

2nd Iberic Meeting on Medicinal Chemistry:

G Protein-Coupled Receptors and Enzymes in Drug Discovery

Porto, Portugal
12 – 15 June, 2011

http://2immc.fc.up.pt

Program and Abstracts


International Year of
CHEMISTRY
2011


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G Protein-Coupled Receptors and Enzymes in Drug Discovery

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Content

Preface

The aim of this meeting is to provide a forum for discussion of recent results in the field of Medicinal Chemistry, particularly concerning the recent developments and trends in the field of promising targets such as GPCRs and enzymes. A strong interplay is expected between academic researchers and clinical/industry practitioners, in a feed-back aimed at translating elemental research into innovative therapeutic protocols.

The meeting is thus intended to assemble multidisciplinary scientists from different areas (Bioorganic and Bioinorganic Chemistry, Biophysics, Biochemistry, Biology, Bioinformatics, Pharmacology, Pharmacy and Medicine) representing distinct perspectives related to molecular aspects of the therapy. Its intimate character and social program is intended to stimulate interactions and discussion among academic and non-academic researchers.

It is the goal of the organizers to make this meeting an event of scientific excellence, attractive to both industrial and academic scientists in Medicinal Chemistry and related fields of research.

Committee

Members

SCIENTIFIC COMMITTEE

Amílcar Falcão (Univ. Coimbra, Portugal)
Carmen Terán (Univ.Vigo, Spain)
Claudio Sunkel (Univ. Porto, IBMC, Portugal)
Eugenio Uriarte (Univ. Santiago Compostela, Spain)
Fernanda Borges (Univ. Porto, Portugal)
João José Sousa (Univ. Coimbra, CEF/UC, Portugal)
Joaquin Campos (Univ. Granada, Spain)
Jorge Gonçalves (Univ. Porto, SPF, Portugal)
Manuel A. V. Ribeiro da Silva (Univ. Porto, CIQ/UP, Portugal)
Manuel Sobrinho Simões (Univ. Porto, IPATIMUP, Portugal)
Maria João R. P. Queiroz (Univ. do Minho, CQ/UM, Portugal)
Maria Luz López Rodríguez (Univ. Complut. Madrid, SEQT, Spain)
Marina Gordaliza Escobar (Univ. Salamanca, Spain)
Natércia Teixeira (Univ. Porto-SPB, Portugal)

ORGANIZING COMMITTEE

Elisiário Tavares (Univ. Coimbra, Portugal)
Fernanda Borges (Univ. Porto, Portugal)
Fernanda Roleira (Univ. Coimbra, Portugal)
Francisco Silva (ISCS-N/CESPU, Porto, Portugal)
Jorge Garrido (ISEP-IPP, Porto, Portugal)
Lourdes Santana (Univ. Santiago Compostela, Spain)
Marta Teijeira (Univ. Vigo, Spain)
Natália Cordeiro (Univ. Porto, Portugal)
Rita Catarino (FCS-UFP, Porto, Portugal)

LOCAL ORGANIZING COMMITTEE

Alexandra Gaspar - Responsable
Carla Varela
Catarina Oliveira
Fernando Cagide
Filipe Teixeira
Joana Reis
Nuno Milhazes
Paulo Ferreira
Sofia Benfeito
Tiago Silva

General Information

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VENUE

Faculty of Science of the University of Porto
Anfiteatro 0.41 do edifício FC4 (Edifício do Departamento de Biologia) da Faculdade Ciências do Porto; Rua do Campo Alegre, s/n, Edifício FC4.
Porto
Portugal

The responsability of abstract preparation rests with the authors.

Scientific Program

Sunday, June 12

17:00 Registration (Círculo Universitário)

19:00 Welcome Reception (Círculo Universitário)

Monday, June 13

09:30 Opening session (FCUP)

Prof. António Fernando Sousa da Silva, Dean of FCUP.

Prof. Manuel R. Falcão Moreira, President of the Scientific Council of FCUP.

Prof. Amílcar Celta Falcão Ramos Ferreira, Dean of FFUC and Vice-Rector for Research and 3rd cycle, University of Coimbra.

Prof. Fernanda Borges (University of Porto)

Prof. Elisiário Tavares (University of Coimbra)

Prof. Paula Gameiro, Vice-President of DQB.

Session 1: Symposium 1 on G Protein-Coupled Receptors in Drug Discovery

Chairperson(s): Carmen Terán and Natália Cordeiro

10.00 Plenary Lecture 1: "Adenosine receptors - new ligands for an old target"

*Speaker:*Karl Norbert Klotz

Department of Pharmacology and Toxicology, University of Würzburg, Germany

11.00 Coffee Break * **Poster session**

11.30 Oral Communication (Invited): "Fine-tuning modulation of purinoceptors activity by ectoNTPDases in human diseases"

*Speaker:*Paulo Correia de Sá

Department of Imuno-Physiology and Pharmacology, ICBAS, University of Porto, Portugal

12.00 O.C. 1: "QSARDW, a web based workflow for databases creation. Cleaning adenosine A2B antagonist ligands"

Speaker: Virginia Rivero-Buceta.

12.15 O.C. 2: "Response of human fibroblasts to inflammatory mediators: involvement of hemichannels and purinoceptors activation"

Speaker: Ana Rita Pinheiro.

12.30 O.C. 3: "Synthesis and binding affinity of conformationally constrained butyrophenone analogues of haloperidol as potential atypical antipsychotics"

Speaker: Laura Carro

13.00 Lunch (Círculo Universitário)

Session 2: Symposium 2 on G Protein-Coupled Receptors in Drug Discovery

Chairperson(s): Marta Teijeira and Lourdes Santana

15.00 Plenary Lecture 2: "In silico engineering of novel adenosine receptors antagonists"
Speaker: Stefano Moro
Molecular Modeling Section Università degli Studi di Padova, Italy

16:00 Oral Communication (Invited): "Pharmacological meaning of 5-HT2A receptors dimerization"
Speaker: Maria Isabel Loza Garcia
Department of Pharmacology, Faculty of Pharmacy, University of Santiago Compostela, Spain

16.30 Coffee Break *** Poster session**

17:00 O.C. 4: "Ecto-Ntpdase activity and P2y6 receptors involvement in osteogenic differentiation of human bone marrow stromal cells"
Speaker: J.B. Noronha-Matos

17:15 O.C. 5: "Fibroblasts activation by inflammatory mediators involves signaling via P2 purinoceptors in the rat subcutaneous connective tissue"
Speaker: Mariana Certal

17.30 O.C. 6: The G-quadruplex fold of nucleic acids: a challenging target in anti-cancer drug design
Speaker: Stefano Alcaro

18.00 Social programme (**CITY TOUR**)

Tuesday, June 14

Session 3: Symposium 1 on Enzymes in Drug Discovery

Chairperson(s): Natércia Teixeira and Fernanda Roleira

9.30 Plenary Lecture 3: "New drugs to overcome endocrine resistance in breast cancer"
Speaker: Shiuan Chen
Department of Surgical Research, Beckman Research Institute of the City of Hope, USA

10.30 Oral Communication (Invited): "Tuning the reactivity of the beta-lactam scaffold as a tool to improve inhibitory potency against human neutrophil elastase"
Speaker: Rui F. A. Moreira
Faculty of Pharmacy, University of Lisbon, Portugal

11.00 Coffee Break *** Poster session**

11.30 O.C. 7: "New A-ring steroidal olefins and epoxides as aromatase inhibitors. Synthesis and SAR studies"

Speaker: Carla Varela

11.45 O.C. 8: "From a simple chemical model to the real thing: The path to the discovery of the inhibition of thioredoxin reductase by mitomycins"

Speaker: Manuel M. Paz

12.00 O.C. 9: "Biomarkers of redox unbalance and oxidative stress""

Speaker: Chiara De Luca

12.15 O.C. 10: "Leishmanicidal activity of lactoferrin-derived peptides"

Speaker: Tânia Silva

12.30 Lunch

BUSINESS MINI SYMPOSIUM

Medicinal Chemistry Opportunities: from academia to industry

Chairperson(s): Elisiário Tavares and Jorge Garrido

14.00 Plenary Lecture 4: A Success History of Research from Academia.
From concepts to real life in the photodynamic therapy of cancer"

Speaker: Luis G Arnaut

Department of Chemistry of Faculty of Sciences and Technology, University of Coimbra, Portugal

15.00 BIAL Oral Communication: "Computation of binding affinity of protein-ligand complexes; Inhibitors of catechol-o-methyltransferase"

Speaker: Nuno Palma.

15:30 BIOALVO Oral Communication: "Identifying receptor selectivity of naturally occurring opioid-like molecules"

Speaker: Gonçalo Andrade.

16:00 IBMC Technology Transfer Office Oral Communication: "Starting up business in an academic environment"

Speaker: António Parada

16:30 Round Table

Chairperson(s): Fernanda Borge

Coordinators of I&D Centres:
CEF/UC, João José Sousa
CIQUP, Ribeiro da Silva
CQ/UM, Maria Jaão Queiroz
IBMC, Claudio Sunkel
Health Cluster Portugal Executive Director (CEO), Joaquim Cunha
President: Jorge M. M. Gonçalves (Vice-Rector for RD&I - Research, Development and Innovation, University of Porto)

20:00 Gala Dinner - Taylors Port Wine Cellars

Session 4: Symposium 2 on Enzymes in Drug Discovery

Chairperson(s): Eugénio Uriarte and Francisco Silva

9.30 Plenary Lecture 5: “Peptide Prodrugs based on the DPPIV/CD-26 enzyme. A new prodrug approach”

Speaker: Maria Jose Camarasa

Instituto de Química Médica (CSIC)-Madrid, Spain

10.30 Oral Communication (Invited): “Secondary metabolites as inspiration to the discovery of news drugs”

Speaker: Nuria Cabedo

Department o f Pharmacology, Faculty of Pharmacy, University of Valencia, Spain

11.00 Coffee Break *** Poster session**

11.30 O.C. 11: “Phenolic content, antioxidant, cytotoxicity and acetylcholinesterase activities evaluation from in vivo and in vitro Hypericum undulatum”

Speaker: Nuno Rainha

11.45 O.C. 12: “Structural insights into Leishmania infantum endoG active site and analysis of the reaction mechanism”

Speaker: Juan Bueren-Calabuig

12.00 O.C. 13: “New endoperoxide-peptidyl falcipain inhibitors”

Speaker: M. Lurdes S. Cristiano

12.15 O.C. 14: “Enzyme inhibition and cell signalling modulation by sulphur- and selenium-containing flavonoids – an in silico and in vitro approach”

Speaker: Gonçalo C. Justino

12.30 O.C. 15: “Plant polyphenols and tumors: from mechanisms to therapies, prevention, and protection against toxicity of anti-cancer treatments”

Speaker: Liudmila G. Korkina

Closing Session

Social Program

Sunday, June 12

19:00 Welcome Reception (Círculo Universitário)
(Included in the registration fee)

Monday, June 13

18.00 Conference Social Programme (**CITY TOUR**)
(Included in the registration fee)

Tuesday, June 14

20:00 Gala Dinner - Taylors Port Wine Cellars
(Price: 55 EUR under reservation)

Poster Session

The poster sessions will take place during the Coffee Breaks. All sessions will be presented simultaneously. The poster exhibition will be accessible during all the meeting.

Supports

Faculdade de Farmácia, Universidade de Coimbra
Faculdade de Ciências, Universidade do Porto
Centro de Estudos Farmacêuticos, Universidade de Coimbra
Centro de Investigação em Química, Universidade do Porto
Fundação para a Ciência e Tecnologia
Porto Cidade da Ciência
Sociedade Portuguesa de Química
Sociedade Portuguesa de Bioquímica
Sociedade Portuguesa de Farmacologia
Sociedad Española de Química Terapéutica
Reitoria da Universidade do Porto
Infarmed - Autoridade Nacional do Medicamento e Produtos de Saúde, I.P.
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Mini Symposium

Business
Mini Symposium

Medicinal Chemistry Opportunities: from academia to industry

Chairperson(s): Elisiário Tavares and Jorge Garrido

Plenary Lecture 4: A Success History of Research from Academia.
From concepts to real life in the photodynamic therapy of cancer"
Speaker: Luis G Arnaut
Department of Chemistry of Faculty of Sciences and Technology, University of Coimbra, Portugal

Bial - Invited Oral Communication: "Computation of binding affinity of protein-ligand complexes; Inhibitors of catechol-o-methyltransferase"
Speaker: Nuno Palma

Bioalvo - Invited Oral Communication: "Identifying receptor selectivity of naturally occurring opioid-like molecules"
Speaker: Gonçalo Andrade

IBMC Technology Transfer Office - Invited Oral Communication: "Starting up business in an academic environment"
Speaker: António Parada

Round Table

Chairperson(s): Fernanda Borges

President: Jorge M. M. Gonçalves (Vice-Rector for RD&I - Research, Development and Innovation, University of Porto)
Coordinators of I&D Centres:
João José Sousa CEF/UC
Ribeiro da Silva CIQUP
Maria Jaão Queiroz CQ/UM
Claudio Sunkel IBMC
Health Cluster Portugal Executive Director (CEO):
Joaquim Cunha

Plenary Lecture Abstracts

Notes

Adenosine receptors - new ligands for an old target

Karl-Norbert Klotz

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The effects of adenosine on the heart are well known for over 80 years. Several decades passed before the concept of adenosine receptors was developed and at the end of the 1970s adenosine receptor subtypes were described for the first time. As techniques in biochemistry and molecular biology progressed, four subtypes named A_1, A_{2A}, A_{2B} and A_3 were identified by cloning and pharmacologically characterized in great detail. Their tissue-specific distribution aroused interest as to their role as potential therapeutic targets in almost every organ system. Adenosine receptors are considered as promising targets in conditions including CNS degeneration, ischemic injury of brain and heart, asthma, acute renal failure, osteoporosis, wound healing, cancer, and many more.

