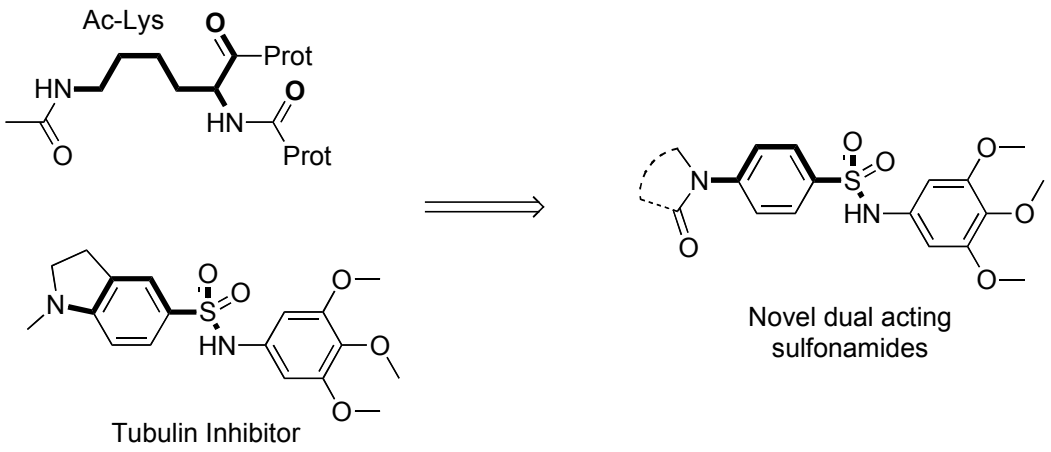
Tubulin is a well established anticancer target. Compounds binding at the colchicine site of tubulin are therefore being actively explored as cytotoxic and vascular disrupting agents.

Recently, bromodomains and extraterminal domain inhibitors (BET) recognizing acetylated lysins in histons have shown interesting antitumor activities.

Based on the structure of antimitotic sulfonamides binding tubulin at the colchicine site and the structure of acetyl lysines, we have designed and synthesized novel hybrid sulfonamides aimed at both targets.



The synthesis and preliminary biological evaluation results of the synthesized compounds will be presented and with the results of molecular docking experiments.

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Novel coumarin-based agents for use in multi-targeting neurodegenerative diseases' therapy

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Neurodegenerative disease (ND) is an umbrella term for a range of conditions that primarily affect the neurons in the human brain. ND are still incurable and debilitating conditions that result in progressive degeneration and/or death of nerve cells. These pathologies cause problems with movement (called ataxias), or mental functioning (called dementias). Alzheimer's and Parkinson's diseases are the most prevalent ND, being considered disorders of multifactorial origin, inevitably progressive and having a long preclinical period. In the last years, the therapeutic alternatives have been focus on the *multitargeting* strategies.[1-3] Cholinesterases (AChE and BuChE),[1] monoamine oxidases (MAO-B),[2] specific adenosine receptors (A_{2A} and A_3)[3] and oxidative stress are actively involved in the biochemical modulation of these disorders.

In recent years, great advances have been made in the ability to design and test new synthetic molecules. Coumarins represent an important class of natural and/or synthetic oxygenated heterocycles, bearing a typical benzopyrone framework, showing a myriad of biological activities. Therefore, the current communication provides an overview about the potential of different substituted coumarins as inhibitors of enzymatic systems (AChE, BuChE and MAO-B), adenosine ligands, antioxidants and neuroprotective agents. The synthetic routes and the most representative experimental results are going to be presented in the communication.

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CB_2 Cannabinoid receptor dimerization: synthesis and identification of novel bivalent ligands by FRET and BRET assays

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Growing evidence suggests that numerous GPCRs, cannabinoid receptors among them, may form hetero- and homodimers. This oligomerization interferes with the receptor function, activation and signal transduction. In this scenario, bivalent ligands, which consist of two pharmacophores connected by a spacer, have emerged in the last years as promising pharmacological entities and potential tools for the biological study of their respective dimeric receptors.

Few homobivalent ligands have been described for the cannabinoid receptors; most of them target CB_1 receptor dimers. Heterobivalent ligands targeting CB_1 and opioid receptors have previously been developed by us. To our knowledge, it is the first time that CB_2 selective bivalent ligands are reported. These new compounds are structurally based on a chromenopyrazole scaffold previously described by us as cannabinoid ligand.

A series of homobivalent chromenopyrazoles containing alkyl chains as spacers and their respective univalent 9-alkoxychromenopyrazole analogs have been synthesized. Their ability to bind to cannabinoid receptors was measured through radioligand assays observing full selectivity towards the CB_2 type eliminating the psychotropic effects related to the CB1 type. Interestingly, their univalent analogs did not bind to cannabinoid receptors. Functional cAMP assays performed in HEK293 cells overexpressing recombinant human CB_2 receptors showed their CB_2 agonist profile. Since we have identified the presence of CB_2-CB_2 homodimers by BRET and FRET techniques in CB_2-HEK293 cells, we have undertaken studies of the action of our bivalent CB_2 ligands on these CB_2-CB_2 homodimers.

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Aminated resveratrol analogues as potential anticancer drugs

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Cancer is a class of *diseases* characterized by out-of-control cell growth. There are over 100 different types of cancer although carcinogenic processes share some common characteristics,[1] including the ability to induce angiogenesis and to regenerate telomeres.[2]

Angiogenesis is the process of formation of new capillaries from pre-existing blood vessels. These new capillaries supply oxygen and nutrients to cells, being a crucial step in the growth and metastasis of tumours.[3] VEGF proteins, present in a large variety of cancers, play a crucial role in tumour angiogenesis.[4] Telomeres, non-coding nucleoprotein structures located in the end of chromosomes, act preserving DNA integrity and stability.[5] Telomerase is an enzyme that prevents aging and cell death by telomere elongation.[6] Telomerase is formed by a RNA subunit (hTR or TERC) and a catalytic protein subunit with reverse transcriptase activity (hTERT)[7] that represents the limiting stage in telomerase activation[8] and is regulated by some transcription factors including c-Myc.[9]

Efficient drugs attack proteins present in tumour cells but not present in normal cells, or present in low concentrations.[10] For that reason, the inhibition of expression of *VEGF*, *hTERT* and *c-Myc* genes are good targets against cancer. Resveratrol (*trans*-3,5,4'-trihydroxystilbene) is a natural product with several therapeutic applications, like cancer-preventive or antiangiogenic and antitelomerase activity.[11]

In this communication the synthesis of aminated resveratrol analogues will be presented as well as the cytotoxicity, the VEGF protein secretion, and the inhibitory effect on the expression of *VEGF*, *hTERT* and *c-Myc* genes.

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The binding of a poly-cationic peptoid (SICHI) to glycosaminoglycans suggests a putative mechanism for its Sema3A pathway inhibitory activity through electrostatic interactions

Miriam Corredor, Roman Bonet, Alejandra Moure, Cecilia Domingo, Jordi Bujons, Yolanda Pérez, Ignacio Alfonso and Angel Messeguer

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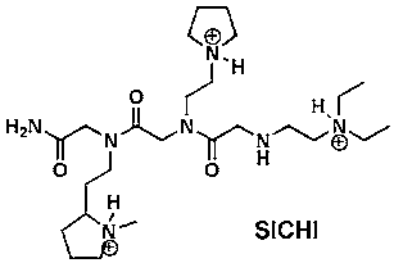
Sema3A is a member of class 3 secreted semaphorins and in the central nervous system acts as canonical repulsive axon guidance molecule, inhibiting CNS regenerative axonal growth and sprouting. Interfering with Sema3A signaling is an important therapeutic goal for achieving functional recovery after CNS injuries.[1,2]

A peptoid named SICHI (Semaphorin Induced Chemorepulsion Inhibitor) was first identified by our group as being able to block semaphorin3A chemorepulsion and growth-cone collapse in axons at extracellular level.[3]



In the present work, we decided to confirm the direct target for SICHI inhibitory effect in the Sema3A signaling pathway. The prime candidates were secreted Sema3A or Nrp1 receptor. In addition to these obvious SICHI protein targets, recent studies have suggested a role for proteoglycans (PGs) in Sema3A and its Nrp1 receptor function.[4]

After confirming that SICHI does not bind directly to the Sema3A domain or to Nrp1 extracellular domain, we proposed a new hypothesis: SICHI could exert its function by binding to the GAG side in the Sema3A-GAG interaction. To evaluate whether a small molecule like SICHI, positively charged at physiological pH, is able to disrupt Sema3A-GAG interaction, we have used different biophysical techniques like NMR, SPR, ITC and UV-VIS spectroscopy. To get further insight about the interaction of SICHI and GAGs, MD simulations were also carried out.

We have shown that SICHI and two peptides mimicking the C-terminal basic region of Sema3A interact with GAGs in the micromolar range. Moreover, we have demonstrated that SICHI competes with the Sema3A peptides for the binding to GAGs.

These results widen the possibility to optimize the pharmacological properties of SICHI structure addressed to ameliorate neurodegenerative disorders.

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Nature-inspired oxacycles as antioxidant agents: pharmacological applications

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Antioxidants are substances often regarded as potential therapeutic agents being recognized beneficial health effects in awide range of oxidative stress related illnesses, namely neurodegenerative and inflammatory diseases, cancer and ageing. Antioxidants display pharmacological activity throughout different mechanisms such as scavenging reactive species, binding to pro-oxidant transition metals (mainly Cu and Fe) and inhibiting free radical generating enzymatic systems. In this context, antioxidants have received considerable attention over the last years as a promising approach to delay or slow the oxidative stress. Over the years, numerous compounds have been screened as antioxidants, in cell cultures and/or animal models, and in general interesting outcomes have been obtained.[1] In recent years and due to the advances in synthetic chemistry, great developments have been made in the ability to design and test new synthetic molecules. Coumarins and chalcones are an important class of natural and/or synthetic compounds that present innumerable biological activities.[2-4] In the present communication, the antioxidant properties and the pharmacological evaluation on different targets (Adenosine Receptors and MAO) of a variety of coumarin-chalcone hybrids is presented. Both the synthetic methodologies and the most representative results will be presented in the communication.

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Dream-modulators for dreaming of novel drugs for neurodegenerative diseases

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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, Down syndrome and Huntington's disease. DREAM (Downstream Regulatory Element Antagonist Modulator, also known as calsenilin or KChIP-3 (potassium channel interacting protein-3), is a multifunctional calcium binding protein that controls the expression level and/or the activity of several proteins related to calcium homeostasis, neuronal excitability and neuronal survival. 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.

The interest in DREAM is based on its key role in the regulation of intracellular calcium levels. As a calcium-dependent transcriptional repressor, DREAM is a master regulator of activity-dependent gene expression and controls genes important for calcium homeostasis. As an auxiliary protein in the plasma membrane, DREAM interacts with and regulates the gating of Kv4 potassium channels, L- and T-type voltage-dependent calcium channels and NMDA receptors. 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 modulate its physiological functions could be candidates for drugs to treat neurodegenerative diseases. Moreover, up to now, only two DREAM-binding molecules have been identified.

In this communication we report the rational design and the synthesis of novel DREAM-binding molecules and their effects on the modulation of DREAM/protein interactions

ACKNOWLEDGEMENTS

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Anti-HIV entry inhibitors based on tripodal scaffolds with modified tryptophans on the periphery

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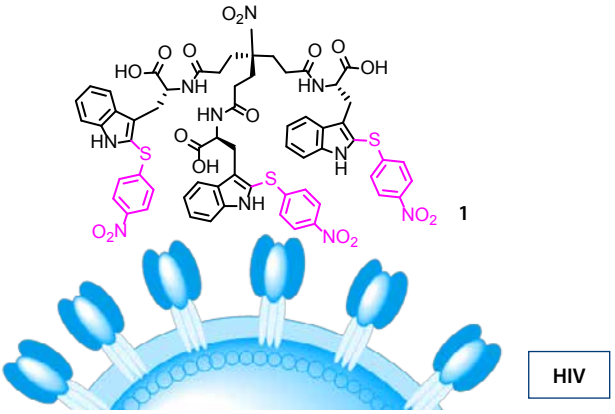
The entry of Human Inmmunodeficiency Virus (HIV) into its target cells represents an attractive target for the development of anti-AIDS therapy.[1] In fact, drugs that interfere with this early event may represent an advantage over other existing therapeutic approaches that target the viral enzymes such as reverse transcriptase or protease, as they may show remarkable efficacy against viruses resistant to these anti-HIV inhibitors and prevent the uptake of the virus by uninfected CD4-positive cells.[1]

As part of our ongoing work directed to the synthesis of anti-HIV entry inhibitors[2] we found that compounds with a tripodal scaffold and multi-branched spacers connecting the scaffold with the periphery (9 tryptophans) showed anti-HIV activity. Moreover time of addition experiments revealed that these compounds inhibit an early step of the HIV replicative cycle.[2] Structure activity relationship-studies showed that the activity is lost when only 3 Trps were present on the periphery while it was restored when the C2 position of the 3 Trps was substituted by a 4-nitro thiophenyl moiety (compound **1**).

The anti-HIV activity of **1** prompted us to perform systematic modifications on this prototype with the aim of determining the minimal structural features essential for HIV-1 inhibition. This structure-activity study, together with biological experiments to determine if **1** and their analogues might act as entry inhibitors constitutes the subject matter of this presentation.



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Acknowledgments: The Spanish MINECO (Projects: SAF 2012-39760-C02-01, co-financed by the FEDER programme; Plan Nacional de Cooperación Público-Privada, Subprograma INNPACTO IPT-2012-0213-060000, co-financed by the FEDER programme), Comunidad de Madrid (Project: BIPEDD2-CM-S2010/BMD-2457), K.U.Leuven (GOA 10/14; PF-10/18) and the FWO (G-0528.12N) are acknowledged for financial support. The Spanish MINECO is also acknowledged for a FPI predoctoral contract to B. Martinez-Gualda

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Synthesis and evaluation of novel antioxidant compounds based on CR-6

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Oxidative stress is one of the most crucial factors involved in the development of degenerative diseases like cancer and Alzheimer, and of ischemia episodes as well. This stress encompasses the generation of oxygen (ROS) and nitrogen (RNS) reactive species that result in cell damage[1].

Antioxidant and neuroprotective agents are reported to reduce the levels of oxidative damage. For selected applications, these agents should cross the highly selective blood-brain-barrier (BBB) at the central nervous system. Therefore, the development of antioxidants and neuroprotective agents with good penetration across the BBB is a subject of interest in drug discovery.

We previously reported the antioxidant compound CR-6[2], which is currently used in dermopharmacy. In the present work, we synthesized fourteen novel CR-6 analogues (Figure 1) with the aim of improving their penetration across biological membranes and particularly BBB. We have attempted to covalently link the CR-6 scaffold to selected endogen substrates that are able to pass through these membranes. DPPH[3] and cellular antioxidant[4] assays have been performed to test the antioxidant activity of these compounds. Their permeability through the BBB has been evaluated using the PAMPA membrane model[5].

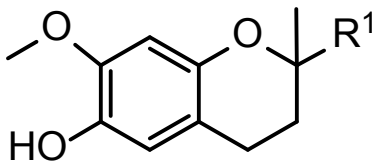

Figure 1. General structure of novel CR-6 analogues.

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New insights into colchicine site-binders with a cyclohexane-dione scaffold based on X-ray crystallography

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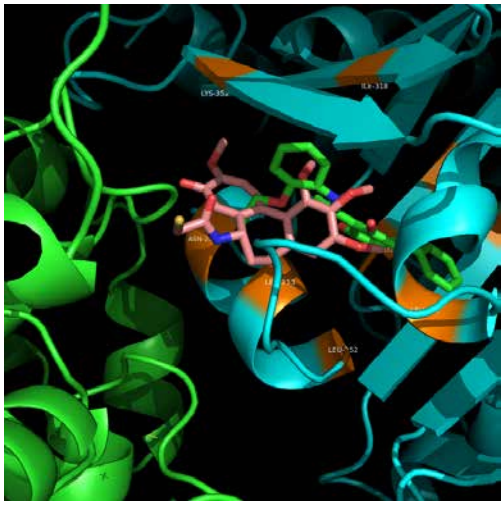
Vascular Disrupting Agents (VDAs) constitute an innovative anticancer therapy approach complementary to other existing therapies.[1] VDAs act directly and selectively at the tumor endothelium, inducing crucial morphological and functional changes that lead to tumor hypoxia and finally to necrosis.[2] The best studied VDAs are microtubule-destabilizing agents that bind the α/β tubulin heterodimers at the colchicine binding-site, combretastatine A4 (CA-4) being the most representative example. Moreover, VDAs with a colchicine-like profile also behave as antimitotic agents affecting tumor cells. This dual mechanism, that is, antivascular (against endothelial cells) and antimitotic (against tumor cells), turns these compounds into very promising anticancer drugs.

We have recently described a novel prototype of colchicine-site binders with anti-proliferative activity against tumor and endothelial cells. This novel chemotype with a cyclohexadione scaffold was identified through a ligand-based virtual screening protocol. Binding at the colchicine-site in tubulin of these cyclohexanediones was confirmed by a competition assay with *N,N'*-ethylene-bis(iodoacetamide) (EBI) and by fluorescence spectroscopy.[3]



We have now determined the crystal structure of our lead compound (namely TUB015) in complex with α/β tubulin (Figure). The analysis of the complex in comparison with that of colchicine has shown the degree of overlapping and subtle differences of both complexes. In addition we have addressed, through a structure-guided design, the synthesis and anti-proliferative evaluation of the second generation of cyclohexanediones whose data will be presented.

Acknowledgements: The financial support of the Spanish MINECO (SAF2012-39760-C02-01) and Comunidad de Madrid (BIPEDD2; ref P2010/BMD-2457) is sincerely acknowledged.

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Characterization of beta-amyloid soluble oligomers as independent drug targets in Alzheimer's disease: an integrated approach

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Alzheimer's disease (AD) is the most common form of dementia and it is defined as a neurodegenerative amyloidosis [1]. AD is considered a multifactorial disease, since it develops as a complex network of events, including low acetylcholine levels and amyloid beta (Aβ) aggregation in the brain, as well as oxidative stress [2]. Despite great efforts, the aetiology of AD is not completely known and more recent studies have focused on soluble Aβ oligomers as potential drug targets [1, 3].While the different oligomeric species that are formed on the fibrillogenic pathway seem to be involved in AD, literature data are still controversial. So far, both low molecular weight oligomers (dimers up to examers) and larger aggregates (dodecamers and >22mers) have been reported to be responsible for the impairments observed in AD patients [3]. Thus, there is a great need to fully characterize and isolate these dynamic and heterogeneous species and to investigate their physiological and pathological role. This should be accomplished in a way so that they can be further easily tested as independent drug targets.

In this work, sample preparation protocols have been evaluated to obtain different aggregation states of synthetic Aβ42: by accurately tuning peptide concentration, type of solvent, pH, ionic strength, temperature, addition of salt and/or surfactants, the formation of different oligomeric species at equilibrium in solution has been obtained [4]. The equilibrium has been monitored over time by capillary electrophoresis (CE), until final precipitation into amyloid fibrils, which were identified by transmission electron microscopy. Oligomers have been isolated and characterised by ultrafiltration followed by CE and/or SDS-PAGE followed by Western Blot. Importantly, this set up allows an independent toxicity test of the isolated oligomeric populations. Consequently, the effects of Aβ42 oligomers on cell fate and on mitochondrial biogenesis have been evaluated on both SH-SY5Y neuroblastoma cells and primary rat hippocampal cultures [5]. This experimental platform can be also applied in presence of small molecules, so to derive, altogether, information on their binding to oligomers, on their anti-aggregation properties and on their effect on cellular fate.