Over the years numerous laboratories have contributed a large selection of agonists with distinct pharmacological characteristics with modifications primarily at the 2-, N^6- and 5'-positions of the purine scaffold. Additional substitutions in 8-, 2'- and 3'-positions may affect the efficacy of compounds leading to partial agonists or antagonists. Prototypical adenosine receptor antagonists are derived from methyl xanthines like theophylline. Based on the xanthine structure numerous antagonists were developed with selectivity for individual receptor subtypes. In addition, various non-xanthine scaffolds were developed that provide a great diversity of high-affinity antagonists with characteristic selectivity profiles. Amongst these are triazoloquinazolines and related structures, adenine derivatives, flavonoids, and dihydropyrimidines, to name a few.

In the light of such a remarkable arsenal of selective high affinity ligands it is surprising that only a few compounds are studied in clinical trials. This may be explained, at least in part, by the fact that adenosine-mediated physiological responses sometimes show pronounced species dependence and, thus, therapeutic success is hard to predict. In particular in the case of the A_3 receptor dramatic species variation occurs between rat and human receptors such that typically high affinity A_3 antagonists do not bind to the rat subtype precluding rat studies required for drug approval. Molecular modelling and receptor mutation may help to uncover the responsible structural determinants of these differences. With further progress in the characterization of ligand-receptor interaction and ligand development adenosine receptors remain an appealing target for treatment of many diseases.

Notes

In silico engineering of novel adenosine receptors antagonists

Stefano Moro, Silvia Paoletta, Magdalena Bacilieri, Davide Sabbadin

Molecular Modeling Section (MMS), Department of Pharmaceutical Sciences, University of Padova,Via Marzolo 5, 35131 Padova - ITALY
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G protein-coupled receptors (GPCRs) form a large protein family that plays an important role in many physiological and pathophysiological processes. Since the sequencing of the human genome has revealed several hundred new members of this receptor family, many new opportunities for developing novel therapeutics have emerged. The increasing knowledge of GPCRs (biological target space) and their ligands (chemical ligand space) enables novel drug design strategies to accelerate the finding and optimization of GPCR leads. The crystal structure of the recently published GPCR extend and support homology modeling studies and structure-based drug design approaches. On the other hand, the classical ligand-based design approaches (for example, virtual screening, pharmacophore modeling, quantitative structure-activity relationship (QSAR)) are still powerful methods for lead finding and optimization. In addition, the cross-target analysis of GPCR ligands has revealed more and more common structural motifs and three dimensional pharmacophores. Such GPCR privileged structural motifs have been successfully used by many pharmaceutical companies to design and synthesize combinatorial libraries, which are subsequently tested against novel GPCR targets for lead finding. In the near future structural biology and chemogenomics might allow the mapping of the ligand binding to the receptor. The linking of chemical and biological spaces will aid in generating lead-finding libraries, which are tailor-made for their respective receptor.

Ever since the discovery of the hypotensive and bradycardiac effects of adenosine in the circulation, adenosine receptors continue to represent promising drug targets. Firstly, this is due to the fact that the receptors are expressed in a large variety of cells; in particular, the actions of adenosine (or, respectively, of the antagonistic methylxanthines) in the central nervous system, in the circulation, on immune cells and on other tissues can be beneficial in certain disorders. Secondly, there exists a large number of ligands, which have been generated by introducing several modifications in the structure of the lead compounds (adenosine and methylxanthine), some of them highly specific. Four adenosine receptor subtypes (A_1, A_{2A}, A_{2B} and A_3) have been identified by molecular cloning. They are encoded by distinct genes. Originally, the receptors were classified based on they belong to the family of G protein-coupled receptors, which transfer signals by activating heterotrimeric G proteins. Adenosine receptors can also be differentiated according to their preferred mechanism of signal transduction: A_1 and A_3 receptors interact with pertussis toxin-sensitive G proteins of the Gi and Go family; the canonical signalling mechanism of the A_{2A} and of the A_{2B} receptors is stimulation of adenylyl cyclase via Gs. A_{2B} receptors can, in addition, also activate phospholipase C and this is thought to be mediated by activation of Gq. The A_3 adenosine receptor, which is the most recently identified adenosine receptor is implicated in a variety of important physiological process. Activation of A_3 adenosine receptors increases the release of inflammatory mediators, such as histamine, from rodent mast cells and inhibits the production of tumor necrosis factor-α. The activation of the A_3 adenosine receptor is also suggested to be involved in immunosuppression and in the response to ischemia of the brain and heart. It is becoming increasingly apparent that agonists or antagonists of A_3 adenosine receptors have potential as therapeutic agents for the treatment of ischemic and inflammatory diseases.

Obviously, the development of agonists and antagonists for the human adenosine receptors has so far been directed by traditional medicinal chemistry. However, the availability of crystallographic information promises to facilitate understanding of the drug-receptor interaction leading to the rational design of a potentially therapeutically important class of drugs.

Reference title

1. Paoletta, S., Federico, S., Spalluto, G., Moro S. *Methods Enzymol.* **2010** (485) 225

Notes

PL3

New drugs to overcome endocrine resistance in breast cancer

Shiuan Chen, Ph.D.

Department of Surgical Research, Beckman Research Institute of the City of Hope, Duarte, CA 91010
schen@coh.org

Approximately 60% of premenopausal and 75% of postmenopausal breast cancer patients have estrogen-dependent carcinomas. Anti-estrogens and aromatase inhibitors (AIs) are the major types of drugs used to treat estrogen-dependent breast cancer. Anti-estrogens (such as tamoxifen) act as antagonists that block the binding of estrogen to estrogen receptor (ER) while AIs (such as the third-generation AIs: anastrozole, letrozole and exemestane) decrease the production of estrogen by inhibiting the aromatase enzyme that catalyzes estrogen biosynthesis. Both premenopausal and postmenopausal patients can be treated by tamoxifen. Considered a feedback mechanism, AIs are mainly used by postmenopausal patients. Based on results from several major Phase-III clinical trials, AIs have been shown to be superior to tamoxifen with regard to disease progression, incidences of locoregional and distant relapses, and contralateral breast cancers in postmenopausal women. These drugs are highly potent, specific, and effective to treat estrogen-dependent breast cancer; however, development of resistance to these inhibitors still occurs.

There are two types of endocrine resistance, *de novo* and acquired resistance. Some patients have tumors that contain functional ER and aromatase, but they do not respond to endocrine therapy, i.e., *de novo* resistance. It is believed that the growth of these tumors is predominantly driven by estrogen-independent mechanisms. In the case of acquired resistance, estrogen-induced tumor growth is suppressed by antiestrogens or AIs. However, as an adaption process, growth factor pathways become dominant after estrogen-dependent pathways are inhibited. These growth factor pathways then activate ER through cross-talk, leading to a ligand-independent activation of ER or stimulation of nongenomic mechanisms of ER.

Endocrine resistance is a clinically important problem. Our laboratory has developed a set of cell lines as models of the two types of endocrine resistance. Using these models, we have found that both HSP90 inhibitors and HDAC inhibitors are potentially useful to treat patients who do not respond well to endocrine therapy. While HSP90 inhibitors are shown to be effective for both *de novo* and acquired resistant cancers, efforts are underway to reduce the toxicity associated with the treatment. One HDAC inhibitor, LBH589, has been found to selectively suppress aromatase expression in breast cancer cells. Based on our novel findings, a clinical trial has been initiated to evaluate the synergistic action between LBH589 and letrozole.
Results from these studies will be presented and discussed.

Notes

From concepts to real life in the photodynamic therapy of cancer

Luis G. Arnaut

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This is a story of a therapeutic concept that emerged from academic research and found its way to an entrepreneurial adventure. The concept was the use of bacteriochlorins for photodynamic therapy (PDT), and was formulated at a time the established scientific consensus considered bacteriochlorins too unstable for clinical use. The synthesis of the first stable bacteriochlorins was criticized by the lack of synthetic flexibility. Environmentally-friendly and flexible synthetic methods later opened the way for a wide variety of stable and amphiphilic bacteriochlorins.[1,2] The optimization of bacteriochlorin structures eventually lead to a series of photosensitizers with nearly ideal properties for PDT.[3] *In vitro* screening revealed phototoxicities two orders of magnitude higher than clinically accepted photosensitizers.[4] *In vivo* studies revealed low dark toxicities, including low skin phototoxicity, and favourable pharmacokinetics.[5] This was the used to obtain substantial tumour growth delays in animal-models and, in some cases, complete tumour regression. Proof-of-principle was also given for the application of these bacteriochlorins in the diagnostic of tumours.[6] In summary, this story illustrates the role of the academic concepts in the development of new drugs and how the academic work find continuity in the pharmaceutical industry.[7]

1. **Synthesis and Photophysical Properties of Amphiphilc Halogenated Bacteriochlorins: New Opportunities for Photodynamic Therapy of Cancer**
M. M. Pereira, C. J. P. Monteiro, A. V. C. Simões, S. M. A. Pinto, L. G. Arnaut, G. F. F. Sá, E. F. F. Silva, L. B. Rocha, S. Simões, S. J. Formosinho *J. Porphyrins Phthalocyanines*, **13** (2009) 567-573.
2. **Synthesis and photophysical characterization of a library of photostable and halogenated bacteriochlorins: An access to near-infrared chemistry**
M. M. Pereira, C. J. P. Monteiro, A. V. C. Simões, S. M. A. Pinto, A. R. Abreu, G. F. F. Sá, E. F. F. Silva, L. B. Rocha, J. M. Dabrowski, S. J. Formosinho, S. Simões, L. G. Arnaut, *Tetrahedron*, **66** (2010) 9545-9551.
3. **Mechanisms of singlet-oxygen and superoxide-ion generation by porphyrins and bacteriochlorins and their implications in photodynamic therapy**
E. F. F. Silva, C. Serpa, J. M. Dabrowski, C. J. P. Monteiro, S. J. Formosinho, G. Stochel, K. Urbaska, S. Simões, M. M. Pereira, L. G. Arnaut, *Chem. Eur. J.* **16** (2010) 9273-9286
4. **New Halogenated Water-Soluble Chlorin and Bacteriochlorin as Photostable PDT Sensitizers: Synthesis, Spectroscopy, Photophysics, and in vitro Photosensitizing Efficacy**
J. M. Dabrowski, L. G. Arnaut, M. M. Pereira, C. J. P. Monteiro, K. Urbanska, S. Simões, G. Stochel, *ChemMedChem*, **5** (2010) 1770-1780.
5. **Biodistribution and Photodynamic Efficacy of a Water-Soluble, Stable, Halogenated Bacteriochlorin against Melanoma**
J. M. Dabrowski, K. Urbanska, L. G. Arnaut, M. M. Pereira, A. R. Abreu, S. Simões, G. Stochel, *ChemMedChem*, **6** (2011) 465-475.
6. **Infrared Absorbing Dyes Tailored for Detection and Therapy of Solid Tumors**
F. A. Schaberle, L. G. Arnaut, C. Serpa, E. F. F. Silva, M. M. Pereira, A. R. Abreu, S. Simões, *Proc. SPIE* **7376** (2010) 73760X-1-7.
7. **Design of Porphyrin-Based Photosensitizers for Photodynamic Therapy**
L. G. Arnaut, *Adv. Inorg. Chem.*, **63** (2011) in press.

Notes

PEPTIDE PRODRUGS BASED ON THE DPPIV/CD-26 ENZYME. A NEW PRODRUG APPROACH

María-José Camarasa

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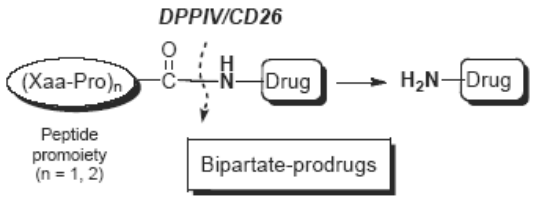
e-mail: mj.camarasa@iqm.csic.es

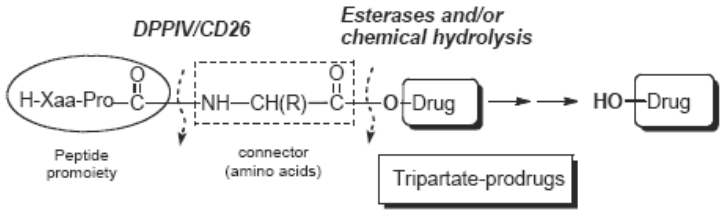
Many prodrug technologies have already been developed depending on the kind of drug that has to be converted. The prior art for ameliorating solubility and bioavailability reveals however only amino acid prodrugs (only one amino acid coupled) of small organic molecules whereby the amino acid is usually coupled through ester bonds, allowing easy back conversion to the free therapeutic agent by esterases. However, these prodrugs have low stability at physiological pH and their delivery is often not optimal.

The glycoprotein CD26, belongs to a unique class of membrane-associated peptidases and it is identical to dipeptidyl-peptidase IV (DPP IV). DPP IV hydrolyzes a prolyl peptide bond. CD26 is expressed on the surface of certain cells. A soluble form of this enzyme also exists in plasma and cerebrospinal fluid at lower amounts. DPP IV truncates several bioactive peptides of medical importance. CD26 has high selectivity for peptides with a proline or alanine at the penultimate position of the N-terminus of a variety of natural peptides. A free amino group on the ultimate amino acid is a prerequisite for substrate activity by the enzyme.

Recently, we have developped a novel enzyme-based (DPPIV/CD26) prodrug approach that provides conjugates of therapeutic agents with a di- (or oligo) peptide moiety that allows to ameliorate the solubility and/or bioavailability of them in a potentially more successful manner. The conjugates contain a proline at the penultimate position of the N-terminus, as a carrier, wherein the conjugate (biipartate prodrugs) is specifically cleavable by the endogenous DPP IV/CD26. Moreover, the therapeutic drug is linked to the peptide through a more stable amide bond (instead of an ester bond) which is specifically cleaved by DPP IV/CD26. The presence of a proline near the N-terminus may also serve as efficient structural protection against nonspecific proteolytic degradation.

The approach was applied to a variety of drugs containing a free amino group (of different nature) that was directly coupled with the carboxyl group of amino acids *via* an amide bond (bipartate prodrug). With these conjugates It was possible to modulate the hydrolisis rate (half-life) and the physicochemical properties of the compounds by modifying the nature and length of the peptide (di- or tetrapeptides) of the prodrug moiety.





Also, we expanded, our prodrug strategy to a variety of hydroxy-containing compounds (of different nature). The synthesized prodrugs contain a dipeptide moiety (cleavable by CD26), a heterobifunctional linker [released by chemical or enzymatic (esterases) hydrolysis of the ester bond] and the drug (tripartate prodrugs). Several of them showed a high improvement in aqueous solubility, in oral bioavailability, together with an efficient conversion to the biologically active parent drug in the presence of DPPIV/CD26 and in serum. The results support the wide applicability of our prodrug approach.

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Notes

Invited Oral Communications Abstracts

Notes

Fine-tuning modulation of purinoceptors activity by ectoNTPDases in human diseases

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Membrane compartmentalization of ecto-enzymes and other components of purinergic signaling cascade have been demonstrated. Nucleotide-inactivating ecto-enzymes are located in close proximity with each other and next to nucleotides permeation sites, releasable stores and purinoceptors, presumably associated with lipid rafts and caveolae. It seems reasonable to accept that released extracellular nucleotides and their derivatives are mainly concentrated on the cell surface, where they are subsequently "hand-to-hand" delivered for the succeeding phosphatase reactions. This important feature permits divergent cellular functions mediated by subtype specific P2 purinoceptor agonists (e.g. ATP vs ADP, UTP vs UDP) to take place at specific microdomains on the cell surface.
Endogenously released adenine nucleotides control human bone cells dynamics. ATP and ADP exert striking in vitro actions at concentrations in the low micromolar range to stimulate the formation and resorptive activity of osteoclasts, whereas ATP decrease ALP activity and inhibit mineralized bone formation by osteoblasts. Our data show for the first time that uracil nucleotides, both UTP and UDP, enhance intracellular [Ca^{2+}] and osteogenic differentiation of human primary bone marrow stromal cells (BMSCs) from post-menopausal women undergoing hip arthroplasty, yet their effects decrease in magnitude upon cells maturation. Paradoxically, these changes were notably accompanied by an increase in the expression of UTP-sensitive $P2Y_2$ and $P2Y_4$ receptors in differentiated osteoblasts, whereas the expression of UDP-sensitive $P2Y_6$ receptor remained fairly constant. Given that (1) UDP was more potent than UTP and that (2) the enzymatically stable UDP analogue, PSB0474, enhanced ALP activity at all stages of BMSC cultures whereas UTPγS was devoid of effect, it is reasonable to admit that the $P2Y_6$ receptor exerts a predominant role on osteogenic differentiation of BMSCs. Interestingly, activation of $P2Y_6$ receptors by UDP originated from the catabolism of UTP in bone microenvironment is balanced through the action of type-specific membrane-bound NTPDases (1, 2 and 3) whose expression varies along human BMSCs maturation.
Purinergic transmission is also important for initiation and maintenance of human penile erection. ATP is a powerful regulator of vascular hemodynamics through the activation of vasoconstrictor P2X and vasodilator P2Y and adenosine receptors. Nucleotide effects may be cut-short by extracellular hydrolysis via the ectonucleotidase cascade. Human corpora cavernosa have a high NTPDase1/CD39 activity converting ATP directly into AMP, without a significant ADP formation. Extracellular ATP hydrolysis is slower in impotent patients with endothelial dysfunction. Our findings demonstrate that decreased NTPDase1/CD39 activity leads to desensitization of P2 purinoceptors and poor adenosine formation, thus contributing to impair relaxation of corpora cavernosa in impotent men.

Inflammation and mechanical perturbations (e.g. shear stress, stretching) stimulate ATP release from urinary bladder cells. Urodynamic and in vitro studies demonstrated that uroepithelial cells and cholinergic nerves from overactive human bladders (OAB) exhibit a 3-fold increase in ATP release followed by slower inactivation kinetics of the nucleotide compared to cadaveric controls. Clinical studies led us to propose that urinary ATP may be a non-invasive biological marker of overactive bladder syndromes, exhibiting higher sensitivity and specificity (AUC: 83.2%, CI 95%: 69.7-96.7) than other experimental markers (e.g. NGF). Increased ATP bioavailability might explain hyperactivity of bladder nerves (via P2X3 receptors) and detrusor contractions (via P2X1); implication of changes in the activity of co-localized inhibitory metabotropic P2Y and P1 receptors by ATP hydrolysing products, ADP and adenosine, will also be discussed. In this respect, immunolocalization studies showed that human urothelial cells are highly enriched in ecto-NTPDase3 enzyme, whereas the detrusor smooth muscle layer exhibits predominantly the ecto-NTPDase1/CD39 marker. These findings were confirmed by HPLC kinetic experiments and may explain the neurochemical differences observed in patients with OAB.

Altogether, these previously unrecognized targets for local regulation of extracellular accumulation of adenine and uracil nucleotides may prompt for novel therapeutic strategies to control human diseases.

Work supported by FCT (FEDER funding, PTDC/SAU-OSM/3576/2006), Soc.Port.Andrologia and UPorto/SantanderTotta.

Notes

5-HT_{2A} RECEPTORS FUNCTIONAL SELECTIVITY IN DRUG DISCOVERY

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G protein-coupled receptors (GPCRs) constitute the major family of cell surface proteins involved in cell signaling cascades, and are the target of ≈ 50% of clinical drugs.[1] It is well accepted that agonist (full and partial) ligands and allosteric positive regulators can invoke different active conformations of GPCRs and that these may allow differential agonist-dependent regulation of signaling pathways. Such effects have been described as 'agonist-directed trafficking of receptor stimulus'[2], 'biased agonism'[3], 'functional selectivity'[4] or 'collateral efficacy'.[5]

The 5-HT_{2A} receptor is a class A GPCR whose antagonists have important applications in the treatment of disorders of the cardiovascular and central nervous systems,[6] and also in virology.[7] 5-HT_{2A} receptors regulate inositol phosphates (IP) accumulation mediated by phospholipase C and arachidonic acid (AA) release mediated at least partially by phospholipase A_2, and different 5-HT_{2A} receptor agonists, for instance serotonin and the hallucinogenic (±)DOI (2,5-dimethoxy-4-iodoamphetamine), show functional selectivity discriminating between these two signaling pathways.[8]

5-HT_{2A} receptors are target of antipsychotics and it was recently shown that the antipsychotic clozapine but not haloperidol down-regulates the heterodimer $mGlu_2R$-5-HT_{2A} receptor through a 5-HT_{2A}-dependent process.[9] These two drugs also differentiate themselves by blocking 5-HT_{2A} responses by two distinct mechanisms. When we studied the binding of different drugs at these receptors, some antagonists like the atypical antipsychotic drugs clozapine and risperidone, differentiate from others, like the classical antipsychotic drug haloperidol, since they displayed biphasic binding competition curves for agonist- ([^{3}H](±)DOB)- labeled human 5-HT_{2A} receptors, whilst haloperidol displayed monophasic competition curves. These differences were also observed for other 5-HT_{2A} antagonists (ketanserin and MDL100,907 are biphasic inhibitors while mesulergine is a monophasic one).

We established an experimental design mimicking that of the binding competition studies, and generated concentration-response curves for both ligands at two non-interlinked 5-HT_{2A}-mediated signaling pathways, IP accumulation and AA release. While haloperidol showed a monophasic inhibition of the 5-HT-dependent activation of both effector pathways, clozapine inhibited the 5-HT-stimulated IP accumulation in a monophasic manner, but, intriguingly, its inhibition of the 5-HT-stimulated AA release was biphasic with a strong parallelism with the negative cooperative binding observed for this ligand in radioligand binding assays, even in the fraction of high and low affinity populations and this behavior was better described by a dimer receptor model than by a monomer receptor model.

These results therefore provide evidence of a functional antagonist-dependent negative cooperativity in the inhibition of the AA pathway that mirrors the antagonist-dependent negative cooperative binding indicative of 5-HT_{2A} receptor homodimers. This specific dimer-effector antagonism opens up a new avenue for obtaining new compounds with different antagonistic functional selectivity in order to ascertain the relevance of the 5-HT_{2A} homodimers in therapeutics.

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Notes

Tuning the Reactivity of the β-Lactam Scaffold as a Tool To Improve Inhibitory Potency Against Human Neutrophil Elastase

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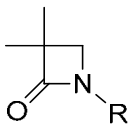
β-Lactams, **1**, are moderate time-dependent inhibitors of human neutrophil elastase (HNE), a serine protease involved in inflammatory diseases.[1] The mechanism of action of β-lactams involve acylation of the catalytic serine residue via ring-opening. The driving force for β-lactam reactivity towards nucleophiles lies in the leaving group ability of the amine formed from the decomposition of the tetrahedral intermediate, rather than the strain energy in the four-membered ring or reduced amide resonance. In contrast to amine leaving groups, which require general-acid assistance, amide leaving groups are anionic and do not require any assistance from HNE's catalytic machinery, and thus we rationalized that 4-oxo-β-lactams, **2**, could lead to improved inhibitory potency. Compounds **2** are potent time-dependent active-site directed inhibitors of HNE and related serine proteases.[2,3] The effect of different amide leaving groups by C-N fission on the kinetics of alkaline hydrolysis and enzyme inhibition was evaluated. Substituent effects indicate that the enzyme catalyzed attack of serine occurs at an earlier position along the reaction coordinate leading to formation of the tetrahedral intermediate, when compared with the hydroxide-ion-catalyzed hydrolysis. These results suggest that the 4-oxo-β-lactam scaffold promotes the enzyme's ability to use its catalytic apparatus to stabilize the transition state and increase the rate of serine acylation. Implications for the design of novel HNE inhibitors will be discussed.



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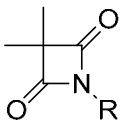

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Ackowledgements
This work was supported by Foundation for Science and Technology (FCT) through the project PTDC/QUI/64056/2006 and the PhD fellowship SFRH/BD/17534/2004 to JM.

Notes

Secondary metabolites as inspiration to the discovery of news drugs

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Natural Products play a highly significant role in the drug discovery. Complex chemical skeletons such as morphine and penicillin are not able to be invented in any laboratory. Nowadays, the pharmacological activities displayed by several molecules of natural origin are still inspiring worldwide chemists and pharmacologists to synthesize new therapeutic agents.

Recently in our group, series of 1-substituted-7-chloro-6-hydroxy-tetrahydro-isoquinolines (1-butyl-, 1-phenyl- and 1-benzyl derivatives) were prepared to explore the influence of each of these groups at the 1-position on the affinity for dopamine receptors. All the compounds displayed affinity for D_1-like and/or D_2-like dopamine receptors in striatal membranes, and were unable to inhibit [^{3}H]-dopamine uptake in striatal synaptosomes. Different structure requirements have been observed for adequate D_1 or D_2 affinities. Moreover, 1-butyl-7-chloro-6-hydroxy-tetrahydroisoquinoline with the highest affinity towards D_2-like receptors (Ki value of 66 nM) and the highest selectivity (49-fold D_2 *versus* D_1) by *in vitro* binding experiments was then evaluated in behavioural assays (spontaneous activity and forced swimming test) in mice. This compound increased locomotor activity in a large dose range (0.04-25 mg/kg). Furthermore, this lead compound produced reduction in immobility time in the forced swimming test at a dose (0.01 mg/kg) that did not modify locomotor activity. The haloperidol (0.03 mg/kg), a D_2 receptor preferred antagonist, blocked the antidepressant-like effect of compound.

Notes

Invited Oral Communications
Business Mini-Symposium
Abstracts

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COMPUTATION OF BINDING AFFINITY OF PROTEIN-LIGAND COMPLEXES;
INHIBITORS OF CATECHOL-*O*-METHYLTRANSFERASE

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The calculation of the free energy changes associated with biochemical events is of major importance, as it provides a link between the microscopic structure and dynamics of the system, which can be obtained from atomic-level molecular simulations, and the macroscopic thermodynamic properties, that can be measured experimentally. In other words, it enables to bridge the gap between theory and experiment.

We present a theoretical study of the energetics and dynamics of the molecular interactions of a novel antiparkinsonian drug candidate (BIA 9-1067) with its biological target, the human enzyme Catechol-*O*-methyltransferase (COMT, EC 2.1.1.6). We have computed the change in free energy of complex formation, which is directly related to the thermodynamic binding affinity, and discuss the dynamic aspects of the inhibition mechanism.