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Flavonoid-like compounds as antitrypanosoma candidates

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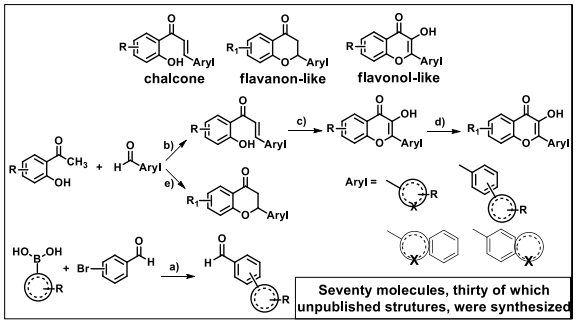
The group of infections known as neglected tropical diseases (NTDs) collectively affects one billion people worldwide and represents an important burden in terms of human suffering. Parasites of the family of Trypanosomatidae are agents of serious human diseases, including African sleeping sickness, Chagas disease and Leishmaniasis. Drugs currently in use against *Leishmania* and *Trypanosoma* infections have limitations in terms of efficacy, safety, duration of treatment, toxicity and resistance. For these reasons, there is an urgent requirement for new effective drugs.

The folate pathway is a successful target for the treatment of bacterial infections and some parasitic diseases, such as malaria. In theory, folate-dependent enzymes targeting drugs should provide useful candidates to face trypanosomatidic infections. However, the classical inhibitors of dihydrofolate reductase (DHFR) are ineffective against *Leishmania* and *Trypanosoma* because of Pteridin Reductase 1 (PTR1). PTR1 is an enzyme responsible for the salvage of pterins in parasitic trypanosomatids and it overlaps the activity of DHFR providing a metabolic bypass to alleviate DHFR inhibition[1],[2]. Therefore, PTR1 is considered a promising target for the development of improved therapies.



Computational studies were performed to screen a library of 90 natural compounds from plants of different origins. Flavonoids turned out to be an interesting class to be explored as PTR1 inhibitors. Even though flavonoids have been widely explored and often pleiotropic properties can be observed leading to promiscuous inhibition, they represent an interesting starting point for our work. Aiming to explore the flavonoid scaffold, we have synthesized seventy-one compounds: 25 chalcones, 9 flavanone-like compounds and 37 flavonol-like compounds. X-ray crystallographic structures of some of the synthesized compounds were solved and used for further structure-based drug design. Fifty-two compounds were investigated for their activity towards *Trypanosoma brucei* and *Leishmania major* PTR1. According to enzymatic inhibition assays, many molecules showed IC_{50} values lower than 30 μM, with IC50 = 4.3 μM being the best one. The compounds were assessed also for *in vitro* toxicity and ADME. In details, the inhibitory activity towards Aurora B kinase, hERG and cytochromes (CYP1A2, CYP2C9,CYP2C19, CYP2D6 and CYP3A4) was estimated. The mitochondrial toxicity and the cytotoxicity in primary human hepatocytes were evaluated. The percentage of A549 cell growth was evaluated and no molecule turned out to be cytotoxic. Ten compounds have been tested towards *Trypanosoma brucei* and two turned out to have IC_{50} lower than 10 μM. Aiming to overcome the flavonoids instability problem, we are using drug delivery systems for *in vivo* tests.

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Pyrrole- and indole-containing tranylcypromine derivatives as novel lysine-specific demethylase 1 inhibitors active on cancer cells

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On the basis of previous research showing the capability of *N*-carbobenzyloxy-(Z-)amino acid-tranylcypromine (-TCPA) derivatives to inhibit LSD1,[1] we inserted at the 4-amino-TCPA moiety first a Z-Pro (**9**) and a Z-Gly (**10**) residue and then, after the encouraging data obtained for **9**, a pyrrole and an indole ring in which the relative N_1 position carried a acetophenone, a *N*-phenyl/benzylacetamide, or a Z chain (**11a-f** and **12a-f**, respectively). In both series, the Z-pyrrole and indole derivatives **11e**, **f** and **12e**, **f** displayed high LSD1 inhibitory activity. The compounds are able to inhibit LSD1 in NB4 cells, increasing the expression of two related genes, GFI-1b and ITGAM, and to induce cell growth arrest in the AML MB4-11 and APL NB4 cell lines.[2]

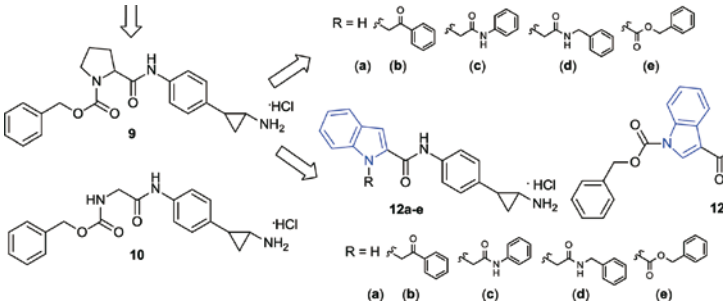

Fig. 1 Novel TCPA analogues based on Z-Pro (**9**), Z-Gly (**10**),pyrrole (**11a-f**) and indole (**12a-f**) structures.

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1,2-dithioles derivates as inhibitors of *Trypanosoma cruzi*: Biological evaluation, and mechanism of action studies

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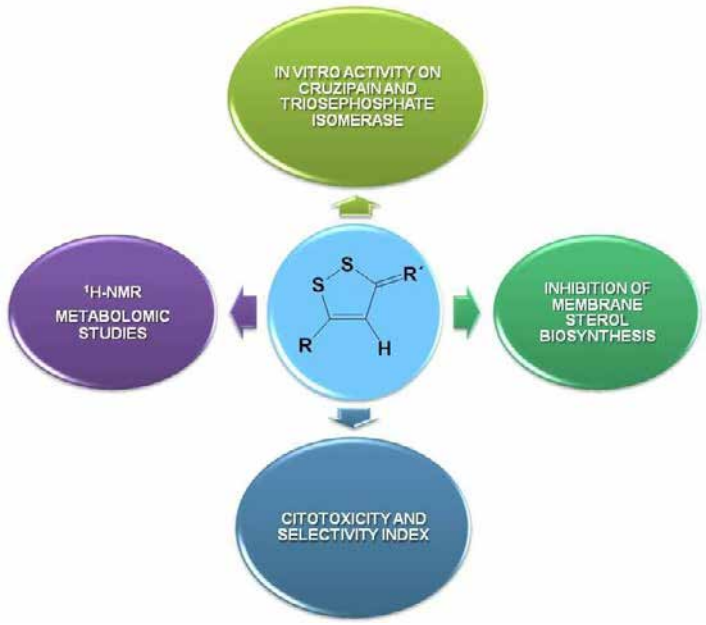
Chagas disease (CD) is a chronic systemic parasitic infection caused by the protozoa Trypanosoma cruzi, which constitutes a public health problem in many Latin American countries, with an estimated of 9.8 to 11 million people infected, leaving 60 million people at risk. Currently the treatment of CD is limited to nifurtimox and benznidazole, which are toxic and mutagenic and present common side effects, including anorexia, vomiting, peripheral polyneuropathy and allergic skin disease may in some cases lead to treatment discontinuation. Thus, the development of new drugs has become an urgent need [1]. The aim of this work was the biological evaluation of nineteen 3H-1,2-dithiole derivates as antitrypanosomal agents. Scheme 1 shows different studies that were carried out.



Scheme 1

Seven derivatives displayed anti-*T. cruzi* activity (IC_{50} between 25 and 5 μM) with selectivity to parasitic cells between 2 and 12 times. Also, the most active derivatives could be considered as multitarget agents because they inhibited TcTIM, cruzipain, and the sterol biosynthesis.

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Activity assay of purine nucleoside phosphorylases by LC-MS/MS

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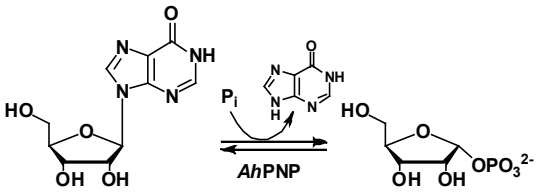
In the search for new therapeutics, fast and automated screening tools of chemical libraries are required for hit selection.

Nucleoside phosphorylases (NPs, E.C. 2.4.2) are among the key enzymes in nucleotide salvage/recycling pathway. NPs catalyze the reversible cleavage of the glycosidic bond of (deoxy) ribonucleosides in the presence of inorganic orthophosphate (P_i) to generate the nucleobase and α-D-(deoxy)ribose-1-phosphate (see Scheme 1).[1]

NPs are also essential for the metabolism of nucleotides in bacteria and other organisms. Nucleotide metabolic pathways in lower organisms represent reasonable targets for chemotherapy as they usually differ from the human counterparts.[1b] Inhibition of pathogen purine nucleoside phosphorylases (PNPs, E.C. 2.4.2.1) might result in the impairment of replicative processes, thus providing a new potential route to infection control.[2]

Here we describe a novel LC-MS/MS method for the assessment of the activity of PNPs as an alternative to routinely used assays.[1b] Enzymatic activity was assessed by phosphorolysis of inosine to hypoxanthine (Scheme 1). Kinetic parameters (K_m, V_{max}, K_{cat}) were determined with respect to inosine and P_i.

Scheme 1 Phosphorolysis of inosine



The assay was performed in a 96 well plate format with an overall reaction time of about 15 minutes per plate, followed by the application of HILIC-LC-MS/MS method for the rapid quantification of the produced hypoxanthine (less than 2 minutes for sample).

For method development and validation, a PNP from *Aeromonas hydrophila* was used due to accumulated data on this enzyme by our team over the years.[3]

The newly developed LC-MS/MS assay will be applied to the screening of potential inhibitors against pathogenic PNPs.

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From garcinol to barbituric acid derivatives: development of a novel cell-permeable, selective, and noncompetitive inhibitor of KAT3 histone acetyltransferases

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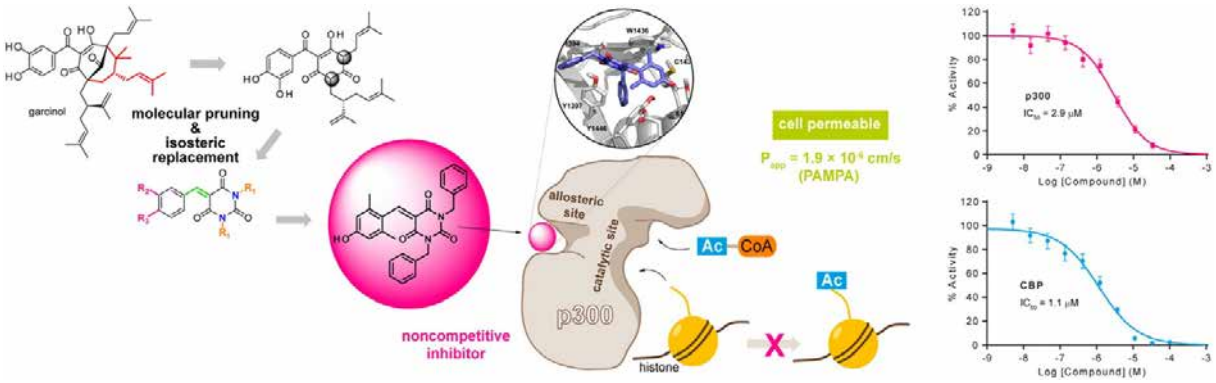
Lysine acetylation is among the prominent posttranslational modifications in eukaryotic cells. Protein acetylation level is a consequence of the balance between the opposite activities of protein acetyltransferases (KATs) and deacetylases (KDACs), and its deregulation has been linked to several diseases, including cancer, inflammation and neurodegenerative diseases. At present, only a small number of KATs modulators have been reported and just a few of them show selectivity between KATs isoforms. Among these, a polyisoprenylated benzophenone, garcinol, isolated from *Garcinia Indica*, is a potent inhibitor of histone acetyltransferases p300 and PCAF[1]. Starting from the garcinol hardly optimizable and not very cell-permeable core structure, we applied a molecular pruning approach and prepared many analogues that were screened for their inhibitory effects using biochemical and biophysical (SPR) assays. Further optimization led to the discovery of the benzylidenebarbituric acid derivative **7h** (EML425) as a potent and selective reversible inhibitor of CBP/p300, non-competitive versus both acetyl-CoA and a histone H3 peptide, and endowed with good cell permeability. Furthermore, in human leukemia U937 cells, it induced a marked and time-dependent reduction in the acetylation of lysine H4K5 and H3K9, a marked arrest in the G0/G1 phase and a significant increase in the hypodiploid nuclei percentage[2].



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2-Oxo-1,2-dihydro-pyridine-3-carboxamide derivatives as a source of CB_2 receptor modulators with polypharmacological features

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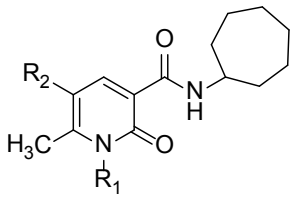
Cannabinoid receptors (CB_1 and CB_2) are key components of a ubiquitous complex lipid signaling system known as endocannabinoid system (ECS), which also consists of the endocannabinoids anandamide (AEA) and 2-arachidonoilglycerol (2-AG), the enzymes implicated in their biosynthesis, and inactivation (MAGL and FAAH) and several other components (membrane transporter, intracellular carrier proteins, and other catabolic enzymes) [1]. As CB_1 receptor probably mediates most of the psychotropic effects of cannabinoids [2], CB_2 receptor selective ligands are attractive as therapeutics for treating inflammation, pain, neurodegenerative disease and cancer [3] because they would presumably lack this psychoactivity.

In a research program aimed at obtaining CB_2 receptor selective ligands, a series of 2-oxo-1,2-dihydropyridine-3-carboxamide derivatives was developed. These derivatives exhibited good CB_2 receptor affinity and selectivity. Furthermore, it was showed that the nature of the substituent in position C5 of the heterocyclic nucleus controls the switch among the different types of pharmacological modulation (agonism, inverse agonism and antagonism) of the receptor [4]. To further study the structure-activity relationship of this class of CB_2 ligands, the insertion of a methyl group in position C6 of the 2-oxo-1,2-dihydropyridine ring was investigated.



6-methyl-2-oxo-1,2-dihydropyridine derivatives

The results indicate that the presence of a small group in C6 position does not affect the CB_2 receptor affinity and selectivity of this class of compounds. Moreover, the 6-methyl-2-oxo-1,2-dihydropyridine derivatives were also tested on all the main targets of the ECS. Interestingly, some of the compounds, which showed also the best binding properties at CB_2 receptors, showed potent inhibition of AEA and 2-AG uptake with IC_{50} values in the nanomolar range. Therefore, our library of compounds which contains the full repertoire of CB_2 receptor modulators (agonist, inverse agonist and antagonist) could be useful to elucidate the still unclear role of the CB_2 receptor in certain physio-pathological conditions. In addition, the presence of compounds, which modulate different targets of the ECS beyond the CB_2 receptors, will allow investigating the pharmacological relevance of these multi-target approaches in different cellular and animal models.

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New allosteric modulators of alpha7 nAChRs with analgesic and antioxidant properties

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The α7 nicotinic acetylcholine receptors (α7 nAChRs) are ligand-gated channels widely expressed in the nervous system that are involved in cognition disorders and inflammatory processes. Many efforts have been focused in compounds able to interact with the receptor at allosteric binding sites. This would permit the selective regulation versus other nicotinic receptors, avoiding many of the secondary effects associated to ligands that target the highly conserved acetyl choline binding site.

Recently, we have described a new series of polyhydroxy-substituted chalcones as potent and selective positive allosteric modulators of α7 nAChR. Trying to mitigate the risk of adverse effects that could be associated to the high chemical reactivity showed by the α,β–unsaturated carbonyl system of the chalcone structure, we have performed modifications of this linker chain. This has permitted the identification of new allosteric modulators of the α7 nAChRs by chemoselective reduction of the conjugated double bond.

The synthesis, antinociceptive and antioxidant properties of the new series of compounds will be commented in detail.

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Synthesis, biological evaluation and study of the mechanism of action of new compounds against Chagas disease

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Chagas disease, caused by *Trypanosoma cruzi*, is endemic in 21 Latin American countries and affects about 8 million people, causing 12,000 deaths annually. Currently, there are two drugs for treating the disease that present severe toxicity problems and inefficiency during the chronic phase and are not approved by the FDA.[1] This situation justifies the urgent need to develop new drugs. With this aim, we have designed and synthesized a total of 112 novel compounds as anti-*T. cruzi* agents. 92 of the evaluated compounds presented IC_{50} values in epimastigote lower than the reference drugs. Moreover, 16 of the derivatives showed IC_{50} values lower than 1.5 μM. According to Selectivity Indexes (SI), 14 of the compounds showed SI>20. 8 out of 14 had SI>50, outstanding 2 of them with SI higher than the reference drugs.[2] Therefore, this family of compounds stands out as promising antichagasic agents. The selected compounds were no mutagenic in the SOS/umu mutagenicity screening assay. Meanwhile, the reference drugs showed mutagenicity at this level. At present, the selected compounds are under further studies. In addition, some of the evaluated compounds were selected for additional studies to gain insight into the mechanism of action of these novel derivatives. In the cruzipain inhibition assay at pH 5.5, 12 out of the 23 evaluated compounds exhibited IC_{50} values between 9.3 and 34.6 μM.[3] 9 out of 20 tested compounds showed a possible inhibition of squalene epoxidase enzyme as observed by accumulation of squalene and lanosterol depletion. None of the 3 compounds tested in the metabolomics assay, showed significant changes on the metabolic profile of *T. cruzi* epimastigotes.[4] We will continue with further studies out to determine their mechanism of action. References:

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Visualizing melatonin receptors using the Click-Chemistry strategy

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Over the last years, fluorescence-based techniques have become essential tools in the study of pharmacological and biochemical processes. In the case of G-protein-coupled receptors (GPCRs), these methodologies have been of major importance for understanding the pharmacology of this family of membrane receptors and their expression.[1] In this regard, fluorescence labelling lacks most of the inconveniences associated to radioactive-isotopic labelling. Melatonin is an endogenous molecule that exerts a plethora of physiological and pharmacological actions (circadian and seasonal regulation, immune and endogenous antioxidant systems, promotion of brain neurogenesis, etc), mainly mediated by two GPCRs, MT_1 and MT_2. Recently, in our research group we characterized a small series of alkyne-based melatonin analogues displaying nanomolar affinities for human MT_1 and MT_2 receptors.[3] Herein we present the ongoing efforts aiming to visualize melatonin receptors in *Xenopus laevis* melanophores by *in situ* click reaction of the alkynes with azide-bearing fluorophores.

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Serendipitous discovery of indole derivatives as potent tyrosinase inhibitors

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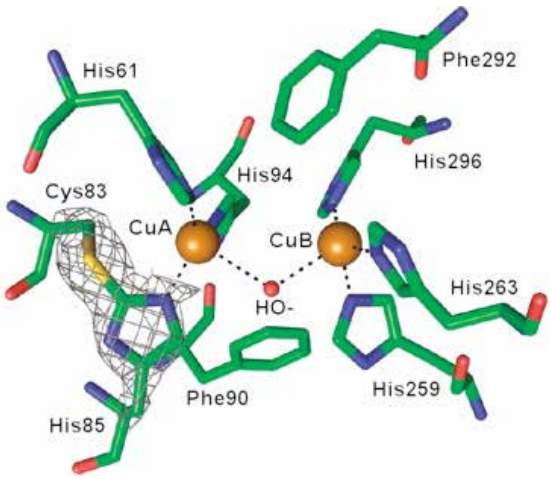
Tyrosinases (TYs, EC 1.14.18.1) catalyze the oxidations of both monophenols and o-diphenols and are physiologically involved in melanin biosynthesis; however they are also closely associated with hyperpigmentation disorders, cancer, and neurodegenerative diseases, and as such, it is an essential drug target. TYs are metalloenzymes utilizing two copper atoms as cofactors able to incorporate molecular oxygen. The anti-tyrosinase activity can be achieved by several ways: (a) by reducing agents or by introducing o-dopaquinone scavengers; (b) by employing alternative tyrosinase substrates; (c) by denaturating the enzyme with non-specific or specific inhibitors. To date a large series of TY inhibitors (TYIs) from natural and synthetic sources have been identified. However, known TYIs possess adverse side effects, so it is necessary to develop new agents. Even if the TYIs from natural sources have inspired the design of efficient synthetic analogs, many synthetic inhibitors with novel scaffolds have been prepared. Continuing the efforts for the identification of novel synthetic TYIs we carried out a screening campaign of our library of compounds which have been designed to target other enzymes. By testing the inhibitory effects toward mushroom TY, we found that two indoles showed inhibitory effects [1]. So we decided to widen the screen thus enclosing other indoles. We discovered an unexpected series of TYIs being efficacious agents at lower concentration (*e.g.* IC_{50}=5.0 micromolar). We believed these compounds to be a good starting point for identification of new scaffolds. Herein we report the synthesis, structure-activity relationships and anti-tyrosinase effects; for the most potent agents we explored the mechanism of action by examining inhibitory effects in the presence of TY or DOPA as substrates.