Our results reveal an exceptionally high binding affinity of BIA 9-1067 for human COMT and provide insightful clues on the nature of the molecular interactions. Moreover, the inhibitor, itself, is a slowly reacting substrate of the target enzyme, being released in the form of *O*-methylated product. By comparing the catalytic rate (k_{cat}) and the calculated dissociation rate (k_{off}) constants of the complex, one can conclude that the observed inhibition strength (K_i) and enzyme inhibition half-life are primarily dependent on the catalytic rate constant of their *O*-methylation, rather than the rate of dissociation of the complex. This observation has implications for the pharmacological profile of BIA 9-1067.

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Identifying receptor selectivity of naturally occurring opioid-like molecules

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Opioid receptors are a group of G protein-coupled receptors with opioids as ligands[1]. The endogenous opioids are dynorphins, enkephalins, endorphins, endomorphins and nociceptin. Opiate receptors are distributed widely in the brain, and are found in the spinal cord and digestive tract. These G-protein coupled receptors have been involved in the modulation of analgesia. Therapeutic solutions for pain management have been based on the opioid G-protein coupled receptors, Mu (MOP), Delta (DOP) and Kappa (KOP) receptors, and include plant derived morphine and its derivatives, to name a few.
Although several SAR approaches have been successful at designing compounds that modulate these targets, the fact that prolonged use can lead to abuse, dependence and habituation has brought into the spotlight naturally occurring opioid and opioid-like compounds. These include kyotorphin, a dipeptide with analgesic properties[2,3], opiorphin, a pentapeptide also with analgesic and anti-depressant properties[4,5] and others.

This work aims at identifying to which GPCRs naturally occurring opioid-like compounds bind to and at elucidating receptor selectivity. In order to achieve this, protein models of Mu, Delta, Kappa opioid GPCR were built, based on the already available X-ray crystallography solved 3D structures, namely D3 dopaminergic receptor, CXCR4 chemokine receptor, bovine rhodopsin, Beta2-adrenergic receptor, Beta1-adrenergic receptor, using the protein modelling tool Modeller. These models were refined and evaluated using Procheck. Following this analysis, the 3 best models for each GPCR were selected for molecular docking using GOLD 3.2 and both Goldscore (a force-field based algorythm) and Chemscore (an empyrical based algorythm) as docking scoring functions. A library of naturally occurring opioid-like molecules was docked to the binding pockets of the modelled GPCRs, using known ligands as templates for binding pocket definition. Both scoring fitness and docking pose were evaluated and analysed.
After this comparative analysis the opioid-like molecules were ranked and their selectivity assessed.

The selectivity profile of these naturally occurring molecules can be improved by directed medicinal chemistry efforts, addressing specific residues in the binding pockets of each receptor. This can turn these opioid-like molecule derivatives into a strong and efficacious therapeutic solution for pain management without the dependence and habituation issue associated to current opioid-based therapeutics.

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Starting Up Business in an Academic Environment

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IBMC has a growing focus on commercialization of its Intellectual property rights. We have sold and license patents to international businesses, collaborated in R&D programs with access to royalties and other ways to generate a cash inflow. However, the spinning of commercial activities and the attraction of foreign direct investment are the preferred ways to conduct business. The reasons are the direct creations of jobs and the development of a stable partnership.
Ablynx is a stock listed company based in Belgium. It has a centre of excellence in phage display in Porto. This unit with 10 PhDs and a total work force of 26, is a Portuguese company fully owned by Ablynx N.V. It has a remarkable position in the ranking of technology based companies, according to the Observatory of the Ministry of Science. The history, milestones and forms of collaboration of its presence in Portugal will be addressed at the presentation

Notes

Oral Communications Abstracts

Notes

QSARDW, a web based workflow for databases creation. Cleaning adenosine A_{2B} antagonist ligands

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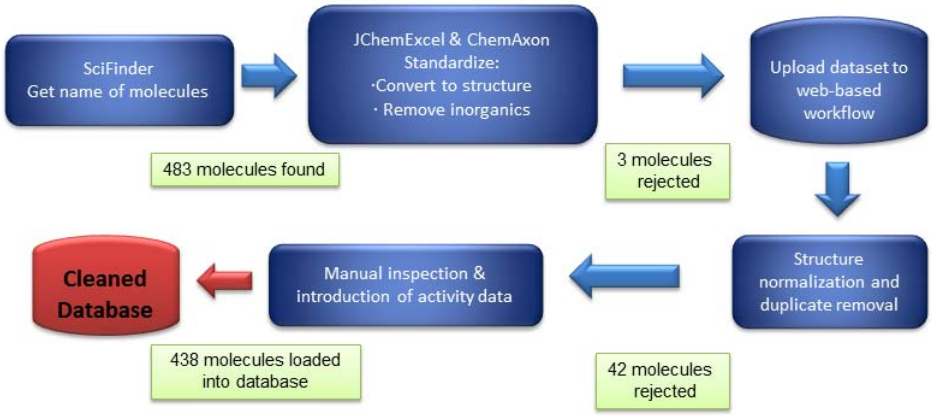
Obtaining good results in QSAR models mainly depends on working with a cleaned database. For this reason one of the most important steps of our work is to develop a workflow for cleaning it.[1]
Following our *in silico* research concerning A_{2B} adenosine receptor antagonist (A_{2B}AR) ligands,[2] our first aim at this stage is to build a rigorous A_{2B} adenosines database in such a way that references are taken manually from SciFinder. From the selected papers we only extract the name of molecules with biological data into a file in txt format. This archive is imported into Microsoft Excel and with ChemAxonJChem each name is converted to its structure. After that we use ChemAxon Standardize to eliminate inorganics from the initial dataset.
To simplify and automate the process of normalize structures and remove duplicates in the dataset, a custom-made web application has been developed using open source tools like Open Babel and CDK[3] projects. This application also allows us convert the molecules into all needed formats and load them into a relational database to simplify the querying. The web workflow is also used for doing manual inspection of the dataset and inserting the activity data of each molecule. Once all this steps are completed, the database is ready to work with.
This methodology was put into practice for creating an A_{2B} adenosine database. The first step was to search for references with the suitable protocol (radioligand, cell line and specie). The workflow was carried outwith an initial number of 427 molecules, obtaining at the end a cleaned database with 383 molecules which were used to get a QSAR model.



Ackowledgements
We are grateful to the University of Vigo and Xunta de Galicia (IN845B-2010/020) for the financial support.

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Response of human fibroblasts to inflammatory mediators: involvement of hemichannels and purinoceptors activation

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Propagation of intracellular calcium waves to neighboring cells is a common feature mediating cell-to-cell signaling. Translocation of intracellular mediators, like Ca^{2+} and IP_3, through gap-junctions has been implicated in this communication pathway. Recently, attention has been given to the role of purines as ubiquitous autocrine/paracrine signaling mediators on Ca^{2+} wave propagation via mechanisms unrelated to gap-junctions[1]. Besides massive leakage of nucleotides that occurs upon cell lysis, nucleotides appearance in the extracellular milieu may be due (1) to electrodiffusional movement through membrane ion channels, like hemichannels, (2) to facilitated diffusion by nucleotide-specific ATP-binding cassette transporters, and (3) to cargo-vesicle trafficking and exocytotic granule secretion[2]. Since connective tissue is richly innervated and fibroblasts may release purines, by a yet unknown mechanism, thereby triggering intracellular calcium waves in response to stressful conditions, these cells become preferential in the modulation of afferent sensory inputs, thereby contributing to musculoskeletal pain. Therefore, we hypothesized that such mechanism may be involved in the sensitization of myofascial nociceptors leading to chronic musculoskeletal pain. So, we investigated the sensitivity of human fibroblasts to inflammatory mediators in order to test the involvement of connexin (Cx) hemichannels and purinoceptors activation.

Experiments were performed on primary cultures of human fibroblasts from subcutaneous connective tissue (approved by the Ethics Committee). Intracellular $[Ca^{2+}]_i$ oscillations were monitored using a microplate reader (Sinergy HT, BioTek) after loading the cells with Fluo-4NW (2.5 μM, 45 min at 37ºC). ATP release was inferred by bioluminescence using the luciferin/luciferase assay and by confocal microscopy (FluoView 1000, Olympus) measuring the destaining of cells loaded with quinacrine (30 μM, 60 min at 37ºC).

Incubation of human fibroblasts in culture with bradykinin (BK, 30 μM) and histamine (Hist, 100 μM) caused a transient rise of intracellular $[Ca^{2+}]_i$ which reached a maximum of 47±3% (*n*=24) and 27±3% (*n*=11), respectively. Both responses seem to depend on Ca^{2+} released from intracellular stores, since its depletion induced by thapsigargin completely abolished Ca^{2+} transients, contrary to what we observed when extracellular fluid was Ca^{2+} free. The effect of BK (30 μM) was significantly ($p<0.05$) attenuated in the presence of the Cx36 and Cx50 inhibitor, mefloquine (3 μM, 4±1%, *n*=6), but not in the presence of the pannexin inhibitor, ^{10}Panx (100 μM, 42±3% (*n*=3). Blockade of vesicular transport with brefeldin A (3-20 μM) concentration-dependently reduced $[Ca^{2+}]_i$ transients produced by BK (30 μM) to 5±3% (*n*=4). Also, BK and Hist-induced $[Ca^{2+}]_i$ transients were partially attenuated in the presence of the non-selective P2 purinoceptor antagonist PPADS (300 μM) to 27±5% (*n*=10) and 9±3% (*n*=4), respectively. In addition to $[Ca^{2+}]_i$ oscillations, BK and Hist induced the release of ATP (revealed either by bioluminescence or quinacrine destaining). Selective antagonists of ADP-sensitive $P2Y_1$, $P2Y_{12}$, $P2Y_{13}$ (MRS2179 300 nM, AR-C66096 100 nM and MRS2211 10 μM) showed no involvement of these receptors both in BK neither in Hist-induced $[Ca^{2+}]_i$ responses.

Data suggest that inflammatory mediators, like BK and Hist, cause fluorescent $[Ca^{2+}]_i$ oscillations and the release of ATP from human fibroblasts in culture. BK-induced $[Ca^{2+}]_i$ transients seem to be partially mediated by endogenously released adenine nucleotides. Still, which purinoceptors might be involved requires further investigation. The involvement of Cx36 and Cx50 hemichannels and vesicular translocation in the release of ATP also deserves future studies.

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Work supported by FCT (FEDER funding, PTDC/SAU-FCF/108263/2008 and UMIB-215/94).ARP is in receipt of a PhD Studentship funded by FCT (SFRH/BD/47373/2008).

Synthesis and Binding Affinity of Conformationally Constrained Butyrophenone Analogues of Haloperidol as Potential Atypical Antipsychotics

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Schizophrenia is a complex disorder affecting approximately 1% of the population. For its treatment, classical (typical) neuroleptics, such as haloperidol (**Figure 1**), are currently used but their use is limited due to their severe mechanism-related side effects, such us induction of acute extrapyramidal symptoms (EPS) and their inefficacy against the negative symptoms of the syndrome.[1] The introduction of clozapine (**Figure 1**) for treatment-resistant schizophrenia gave rise to a new group of atypical or non-classical antipsychotics which have no EPS and are effective against negative symptoms. These drugs exhibit potent antagonism at multiple receptor subtypes including serotonin and dopamine receptors, suggesting the implication of the serotoninergic system in this pathology.[2]

Meltzer et al. related the special clinical profile of clozapine and other atypical neuroleptics with an empirical ratio, the so-called Meltzer Index, between pK_i for 5-HT_{2A} and pK_i for D_2. They proposed that this ratio may be used to discriminate atypical antipsychotics (ratio >1.12) from classical ones (<1.09).[3]

As part of our ongoing work to develop multi-target ligands for potential use as treatments for schizophrenia, we have studied the modulation of the butyrophenone system, with the aim of achieving a single molecule that can antagonize receptors of the 5-HT_2 and D_2 families and would have fewer side effects than currently available pharmacotherapies.[4]

In this communication, we will describe our recent efforts to discover novel templates in the area of selective dual 5-HT_{2A}/D_2 antagonists synthesizing a variety of different butyrophenone and butyrophenol analogues in which the rigidity of the haloperidol butyric chain has been increased by its inclusion into a tetralone or a tetralol core (**I** and **II**, **Figure 1**) and a quinazolinone scaffold (**III**, **Fig. 1**).

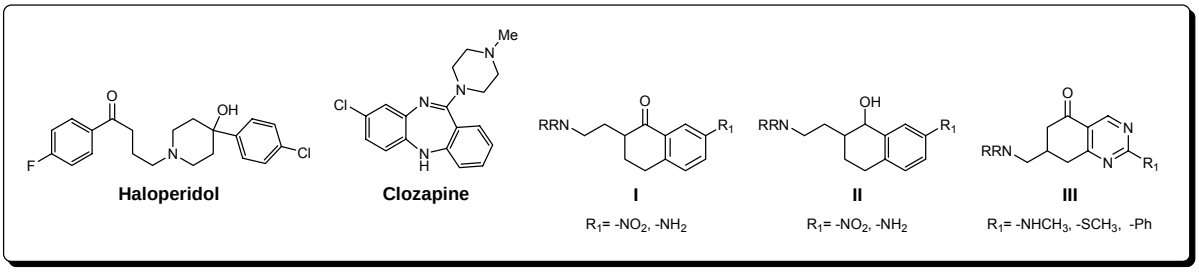

Figure 1

The synthetic routes and the binding affinities on dopamine D_2 and serotonin 5-HT_{2A} human receptors of these new compounds will be further discussed in the presentation.

Acknowledgments:

We would like to thank the Spanish *Ministerio de Ciencia e Innovación* for the financial support of this work (**Ref. SAF2009-13609-C04**). Laura Carro is gratefully acknowledged to *Diputación da Coruña* for the research grant.

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ECTO-NTPDASE ACTIVITY AND $P2Y_6$ RECEPTORS INVOLVEMENT IN OSTEOGENIC DIFFERENTIATION OF HUMAN BONE MARROW STROMAL CELLS

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Extracellular ATP have been shown to increase during bone loading, bone fracture, and bone repair.[1] Once released, the action of nucleotides may be rapidly terminated by cell-surface-located ecto-NTPDases producing di- and monophosphate derivatives. In contrast to the compelling evidence for the extracellular signalling role of ATP, the hypothesis that uracil nucleotides may also fulfil an autocrine/paracrine role has only recently gained attention. Metabotropic $P2Y_2$ recognizes both ATP and UTP as the most potent agonists, while $P2Y_4$ and $P2Y_6$ receptors were recently identified and characterized as UTP- and UDP-selective receptors in humans, respectively. This study was designed to investigate the role of uracil nucleotide-sensitive receptors on osteogenic differentiation of bone marrow stromal cells from postmenopausal women.

Human bone marrow specimens were obtained from postmenopausal female patients (68±5 years old, *n*=8) undergoing total hip arthroplasty. Ethical approval and informed consent to use the biological material that would be otherwise discarded, was obtained. Primary osteoblast cell cultures were characterized for proliferation (MTT assay), total protein content (method of Lowry) and alkaline phosphatase (ALP) activity during 30 days. The catabolism of uridine nucleotides (30 μM) was evaluated by HPLC.[2] Single-cell $[Ca^{2+}]_i$ imaging was performed after loading the cells with Fluo-4NW (2.5 μM, 45 min at 37ºC). The time-course of expression of uridine-sensitive P2 purinoceptors ($P2Y_2$, $P2Y_4$ and $P2Y_6$) and ecto-NTPDase subtypes (1, 2 and 3) was undertaken by immunofluorescence labeling using a confocal microscope (FluoView 1000, Olympus).

At day 7, UTP and UDP (100 μM) facilitated osteogenic cell differentiation measured by increases in ALP activity. These compounds caused concentration-dependent rises in osteoblast-cell $[Ca^{2+}]_i$ signals, which were more intense in the case of UDP. In contrast with UTPγS (100 μM), the enzymatically stable UDP analogue PSB 0474 (10 μM), which selectively activates $P2Y_6$ receptors, mimicked the effects of the two native uracil nucleotides; ALP activity increased progressively with time in the presence of PSB 0474 (10 μM). Selective blockade of $P2Y_6$ receptors with MRS 2578 (100 nM) prevented $[Ca^{2+}]_i$ rises and induction of ALP activity caused by UDP (100 μM). The presence of $P2Y_2$, $P2Y_4$ and $P2Y_6$ receptors on human primary osteoblast cells was confirmed by immunofluorescent labeling. While the expression of $P2Y_6$ receptors remained fairly constant at all culture time points (7~21 days), $P2Y_2$ and $P2Y_4$ become more evident in less proliferative and more differentiated cultures (7<21 days). The rate of extracellular UTP and UDP inactivation was higher in less proliferative and more differentiated cell populations (UTP: 1.13±0.06 *vs* 0.21±0.18 μM/μg protein/min and UDP: 1.52±0.03 *vs* 0.80±0.18 μM/μg protein/min at days 21 and 7, respectively, *n*=3). These findings are positively correlated with a rise in the imunoreactivity against ecto-NTPDases 1, 2 and 3 as cells differentiate (7<21 days).

Data show that uracil nucleotides, in particular UDP, are important regulators of osteogenic cell differentiation predominantly through the activation of $P2Y_6$ receptors coupled to increases in $[Ca^{2+}]_i$ accumulation. Activation of uracil-sensitive receptor subtypes (particularly the most expressed $P2Y_6$ form) may be balanced through the presence of specific ecto-NTPDases determining whether osteoblast progenitors are driven into proliferation or differentiation.

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This work was supported by FCT (FEDER funding, grants PTDC/SAU-OSM/3576/2006 and UMIB-215/94)

Fibroblasts activation by inflammatory mediators involves signaling via P2 purinoceptors in the rat subcutaneous connective tissue

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Purines acting through distinct signaling mechanisms are involved in pain perception.[1] Data from the literature indicate a role for ATP in the excitation / sensitization of myofascial receptors. Limited information is, however, available on whether uracil nucleotides are also capable to mediate cell-to-cell communication[2]. Considering that fibroblasts from the subcutaneous connective tissue can release adenine and/or uracil nucleotides which are responsible for intracellular Ca^{2+} waves in response to stressful conditions, these cells may play a key role in propagation/amplification of signals to primary nerve afferent.[3] Thus, we aimed at investigating the involvement of P2 purinoceptors on the response of fibroblasts from the rat subcutaneous connective tissue to inflammatory mediators, such as bradykinin (BK).

Experiments were performed in the first subculture of fibroblasts isolated from the rat subcutaneous connective tissue. Intracellular $[Ca^{2+}]_i$ oscillations were monitored using a microplate reader (Sinergy HT, BioTek) after loading the cells with Fluo-4NW (2.5 μM, 45 min at 37ºC). Results are expressed as percentage of the maximal $[Ca^{2+}]_i$ responses triggered by the calcium ionophore, ionomycin (5 μM).

BK (30 μM) caused a transient rise of $[Ca^{2+}]_i$ (28±2%, *n*=22), which was partially attenuated by inhibiting the enzymes responsible for extracellular inactivation of endogenous nucleotides, ecto-NTPDases, with POM-1 (20 μM, 16±4%, *n*=7). On the contrary, apyrase (2U/mL), the enzyme that hydrolyses ATP/UTP directly into AMP/UMP with little formation of ADP/UDP, was devoid of effect (26±5%, *n*=8) on BK-induced $[Ca^{2+}]_i$ transients. The involvement of distinct subtypes of P2 purinoceptors and/or of P1 receptors, the latter being activated by adenosine originated via dephosphorylation of AMP by ecto-5'-nucleotidase, deserves further investigation.

On their own, adenine and uracil nucleotides concentration-dependently enhanced intracellular $[Ca^{2+}]_i$ in rat fibroblasts. The rank order of potency for the nucleotides applied at 100-μM was the following: ATP (54±2%, *n*=4) > UTP (46±2%, *n*=22) ≈ ATPγS (44±3%, *n*=9) ≥ ADPβS (39±2%, *n*=16) >> UDP (15±3%, *n*=4). The selective $P2Y_1$, $P2Y_2$ and $P2Y_6$ receptor agonists, respectively MRS2365 (10 nM), MRS2768 (3-10 μM) and PSB0474 (10 μM), were virtually devoid of effect. Reactive blue-2 (RB-2, 100 μM) was more effective than suramin (100 μM) and PPADS (100 μM) in decreasing ($p<0.05$) $[Ca^{2+}]_i$ transients caused by 100 μM UTP and ATPγS. Given that the rat $P2Y_2$ receptor is virtually insensitive to RB-2 and that UDP was less potent than UTP in these cells, it seems like that intracellular $[Ca^{2+}]_i$ transients caused by UTP and ATPγS are mediated predominantly by the $P2Y_4$ receptor subtype. On the other hand, $[Ca^{2+}]_i$ transients induced by ADPβS (30-300 μM) were significantly ($p<0.05$) attenuated by the selective $P2Y_{13}$ antagonist, MRS 2211 (10 μM), without being affected by other ADP-sensitive P2 receptor antagonists, namely MRS2179 (0.3 μM) and AR-C66096 (0.1 μM) which were designed to block $P2Y_1$ and $P2Y_{12}$ receptors, respectively.

Data suggest that fibroblast $[Ca^{2+}]_i$ oscillations triggered by inflammatory mediators, like BK, involves signaling via P2 purinoceptors by endogenous released nucleotides in the rat subcutaneous connective tissue. The mechanism by which purines and/or pyrimidines are released from activated fibroblasts requires further investigation. Our findings indicate that stimulation of $P2Y_4$ receptors by ATP and/or UTP, as well as of $P2Y_{13}$ receptor by ADP, are implicated in fibroblast $[Ca^{2+}]_i$ transients. Via this mechanism, fibroblasts of the subcutaneous tissue may ultimately contribute to sensitization of sensory neurons under painful situations. We, therefore, hypothesized that fibroblast-located purinoceptors may be a target for therapeutic intervention in chronic painful conditions (e.g. fibromyalgia).

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Work supported by FCT (FEDER funding, PTDC/SAU-FCF/108263/2008 and UMIB-215/94).ARP is in receipt of a PhD Studentship funded by FCT (SFRH/BD/47373/2008).

The G-quadruplex fold of nucleic acids: a challenging target in anti-cancer drug design

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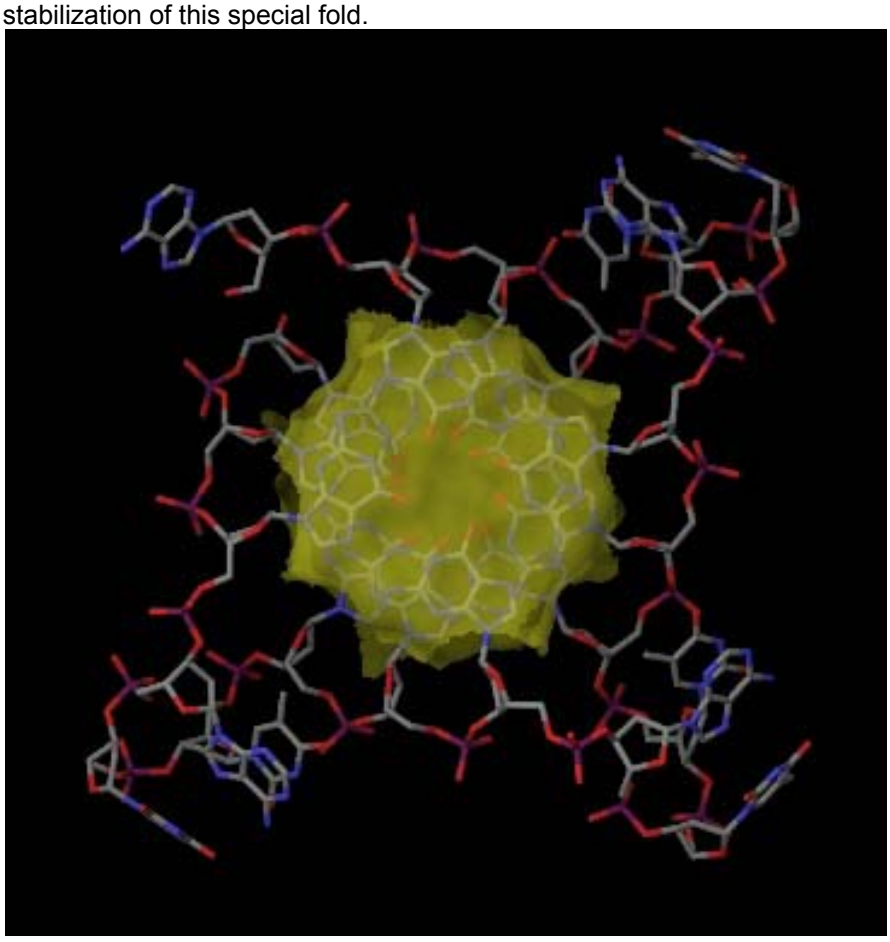
The G-quadruplex fold is an unusual DNA and/or RNA conformation adopted in physiological and pathological conditions.[1] The hyper-elongation of the human repeated telomeric sequence folding into this conformation is related to the hyper-proliferation of neoplastic cells.
Recently the rationale of blocking the elongation process has been proposed as new and selective mechanism to develop antitumor agents.[2] The conformational properties of the G-quadruplex target will be discussed as well as the opportunity to carry out rational design of novel antitumor agents based on the stabilization of this special fold.



New computational tools based on different approaches developed in our laboratory will be described with some G-quadruplex case studies.[3]
This research work is supported by a FIRB_IDEAS grant of the Italian Ministry of Education (code RBID082ATK).

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New A-ring Steroidal Olefins and Epoxides as Aromatase Inhibitors. Synthesis and SAR Studies.