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2-methyl-1H-indol-5-yl-1-naphthalenesulfonamide: a novel reversible antimitotic agent inhibiting cancer cell motility

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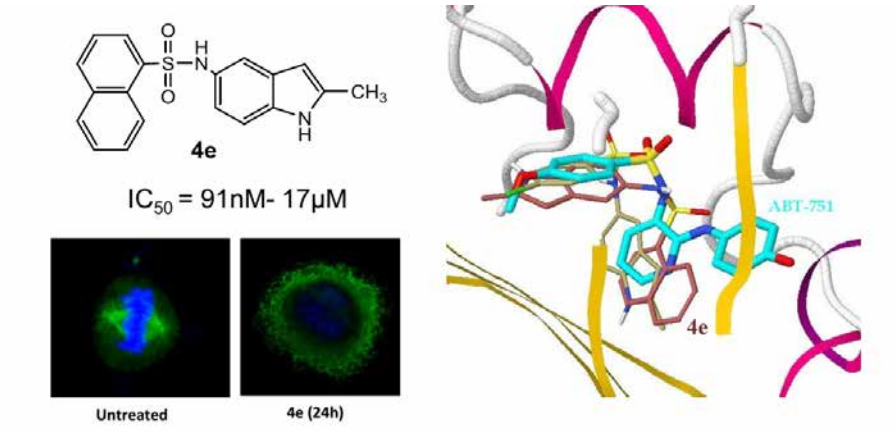
Based on the increasing biological importance of sulfonamides in the field of anti-tumoral chemotherapy,[1] a series of compounds containing the sulfonamide scaffold were synthesized and screened for their *in vitro* activity against a representative panel of human cancer cell lines. 2-methyl-1H-indol-5-yl-1-naphthalenesulfonamide **4e** showed a remarkable activity across the panel, with IC_{50} values in the nanomolar range. Cell cycle distribution analysis revealed that **4e** promoted a severe G2/M arrest, which was followed by cellular senescence. Importantly, tubulin staining showed that **4e** promoted a severe disorganization of microtubules and mitotic spindle formation was not detected in **4e**-treated cells. **4e** inhibited tubulin polymerization *in vitro* in a dose-dependent manner and docking analysis revealed a compatible interaction with the colchicine-binding site of tubulin. These cellular effects were reversible since disruption of treatment resulted in the reorganization of microtubules, cell cycle re-entry and a loss of senescent markers. Interestingly, **4e** is also a robust inhibitor of cancer cell motility. Our data suggest that **4e** may be a promising new anticancer agent capable or both reducing cancer cell growth and cell motility.



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Dissecting the role of the S2 noncanonical binding pocket on farnesoid X receptor activity

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Farnesoid X Receptor (FXR) has been an actively pursued drug target in recent years. Often referred to as the "bile acid sensor", the modulation of this metabolic nuclear receptor represents an attractive area in drug discovery to develop novel therapeutic agents for liver and metabolic disorders.[1] Much of the current knowledge on FXR has been achieved through the availability of potent and selective FXR agonists.[2] Some of them have been derived from the chemical manipulation of the bile acid scaffold as the first-in-class 6α-ethyl-chenodeoxycholic acid (obeticholic acid, OCA),[3] that is demonstrating to possess hepatoprotective effects in patients with primary biliary cirrhosis, type 2 diabetes with non-alcoholic fatty liver disease and non-alcoholic steatohepatitis.

FXR activation promotes the expression of a number of target genes, thus raising the point of the molecular determinants linking ligand binding, coactivator recruitment and target gene transcription. In this respect, it has been demonstrated that guggulsterones (GSs), the active steroidal components of guggulipid, are able to selectively modulate FXR target genes and are classified as Selective Bile Acid Receptor Modulators (SBARMs).[4] According to our docking studies,[5] this peculiar activity can be ascribed to the occupancy by GSs, or bile acids characterized by an extended side chain, of a noncanonical binding site (S2) ("back door" FXR pocket) located at the loop region between helix H1 and helix H2.[6] Remarkably, this model of interaction has been recently confirmed in an experimental study that used amide hydrogen/deuterium exchange (HDX) coupled with mass spectrometry.[7]

In this work, we report our ongoing endeavours in confirming the hypothesis of the S2 binding pocket by determining the biochemical response of the co-administration of GS and OCA using AlphaScreen® assay. Furthermore, to elucidate the complex mechanisms of FXR modulation through S2 interaction, complex computational study composed by docking and molecular dynamic simulations will be discussed in view of the amino acidic residues involved in this 'alternative' FXR modulation. The readout validation combined with the use of steroidal chemical tools will be very useful to design a new class of FXR modulators that might be used for the treatment of metabolic or inflammatory disorders.

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Disclosing the importance of individual residues and conformation in the analgesic peptide DD04107

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DD04107 compound (Palmitoyl-EEMQRR-NH_2), which corresponds to the *N*-terminal sequence of SNAP25, a protein of the SNARE complex, blocks the inflammatory recruitment of ion channels to the plasma membrane of nociceptors and the release of calcitonin gene-related peptide from primary sensory neurons.[1,2] Subcutaneous administration of DD04107 produces dose-dependent, long-lasting in vivo antihyperalgesic and antiallodynic activities in chronic models of inflammatory and neuropathic pain.[3]

To evolve this peptide into small-molecule peptidomimetics is necessary to know the contribution of each individual residue to the activity, as well as its possible bioactive conformation. For the first aim, we performed an Ala scan, preparing a collection of six peptides in which each residue was changed by Ala. For solubility reasons, the *N*-terminal palmitoyl group was substituted by an acetyl group in all cases. To gain insight into the conformational behavior, CD and NMR studies were performed, both on the above linear peptides as well as in side-chain to side-chain cyclic analogues. The solid-phase synthesis, conformational studies and biological evaluation of all these peptides will be described.

This work was supported by the Spanish Ministerio de Economía y Competitividad (BFU2012-39092-C02)

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New TRPM8 antagonists from heterocyclic libraries

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TRPM8 is considered an attractive target for therapeutic intervention, mainly in the search for new analgesics and antitumor agents.[1] Cumulative evidences point to the increased TRPM8 expression in sensory neurons after nerve injury or inflammation, resulting in enhanced sensitivity to cold allodynia and hyperalgesia, while activation of TRPM8 appears also important in attenuating pain in certain acute and inflammatory pain states.[2] Also worth of mention, TRPM8 expression is up-regulated in different tumor cells (i.e. androgen-sensitive prostate and skin melanoma cancers).[3,4]

The crucial role of TRPM8 in human pathologies is behind the intensive drug-discovery programs that are being developed in recent years around this channel.[1] Our contribution in this field is related to the discovery of new, potent TRPM8 antagonists after HTS screening of our in house library of compounds. From the initial results we choose some derivatives for further pharmacological characterization and optimization. These compounds bear two chiral heterocyclic central moieties that fall within the so called privileged scaffolds. This communication will deal with the results of the biological evaluation of the indicated in house library, and the preliminary pharmacological studies on selected hits. A docking model of interaction will also be proposed.

This work was supported by the Spanish MINECO (BFU2012-39092-C02)

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New melatonin-based compounds: synthesis and pharmacological evaluation as melatonergic agonists and neurogenic agents

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Melatonin is an endogenous molecule that exerts a plethora of physiological and pharmacological actions, like circadian and seasonal regulation, immune and antioxidant systems, and promotion of endogenous brain neurogenesis. Recently, we developed a new family of melatonin-based compounds, in which the *N*-acetyl chain of melatonin has been bioisosterically replaced with different azoles. These compounds bind to melatonergic receptors and are potent neurogenic stimulants.[2] A melatonin agonist from the above series was superimposed with the bioactive conformation of melatonin and based on this study, now we wish to broaden the above family by obtaining new 5-methoxiindole derivatives containing different azoles. New compounds behave as full or partial agonists in melatonin receptors from *Xenopus laevis*, with EC_{50} in ranges from submicro- to nanomolar. In *in vitro* experiments, some of these melatonin-based compounds promote differentiation of rat neural stem cells into a neuronal phenotype. This unique pharmacological profile, in special their neurogenic potential, highlights these melatonin-based compounds as useful prototypes for the development of brain-reparative agents for the potential treatment of neurodegenerative diseases.

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Synthesis and antiproliferative effect of novel 13-(di)arylalkyl berberines in malignant mesothelioma cells

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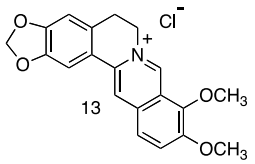
Berberine (**1**) is an isoquinoline quaternary plant alkaloid which has been used in the Ayurvedic and Chinese medicines since hundreds of years.[1] The diverse pharmacological properties exhibited by berberine indicate that the alkaloid has a definite potential in a wide spectrum of clinical applications. It represents a biologically interesting skeleton by providing an attractive natural lead compound for the introduction of chemical modifications in search for more selective and specific medical indications.[2,3] Anticancer properties of berberine have also been reported.[4,5] In this respect we discovered novel 13-(di)arylalkyl berberines (**2**) with improved anticancer properties,[6] several of which show remarkable antiproliferative effects on a variety of human cancer cell lines refractory to chemotherapy. The derivatives can be prepared starting from 7,8-dihydroberberine precursor (**3**) by using various synthetic methodologies, including an uncommon aldehyde-enamine condensation. Although the precise molecular bases of the many biological activities of berberine are still debated, we present informations and data regarding downregulation of thymidylate synthase (TS) protein expression as the putative major biological effect of these berberine derivatives. The findings could be exploitable in therapeutic regimens targeting TS in malignant mesotheliomas and in poorly responsive tumours characterized by abnormal level and activity of TS.



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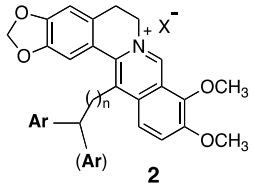

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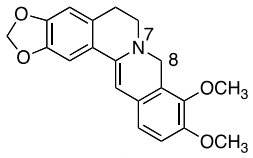

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A new family of multitarget directed compounds with potential application in Alzheimer's disease treatment

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Given the complexity of neurodegenerative processes such as Alzheimer's disease (AD), a promising strategy for the search of new drugs, is the design and synthesis of new Multitarget Directed Compounds[1] able to interact simultaneously with different proteins related to disease onset and progression.

In this work we have targeted Acetylcholinesterase (AChE), β-secretase (BACE-1), the sigma-1 receptor protein[2] and the oxidative stress characteristic of neurodegenerative diseases.[3] These new compounds are Sigma-1 agonists and AChE inhibitors in the nanomolar range and BACE-1 inhibitors in the low micromolar range. At the same time, they have shown a good antioxidant profile according to the Oxygen Radical Absorbance Capacity method (ORAC).

According to our results we have obtained a family of new molecules able to target three important enzymes related to AD, counteract simultaneously the oxidative stress and with the ability to cross the blood brain barrier, according to the predictive Parallel Artificial Membrane Permeability Assay (PAMPA).

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Carbonic anhydrase inhibitors: design and synthesis of new heteroaryl-N-carbonylbenzenesulfonamides targeting druggable human carbonic anhydrase isoforms (HCA VII, HCA IX, and HCA XIV)

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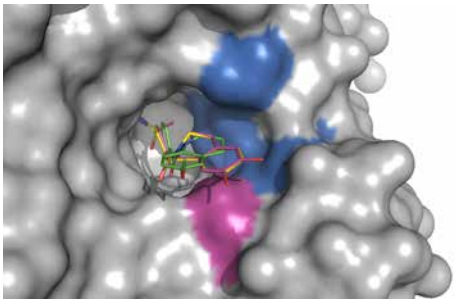
The family of carbonic anhydrases (CAs, EC 4.2.1.1) includes several metalloenzymes highly abundant in prokaryotes and eukaryotes. There are 16 human CA (hCA) isoforms having different tissue and cellular localizations. Their catalytic domain is formed by zinc(II) ion reacting with carbon dioxide to give bicarbonate that is an essential physiological reaction, which controls many physiological processes. Among the different hCA isoforms, hCA VII, hCA IX, and hCA XIV have recently been shown to be druggable targets, therefore the current research on CAIs is focused on the design of new molecules displaying high selectivity towards these three isoforms over that cytosolic hCAII and hCA I.

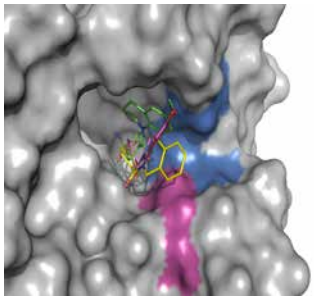
By means of a computational approach some arylsulfonamides have been recently disclosed as potent carbonic anhydrase inhibitors.[1,2]

A set of novel heteroaryl-N-carbonylbenzenesulfonamides has been designed, synthesized, and screened as inhibitors of hCAs. Six compounds were low nanomolar inhibitors of tumor-associated hCA IX isoform (Ki values <10 nM); among them we identified three arylsulfonamides showing unexpected selectivity over brain distributed hCA VII isoform (hCa IX/ hCAVII selectivity ratio > 1,500).

X-ray studies for selected compounds co-crystallized with hCA II highlighted the main interactions between the arylsulfonamide moiety and catalytic pocket thus confirming the mechanism of inhibitory effects while molecular docking studies attempted to the reason of this unexpected behavior.



X-ray data on hCA II



Docking results into hCA VII

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Synthesis, anti-fungal activity and theoretical prediction of NMR chemical shifts for novel thiazole derivatives

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For several years, azoles and their derivatives have been widely studied in medicinal chemistry because of their varied biological activities such as antimicrobial,[1] antitumour,[2] and anti-HIV.[3] In the present project the 2,4-disubstituted 1,3-thiazoles and 1,3-selenazoles were developed and evaluated for their antifungal activity against a panel of Candida spp. Structure of the novel compounds is determined using the nuclear magnetic resonance (NMR) spectroscopy. To assist experiment, we additionally evaluate NMR chemical shifts of the investigated systems using density functional theory (DFT). On the basis of test calculations carried out for a set of small molecules: methane, benzene, thiazole, aminothiazole, using a wide range of basis sets, the B3LYP/6-311++G** approximation is chosen for evaluation of the NMR shifts of new thiazole derivatives. Results are compared to the experimental values.

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Theoretical prediction of specific rotation of flexible biologically active molecules

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Synthesis of new chiral drugs requires determination of their absolute configuration (AC), which can be achieved using *e.g.* the X-ray crystallography or chemical correlation. An attractive alternative to the often difficult experimental evaluations is theoretical determination of AC based on calculation of optical rotation (OR) of chiral molecules and comparison of the result with experimental value. The present work attempts to evaluate quality of specific rotation values yielded by the ORP basis set[1] in the case of conformationally flexible molecules. Calculations are carried out for a set of biologically active molecules for which experimental OR data is available. Due to the large size and conformational flexibility of these systems, investigation is limited to the ORP set and the smaller among the Dunning's basis sets. Results are compared to experimental data. Next, we choose three chiral azido alcohols – potential precursors of amino alcohols – and evaluate their specific rotation. To the best of our knowledge, these are the first estimates of their specific rotation.

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Design of new transthyretin-Aβ sequestration enhancers for the study of Alzheimer's disease

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Alzheimer's disease (AD) is a progressive neurodegenerative disorder strongly associated to the amyloid peptide (Aβ) self-assembly, forming fibrils and being the main cause of neuronal death. Targeting the amyloid fibril pathway through the sequestration of the amyloid Aβ peptide using Transthyretin protein (TTR) has been proposed as a new approach to AD study owing that TTR is the major Aβ binding protein in the cerebrospinal fluid (CSF), and that this transport protein seems to arrest the aggregation of the amyloid peptide, playing a protective role against AD in an animal model[1].

On an ongoing project[2] and by using bioinformatics methodologies we have developed a possible structural model of interaction between TTR and Aβ peptide; our computational results evidence the importance of the external regions and the α-helix section of TTR as a possible zone to bind the Aβ peptide. The computational model obtained sheds light on the structural features of the TTR-Aβ interaction, and allows us to perform a virtual screening to identify new Aβ sequestration enhancers.

The selection of discrete subsets of registered drugs or small compounds has been performed applying three methodologies, in order to enrich our final set and increase our product scope. First, a network analysis has revealed a common scaffold interacting with both proteins, TTR and Aβ, which has been used for a virtual screening campaign against target combinatorial and repurposing libraries. Second, a pharmacophore model for different TTR interacting ligands has been generated, in order to optimize the appropriate features, and a virtual screening based on the pharmacophore has been carried out against the same libraries. Finally, this last approach has been applied to filter compounds related with AD cascade proteins, to find multitarget hits.

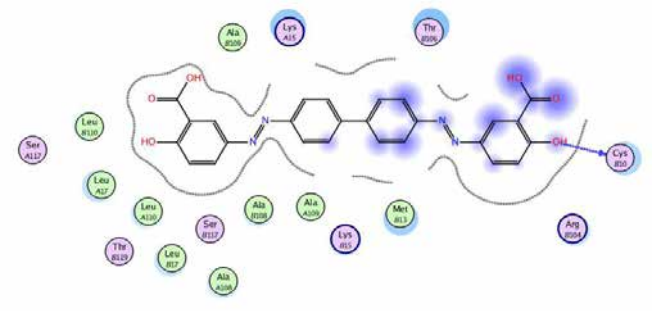

Figure 1. Small molecule from our Virtual Screening approach interact with TTR-Aβ optimized system

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Docking studies justifying the choline kinase inhibition of a series of 6-benzylthio-9H-purin-9-yl-pyridinium derivatives

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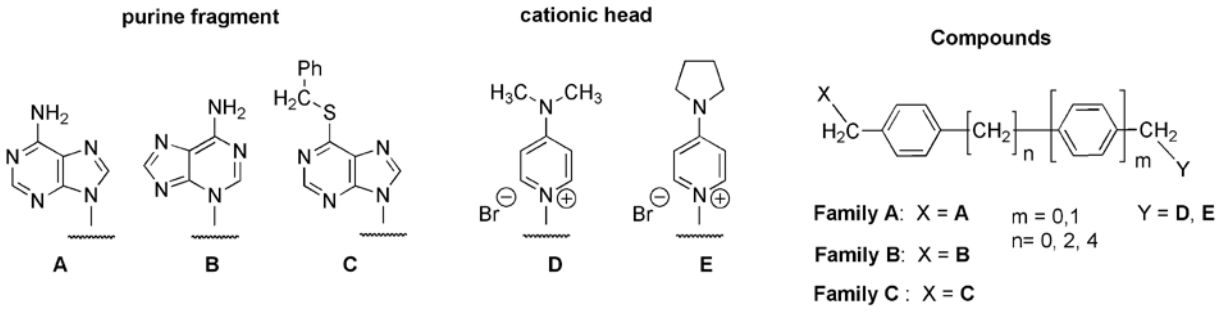
Choline kinase (ChoK) is the first enzyme of the Kennedy pathway for the biosynthesis of phosphatidylcoline, the mayor phospholipid component of mammalian cells and a precursor of second messengers that can modulate growth or survival pathways. In human tumours, the enzyme Choline Kinase (ChoK) is overexpressed and, consequently, ChoK inhibitors have proven to be effective antitumoral drugs *in vitro* and *in vivo*.

Recently, the rational design, synthesis and biological evaluation of a series of non-symmetrical ChoK inhibitors bearing a purine moiety (**A, B or C**), a 4-substituted pyridinium ring (**D** or **E**), and a linker (**F**, **G**, **H** or **I**) that connects both fragments has been published. [1-3] In the first paper, [1] two families of these compounds were obtained (Families **A** and **B**). In the first family (Family **A)** the linker is connected to the adenine *N*-9 nitrogen atom, while in the second family (Family **B**) the linker is connected to the adenine *N*-3 nitrogen atom. In the second paper,[2] a new series of non symmetrical ChoK inhibitors was published (Family **C**). In this new family, the 6-NH_2 amino group was substituted by a 6-benzylthio fragment in order to increase the lipophilicity of these new molecules to obtain better antiproliferative activity, obtaining really good activity against HeLA cell line. In the third paper, the ChoK inhibition activity of compounds of Family C has been published.

Docking studies were performed in order to design these compounds and to justify the ChoK inhibition activity. Two of these molecules have been crystalized in complex with ChoK-α1. [4,5]



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Pharmacophore-based virtual screening to discover new active compounds for human choline kinase α1

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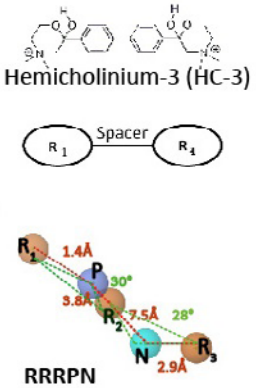
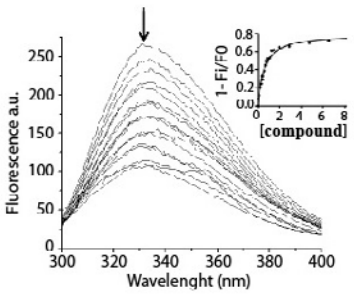
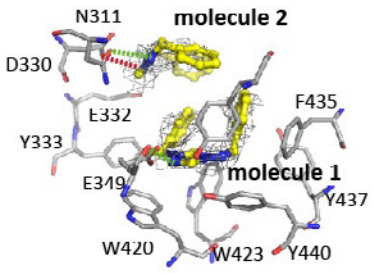
Choline kinase (CK) catalyses the transfer of the ATP γ-phosphate to choline to generate phosphocholine and ADP in the presence of magnesium leading to the synthesis of phosphatidylcholine. Of the three isoforms of CK described in humans, only the α isoforms are strongly associated to cancer and have been validated as drug targets to treat this disease[1]. Over the years, a large number of HsCKα biscationic inhibitors based on Hemicholinium-3 (HC-3) have been developed[2], though the relevant common features important for the biological function have not been defined. Here, selecting a large number of previous HC-3-based inhibitors[3], we discover through computational studies a pharmacophore model that is formed by five moieties that are included in the 1-benzyl-4-(N,N-phenylmethyl)pyridinium fragment. Then, using a pharmacophore-based virtual screening, we identified 6 molecules that showed binding affinities to HsCKα1 in the low μM range. Finally, protein crystallization and growth inhibition assays of tumor cells with these compounds were performed suggesting that one of these molecules is bound to the choline and ATP-binding site while the compound with better affinity for the enzyme shows EC50 values in the low μM range. In conclusion, we show a pharmacophore model that not only has allowed us to dissect the structural important features of the previous HC-3 derivatives but also to discover small monocationic-compounds with good ligand efficiencies.







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Development of novel substituted naphthyl amino benzoic acid derivatives as CDC25 phosphatase inhibitors: design, synthesis and biological evaluation

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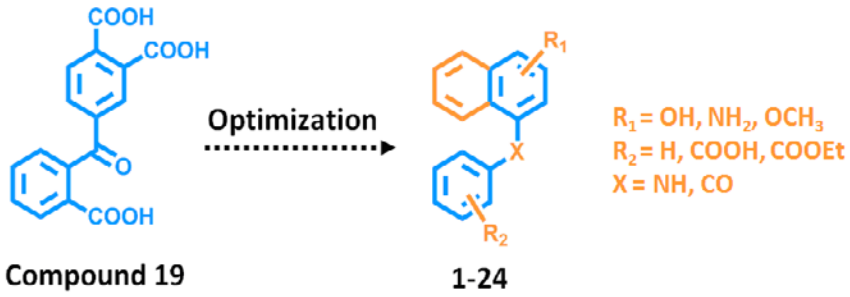

Cell division cycle 25 (CDC25) proteins are key phosphatases regulating cell cycle transition and proliferation by regulating CDK/cyclin complexes. Overexpression of these enzymes is frequently observed in cancer and is related to aggressiveness, high-grade tumors and poor prognosis. Thus, targeting CDC25 by compounds, able to inhibit their activity, appears a good therapeutic approach [1]. Recently, we identified compound **19** (NSC 28620) as a novel inhibitor of CDC25 phosphatase via a structure-based virtual screening approach [2]. This compound displayed reversible inhibition kinetics with in vitro K_i values for CDC25A and -B of 2.3 and 5.3 **μ**M, respectively. To obtain a structural insight into the molecular properties of this new type of CDC25 phosphatase inhibitor and to optimize its potential, herein, computer-assisted optimization of the initial hit **19** by synthesis and preliminary screening of a focused compound library led to the identification of a set of 24 novel derivatives. Among them, 15 compounds showed notable inhibitory activity against CDC25B. The most efficient ones in this subset, i.e. **5**, **10**-**13**, **20** and **23**, improved the potency compared to hit **19**, as evaluated through the IC_{50} values. In order to provide insight into the binding mechanism of the designed compounds, we adopted the relaxed complex scheme (RCS) method, an advanced computer-docking methodology that accounts for protein receptor flexibility using molecular dynamics simulations.

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New more polar symmetrical bipyridinic compounds: study of setting up a new scaffold for the inhibition of choline kinase α1 (ChoKα1)

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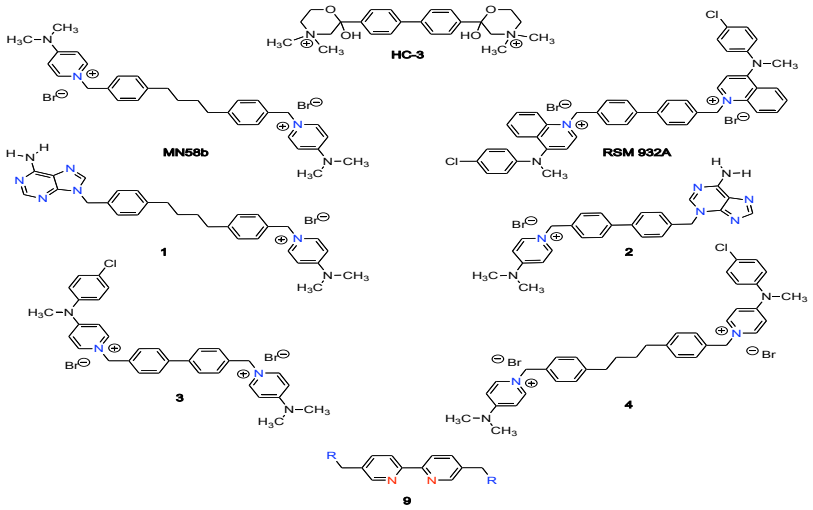
Choline kinase (ChoK) is the first enzyme of the Kennedy pathway for the biosynthesis of phosphatidylcoline, the mayor phospholipid component of mammalian cells and a precursor of second messengers that can modulate growth or survival pathways. In human tumours, the enzyme Choline Kinase (ChoK) is overexpressed and, consequently, ChoK inhibitors have proven to be effective antitumoral drugs *in vitro* and *in vivo*.

Research of the anti-tumor properties of biscationic compounds has received significant attention over the last few years. A novel family of 1,1'-([2,2'-bipyridine]-5,5'-diylbis(methylene))bis-substituted bromide (**9a-k**), containing two nitrogen atoms in the spacer of the linker that connect the biscationic compounds, considered as hypothetical hydrogen bond acceptors, were synthesized and evaluated as choline kinase inhibitors and their antiproliferative activity against six cancer cell line. Just as we predicted, the most promising compounds in this series are 1,1'-([2,2'-bipyridine]-5,5'-diylbis(methylene))bis(4-(methyl(phenyl)amino)-quinolinium bromide derivatives **9g-l**, that significantly inhibit cancer cell growth at even submicromolar concentrations, especially against leukemia cells, and also inhibit the ChoKα1 with good or moderate values, as predicted initials docking studies. In addition the most active compound **9h** remarkably induce apoptosis in two cells lines following the mitochondrial pathway.



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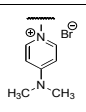
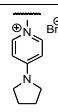
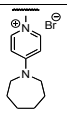
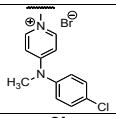
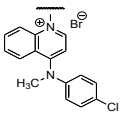
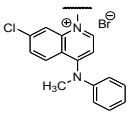
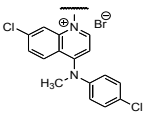
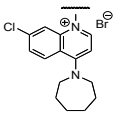
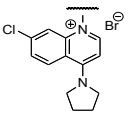
R						
Comp.	**9g**	**9h**	**9i**	**9j**	**9k**	
R						

Cladosporol B, a PPARgamma partial agonist, displays antiproliferative and proapoptotic properties against HT-29 colon cancer derived cells

Antonio Lavecchia,[b] Diana Zurlo,[a] Carolina Votino,[a] Carmen Cerchia,[b] Gemma Assante,[c] Salvatore Moricca,[d] Ettore Novellino,[b] Vittorio Colantuoni,[a] Angelo Lupo,[a]

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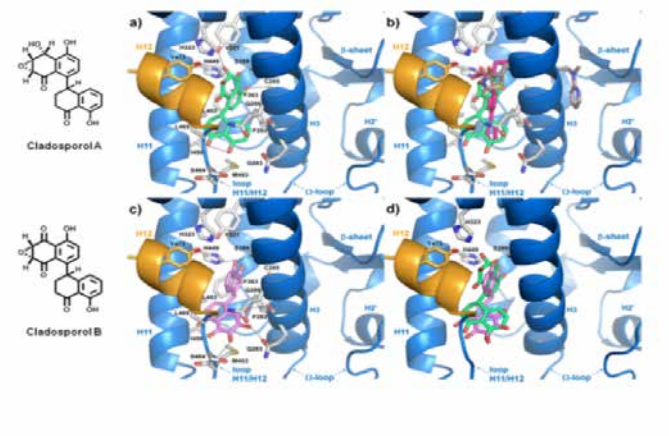

Cladosporols are secondary metabolites isolated from *Cladosporium tenuissimum* and functionally characterized for their ability to control cell proliferation [1]. Specifically, cladosporol A inhibits proliferation of various human colon cancer cells through PPARγ-mediated modulation of gene expression of several cell cycle gatekeepers and inhibition of the nuclear β-catenin/TCF pathway [2]. In this work we investigated the antiproliferative and proapoptotic properties of cladosporol B, an oxidate form of cladosporol A. We demonstrate that cladosporol B inhibits proliferation of HT-29 cells more than cladosporol A due to a robust G1-phase arrest, and an early overexpression of p21. In transient transfections of HEK293 cells cladosporol A displays the same potency and efficacy as rosiglitazone, a PPARγ full agonist, while cladosporol B shows a reduced transactivation potential acting as a partial PPARγ agonist. Docking studies were performed to highlight the interaction of both cladosporols A and B with the PPARγ ligand-binding domain (LBD). The distinctive biological properties of the compounds appear to reside in their differential binding to the PPARγ LBD.

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Nanostructured materials in the antiretroviral therapy

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Since many years our research group is engaged in the development of new anti-HIV agents acting as reverse trascriptase [1], integrase [2], and IN-Ledgf/p75 interaction [3] inhibitors. It is now widely known that in spite of the biological activity the development of new drugs is very often obstructed by unfavorable physical-chemical properties. In the light of these considerations we recently interested to the use of nanotechnology which has demonstrated its potential in medicine field with the design of new functional nanomaterials characterized by good toxicity profile, the possibility of drug-release modulation, low cost, active and passive targeted delivery and the possibility to include different antiretroviral drugs in the same delivery system. Among the different classes of nanomaterials carbon allotropes, fullerenes and carbon nanotubes have induced particular attractiveness in antiviral therapy.

In this context we have reported for the first time the in vitro anti-HIV activity of differently functionalized multi-walled carbon nanotubes, highlighting the pivotal role of the nano-systems properties which critically influence the pharmacokinetic profiles of the molecules and their biological interactions [4].

As results of this study, the presence of free carboxylic groups, inducing a better water dispersibility of the nanomaterial, appeared to be relevant for the interaction with biological counterparts by means of electrostatic or hydrogen bonds. So, another type of carbon nanomaterials, named carbon nanodots (C-dots), because their excellent water solubility, possibility to be revealed in a biological medium, chemical inertness, and ease of functionalization have drawn our attention. Synthesis and biological evaluation results of the new designed conjugated systems will be presented and discussed.

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Modelling drug binding to the M2 channel: structural and energetic insights gathered using enhanced sampling methods

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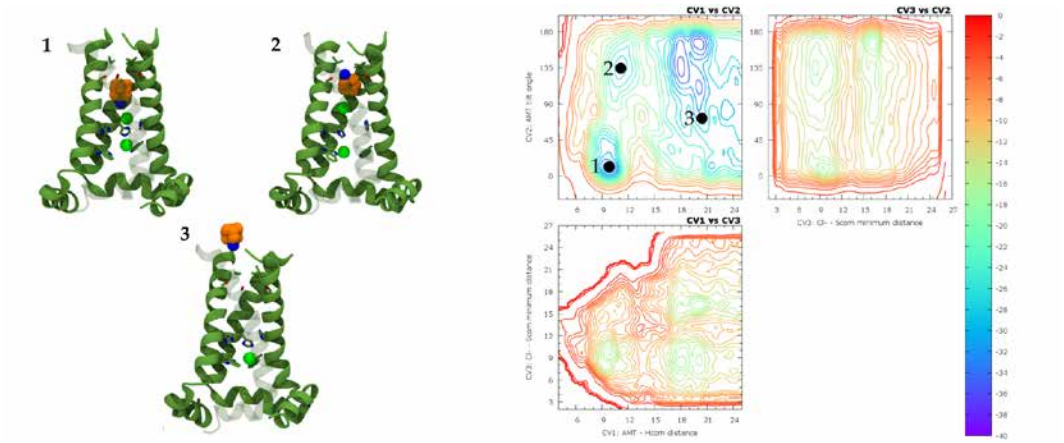
The M2 protein is a 97 residue long protein that, once assembled as an homotetramer in the virion surface, serves as a pH-gated proton channel that allows the acidification of the viral interior. Since such process is essential for the release of genetic material to the intracellular media, the M2 channel plays a critical role in the life cycle of the influenza A virus [1-2]. The FDA approved drugs Amantadine (Amt) and Rimantadine (Rmt) effectively inhibit the wild type (WT) form of the channel. However, widespread of Amt resistance mutations found in latter flu circulating strains (including pandemic 2009 H1N1 swine flu and the highly lethal H5N1 chicken flu) impelled the CDC to advise against its use as flu treatment [3], dramatically decreasing the available therapeutical options.

How mutations affect the energetic and structural determinants of drug binding to the M2 channel is a key question to address the design of new compounds that can target Amt-resistant flu strains. In order to better understand such process, we have combined a set of unrestrained Molecular Dynamics simulations with enhanced sampling techniques (i.e., Metadynamics) that provided meaningful insights in the binding and unbinding of M2 channel blockers. The results provide valuable guidelines for the development of new inhibitors of potential therapeutical interest.



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Targeting kinases with anilinopyrimidines: discovery of *N*-phenyl-*N'*-[4-(pyrimidin-4-ylamino)phenyl]urea derivatives as selective inhibitors of Type III receptor tyrosine kinase subfamily

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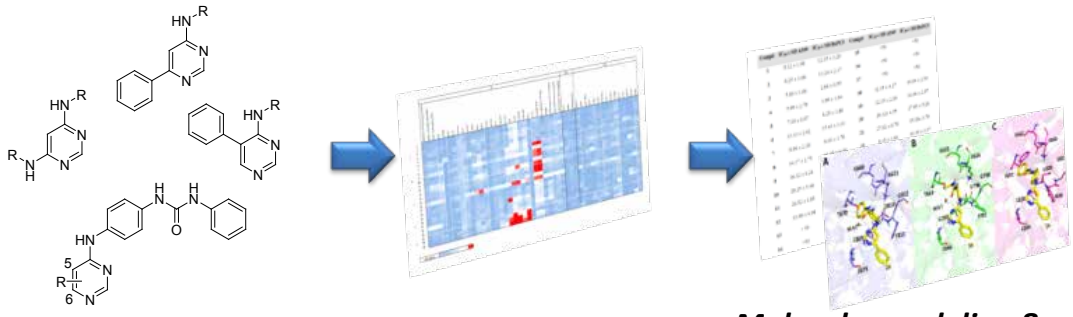
Kinase inhibitors are attractive drugs/drug candidates for the treatment of cancer [1]. The most recent literature has highlighted the importance of multi-target kinase inhibitors [2], although a correct balance between specificity and non-specificity is required. In this view, the discovery of multi tyrosine kinase inhibitors with subfamily selectivity is a challenging goal [3]. Herein we present the synthesis the kinase profiling and the biological evaluation of a set of novel 4-anilinopyrimidines as promising anticancer compounds. Molecular modeling simulations were used in order to rationalize the behavior of the title compounds.



Among synthesized compounds, *N*-phenyl-*N'*-[4-(pyrimidin-4-ylamino)phenyl]urea derivatives targeted some members of type III receptor tyrosine kinase family. In particular, compound **24** was identified as a selective dual KIT/PDGFRbeta inhibitor. The compound was more cytotoxic than sunitinib against A549 and PxPC3 human cancer cell lines, and showed a preferential antiproliferative activity toward neoplastic rather than HEK293 non-tumor cells.

Overall, our data suggested that the 4-anilino-6-phenylpyrimidines constitute a promising class of subfamily selective inhibitors of Type III RTKs subfamily. These results are of remarkably importance since, despite the huge interest in identifying subfamily selective kinase inhibitors, poorly toxic and highly active as antitumor agents, nowadays there is still a paucity of reports investigating their antitumor activity.

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Biphenylaminoquinazolines as novel dual inhibitors of tyrosine kinases and tubulin polymerization: synthesis, SARs and anticancer properties

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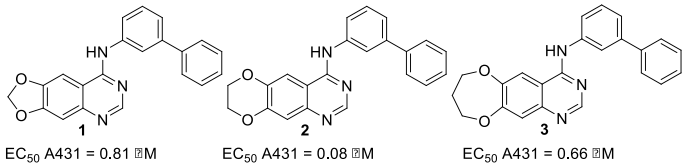
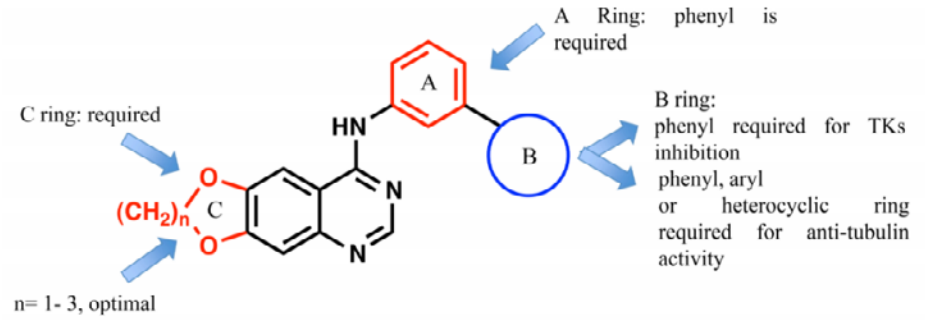
The discovery of the anticancer drugs erlotinib [1] and gefitinib [2] in the early 2000's prompted intensive research on 4-anilinoquinazoline compounds. The main biomolecular target for this class of compounds remains the Epidermal Growth Factor Receptor (EGFR), although some compounds do not show high selectivity for it. Cell cycle experiments with our previously reported 4-biphenylaminoquinazolines (**1**-**3**) multi-tyrosine kinase inhibitors [3] revealed an activity profile resembling that of known tubulin polymerization inhibitors.