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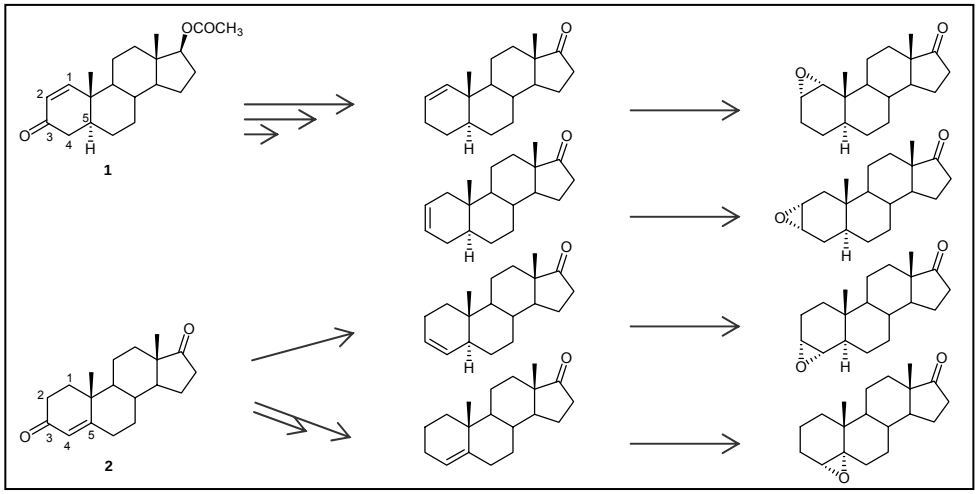
Estrogens, which are biosynthesised in the human body by the enzyme aromatase, play an important role in breast cancer development. The binding of estrogen to its receptor leads to the proliferation of estrogen-dependent cancer cells. Two main chemical approaches have been successfully applied as endocrine therapy of estrogen-dependent breast cancer: one is directed to the estrogen receptor by use of selective estrogen receptor modulators (SERMs) (like tamoxifen) and the other is directed to aromatase (like exemestane). Nowadays, SERMs are being taken over by aromatase inhibitors (AIs) due to their higher efficacy and favourable safety profile. In the course of our previous studies on the structure-activity relationships (SAR) for steroidal AIs, it was found that some planarity in the A-ring and A/B-ring region is usually required for the interaction with the active-site of the enzyme and for anti-aromatase activity[1-3]. This planarity can be achieved by a double bond, as in the natural substrate of the enzyme androstenedione, or by an epoxide function. In the present work, we introduce a study on the influence of the double bond/epoxide position along the steroidal A-ring, in aromatase inhibitory activity. Therefore, we designed, synthesised and studied the inhibitory activity towards aromatase of a series of A-ring C-1, C-2, C-3 and C-4 olefins as well as C-1/C-2, C-2/C-3, C-3/C-4 and C-4/C-5 epoxides. C-1 olefin was synthesised through a four-step reaction from **1**, while C-3 and C-4 were prepared from **2** through a one- and two-steps reaction, respectively. The synthesis of epoxides was performed through oxidation of the olefins with performic acid in dichloromethane. The biochemical report suggests that, in general, olefins are better aromatase inhibitors than their respective epoxides. On the other hand, shifting the double bond from the C-4 position to the C-1, that is, moving it away from the A/B-ring fusion, a decrease in the aromatase inhibitory activity is observed.



Acknowledgements
This work was supported by FCT (Fundação para a Ciência e Tecnologia). C. Varela and C. Amaral are recipients of a PhD grant of FCT (SFRH/BD/44872/2008 and SFRH/BD/48190/2008, respectively).

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From a Simple Chemical Model to the Real Thing: The Path to the Discovery of the Inhibition of Thioredoxin Reductase by Mitomycins

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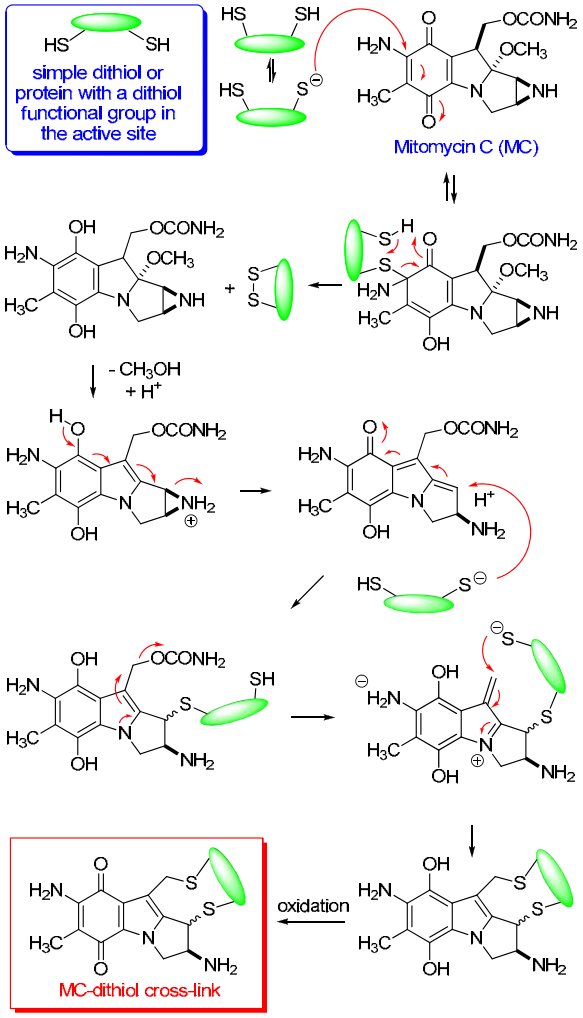
Mitomycin C (MC) is a natural antitumour antibiotic used in anticancer therapythat requires a reductive activation to exert its biological activity.[1] MC is inert in its original structure but after bioreduction it generates a bifunctional electrophile that alkylates cellular nucleophiles, in particular the complementary strands of DNA. A few years back we found that dithiols efficiently activate mitomycin A (MA), a highly toxic member of the mitomycin family.[2] We found later that dithiols also activate MC, but the activation was rather less efficiently than that of MA.[3] We also found that MC, after being reductively activated by dithiols, further reacts with additional diithiol to form bis-alkylated 1:1 adducts, that were characterized as MC-dithiol cross-links[4] (see accompanying scheme). The ease of formation of MC cross-links with simple dithiols led us to speculate that MC could be a substrate for r biological dithiols and that the resulting activated MC could afterwards alkylate the dithiol site, thus inactivating the enzyme. To test this possibility we considered thioredoxin reductase (TrxR) as the ideal choice. TrxR, a reductase containging a selenolthiol active site, is a known biological target for anticancer (cisplatin, mustards).[5]



Mechanism for the reductive activation of MC by dithiols and posterior alkylation of the dithiol by activated MC

Both the reductive activation of MC and the formation of dithiol cross-links occur optimally at a pH value averaging the pKa_1 and pKa_2 values of the dithiol. In the case of simple thiols, this means an optimum pH of 9.5–10, that may seem of no biological significance. However, in TrxR the average pKa of the selenotlhiol group is around 7, and therefore the reactions observed for MC with simple thiols at pH 9.5–10 may be expected to occur at physiological pH values with TrxR. Further, the selenol group of TrxR is known to behave as a good nucleophile at physiological pH in reactions with electrophiles. If our hypothesis proved correct, then MC would behave as a suicide (mechanism-based) inhibitor for TrxR. With this hypothesis in hand, the potential inhibition of TrxR by mitomycins was investigated and we found that both MA and MC are, indeed, mechanism-based inhibitors of TrxR.[6]

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Biomarkers Of Redox Unbalance And Oxidative Stress In Environmental Pathologies

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A severe imbalance in the homeostatic control of the physiological red-ox *status*, and in the capability to utilize antioxidants to modulate endogenous and environmental-driven free radical formation is a common feature of environmental-borne syndromes, including chronic inflammatory, degenerative and metabolic diseases. Uncontrolled oxidative damage is also a common biochemical marker following invasive pro-inflammatory treatments, notably radio-, photo- and chemo-therapy protocols. To address this functional impairment, the administration of antioxidant / chelator / natural immuno-modulating treatments is currently well established, although routine protocols for monitoring and follow-up of oxidative stress biomarkers through non-invasive blood testing procedures are still generally unavailable in the clinical practice at both Italian and European levels.
The leading features of oxidative imbalance can in fact be monitored, both in blood components and at the skin level, in the Clinical Chemistry Laboratory by means of standardized and validated high performance chromatograpy, spectrophotometry, chemiluminescence, fluorimetry and microarray techniques. This approach includes metabolomic determination of a) lipophilic/hydrophilic low-molecular weight antioxidants (AO) and AO enzyme expression/activity b) lipoperoxidation products, namely malonyl aldehyde (MDA) and 4-hydroxy-nonenal (4-HNE); c) reactive oxygen and nitrogen species (ROS and RNS), i.e. nitric oxide, superoxide anion, hydrogen peroxide production; d) plasma and cell pro-inflammatory cytokine profiles, strictly interrelated with free radical status. The lipidomic analysis of red/white cell or plasma membranes provides nutritional and functional information, also relevant for immunological status; red blood cell or platelet energy production (ATP) allows a rapid evaluation of the energetic *status* of the individual patient.
In the area of environmental medicine, specific profiles of oxidative unbalance have been described for chronic dermatologic diseases with nutritional or environmental etiologic involvement, such as psoriasis[1,2], vitiligo, atopia. As for professional categories exposed to severe ambient stress, cosmonauts, long-haul aviation pilots, or emergency operators suffering from burn-out syndrome, represent an excellent example of a redox-imbalance condition, where most part of the above listed blood biochemical parameters show significant alterations, with a general increase of oxidative markers and depleted antioxidant defence levels, due to both the wearing working schedules and the frequently unregulated diet and lifestyle[3]. A far more defined pattern of redox unbalance has been shown by our research group[4] in a wide case-history of 226 multiple chemical sensitivity (MCS) patients, demonstrating specific features of oxidative stress, with reduced enzymatic antioxidant and detoxification capacity of catalase, glutathione peroxidase and glutathione transferase enzymes, reduced glutathione levels, increased RNS (peroxynitrite) and lipid oxidation stable-products, and a trend-to-excess levels of the genotoxic 4-hydroxy-2-nonenal (HNE)-protein adducts, consistent with depleted levels of polyunsaturated fatty acids of red cell membranes, and a specific profile of up-regulated pro-inflammatory cytokines. These patterns have proven to be selectively relevant for the MCS condition, and for the relative individual treatment-response profile. These data bear promising implications also for other environmental-borne disabling conditions, such as fibromyalgia, chronic fatigue syndrome, and other disabling conditions often still ascribed to the "medically unexplained symptoms", including irritable bowel syndrome, sick building and Persian Gulf syndromes, amalgam disease, and electric hypersensitivity[5].
Established analytical protocols, based on a standardized validated battery of clinical chemistry blood measurements, are now able to provide the Environmental Medicine clinicians with a powerful tool for reliable diagnosis and follow-up. In association with toxico- and pharmaco-genomics, they are bound to offer a solid rationale for evidence-based individualized therapy.

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Leishmanicidal activity of lactoferrin-derived peptides

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Currently, there is an urging need to develop new therapies to fight infectious diseases, mainly due to the increasing resistance of the pathogens in the past years to the current therapies. For parasitic diseases an additional problem is the scarcity of available drugs to treat these diseases, mostly due to the lack of interest of the pharmaceutical corporations to invest in this area and to develop and test new leads. Still, this need is prompting an active and extensive search for new types of strategies to fight also these pathogens.
One potential and interesting alternative is the use of antimicrobial peptides (AMPs). These agents are active on a large variety of pathogens, and although they widely differ in their primary sequence, a high cationic character and the adoption of amphipatic structures are common traits for most of them. It is widely accepted that their mechanism of action mostly relies on disruption of the pathogen membrane, by peptide insertion after an initial electrostatic interaction with the anionic lipids at the surface of the membranes. For some of them intracellular targets were also described. Induction of resistance against AMPs is an unlikely event since it would require an overall reorganization of the cell membrane structure, namely the phospholipid composition, affecting simultaneously the pleiade of transport systems and enzymes embedded in the phospholipid matrix. The protozoan parasite *Leishmania* is a causative agent for a set of clinical infections collectively known as leishmaniasis, ranking as protozoal scourge only second to malaria. More than 12 million people are infected worldwide, outbreaks as those in Sudan in the last years caused 50,000 deaths. This disease is endemic to all European Mediterranean countries and Southern Portugal, mostly as canine leishmaniasis, although as an opportunistic parasite co-infection with HIV is of special relevance. Aside from the clinical, *Leishmania* is an interesting model as a target for AMPs; as the plasma membrane is devoid of external barriers as the ones in Gram-negative bacteria, although the promastigote form of the parasite is endowed with an anionic glycocalix and abundant proteolytic activity at the surface. Secondly, endo- and exocytosis are confined into a special area accounting for 2% of the plasma membrane known as the flagellar pocket, hampering repair mechanism for damaged membrane.
In this study we have been assessing the activity of three AMPs derived from bovine lactoferrin, namely lactoferricin 17-30 (LFcin 17-30), lactoferrampin 265-284 (LFampin 265-284), and LFChimera, a hybrid peptide that is formed by the first two peptides linked by a lysine, mimicking the spacial proximity of the two microbicidal domains in intact lactoferrin as a new leishmanicidal agent. To this end, the EC50 of each peptide was determined on *L. donovani* promastigotes and *L. pifanoi* amastigotes. Also, in the promastigote form of the parasite plasma membrane several permeabilization assays were performed – entrance of the vital dye Sytox Green (MW=600 Da), plasma membrane depolarization (Bisoxonol fluorescence), decrease of free ATP levels in living parasites and microscopic techniques.

Altogether, both LFcin 17-30 and specially LFchimera are leishmanicidal at low micromolar range, inducing plasma membrane permeabilization and bioenergetic collapse of the parasites. The activity of LFchimera is much higher than the sum of its separate components, stressing the importance of this surrogate as imitation of their spatial topology in nature. The feasibility of these peptides as new leishmanicidal agents will be discussed.

Financial support was obtained from FCT through CIQ (UP) and the Spanish–Portugal cooperative project “Acção nº E-40/90”.