Novel 4-biarylaminoquinazoline analogues of compound **1**and **2** were synthesized and evaluated as inhibitors of several tyrosine kinases and of tubulin. While compounds **1**-**3** acted as dual inhibitors, the heterobiaryl analogues possessed only anti-tubulin properties and targeted the colchicine site. On the contrary, the absence of a dioxygenated fused ring led to compounds inactive against tubulin polymerization.



The most interesting compounds were cytotoxic in both OVCAR-8 (human ovarian carcinoma) and NCI/ADR-RES (human ovarian carcinoma P-glycoprotein overexpressing) cell lines at nanomolar concentration.

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Chemical Biology for drug discovery: working with the Spanish Chemical Biology Initiative Chembiobank

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The Spanish Chemical Biology Initiative (Chembiobank) has built a chemico-biological library and annotated database, which will be described. The library contains nearly 35.000 compounds both from commercial and from academic origins, and keeps growing. 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.

We have used the Chembiobank workflow to: a) discover new therapeutic applications and new mechanisms of action for known drugs (repurposing); b) predict and validate the chemico-biological space for orphan drugs, to repurpose drugs for rare diseases; and c) predict and validate the chemico-biological space for compounds sent to Chembiobank from chemists working at academic laboratories.

We are now offering this Chembiobank workflow and platform to chemists, biologists and computational chemists, in order to: a) predict and validate biological interactions for their synthesized compounds, finding new targets and mechanisms of action leading to possible therapeutic aplications; b) select compounds from chemical libraries that may interact with targets or biological systems of their interest; c) carry out computational studies based on the structures of the compounds of the Chembiobank library and on the virtual and experimental screening data of the Chembiobank database.

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). We propose Chembiobank and EU-OPENSCREEN as integrated chemico-biological platforms to catalyze the interactions of chemists and biologists in the process of drug discovery.

Ligand-based chemoinformatic selection of new Inhibitors of CDC25 dual specificity phosphatases and their In vitro efficacy against melanoma cells

Carmen Cerchia,[a] Alessandra Capasso,[b] Carmen Di Giovanni,[a] Giuseppina Granato,[b] Francesco Albano,[b] Simona Romano,[b] Emmanuele De Vendittis,[b] Maria Rosaria Ruocco,[b] Antonio Lavecchia,[a]

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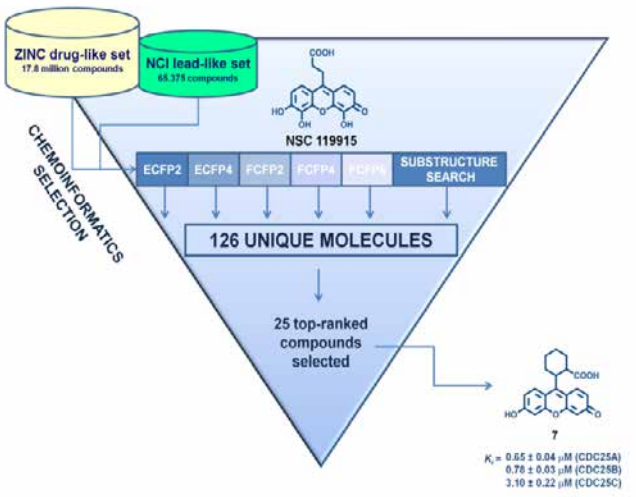
CDC25 phosphatases are important regulators of the cell cycle and represent promising targets for anticancer drug discovery [1]. We recently identified NSC 119915 as a new quinonoid CDC25 inhibitor with potent anticancer activity [2]. In order to discover more active analogs of NSC 119915, we performed a range of ligand-based chemoinformatic methods against the full ZINC drug-like subset and the NCI lead-like set. Nine compounds (**3**, **5**-**9**, **21**, **24**, and **25**) were identified with K_i values for CDC25A, -B and -C ranging from 0.01 to 4.4 μM. One of these analogs, **7**, showed a high antiproliferative effect on human melanoma cell lines, A2058 and SAN. Compound **7** arrested melanoma cells in G2/M, causing a reduction of the protein levels of CDC25A and, more consistently, of CDC25C. Finally, **7** decreased the protein levels of phosphorylated Akt and increased those of p53, thus contributing to the regulation of chemosensitivity through the control of downstream Akt pathways in melanoma cells. Taken together, our data emphasize that CDC25 could be considered as a possible oncotarget in melanoma cells and that compound **7** is a small molecule CDC25 inhibitor that merits to be further evaluated as a chemotherapeutic agent for melanoma, likely in combination with other therapeutic compounds.



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Development of a Smart Therapeutic Nanosystem for the diagnosis and treatment of cancer

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Anticancer therapies face many challenges. Cancer cells can easily mutate and this contributes to the great variation seen both within and between tumours. These factors often result in resistance to therapy. Additionally cancer and normal cells share many biological characteristics which obstruct therapy specificity and increase the occurrence of off-target side effects. Collateral damage to non-targeted cells and resistance to treatment developed by targeted cells constitute two major factors limiting the efficacy with which cancer is currently treated in the clinic. These problems could be overcome if chemotherapeutic drugs and multidrug resistance (MDR) reversal agents were selectively transported to targeted cancer cells in vivo. We are developing Smart Therapeutic Nanosystems (STNs) to achieve this goal. Our systems are constituted by core superparamagnetic nanoparticles that can be easily loaded with a range of chemical and/or biological molecules, each one with a precise contribution to the overall performance of the STN. In this communication we will present ongoing work aiming at developing an STN that: 1) can be loaded with the cytotoxic drug Doxorubicin together plus inhibitors of Doxorubicin-efflux pump proteins over-expressed in drug-resistant breast cancer cells; 2) achieves selective delivery of these cargos to targeted drug-resistant cells, in vivo; and 3) promotes its internalization in the latter cells, and the controlled released of its cargos. The rational behind our design and progress towards its implementation will be presented and discussed.

AMP-activated protein kinase modulators as potential anti-inflammatory agents

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Immunometabolism constitutes a research emerging field. This is due in part to the great deal of data pointing that pathways regulating immune cell function and those regulating metabolism are mutually connected [1-2].

In this context, recent evidences suggest the ability of AMP-activated protein kinase (AMPK), a key player in regulating energy metabolism at the cellular and whole organism level [3], to integrate changes in cellular metabolic demands with inflammatory and immune responses [4]. As example, the production of inflammatory cytokines from key metabolic tissues, including adipose tissue, liver and macrophages, has been shown to increase in obesity and precedes development of insulin resistance, providing a clear relationship between inflammatory and metabolic systems.

In order to have a deeper understanding between these linked processes, we report the study of anti-inflammatory effects shown by some AMPK activators. The results reveal that AMPK activation during spleen T cell activation leads to a reduction of the release of the pro-inflammatory cytokines IL-17 and IFN-γ, while an increase in the production of the anti-inflammatory cytokines IL-4 and IL-10 is detected. The results of this alternative mechanism of action offer not only new opportunities to exploit other therapeutically beneficial effects for AMPK activators but also for developing new therapeutic agents.

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Search for new non-steroidal mineralocorticoid receptor antagonists: structure-based virtual screening

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The mineralocorticoid receptor (MR) belongs to the subfamily of nuclear receptors and is activated by steroid hormones.[1] Gains or loss of MR function can cause disturbances in blood pressure and deleterious cardiovascular and renal effects.[2] Although there are two marketed steroidal MR antagonists, they exhibit undesirable side effects due to interaction with other steroid receptors. To overcome these adverse effects, there is a great interest in the development of non-steroideal MR antagonists.

To identify new compounds able to bind to the ligand-binding domain (LBD) of MR with high affinity and selectivity, structure-based virtual screening has been carried out. Molecular docking studies have been used to screen two compound databases, namely ZINC (the lead-like set around 5 million commercially available compounds) and L1 available at Mount Sinai School of Medicine (MSSM, a set of about 120,000 compounds). After filtering these databases, the final compounds selection was based on their energy score values and the visual inspection of their best poses. Thirty-seven diverse, non-steroidal compounds were selected for biological evaluation. Cellular assays allowed the identification of several hits with moderate MR antagonist activity, which are interesting for hit to lead optimization processes.

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First class of dual inhibitors of REV-ERB-β and autophagy as potential and innovative multi-functional anticancer agents

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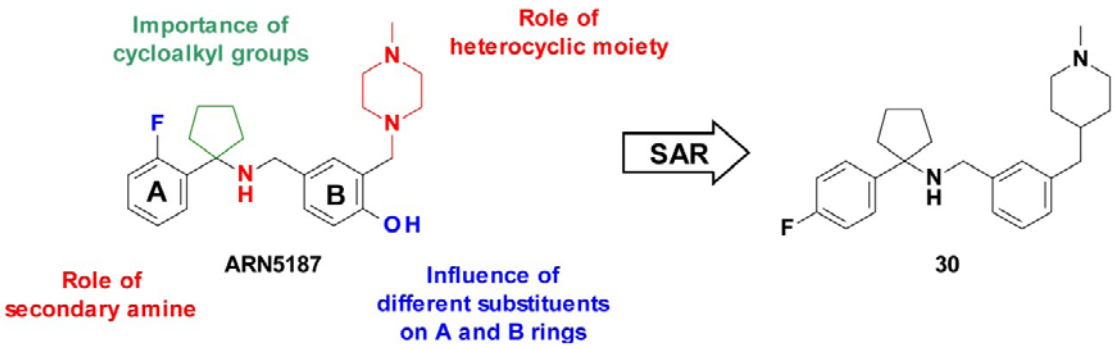
Autophagy inhibition is emerging as a promising anticancer strategy and lysosomotropic autophagy inhibitors, such as chloroquine (CQ), are currently being evaluated in cancer clinical trials. Nevertheless, the high micromolar concentration required by chloroquine to block autophagy in a number of human tissue tumor cells limits the use of this compound as a single anticancer agent.[1]

We recently reported that the circadian nuclear receptor REV-ERBβ plays an unexpected role in sustaining cancer cell survival when the autophagy flux is compromised.[2a] In addition, we identified **ARN5187**,[2b] as a novel cancer cytotoxic lysosomotropic compound with dual inhibitory activity toward REV-ERBβ and autophagy, and improved cytotoxicity against breast cancer BT-474 cells, compared to CQ.



Here, we present our SAR exploration around **ARN5187**, which discloses the first class of dual inhibitors of REV-ERBβ and autophagy. This study led to the identification of novel compounds with improved *in vitro* anticancer activity compared to **ARN5187**, that we demonstrated to be mainly related to an improved inhibitory activity toward REV-ERBβ. Furthermore, we identified compound **30** which is able to decrease the viability of different tumor tissue cells at concentrations from 5 to 50 times lower than CQ, while not affecting the viability of non-cancer human epithelial HMEC cells.

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β-Amino acid morphan scaffolds as hybrid ligands in opioid receptors

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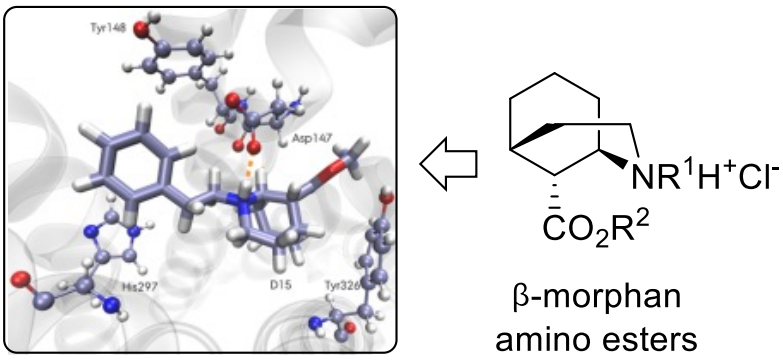
Structure-Activity Relationship (SAR) is a current approach in the design of new pharmacological agents. We previously reported[1] the synthesis of a novel analog of morphine, a 2-azabicyclo[3.3.1] nonane, which contains a β-amino acid functionality. This bicyclic core exhibits two distinctive chemical handles for further elaboration, which allowed us to create a library of morphan-containing compounds by *in silico* molecular docking on the opioid receptor. Lead candidates were synthesized and radioligand binding assays[2] were performed to evaluate their ability to bind to opioid receptors. Four top compounds were found to exhibit inhibition constants in the micro molar range (5-30 μM) for the μ/δ receptor, while one of them shows a nano molar affinity for the δ-receptor. These four lead compounds, three phenyl esters and an *N*-phenylethyl morphan derivative, were selected for Molecular Dynamics simulations to get topological and thermodynamic information. Aromatic morphan derivatives displayed an interacting domain which fits into a hydrophobic cleft and the effect of the substituents in their affinity was explained by the differences in the calculated binding free energies. Our results indicate that the 3D arrangement of the aromatic ring in the morphine derivatives is not a key issue for a specific ligand μ-receptor interaction. Thus, these morphan derivatives represent a new class of opioid receptor ligands which may be of great use in the clinical practice, similarly to the already known 5-phenylmorphan family.[3]



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Triazolopyridopyrimidines: an emerging family of effective DNA photocleavers. DNA binding. Antileishmanial activity

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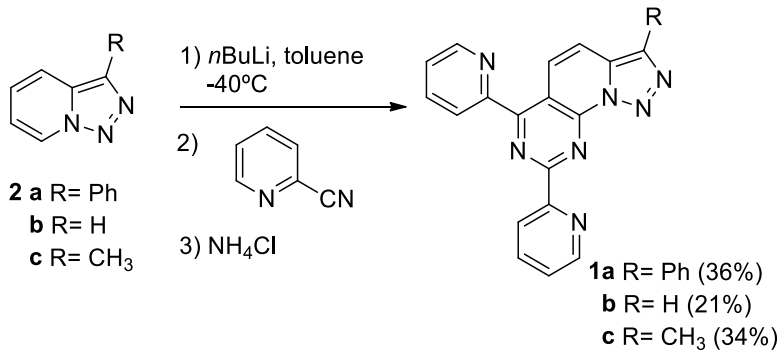
Triazolopyridopyrimidines **1a-c** were synthesized from triazolopyridines **2a-c**,[1] and their electrochemical and luminescent properties were deeply studied. The DNA binding ability of this series of compounds has been investigated by means of UV-vis absorption and fluorescence titrations, as well as viscosity measurements. Results have shown to be strongly influenced by the substituent at the triazol ring. Mechanistic investigations using radical scavengers showed that both **1b** and **1c** generate reactive oxygen species (singlet oxygen, superoxide and hydroxyl radicals) upon irradiation. Furthermore, compounds **1a-c** were tested for their antiprotozoal activity against four different *Leishmania* spp. Triazolo-pyridopyrimidines **1a** and **1c** resulted to be more active and selective than the reference drug (miltefosine) *in vitro* against *L. infantum* amastigotes. Compound **1a** exhibited high leishmanicidal activity against *L. infantum* spleen forms in the *in vivo* test.[2]



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Reaction of benzylamines with diethyl phosphate and triethyl orthoformate as a source of new enzyme effectors

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Aminobisphosponates are a group of stable analogues of inorganic pyrophosphate having nitrogen atom in its structure. Many bisphosphonates have been known as antiosteoporotic drugs in the treatment of diseases associated with dysfunction of calcium metabolism [1]. Most of them are also biologically active and can act as inhibitors of different enzymes, so they might be considered as promising anticancer, antimicrobial, antiviral, antiparasitic [2].

The three-component reaction between derivatives of benzylamine, triethyl orthoformate and diethyl phosphate seems to be simple and leading to preparation af aminomethylenebisphosphonates [3]. However, in frequent cases the reaction affords non-typical products, α-benzylaminophoshonates (**Fig.1**) The formation of these products is unexpected and mysterious. Moreover, the obtained α-benzylaminophosphonic acids have been proved the most effective synthetic inhibitors of human prostatic acid phosphatase in the treatment of prostate cancer and other disease [4][5].The aim of this work are studies on the pathways and mechanisms providing these new scaffolds and optimization of the procedures leading to the most promising compounds having potential biological properties.

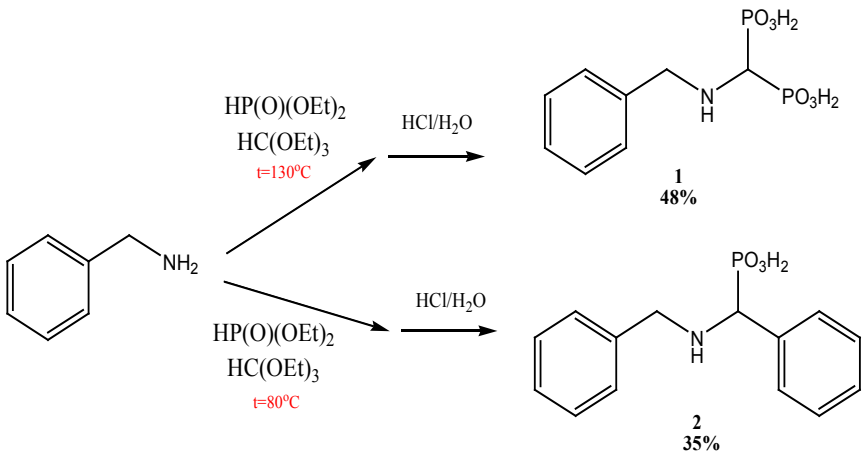

Fig. 1. The reaction leaded in 130°C gave N-benzyl-1-aminomethane-1,1-diphosphonic acid, but reaction in 80°C gave N-benzyl-1-aminobenzylphosphonic acid

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1,2-Ciclohexanodiamines as potent antitumour agents

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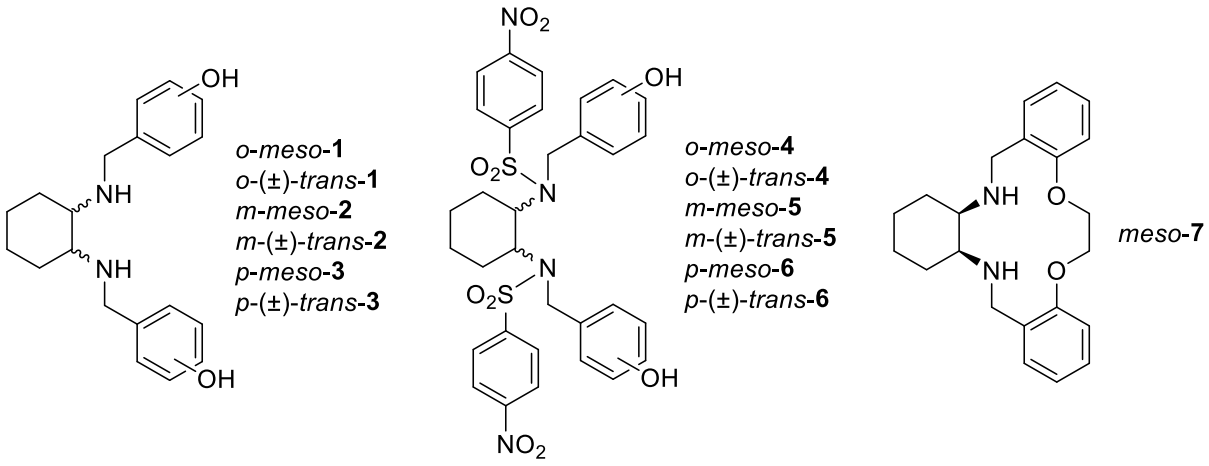
Medical interest has focused on macrocyclic polyamines in the last decades because of their chemical and biological properties as antitumour agents. 1,2-Diamines possess a wide range of bioactivities, such as the anticancer ones. Diamines *meso*- and *trans*-**1** (Figure) show anti-proliferative activity against the MCF-7 cell line [1]. Moreover, we have previously designed different macrocyclic molecules that target cancer [2-4]. Therefore, we present herein the synthesis and biological evaluation of a series of compounds (Figure) designed to study the influence of three structural modifications in the 1,2-diaminocyclohexane derivative (**1**): (a) displacement of the phenol group, (b) introduction of two *p*-nitrobenzenesulfonyl groups and (c), its conversion into a 14-membered macrocycle with an ethylene bridge that links the two phenolic groups of **1**.



Figure

Anti-proliferative, cell cycle and apoptosis analyses were carried out against the MCF-7, HCT-116 and A375 cancer cell lines. Our results show that all the compounds are potent cytotoxic agents against all tumour cell lines assayed, showing the best IC_{50} values against the A375 cell line. The selective activity of the macrocyclic derivative against the melanoma cell line A375, via apoptosis, supposes a great advantage for a future therapeutic use. This exemplifies the potential of 1,2-diaminocyclohexane derivatives to qualify as lead structures for medicinal chemistry campaigns, due to their easy syntheses and noteworthy bioactivity.

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LCAP Biomimetic – 5-HT7 receptor antagonists with antidepressant and pro-cognitive properties

Paweł Zajdel,[a,*] Vittorio Canale,[a] Rafał Kurczab,[b] Anna Partyka,[c] Grzegorz Satała,[b] Magdalena Jastrzębska-Więsek,[c] Anna Wesołowska,[c] Tomasz Kos,[d] Piotr Popik,[d] Andrzej J. Bojarski[b]

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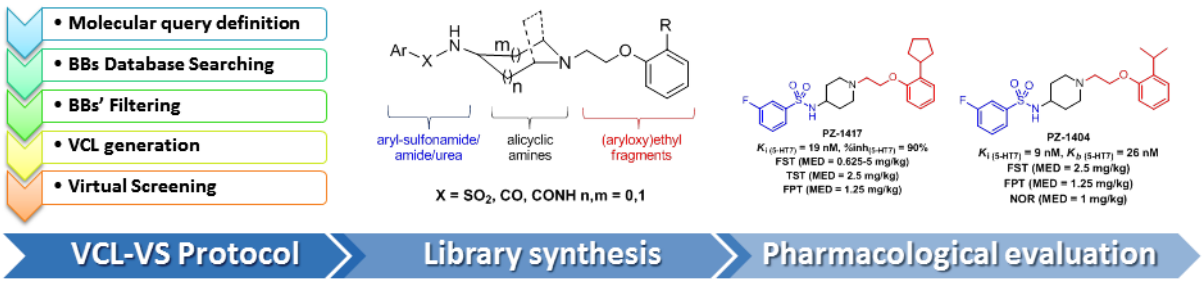
According to the World Health Organization (WHO), it has been estimated that by the year 2020, depression will be the second leading cause of severe disabilities, psychological and physical distress as well as of premature death. Despite progress in pharmacotherapy, efficacy of the clinically-used antidepressants is, however, quite limited. In only about 30–40% their use allow to achieve a remission, often with a risk of relapse, delayed onset of action, tolerance as well as with the sexual dysfunction, weight gain or cognitive impairment.



A growing body of preclinical and clinical data support the hypothesis that 5-HT$_7$R antagonists may be regarded as valid alternative of current drugs for the treatment of depression. Aiming to develop 5-HT$_7$R antagonists, we have recently confirmed the possibility to replace the long-chain arylpiperazine (LCAP) scaffolds, well-known 5-HT$_7$R ligands, with their biomimetic, namely arylsulfonamide derivatives of (aryloxy)ethyl alicyclic amines.[1,2] Virtual Combinatorial Library-Virtual Screening (VCL-VS) protocol integrated with solid-phase methodology has been applied for the selection and the synthesis of the library members. The study identified several 5-HT$_7$R antagonists which displayed distinct antidepressant-like properties in forced swim test (FST) and tail suspension test (TST) in mice and pro-cognitive activity in novel object recognition task (NOR) in rats.

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1-(*P*-Nitrobenzenesulfonyl)-1,2,3,5-tetrahydro-4,1-benzoxazepine as a new scaffold for the design of antitumour compounds

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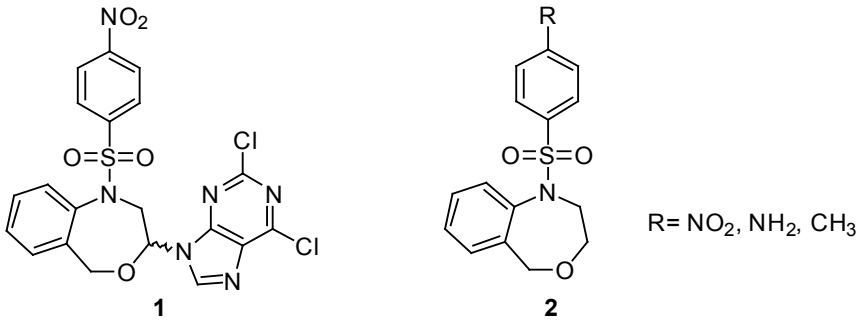
Bozepinib [(*RS*)-2,6-dichloro-9-[1-(p-nitrobenzenesulfonyl)-1,2,3,5-tetrahydro-4,1-benzoxaze-pine-3-yl]-9H-purine] **1** is a potent and selectivity antitumour compound that is able to induce apoptosis in breast cancer cells [1]. We have previously reported the role of the protein kinase PKR as a biological target of **1** involved in the apoptosis of breast and colon cancer cells. Moreover, the specific HER2, JNK and ERKs inhibition, the anti-angiogenic and anti-migration activity together with the *in vivo* anti-tumour and anti-metastatic effect and the non-systemic toxicity of **1** [2,3] has been reported. The chemical structure of bozepinib is made up by the benzo-fused seven-membered ring and the purine moiety. We have previously demonstrated that the purine fragment does not exert anti-proliferative effect *per se* [4]. Thus, in order to study the influence of the benzo-fused seven membered ring in the biological activity of bozepinib we have carried out the synthesis of a series of 1-(*p*-benzenesulfonyl)-4,1-bezoxazepine derivatives **2** (Figure).



Figure

Our results demonstrate that the 1-(*p*-nitrobenzenesulfonyl)-1,2,3,5-tetrahydro-4,1-benzoxazepine is an essential fragment in the anti-proliferative effect against the breast (MCF-7), colon (HCT-116) and melanoma (A-375) cancer cells.

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Control of the regioselectivity and stereochemistry of adenine derivatives

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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 racemic and enantiomers of 3,4-dihydro-2*H*-1,5-benzoxathiepin-3-ol (**1**) and several adenines, such as 6-*N*,*N*-dimethyl-, 2-chloro-6-*N*-methyl-, and 6-*N*-methyl-adenines. A complete inversion of the stereogenic centre of the secondary alcohol was observed when (*S*)-**1** and several adenine derivatives reacted to produce several homochiral contracted six-membered rings (Figure).

The results are interpreted in terms of multident nucleophile reactivity and S_N2 transition states of the Mitsunobu reactions. Alkylation sites have been determined by 2D NMR techniques and for three compounds they 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.

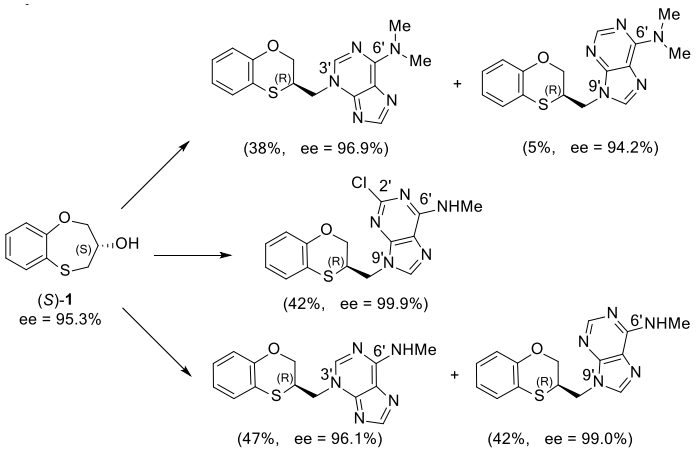

Figure: Alkylated adenine derivatives obtained by the Mitsunobu reaction under microwave-assisted conditions.

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Synthesis and anticancer activitiy of di- and tri-substituted purines with the phenyl glycidyl ether and its nitrogen analogue

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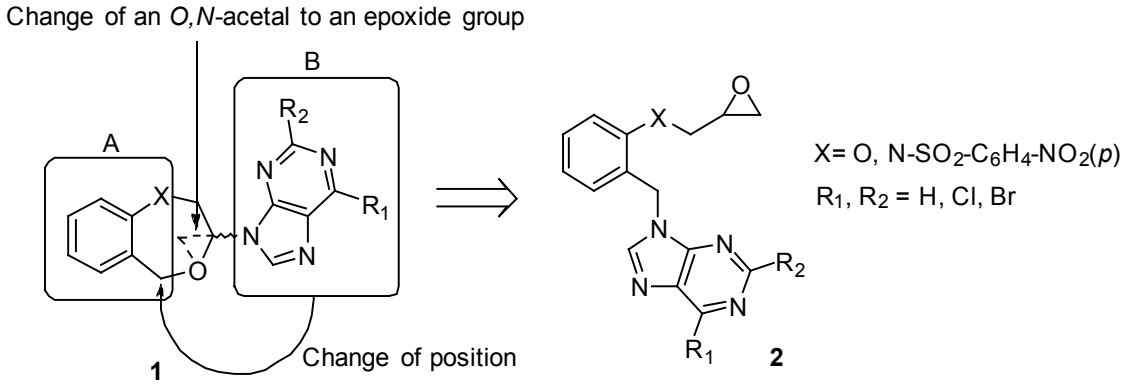
The development of new drugs against cancer belongs among the priorities of the development of science and fundamental research. Alkylating agents have been used for the treatment of cancer for over six decades and yet their repertoire continues to grow.

We have previously reported the synthesis and pharmacological evaluation of (*RS*)-2,6-substituted-9-(2,3-dihydro-5*H*-1,4-benzodiheteroepin-3-yl)-9*H*-purines [1-4]. All compounds show anti-proliferative activity against breast cancer cells.



Figure

Trying to increase the anticancer activity of compounds **1**, their structures were modified as followed: a) The 2-heterobenzyl fragment present in the 2,3-dihydro-5*H*-1,4-benzodioxepine, the 1,2,3,5-tetrahydro-4,1-benzoxazepin fragments (A), and the purines (B) of **1** were maintained; b) The interaction of xenobiotics with DNA and the resulting adducts have been studied extensively, in order to understand how they exhibit their cytotoxic and carcinogenic properties [5]. We have designed compounds in which the purine has been moved to the benzylic position of the benzene ring, and the heteroatom-ethyleneoxy fragment of the seven-membered ring of **1**, has been changed for an epoxymethyl group (Figure).

We describe herein the synthesis and anti-proliferative activity of a series of substituted 9-(2-oxiranylmethylheterobenzyl)-9*H*-purines **2**.

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Enantiospecific synthesis of homochiral compounds with different levels of apoptosis in the human cancerous cell line MCF-7

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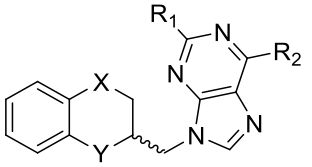
Breast cancer is the most common malignancy amongst women in the United States, and the second leading cause of cancer-related death in women [1]. The issue of chiral drug is now a major theme in the design, discovery and development of new drugs [2]. It has been shown for many pharmaceuticals that only one enantiomer contains the desired activity, and the synthesis of such drugs in their optically pure form is becoming increasingly important.

Racemic compounds **1**-**6** (Figure) 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 ee giving rise to 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 eukaryotic 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 have demonstrated 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

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

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Purines linked to *ortho*-subtituted benzyl groups with anti-proliferative activity

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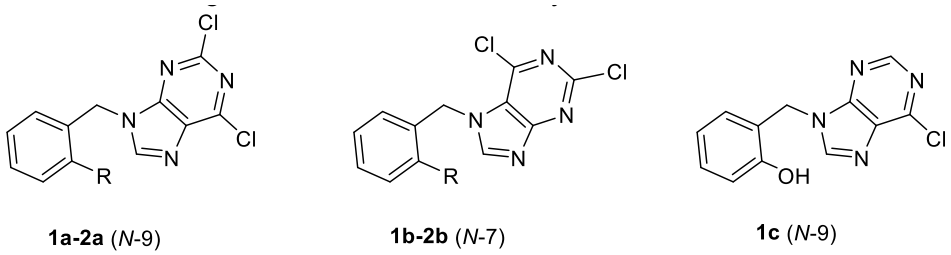
Purine derivatives show better anti-proliferative activitiy than the pirimidine ones; moreover, the presence of at least one halogen atom in the purine ring is necessary to improve the anti-proliferative activity [1].

The main purpose of this communication is to study the effect of the structural simplification in previously synthesized compounds in the research group *i.e.*, if the presence of the 6-chloro- or 2,6-dichloro-purines [2] moiety and the benzyl group is the minimum structural requirement to maintain the anti-proliferative activity against three human cancer cell lines (MCF-7, A-375, HCT-116). These *N*-9 (**1a**, **1c** and **2a**) and *N*-7 (**1b** and **2b**) alkylated purines have been obtained through microwave conditions by Mitsunobu reaction.



1a-2a (*N*-9) **1b-2b** (*N*-7) **1c** (*N*-9)

R= OH (compounds **1**), OMe (compounds **2**)

Figure. 1: Several alkylated purines.

During the reaction of 2-hydroxybenzyl and 2-methoxybenzyl alcohols with 2,6-dichloropurine both *N*-9 and *N*-7 isomers (**1a,b** and **2a,b**) were obtained; nevertheless, if 6-chloropurine was used as the starting heterocycle, the most stable *N*-9 purine was the only obtained regioisomer (**1c**).

IC_{50}	MCF-7	A-375	HCT-116
1a	6.42+/-0.016	3.77+/-0.005	1.84+/-0.013
1b	4.47+/-0.030	5.44+/-0.017	3.95+/-0.014
1c	12.41+/-0.021	4.35+/-0.014	5.25+/-0.009
2a	17.57+/-0.022	2.04+/-0.004	4.66+/-0.017
2b	2.49+/-0.009	1.71+/-0.004	4.20+/-0.006

Table. 1: The anti-proliferative activity of several alkylated purines.

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Discovery of indoleamine 2,3-dioxygenase inhibitors via sequential structure-based virtual screening and *in silico* ligand optimization

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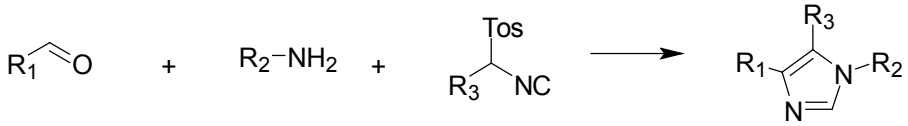
The kynurenine pathway leads to the conversion of tryptophan into kynurenine and other downstream metabolites that can suppress effector T-cell functions and promote T-regulatory cells differentiation. Indoleamine 2,3-dioxygenase (IDO) is a heme containing enzyme involved in the first step of this pathway, converting tryptophan into *N*-formylkynurenine. Cancer cells can overexpress IDO, leading to immunosuppressive microenvironment, tumour escaping and worsening of the disease;[1] hence, IDO inhibitors could represent novel immunotherapeutic candidates for cancer therapy.[2]

4-Phenylimidazole (4-PI) is a non-competitive IDO inhibitor, but is impaired by low selectivity, poor enzymatic and cellular activities, as well as unfavourable properties for pharmaceutical development.[2] As the Van Leusen multicomponent reaction (MCR)[3] is the most versatile access to substituted imidazoles known to date, we decided to exploit this transformation in the discovery of novel imidazole IDO inhibitors.



Scheme 1. Van Leusen MCR.

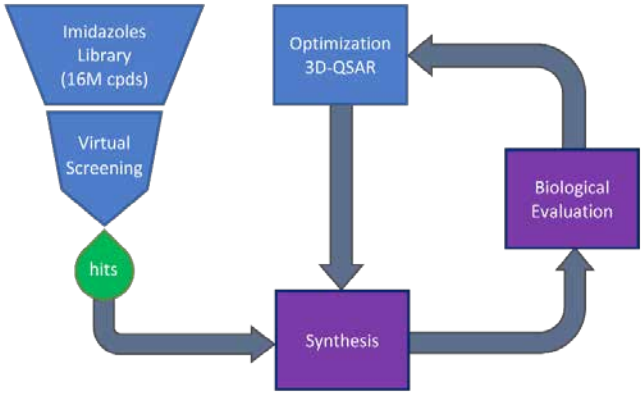


Figure 1. Workflow of the sequential virtual screening approach.

A sequential workflow was set up comprising the following steps: creation of a Van Leusen-accessible imidazole virtual library, IDO structure-based virtual screening, synthesis of the selected hits, evaluation of inhibitory activity, and identification of optimized inhibitors through ligand-based 3D-QSAR approach.

The combination of MCR-type chemistry with *in silico* prediction of drug potential is a new and powerful tool for the medicinal chemist in drug discovery. Experimental details and results of this multidisciplinary approach will be discussed.

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Searching for novel transient druggable pockets in BACE-1. Conformational analysis and definition of new pharmacophores

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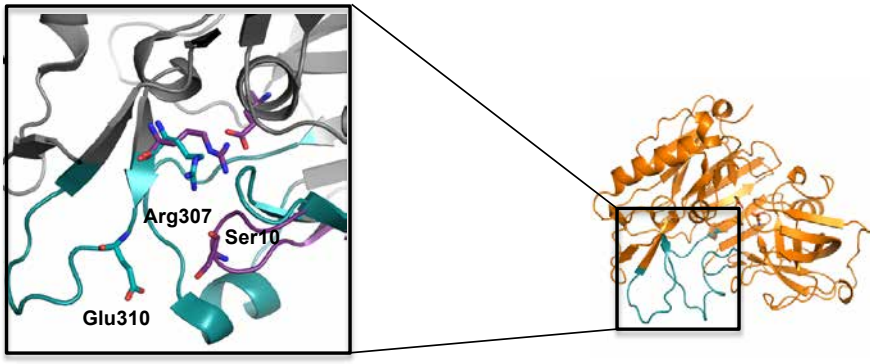
We have recently reported the multitarget pharmacological profile of a novel rhein-huprine hybrid compound, a multipotent disease-modifying anti-Alzheimer agent, which unexpectedly exhibited a potent inhibitory activity against BACE-1 (IC_{50} = 80 nM).[1] Importantly, none of the separate moieties was found to inhibit the enzyme, thus suggesting that their binding acts synergistically to enhance enzyme inhibition. Molecular modeling studies suggested that the huprine moiety should bind the aspartate dyad pocket, while the rhein moiety seemed to fill a "floppy" pocket never described so far. The interaction with Arg307 appeared to be a key feature for the binding of rhein.