Phenolic content, antioxidant, cytotoxicity and acetylcholinesterase activities evaluation from *in vivo* and *in vitro Hypericum undulatum*

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Despite of the remarkable progress in synthetic organic chemistry during the twentieth century, over 25% of prescribed medicines in the industrialised countries derived direct or indirectly from plants. The fast growing population on the planet is causing pressure on the available cultivable land for growing crops and fulfil food needs. The increasing consumption is causing habitat destruction and a rapid loss of medicinal plant supply. Since, the spontaneous growth of medicinal plants is endangered not only by the excess of harvesting but also by misidentification, genetic and phenotypic variability. *In vitro* production of medicinal plants is expanding worldwide, since plants develop faster, the production system offers a homogenous supply of products, uniform quality, higher yield and reliability which is independent of environmental factors such as climate, pests, geographical and seasonal constrains[1].
Hypericum (Guttiferae) is a large genus (ca 450 species) of herbs or shrubs that have been used in folk medicine for centuries. *H. perforatum* (St. John's Wort) the most recognized species is used specially for its anti-depressive and anti-viral properties in a worldwide market estimated of more than 6 billion US dollars[2]. As a consequence high amounts of plant raw material is needed. However, suitable *Hypericum* varieties are the prerequisite for a successful cultivation. *H. undulatum* Schousb. ex Willd is according to some authors very similar both morphological and chemically with *H. perforatum*, and is used in the Portuguese folk medicine as diuretic, anti-inflammatory and for anti-hypertensive issues[3]. Since the objective of our study was to compare: acetylcholinesterase inhibition properties; cytotoxicity on the brine shrimp *Artemia salina*; the antioxidant activity using the free radical scavenging activity (FRSA) and the reducing power assays; the total phenolic content (Folin Ciocalteau method) and the chromatographic profile of the phenolic composition of hot water extracts from *in vitro* regenerated shoots of *H. undulatum* against *H. undulatum in vivo* species.
Two sets of regenerated shoots were produced from *in vitro* plants in two different periods in which six plants of each period were selected for weighting and phytochemical analysis. The results reveal that the two groups of *in vitro* plants are very similar in terms of dry weight and hot water extraction yield. The extraction yield of *in vivo* plants is approximately twice lower in comparison with regenerated *in vitro* shoots. The acetylcholinesterase inhibition and cytotoxicity on the brine shrimp *Artemia salina* of all the *in vivo* and *in vitro* samples were not significant since the values of IC_{50} and LC_{50} were higher than 1.00 mg/mL which are the limit considered for extracts with good enzyme inhibition and cytotoxicity properties.
The total phenolic content was assayed according to the Folin-Ciocalteau method. The results show that *in vitro* extracts are average 25% less rich in polyphenols than *in vivo* extracts. Reverse phase chromatography was used to identify the main phenolic compounds in the extracts. *In vivo* samples are characterized by the presence of chlorogenic acid, hyperoside and isoquercitrin. *In vitro* samples still under study, however the two main compounds chlorogenic acid and quercitrin were already identified. As regards the antioxidant activity, the results are promising for both assays. The EC_{50} on the FRSA was very similar between all samples with the EC_{50} varying between 19.35 to 29.60 µg/mL. A similar behaviour was observed for the reducing power assay, in which the EC_{50} varied within 59.45 to 74.19 µg/mL. In this assay, the *in vivo* samples were more active with an EC_{50} of 53.84 µg/mL. The antioxidant activity was assayed on the four compounds identified in the samples. The results showed that all compounds presented significant antioxidant activity, higher than that found in the sample extracts. These compounds are undoubtedly responsible for the antioxidant properties of these extracts. More research is ongoing in order to identify other compounds within the *in vitro* extracts.

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Structural insights into *Leishmania infantum* endoG active site and analysis of the reaction mechanism

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Leishmaniasis (or 'leishmaniosis') is a complex of mammalian diseases caused by parasitic protozoans classified as *Leishmania* species (Kinetoplastida, Trypanosomatidae). Natural transmission may be zoonotic or anthroponotic, and it is usually through the bite of a phlebotomine sandfly species. Primary skin infections sometimes resolve without treatment, with the host developing acquired immunity through cellular and humoral responses, but the infection can spread to produce secondary lesions in the skin (including diffuse cutaneous leishmaniasis), the mucosa (muco-cutaneous leishmaniasis) and the spleen, liver and bone marrow (visceral leishmaniasis, which is usually fatal if untreated). Most infections occur in tropical or subtropical regions, and only zoonotic *Leishmania infantum*, which causes visceral and cutaneous leishmaniasis in humans and the domestic dog (the reservoir host), is transmitted in both the eastern and western hemispheres.[1]

It is increasingly accepted that *Leishmania* and other single-celled organisms are able to undergo a cell death process that resembles metazoan apoptosis and is induced by a variety of stimuli. However, the molecular mechanisms that participate and regulate this pathway are still very poorly described, and very few of the participating molecules have been identified. Recently our group demonstrated that *L. infantum* promastigotes express a nuclease, LiEndoG, similar to EndoG of higher eukaryotes, that is implicated in the execution phase of programmed cell death.[2] The three-dimensional structure of LiEndoG is currently unknown but theoretical predictions strongly suggest that it belongs to the ββα-metal superfamily of nucleases[3] for which several mechanisms of DNA phosphodiester hydrolysis have been proposed.[4] We have built a homology model of LiEndoG in complex with a short substrate oligonucleotide using as templates the experimentally solved structures of *Vibrio vulnificus* nuclease (Vvn),[5] *Serratia marcescens* endonuclease,[6] *Drosophila melanogaster* EndoG in complex with its inhibitor EndoGI, and the homing nuclease I-PpoI.[7] Of the four, only Vvn and I-PpoI have been co-crystallized with a short piece of DNA representing the substrate or the product of the reaction. To gain insight into the mechanism of DNA cleavage by this family of enzymes we have focused on Vvn nuclease and used a combination of quantum mechanical calculations and molecular dynamics simulations to characterize in atomic detail the different steps of the reaction. This has allowed us to understand the actual role played by both the protein residues in the 'ββα-Mg^{2+}' motif and the assisting water molecules.

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NEW ENDOPEROXIDE-PEPTIDYL FALCIPAIN INHIBITORS

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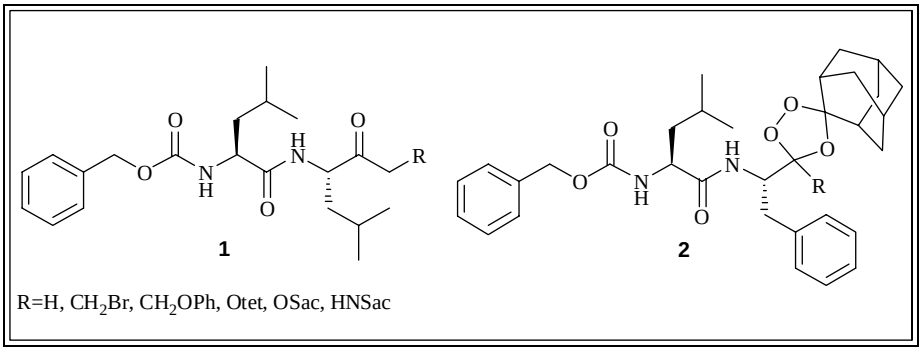
WHO has recently considered Malaria as the world's top-priority tropical disease. Although a number of chemotherapeutic agents are available for the treatment of Malaria, the emergence and spread of multidrug-resistant *P. falciparum* strains is the major obstacle in the control of the disease, urging the need to develop new drugs, preferably addressed to new parasitic targets [1]

It is established that haemoglobin degradation is crucial for the survival of *P. falciparum*. This process relies on the action of several cysteine proteases, among which falcipains-2/3 play a major role.[2] As such, several peptidyl falcipain-2 inhibitors were developed, some of them with activity *in vitro* at low nanomolar range and high potency *in vivo,* in mouse models.[3] However, the peptidic nature of the inhibitors resulted in poor pharmacokinetics due to low bioavailability and enzymatic degradation. Seeking an improvement of the pharmacokinetic profile, we have prepared a range of carbonyl based protease inhibitors **1**.

The hybrid drug concept has recently been applied to Malaria chemotherapy.[4] Hybrid drugs are a potentially useful alternative to classic drug combinations (e.g. ACT's), as they offer the possibility to address different targets or different sites of the same target with a single chemical entity, providing improved efficacy and, most importantly, strongly inhibiting selection of drug resistance. Based on this concept, and considering the high relevance of peroxide-based drugs in malaria chemotherapy, we envisaged the development of endoperoxide cysteine protease inhibitor pro-drug models **2**. The rationale is to combine semi-synthetic or synthetic peroxide-type scaffolds, with a falcipain inhibitor. These peroxide-based hybrids accumulate in the parasite, where they are activated by heme or Fe(II) through reductive cleavage of the peroxide linkage, leading to subsequent formation of alkylating agents and a plasmodial cysteine protease inhibitor. [5]

The rationale behind this investigation and the results obtained are the subject of this communication.



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Acknowledgements

The authors thank FCT for financial support (grants SFRH/BPD/20587/2004 and SFRH/BD/6176/2001)

Enzyme inhibition and cell signalling modulation by sulphur- and selenium-containing flavonoids – an *in silico* and *in vitro* approach

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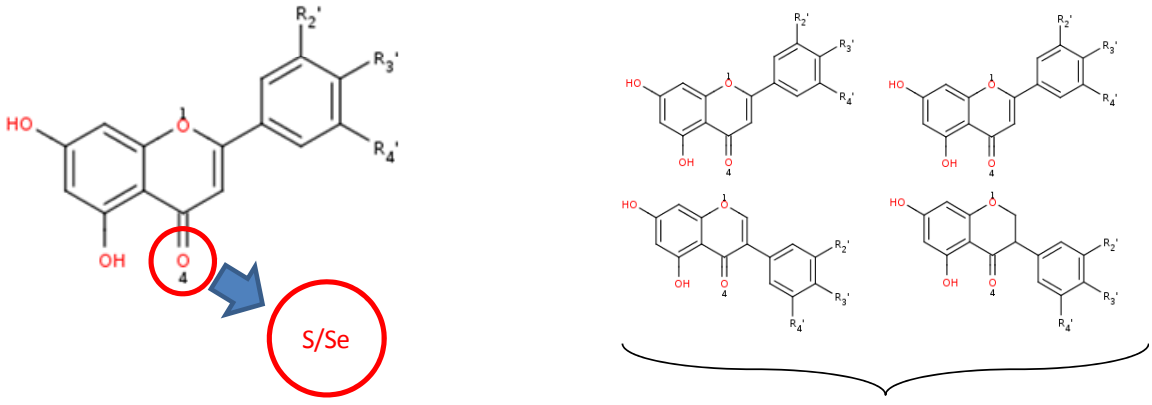
Flavonoids have been studied for decades due to their putative health-promoting effects, initially attributed to their antioxidant capacity as H-atom donors and, more recently, to their ability to inhibit cellular enzymes and to modulate cell signalling pathways. In particular, flavones have been shown to inhibit various cellular kinases and a number of enzymes involved in lipid metabolism; they are also capable of inhibiting cytochrome P450 enzymes, thus interfering with drug metabolism.

We have synthesized sulfur and selenium-containing derivatives of chrysin and quercetin (Figure 1), and evaluated their potential antioxidant activity both experimentally, by assessing their capacity to reduce the DPPH radical, and by using computational methods to probe the energetics of the various mechanisms contributing to their antioxidant activity, namely H atom transfer and sequential electron/proton transfer. Besides those compounds, a library set of flavonoids of different classes, with different chalcogen substitutions on O1 and O4 atoms, was also studied.

A rigid docking approach was used to survey the interaction of the above-mentioned derivatives with various proteins of known structure in order to identify which are the cellular proteins more likely to be modulated by these compounds. A set of about 30 proteins were tested, including protein kinases, various phospholipases, lipoxygenases and cyclooxygenases, a number of ATP-dependent cell-cycle related enzymes, and various isoforms of cytochrome P450. Ligand-protein interactions, discussed in terms of ligand binding to protein residues and inhibition constants, indicate that the chalcogenated derivatives interact more strongly than the precursor flavones with the tested proteins. Also, they maintain the pattern of interaction with the receptors, binding to the same residues that are involved in the binding of well-tested modulators.

In vitro assays are currently being performed, addressing the activity of these compounds in mammal cell lines



Chrysin	$R_{2'}=R_{3'}=R_{4'}=H$	$O_1 \rightarrow O, S, Se$
Morin	$R_{2'}=R_{4'}=OH; R_{3'}=H$	$O_4 \rightarrow O, S, Se$
Quercetin	$R_{2'}=H; R_{3'}=R_{4'}=OH$	

Figure 1 – Structures of the synthetized flavone derivatives (left) and of the library analysed using computational techniques (righty).

Plant Polyphenols And Tumors: From Mechanisms To Therapies, Prevention, And Protection Against Toxicity Of Anti-Cancer Treatments

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Polyphenolic molecules produced by higher plants in response to biotic and abiotic stresses exert numerous effects on tumorigenic cell transformation, and on tumor cells in vitro and in vivo and may interact with conventional anti-tumor therapies. In the presentation, the following aspects will be highlighted:

(i) redox-dependent and redox-independent mechanisms underlying cytotoxic/cytostatic effects of plant polyphenols (PPs) and their metabolites towards tumor cells and cytoprotection of normal cells;
(ii) mechanisms of anti-angiogenic and pro-apoptotic action of PPs;
(iii) PPs-associated phototoxicity against tumor cells and photoprotection of non-tumor cells;
(iv) PPs effects on drug-metabolizing enzymes as a basis for their synergism or antagonism with chemotherapy;
(v) molecular pathways leading to tumor chemoprevention by PPs;
(vi) PPs as protectors against toxic effects of chemo-, radio-, and photodynamic therapies.