To explore the structural and druggability features of the novel pocket, extensive MD simulations have been carried out to examine the conformational flexibility of the protein. In particular, 200 independent 100 ns MD replicas were run for the apo form of the enzyme, and principal component analysis was utilized to examine the conformational flexibility of the binding pocket using the pyPCAzip package.[2] Here, we report the results of the conformational analysis and clustering studies, which have allowed us to characterize the conformational preferences of Arg307 and of the highly flexible neighbouring loops that shape the binding pocket. Furthermore, a new pharmacophore for the above mentioned pocket has been proposed and a virtual screening of a library of 500,000 commercially available fragments has already led to the identification of several scaffolds as potential new hits. Their hybridization with huprine Y, by means of linkers of suitable length, is currently in progress with the aim of obtaining optimized BACE-1 inhibitors.



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Unexpected binding mode of new amantadine-like dual inhibitors of wild type and V27A mutant M2 channels of the Influenza A

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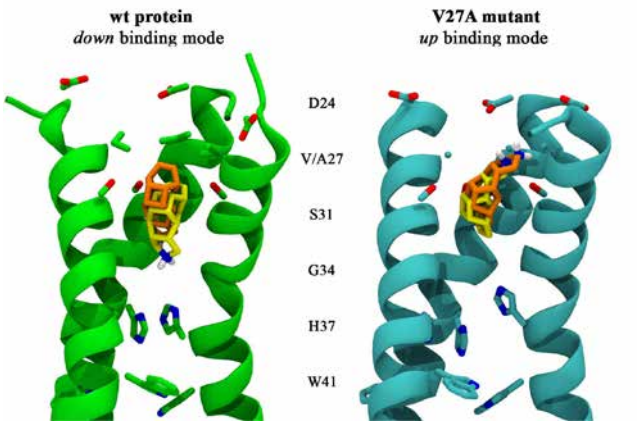
The quest for novel antinfluenza drugs is an urgent medical need, prompted both by the risk of appearance of a novel pandemic flu strains and the impact of drug-resistant associated mutations among already circulating viruses. Thus, while the M2 proton channel is a well-known validated target, there have been a dramatic increase in the number of influenza A strains expressing drug-resistant M2 phenotypes, which in turn has deprived known M2 inhibitors amantadine (Amt) and rimantadine (Rmt) of any relevant role as therapeutic agents.[1]those who have severe, complicated, or progressive illness or who require hospitalization Althoguh M2 mutations have been demonstrated to be viable and Amt-resistant, in 99% of the reported clinical cases viruses expressed one of three M2 variants, namely L26F, V27A or S31N[2–4] .Therefore, developing molecules that target multiple M2 variants is a feasible strategy to counterbalance the resistance mechanisms. In this context, the development of different wt/V27A dual M2 inhibitors has prompted us to study their binding mode by means of Molecular Dynamics simulations, aiming to shed light into the molecular determinants that govern channel inhibition. [5,6]



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STAT3 Direct inhibitors: from MD77 to AC33

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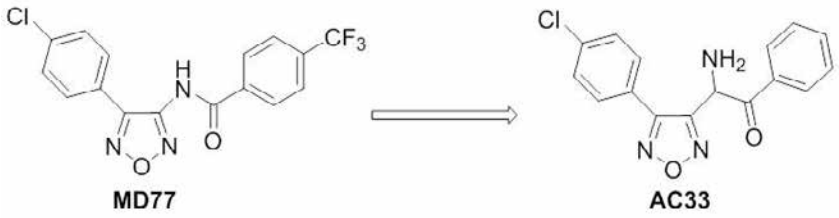
STAT3 (Signal Transducer and Activator of Transcription 3) is a latent cytosolic protein overexpressed in various cancer cell lines which was found to participate in oncogenesis by stimulating cell proliferation and preventing apoptosis [1]. Furthermore, previous studies have shown that blocking constitutively activated STAT3 signaling leads to tumor cell apoptosis, with minimal effect on normal cells [2,3]. This work aims at the synthesis, the structural analysis and the biological evaluation of novel potential antitumor agents, able to inhibit STAT3 protein-protein interaction, starting from MD77 previously identified by our research group [4,5]. Among them, AC33 was selected for further investigations since the AlphaScreen-based assay highlighted its ability to disrupt the STAT3 dimerization, with high selectivity over STAT1 and Grb2. These data induced us to modulate its pharmacokinetic and pharmacodynamic properties. The interesting results obtained will be presented.



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Studies on *Marrubium vulgare* L. leaves: preparation of extracts and in vitro wound healing activity evaluation

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Within a nature aided drug discovery research program targeting future therapies in regenerative medicine, we focus our attention on *Marrubium vulgare* L., an endemic plant distributed in North African area. In the ethnomedical tradition, *M. vulgare* is widely used and the drug extracts are proved to possess antioxidant, anti-inflammatory and antimicrobial properties.[1] Keeping in mind that the above mentioned properties are often related to cell-proliferating activity, and taking into account that chronic wounds represent an important disease of great socio-economic impact, in this work we presented our study on *M. vulgare* leaves extract and its *in vitro* wound healing effect.

Cultivation of wild-collected seed of *M. vulgare* coming from Tunisia was performed, the freshly leaves were sorted out, dried and ground to obtain a homogenous plant material powder. Leaf extracts were then prepared using various extraction procedures.[2] The phytochemical fingerprint of extracts was drawn and a preliminary biological screening performed by testing their free radical scavenging effect and the most active extract (*hit*) was subjected to a deeply investigation, evaluating its ability to promote cell proliferation.[3] A fibroblast cell proliferation assay and an *in vitro* wound-healing test were then performed.[3] Interestingly, *M. vulgare hit* extract showed significant *in vitro* dose-dependent wound healing effect. On the bases of these encouraging results, a bio-guided fractionation of the hit extract will be performed, in order to identify the metabolite/s responsible for the wound healing activity.

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Synthesis and antitumoral evaluation of new aromatic amides, ureas and thioureas

Castillo, Encarnación [a]; Galiana-Roselló, Cristina[a,b]; Aceves-Luquero, Clara[c]; Villalonga-Planells, Ruth[c]; Fernández de Mattos, Silvia[c]; González-Rosende, M. Eugenia[a]; Villalonga, Priam[c].

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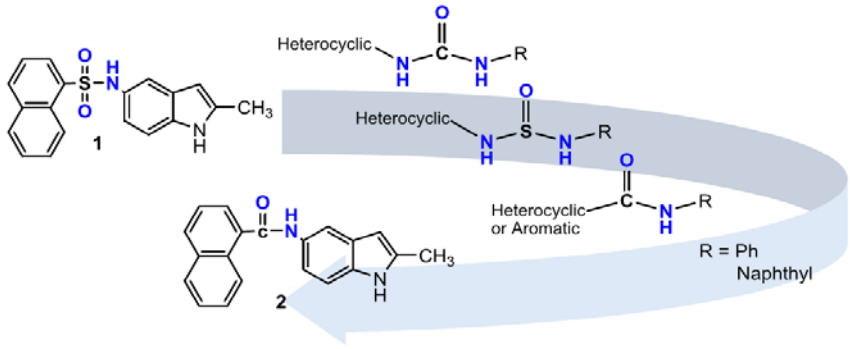
Chemotherapy is the mainstay of cancer therapy; however, the use of available chemotherapeutics is often limited, mainly due to undesirable side effects as well as the constant emergence of drug-resistant and multidrug resistant tumors which clearly underscores the need of developing alternative chemotherapeutic. Amides, ureas and thioureas have been widely used in medicine. Recently, compounds with carboxamide, urea and thiourea linkage have been shown to display promising antitumoral activities.[1]

In order to continue the investigation previously done by our group on antitumoral sulfonamides,[2] we further studied the in vitro activity of structural analogues with amide, urea and thiourea moieties against cancer cell lines MCF-7 and PC3. Compound **2** displayed an interesting antiproliferative activity. However the cell viability was higher than sulfonamide **1**. Our findings indicate the important role of the sulfonamide group. These results provided some hints for further studies on structure modification and new clues for anticancer drug design and development.



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Pharmacophore-based characterization of the Prestwick chemical library

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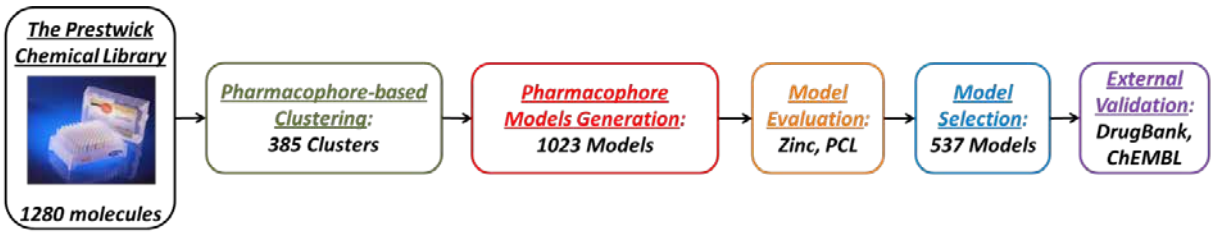
The discovery of novel drug molecules is a long, risky, and costly process and constant efforts are undertaken in order to optimize this task. For hit identification, the inception of smart and focused chemical libraries together with accurate in silico screening methods have proven their efficiency and hence, furthering early drug discovery. The Prestwick Chemical Library (PCL) [1] is a product arising from medicinal chemistry expertise comprising 1280 off-patent drugs, thus presenting a large degree of chemical and pharmacological diversity within a relatively small number of compounds. We were interested in studying the pharmacophore diversity of this library and performed pharmacophore-based (RDF) clustering of the PCL using the LigandScout [2] package and found 385 diverse clusters. From these unique clusters, a total of 1023 pharmacophore models were constructed and 537 were chosen according to their selectivity performance throughout a virtual screening experiment on a subset of the ZINC library. A selection of hand-picked pharmacophores underwent hence external validation using the DrugBank [3] and the ChEMBL [4] databases. Validated examples of our pharmacophore approach are illustrated in the poster. Furthermore, external validation clearly demonstrated that our models were able to return approved drugs connected to the same therapeutic target or therapeutic class. This, likewise, was found to be true for molecules that are still under investigation. We will use the resulting pharmacophore models for extending the PCL into uncovered molecular space.



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New sulfurated cinnamic acid derivatives as multitarget anticancer agents

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It is well known that inflammatory conditions in selected organs increase the risk of cancer. Compounds of the inflammatory tumor microenvironment include leukocytes, cytokines, complement components and are orchestrated by transcription factors, such as STAT-3 (Signal Transducer and Activator of Transcription 3) and NF-kB.[1,2] Therefore drugs able to inhibit one or both transcription factors could be useful tools to treat cancer disease.

Since natural sulfurated compounds (such as dithiolethiones, allicin, diallylpolysulfides and S-methylmethanethiosulfonate) and some cinnamic acid derivatives (in particular ferulic acid and caffeic acid) are endowed with cancer chemopreventive properties, through multiple mechanisms (including STAT3 and/or NF-kB inhibition), we decided to synthesize a set of novel drug hybrids (Fig.1) where the two components of the molecule can act in a synergistic way, thus obtaining new more potent multitarget antiproliferative agents.

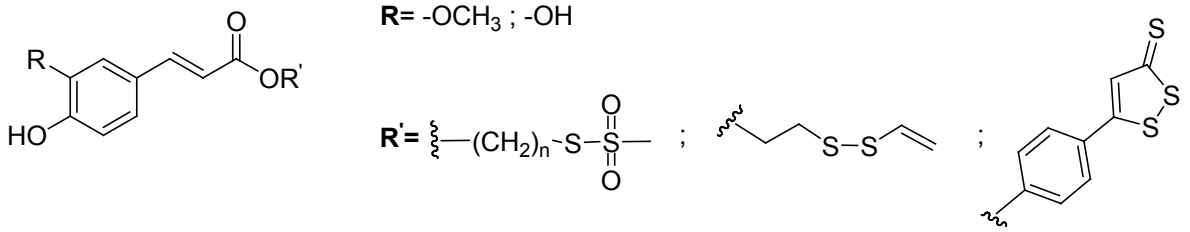

Figure 1. General structure of the synthesized compounds

The new synthesized compounds were submitted to the *AlphaScreen-based assay*, to investigate their ability to bind STAT-3 SH2 domain and to *Luciferase promoter activities assay*, to measure their ability to inhibit NF-kB promoter activity.

Results showed that most of the new hybrid compounds inhibited HCT-116 cell proliferation *in vitro* with IC_{50} in micromolar range. In addition, they were able to strongly and selectively bind STAT-3 SH2 domain (whereas the parent drugs were completely devoid of this ability at the tested concentrations) and were also able to inhibit the NF-kB transcriptional activity in HCT-116 cell line.

Studies are ongoing to better define the profile of our new hybrids as potential dual STAT-3/NF-kB inhibitors.

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Flow synthesis of gold nanoparticles

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Gold nanoparticles (AuNPs) are an interesting and emerging material which has found a wide range of applications including electronics, catalysis and biomedicine [1]. The aim of this work was the preparation of AuNPs with different size using flow apparatus. AuNPs were prepared by reducing Au^{3+} (from $HAuCl_4$) to Au^0 using sodium citrate as reducing and stabilizing agent [2]. The synthesis was performed in a flow synthesizer equipped with two integrated pumps, a loop injection system, a 10 mL PTFE reactor coil, a back pressure regulator and a fraction collector [3]. Two 2^3 factorial designs and two setups were used to evaluate the effect of temperature, flow rate and sodium citrate/$HAuCl_4$ ratio on AuNP size and shape. In the first set-up, a pre-mixed aqueous solution of sodium citrate and $HAuCl_4$ was flowed by the pump through the reactor coil, while in the second the aqueous stock solution of $HAuCl_4$ was injected into the loops and pumped through the reactor heater. AuNP dimensions were estimated by photon correlation spectroscopy and particle size was expressed as mean hydrodynamic diameter. Morphology was characterized using transmission electron microscopy. Designs of experiments were employed to determine factors influencing AuNP size, shape and production yield. The potential advantages in terms of size control, scalability and industrial applications of our method will be illustrated and discussed.

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Design, synthesis and structural characterization of new macrocyclic ligands and their lanthanide complexes

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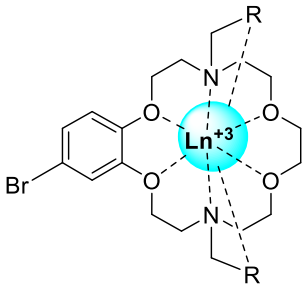
The use of lanthanide binding *tags* (LBTs) to obtain new NMR parameters providing structural information is an interesting tool to study the conformational behavior of carbohydrates and their interaction with protein receptors.[1,2]

We have shown recently that pseudocontact shifts (PCS) and residual dipolar couplings (RDC) are developed when chelating a lanthanide ion to an EDTA or PhDTA unit attached to a carbohydrate. A convenient rigid linker is situated between the metal and the biomolecule in order to decrease the effect of paramagnetic relaxation enhancement (PREs). The paramagnetic effects observed were used to study protein-carbohydrate interactions between lactose and a human galectin.[3]

The design and synthesis of new *tags* that can selectivity recognize trivalent lanthanide ions (Ln^{3+}) giving well defined stable coordinates remains a challenging task.

Herein we will describe our preliminary results on the use of pendant-armed macrocyclic based ligands designed for complexation of Ln^{3+} in aqueous solution.

A new cyclic framework of benzo-4,13-diaza-18-crown-6 bearing pyridyl (L_1) and carboxylate (L_2) pendant groups has been synthesized by the corresponding *N*-alkylation reaction. Metal complexes of L_1 and L_2 using hydrated nitrate salts of lanthanide ions have been synthesized and characterized by microanalysis, mass spectrometry, ATG/DSC, IR and magnetic studies. ^1H and ^{13}C NMR spectra of the complexes formed with Ln^{3+} were obtained in D_2O solution and assigned with the aid of HMQC 2D heteronuclear experiments, as well as standard 2D homonuclear COSY and NOESY spectra. Proton NMR spectroscopic titration confirm the formation of 1:1 complexes.[4]



L_1 = R = 2-pyridyl

L_2 = R = COOH

$LnL_n(NO_3)_3.xH_2O$

Ln^{3+} = diamagnetic La(III), Lu(III) and paramagnetic Eu (III), Tb(III) and Dy (III)

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New polymer-drug conjugates for the treatment of colorectal cancer

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Current chemotherapeutic treatment for metastatic colorectal cancer (CRC) involves high doses of cytotoxic drugs particularly adjuvant combinations of 5-Fluorouracil (5-FU) and Irinotecan (prodrug of SN-38). However, these treatments cause undesirable effects to the patients, which can negatively contribute to their survival. Our efforts to achieve a more specific 5-FU and SN-38 release in tumor tissues are directed towards the synthesis and preclinical evaluation of new polymer-drug conjugates (PDC) based on polyglutamic acid (PGA) or PEGylated systems, loaded with 5-FU or SN38 alone or with both drugs.

Three PDCs designed for colorectal cancer monotherapy treatment, PGA-5FU, SN38-PEG and SN38-PEG-TCP1 will be reported. This last conjugate contains a targeting peptide, TCP1 addressed to colorectal tumor tissues. Their synthesis, physicochemical characterization, *in vitro* efficacy with HT-29 and HCT-116 colorectal cancer cells and *in vivo* tumor accumulation and whole body biodistribution performed with human colon cancer tumor mice models will be described. PGA-5FU and SN38-PEG-TCP1 showed improved tumor accumulation at 25h h compared with the corresponding free drug or with the PEGylated drug without targeting peptide, TCP1.

New polyamine-polyamide ligands as anticancer drugs

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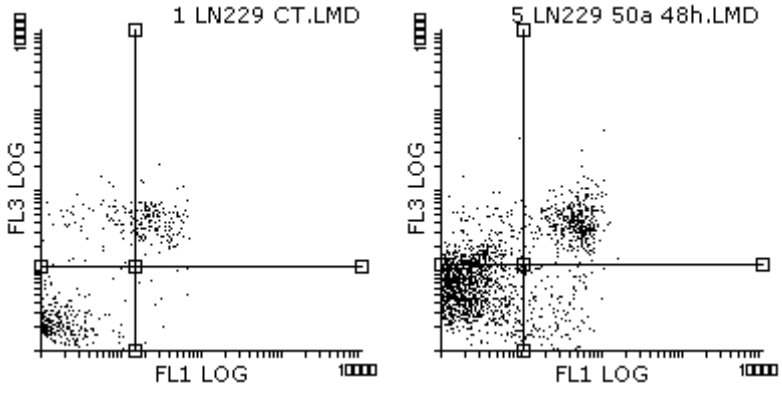
Cancer figure among the leading causes of morbidity and mortality worldwide. It is expected to rise as a result of viral infections and behavioural/dietary risks such as smoking, alcohol consuming, physical inactivity, high body mass index or low fruit and vegetable intake.[1]

Naturally occurring polyamines are ubiquitous compounds found in all eukaryotic and prokaryotic cells. Polyamines play important roles in cell growth, proliferation and differentiation. The biological-polyamine concentrations of putresceina, spermidine and spermine are increased significantly in cancer cells and tissues. At physiological conditions, the polyamines are positive-charged and, in the biological systems, bind to the negative-charged polyanions such as proteins and nucleic acids. These important features prompted to several groups to prepare libraries of biological polyamines derivatives to generate new anticancer drugs.[2]

Herein, it was described the synthesis of several polyamine-amide compounds. The compounds were evaluated for their *in vitro* antiproliferative activities against a panel of eleven human cancer cell lines including Glioblastoma, Colorectal Cancer, non-Hodgkin Lymphoma and Acute T-cell Leukemia cells. The determination of the cell viability was based on ATP quantification using the Cell Titer-Glo Luminiscent assay that outstand the presence of metabolically active cells. In addition, the Cignal Pathway Reporter assay was used to measure the activity of 45 signaling pathways and identified several pathways perturbed by the compounds. In this communication, we report the synthesis and details of the biological data.



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Polyglutamate-based combination therapy in the treatment of advanced breast cancer

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INTRODUCTION

Polymer Therapeutics comprise a variety of complex and rationally designed nanoscale systems in which a water-soluble polymer carrier is provided with covalently bonded bioactive species. A branch of Polymer Therapeutics are Polymer-Drug Conjugates, in which one or more drugs as well as different targeting moieties are chemically bound to the polymer backbone via a biodegradable spacer. The use of Polymer-Drug Conjugates in combination therapy offers the best opportunity to enhance antitumor effect and to reduce the severe side effects[1,2]. Conjugation of two different drugs covalently to the same polymer carriers through protease cleavable or pH sensitive linkers allow to control the release kinetics profile. In our systems, we combine chemotherapeutic agent with endocrine agent in the proper synergistic ratio by binding to the same polymer carrier, ensuring the simultaneous uptake to the target cells and bringing significant advantage versus single treatments. We have previously reported the first polymer drug conjugate of this type: the non-biodegradable HPMA copolymer bearing the combination of the aromatase inhibitor Aminoglutethimide (AGM) with the anthracycline Doxorubicin (Dox)[3,4]. In vivo proof of concept of this conjugate, HPMA copolymer-AGM-Dox has already been achieved in an orthotopic 4T1 murine metastatic breast cancer model and its mechanism of action has been studied[5].In order to further improve the features of our system, we moved to a biodegradable and multivalent carrier poly(L-glutamic acid) (PGA) that allows not only to increase the conjugate molecular weight, being able to enhance the loading possibilities, but also to reduce the immunogenicity and to increase the tumor targeting due to the EPR effect.

RESULTS AND DISCUSSION

A novel PGA-AGM-Dox conjugate family has been developed through amide coupling chemistry. The family includes conjugates with different drug linkers as well as drug ratios, that have been deeply characterized by different physicochemical techniques (NMR, HPLC, DLS, SLS, SANS, etc) in order to elucidate structural and conformational features in solution as physico-chemical descriptors for qualitative determination of structural-activity relationships in biological settings. This family of conjugates has shown the capacity of self-assemble in aggregates around 100 nanometers in aqueous solution, showing differences in either their critical aggregation concentration and shape as evidenced by DLS, SANS and cryo-TEM respectively. *In vitro* evaluation of drug release kinetics, cellular uptake and cell viability allowed a screening for the best candidates achieving drug synergism in both MCF-7ca and 4T1 cell lines. The conjugates showing the greatest *in vitro* activity, were chosen for *in vivo* evaluation in an orthotopic 4T1 murine tumor model and the conjugate PGA-GAGM$_{10}$-Dox$_5$ presented the best antitumor activity. In summary, we demonstrated that structural and conformation features in solution of **Polymer-Drug Conjugates** may play an active role, leading to differences in the biological performance[6].

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Novel inhibitors of the bacterial cell division protein FtsZ: synthesis and antibacterial activity

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Nowadays, bacterial infections are one of the most prevalent health hazard and resistance towards the common antibiotics is in continuous growing. Consequently, the developing of novel antimicrobial agents, having an innovative mechanism of action is now under investigation[1]. In this context in the latest years FtsZ emerged and was proved to be essential in bacterial cell division, because it is able to form a circumferential dynamic Z-ring. In the absence of this Z-ring, the bacterial cell division is blocked[2]. Moreover, FtsZ is conserved in virtually all eubacteria turning out to be actually a potential drug target.

In the latest years, a growing number of small molecules (Figure 1) were developed and revealed to interact with FtsZ and with bacterial cell division; their structures derived from preliminary SAR studies of 3-MBA (3- methoxy benzamide), proven to be an FtsZ inhibitor[3].

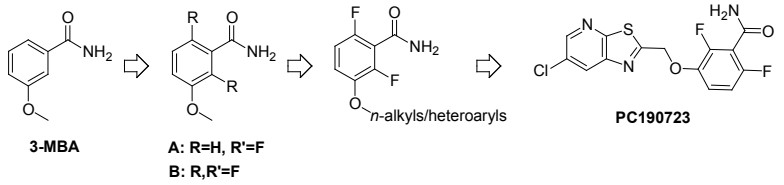

Figure 1

In a recent paper[4], we have demonstrated that the substitution of the tiazolopyridine system of PC190723 with a benzodioxan moiety accomplished promising antibacterial agents, including compound **I**, having a MIC of 0,5 µg/mL.

The present work focused on the synthesis of novel FtsZ inhibitors (Figure 2), where the benzodioxane scaffold is modified:

– introducing different substituents at the aromatic fused ring (Fig **2A**);
– at the dioxane portion, in order to understand the importance of the Oxygen (Fig. **2B**);
– changing the linking point between the methylenic chain and the benzodioxane moiety (Fig. **2C**);

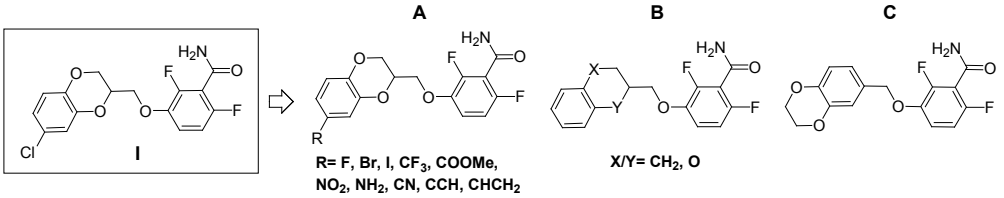

Figure 2

Inhibition of bacterial growth was tested on both Gram positive and Gram negative bacteria, in particular the minimal inhibiting concentration (MIC) and the minimal bactericidal concentration (MBC) were determined.

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Dual modulators of dopamine D3 receptor and fatty acid amide hydrolase: modeling and structure activity relationship studies

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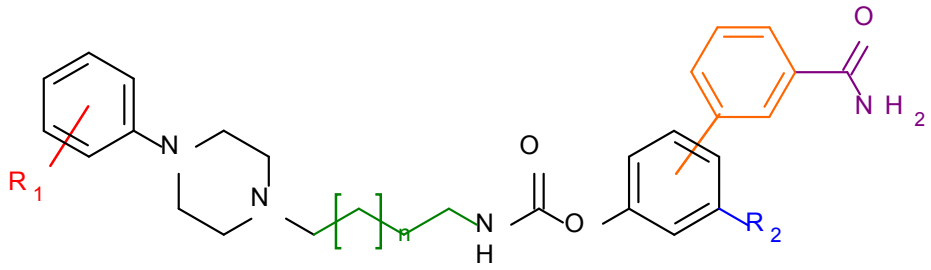
Multi-target directed ligands, namely compounds able to concurrently modulate multiple targets, can hold a great potential toward exerting disease-modifying effects on complex conditions.[1] Recently, we reported the first series of derivatives endowed with potent and balanced activities toward both D3 dopamine receptor and fatty acid amide hydrolase (FAAH) enzyme.[2,3] These two targets contribute through different pathways to initiate and maintain nicotine addiction and engaging them simultaneously could represent a viable strategy toward obtaining truly effective medications against craving and relapse. Once the accuracy of our in silico predictions was experimentally confirmed on prototype compounds, we carried out a synthetic campaign aimed at elucidating the structure activity relationship on this scaffold (Figure 1). Here, some relevant aspects related to the application of computational methods toward tuning selectivity and the partial dopamine D3 partial agonist profile of our molecules are discussed.

Figure 1



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Structure-based lead-optimization of quinoline-based agents as selective non-nucleoside DNA methyltransferase inhibitors

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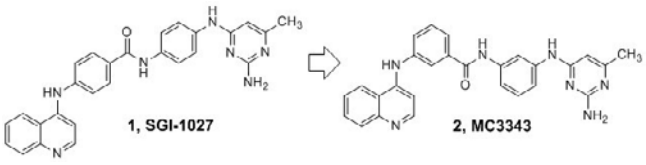
Epigenetics describe the changes happening on a chromosome without altering its DNA sequence, leading to a heritable and stable phenotype [1, 2]. Epigenetic modifications, such as DNA methylation, play an important role in the modulation of gene expression. DNA methylation occurs by transfer of a methyl group from *S*-adenosyl-L-methionine (AdoMet) to the C5-position of cytosine predominantly in the CpG dinucleotides (CpG islands) and is catalyzed by three DNA methyltransferases (DNMTs), DNMT1, DNMT3A and DNMT3B [3]. DNA methylation is a crucial epigenetic modification in modulation of gene expression and it is implied in various physiological processes such as genomic imprinting, chromosome X inactivation, gene silencing, chromosomes stability and repression of transposons. Abnormal activity of DNMTs is described in several diseases including cancer [4]. Disruption of DNMT1 can stop tumour growth and reverse the nondifferentiation state, [5] and the use of specific inhibitors of DNMTs (DNMTi) can reactivate silenced tumour suppressor genes (TSGs) and induce the reprogramming of cancer cells, leading to their proliferation arrest and death [6]. To date only a small number of DNMT inhibitors (DNMTi) has been reported. Among them just two 5-azanucleosides (azacytidine and decitabine) have been approved for the treatment of haematological malignancies but, despite their efficacy, they suffer from poor bioavailability, chemical instability and severe side-effects [7]. Thus, the discovery of novel, potent and selective non-nucleoside chemical entities able to negatively modulate the activity of these enzymes possibly without negative effects is of great interest [8]. Among the non-nucleoside DNMTi, SGI1027 (**1**) was described able to reactivate TSGs in cancer cells [8]. Recently we described MC3343 (**2**), MC3353, MC2838 and MC2835, analogues of SGI-1027, able to inhibit proliferation in several cancer cell lines at low *μM* concentrations. Moreover, tested in PBMC, MC3343 (**2**) was reported to possess a similar potency compared to the SGI-1027, but was more selective trough DNMTs rather than other SAM-dependent methyltransferases and it was able to inhibit proliferation also in cancer stem cells [9].



After this first successful optimization, we will report further quinoline- and/or pyrimidine-containing analogues as novel DNMTi. Such compounds will be tested for their demethylating activity in a luciferase reporter system in cells, and for their antiproliferative effects in a panel of cancer cells.

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New analytical tools to investigate the role of protein glycosylation in Alzheimer's disease

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Glycosylation is one of the most common and the complex forms of post-translational modification of proteins and plays a key role in modulating protein folding, conformation, stability and activity. Changes in the glycosylation pattern of brain proteins have been associated with several neurodegenerative diseases such as Creutzfeldt–Jakob disease [1] and Alzheimer's disease (AD) [2]. In particular, defects in the glycosylation pattern of the Amyloid Precursor Protein (APP), tau and other proteins have been reported in AD patients [2]. As an example, alterations of N-glycosylation of APP has been observed in Swedish and London mutations, which are associated with an increased total amount of amyloid-beta peptide (Aβ) and an increased Aβ42/Aβ40 ratio [3].

Notwithstanding being highly important for the development of improved treatment approaches for AD, the role of protein glycosylation in AD is a relatively low explored topic.

This is partially related to the low amount of pathological samples and complexity of the glycosylation analysis. Indeed, the basic pipeline for glycosylation analysis is time consuming and includes protein deglycosylation and/or glycoprotein digestion (or combination of) followed by the analysis of intact proteins, glycopeptides and free glycans (either not labeled or labeled). This long procedure often hampers the routine screening of the glycosylation pattern and limits the identification of new targets for drug discovery. To overcome low automation rate and long analysis time, glycan release by an immobilized enzyme reactors, i.e., enzymes immobilized on a suitable solid support (IMERs), can be used as a valuable alternative to the in solution assay. Thus, a novel Peptide-N-glycosidase F (PNGase F) immobilized enzyme reactor was designed and prepared by the oriented covalent immobilization of the target enzyme onto the surface of a short bed, high performance monolithic column (epoxy CIMac™ analytical column, Bia Separations, Slovenia). PNGase F is a commonly used enzymatic tool that cleaves N-linked glycans between the innermost GlcNAc and the asparagines of high-mannose, hybrid and complex oligosaccharides, leaving the released glycan intact. Thus, the developed IMER should be suitable for the evaluation of altered N-glycosylation pattern in AD key proteins such as APP.

In this study, the PNGase F-IMER was designed, prepared and first characterized using RNAase B as reference substrate. Furthermore, a liquid chromatography – mass spectrometry (LC-MS) integrated platform consisting in a series of two analytical columns, enabling the separation and analysis of the proteins and the free glycans released by the IMER, was set up. The integrated system offered advantages in terms of automation, reduction of analysis time and investigation of the glycan pattern without need of glycan labeling. This advantages should translate into the possibility of carrying out screening campaigns on larger amount of samples enabling the identification of pathological alterations of the glycosylation pattern of key proteins as well as the investigation of potential new approaches in drug discovery.

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Development of ligands for the validation of the lysophosphatidic acid receptor LPA_1

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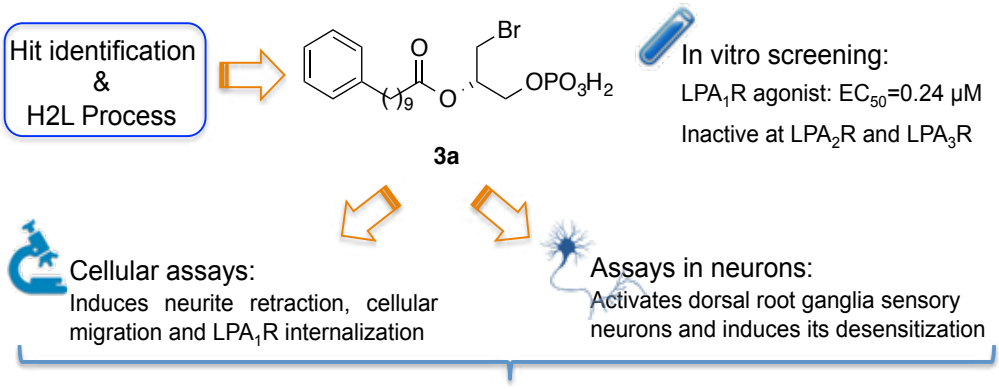
Lysophosphatidic acid (LPA) is a bioactive phospholipid involved in a wide range of biological functions in the nervous, vascular, immune and reproductive systems, and in diseases such as cancer, fibrosis, and obesity. Former orphan Edg2 receptor has been characterized as the LPA receptor of type 1 (LPA_1R).[1] The location of LPA_1R, mainly in the central nervous system (CNS), but also in the reproductive and immune systems, makes it a promising target for therapeutic intervention, and thus, the development of selective and high affinity ligands of LPA_1R is critical for its validation.[2,3] Accordingly, we have started a project aimed at the development of new ligands to validate LPA_1R as a therapeutic target.

In order to find new hits, the structure of the endogenous ligand LPA was considered as starting point. On this basis, a series of compounds were designed, synthesized and assayed, leading to the identification of new LPA_1 agonists. Then, a hit to lead process was carried out. Among them, derivative **3a**, with an EC_{50} of 0.24 μM at LPA_1R, stands out as the first agonist structurally different from the endogenous ligand LPA. This derivative is selective against other LPA receptor subtypes, and it is active in different cellular systems, including neurons in primary culture. These results have prompted us to study the effect of the compound in a pain model, experiments that are currently ongoing.



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Automating computer simulations in drug discovery

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Performing computational experiments using molecular dynamics simulations is still too difficult and the recent capability of running thousands of simulations has further exacerbated the problem. We present here HTMD, a Matlab-like programmable environment that provides the power of system preparation, molecular dynamics, Markov state model analysis and visualization at once. Thus, a single short script can lead from the PDB structure to useful quantities such as relaxation timescales, equilibrium populations, conformations and kinetic rates. This facilitates scientists with minimal background to easily integrate MD into their discovery workflow, drastically reduce errors and improve reproducibility. In addition, we have developed AceCloud, an on-demand service for molecular dynamics simulations designed to facilitate the secure execution of large ensembles of simulations on an external cloud computing service (currently Amazon Web Services). The AceCloud client, integrated into the ACEMD molecular dynamics package, provides an easy-to-use interface that abstracts all aspects of interaction with the cloud services. This gives the user the experience that all simulations are running on their local machine, minimizing the learning curve typically associated with the transition to using high performance computing services. All in all, HTMD and AceCloud make a step forward towards building a robust simulation-based environment for Drug Discovery.

New approaches to oxirane containing natural products: stereoselective total synthesis of the antitumor agents gummiferol and depudecin

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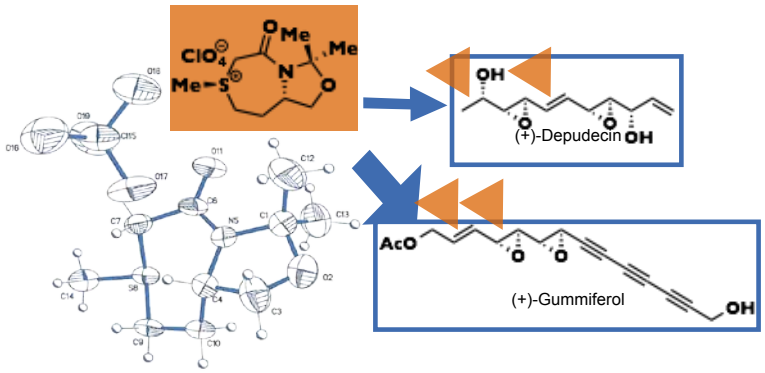
The present work deals with the total synthesis of the natural compounds gummiferol, [1] isolated from the leaves of *Adenia gummifera* and depudecin, [2] isolated from the fungus *Alternaria brassiciola*.

The target molecules, selected in virtue of their prominent antitumor and antiangiogenic activities are featured by the presence of two oxirane rings and have been synthesized by application of a novel methodology of asymmetric epoxidation, based on the use of a new class of chiral sulfur ylides.[3] This strategy has been successfully applied to the synthesis of natural products of biological interest, such as the case of bengamides [4], the cyclodepsidpeptides globomycin and SF-1902 A5 [5], or the sphingoid-type bases clavaminol H, phytosphingosine, sphinganine or sphingosine [6].

The proposed synthetic routes employ cheap and readily available starting materials and additionally provide access to different analogues that may lead to the establishment of SAR studies and the design of potential drug candidates.



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Validating with small-molecules the VHL:HIF-1α protein:protein interaction as a new target in the hypoxia pathway

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Hypoxia Inducible Factor 1α (HIF-1α) is an oxygen-sensitive transcription factor that regulates over 2% of human genes, specially the ones linked to processes such as angiogenesis, cell proliferation and glucose uptake.[1] Under normoxia HIF-1α it is targeted for proteasomal degradation by the $CRL2^{VHL}$ ligase *via* its hydroxylation by prolyl hydroxylase domain (PHD) enzymes. Pharmacological stabilization of HIF-1α has raised significant attention, particularly, progress has been made in the development of PHD inhibitors, with 6 examples of compounds in on-going clinical trials.[2,3]

Crucial for the HIF-1α levels is its recruitment by von Hippel Lindau protein (pVHL), the recognition unit of the $CRL2^{VHL}$. Therefore, the development of inhibitors of the pVHL:HIF-1α interaction could provide an alternative approach by allowing chemical intervention in the pathway downstream, directly blocking the HIF-1α degradation. Such an approach may avoid the HIF-independent *off*-target effects observed with PHD inhibitors.

Using a rational design we have generated nanomolar inhibitors of the VHL:HIF-1α protein-protein interaction.[4] These new inhibitors have been characterized by isothermal titration calorimetry (ITC) and fluorescence polarization (FP) and the binding mode with pVHL elucidated using X-ray crystallography. We have determined their target selectivity and target engagement with a chemoproteomic approach. Moreover, the intracellular unbound concentration of the compounds has been measured. Immunoblotting and immunoprecipitation experiments have been performed to demonstrate downstream action. Finally, luciferase assays and mRNA expression of HIF1α-target genes have been also assessed. Our data shows for the first time that small-molecule inhibition of the pVHL E3 ubiquitin ligase leads to stabilisation of transcriptionally active hydroxylated HIF1α, providing an alternative therapeutic target in the hypoxia signalling pathway, as well as, a useful tool for the study of the unknown VHL and HIF functions.

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