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Poster

Communications Abstracts

Notes

Synthesis and Docking Studies of Carbamates and Esters Based on Coumarin Scaffold as Multi-Target-Directed Drugs for Neurodegenerative Diseases

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At the moment, developed countries' society face, with the increase of the population life expectancy, a major problem related with neurodegenerative diseases (ND), such as Parkinson's and Alzheimer's diseases, and so a need for new drugs targeting these diseases emerges. Actually, one of the few accessible treatments use monoamine oxidases inhibitors (MAOI-A and MAOI-B) and acetylcholinesterase inhibitors (AChEI).

Coumarins are a large family of compounds of natural and/or synthetic origin that have been shown to have numerous pharmacological properties.[1] In our group, we already synthesized and evaluated new coumarins that reveal to be potent iMAO-B.[2-4]

In this study, we developed new synthetic methodologies to create novel multitarget inhibitors for ND focused on the coumarin scaffold (figure1).

The drug development was accomplished by docking studies of the previously prepared coumarins (figure 2). The results of this research will be presented in this communication.

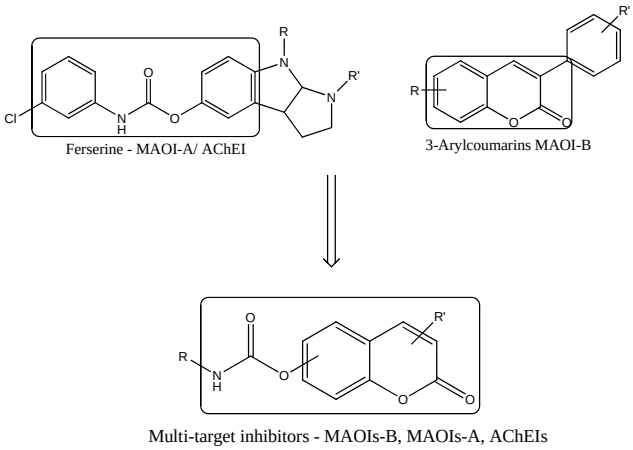

Figure 1 – Obtention of carbamates derived from coumarin as multi-target inhibitors.

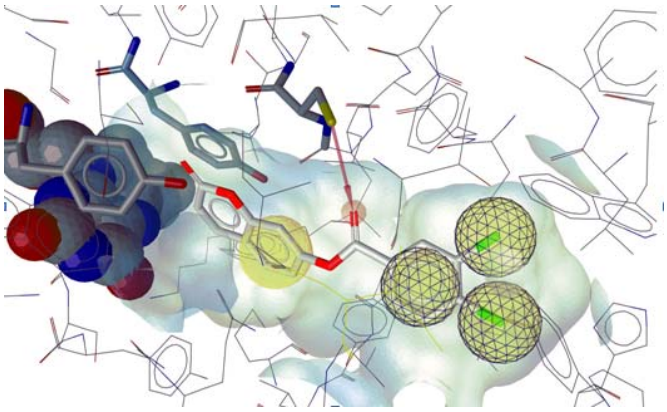

Figure 2 – Docking studies performed with Glide, visualisation by LigandScout.

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Chromone-based Adenosine Receptor Ligands: a New Challenge in the Anticancer Drug Discovery Scenario

Alexandra Gaspar[1], Joana Reis[1], Fernando Cagide[1], Tiago Silva[1], Maria João Matos[1,2], Eugenio Uriarte[2], Karl Norbert Klotz[3], Stefano Moro[4], Fernanda Borges[1].

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Despite the advances in medical and pharmaceutical sciences, there are still many diseases which are incurable maladies. Therefore, there is still a great need for more active and selective drugs with fewer undesired or toxic side-effects[1].
Cancer is a very complex disease, which can be fatal or cause great suffering and disability. This disease is linked with different initiating causes, cofactors and promoters, and several types of cellular damage. Advancing knowledge on the cellular and molecular biology of the processes that regulate cell proliferation, cell differentiation and cellular responses to external signals make a number of potential targets available for new approaches to treat cancer. During the last decade different approaches to treating cancer have been established based mainly on specific targets that are mostly expressed in tumour but not in normal cells. Adenosine is a purine nucleoside found within the interstitial fluid of tumors at concentrations that are able to modulate tumor growth by interacting with four G-protein-coupled adenosine receptor (AR) subtypes, designated A_1, A_{2A}, A_{2B} and A_3. AR levels in various tumor cells are upregulated, a finding that suggest that AR subtypes may function as biological markers and also as targets for specific ligands that in turn led to cell growth inhibition[2].
As chromone scaffold has been recognized as a pharmacophore of a large number of bioactive compounds a project focused on the discovery of new chemical entities (NCEs) that incorporate benzo-γ-pyrone [benzopyran-4-one] substructure as potential AR ligands has been performed. Therefore, in this communication the rational synthesis of a chromone-based library designed to develop novel antagonist adenosine ligands as well as their drug efficacy and selectivity profile towards A_1, A_{2A}, A_{2B} and A_3 AR will be described. In order to identify the hypothetical binding modes at both the crystallographic structure of human AR a molecular modelling investigation of the newly synthesized analogues was also performed. The mentioned analysis was also extended to docking simulations and per residue electrostatic and hydrophobic contributions. The overall data will be presented in this communication.

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This work was supported by the Foundation for Science and Technology (FCT), Portugal (PTDC/QUI-QUI/113687/2009). A. Gaspar (SFRH/BD/43531/2008) and F. Cagide (SFRH/BPD/74491/2010) thank FCT grants.

New Ensemble Machine Learning Method for Classification and Prediction of the Affinity A_{2B} Adenosine Receptor Antagonists

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Combinations of multiple classifiers have been found to be consistently more accurate than a single classifier [1]. The construction of multiple independent classifiers, however, is typically a non-trivial problem.
In this work, a new ensemble machine learning algorithm is proposed for classification and prediction of the affinity A_{2B} adenosine receptor antagonists. So, a set of antagonist compounds, previously synthesized and screening in a consistent binding assay was assembled. With the desire to build a reliable predictive model, the use of several molecular descriptors (e.g. 0D, 1D, 2D and 3D) along with different Artificial Intelligence techniques and feature selection algorithms have been examined [2].
Initially, the database was segmented in training and test sets using cluster analysis. The resulting training set was used to develop single classifier models, which were generated with and without previous feature selections. Then, a classification method was proposed: the combination of diverse classification models. To this end, we used several diversity measures [3] to select the best base classifiers, which use different features subsets. The final multi-classifier predictions result from the combination of base classifiers output using different methods like as: majority vote, average, product, min and max, etc. The strategy led to the following results i) The single classifiers together with previous features selections allow to obtain the best good overall accuracy, internal 10-fold cross-validation and predictability on the test set, ii) The comparison between single classifiers and their combination in the multi-classifier model have shown that multi-classifier model have a better performance than the single classifier model. The developed model allows showing the supremacy of the use of classifiers in the development of QSAR model, specifically to predict the affinity of A_{2B} adenosine receptor antagonists.

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Application of Predictive QSAR Models to the discovery of human monoamine oxidase inhibitors

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Due to their role in the metabolism of monoamine neurotransmitters monoamine oxidases (MAOs), namely MAO-A and MAO-B, present a considerable pharmacological interest. In fact, MAO-B inhibitors are considered useful tools for the treatment of Parkinson Disease. However, a large number of MAO-B inhibitors introduced into clinical practice were abandoned due to adverse effects. Therefore, the rational design of new MAO-B inhibitors is still assumed to be of great significance for the development of new and more effective treatments of PD.
For the development of the QSAR models, a suitable dataset of nitrogen and oxygen heterocycles, such as: chromones, xanthones, coumarins, chalcones and thiazolylhydrazones was selected. These compounds were previously synthesized in one of our laboratories or elsewhere [1-8] and measured in a particular and consistent inhibition assay. From the acquired data several reliable predictive QSAR models were built to be applied to classify the inhibition activity on both isoforms of chemical compounds. For this purpose, a linear discriminant analysis and feature selection algorithms using as input predictors 0D, 1D and 2D molecular descriptors was applied. The final models showed the best good overall accuracy, internal cross-validation and predictability on the test set. In this present communication it will be also shown how the combination of the before models makes better predictions than the single ones.

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PHENYLBENZOFURANS: INHIBITORS AND/OR ACTIVATORS FOR MUSHROOM TYROSINASE?

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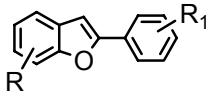
Tyrosinase (EC 1.14.18.1) is an enzyme catalysing the rate-limiting reaction in melanin biosynthesis[1]. The enzyme catalyses two different reactions. The first one is hydroxylation of monophenol to *o*-diphenol (monophenolase activity), and the second is oxidation of *o*-diphenol to *o*-quinone (diphenolase activity). Therefore, tyrosinase is known as a key enzyme implicated in the anabolism of melanin in melanocytes, and its inhibitors have become increasingly important in medicinal and cosmetic products in relation to hyperpigmentation[2, 3].
In previous studies, phenylbenzofurans were isolated from Morus Ihou, belonging to the family of Moraceae, plant that is one of the most ubiquitous traditional herbal medicines in East Asia[4]**.** Interestingly, a phenylbenzofuran *Moracin M* displayed significant inhibitory activities against tyrosinase. For this reason, we have synthesized the new phenylbenzofurans like inhibitors of the mushroom tyrosinase.
The preparation of phenylbenzofurans was successfully achieved by a Wittig reaction between the appropriate triphenylphosphonium salt and the corresponding benzoyl chloride[5].
Tyrosinase activity assays were performed with L-DOPA as substrate, as previously described[6] with slight modifications.



R = H, Me, Br, OMe, OEt
R1 = H, OMe,OH

The compound 2-(4'-metossiphenyl)-7-bromobenzofuran, is the most active compound of this series. Surprisingly, the compound 2-(4'-hydroxyphenyl)-7-bromobenzofuran that has a hydroxyl group instead of a methoxy group shows a different activity.
This preliminary result indicates that the type and the position of groups may play an important role in determining the kind of activity.
The different activities of these compounds that differ in only one group require further study.

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Polyphenols as catalytic inhibitors of human topoisomerase II

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Ellagic acid (EA) is the main metabolite of ellagitannins (ETs), natural compounds abundant in some fruits and nuts. ETs are the main pharmacologically active substances in several traditional herbal products used in folk medicine (1), and their metabolites are responsible for a wide variety of pharmacological activities (2). The main product of the ETs degradation is the ellagic acid (EA), which is further metabolized in urolithins (UL) by the colonic microflora (3).

EA inhibits the human topoisomerase II but its mechanism of action has not been elucidated in details. To elucidate its distinct antitumor properties and to obtain structure-activity relationship (SAR) information, we have characterized the effects of EA, UL and several synthetic derivates on human topo II using enzymatic assays. Specific EA and UL derivatives were highly active on the human topo II α and β, with IC_{50} ≤1μM. The results for the active compounds point out to the importance of the number and relative position of hydroxyl groups on both scaffolds to gain activity on human topo II.

The SAR analysis on EA and UL revealed how the minimal pharmacophore resemble structural features of resveratrol, a natural compound with a wide variety of pharmacological activities, whose weak anti-topo II activity was also reported (4, 5). Therefore, in an effort to enlarge our structure-activity analysis on natural and related synthetic polyphenols with antiproliferative and chemoprotective activities, we have examined a novel set of coumarin derivatives formally derived from resveratrol for their anti-topo II inhibition properties. We report here the enlarged SAR and the putative mechanism of action of this class of catalytic inhibitors of human topisomerase II.

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Effects of A- and D-ring modified steroids in aromatase activity

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Estrogens are required for development of various tissues, however they are pivotal in the growth of neoplastic mammary tissue estrogen-receptor-positive. Nowadays, there are several therapeutic approaches to block estrogen actions, one of them, is based on the inhibition of aromatase, a cytochrome P-450 enzyme (CYP19), responsible for catalyzing the conversion of androgens to estrogens. The aromatase inhibitors (AIs), correspond to an effective alternative to the classical tamoxifen for the treatment of postmenopausal women with ER-positive breast cancer. In a previous work, we have designed and synthesized new steroids with several chemical features that proved to be potent AIs[1,2,3]. In this work we present structure-activity relationship (SAR) results of a series of 14 aromatase inhibitors, obtained from chemical modifications in the A- and D-rings of the natural substrate of aromatase, androstenedione. The inhibition of aromatase activity was evaluated in human placental microsomes and in an ER-positive aromatase-overexpressing breast cancer cell line (MCF-7aro), by a radiometric assay. For the most potent AIs, 5α-androst-2-en-17-one (**18**), 3β-hydroxyandrost-4-en-17-one (**20**), 2α,3α -epoxy-5α-androstan-17-one (**26**) androst-4-en-17-one (**30**) and 4α,5α-epoxy-androstan-17-one (**31**) which presented more than 70% of aromatase activity inhibition, the IC50 values in microsomes were determined. For compounds **18**, **20** and **31** enzyme kinetics studies and aromatase activity in MCF-7aro cell line were evaluated. Compound **30**, a steroid already described by Numazawa *et al.*[4] was also tested in the referred cell line. The results showed that the most potent AIs of our series are the compounds **20** and **31**, which have a hydroxyl group in C-3β position (**20**) and an epoxy group in C-4α,5α position (**31**). In addition, the replacement of the C-17 ketone group by an acetyl, an hydroxyl or a thiol group dramatically reduce the ability to inhibit aromatase activity. These results confirm the importance of certain chemical groups in the A- and D-ring steroid structure for the anti-aromatase activity. This study provides new insights at the structural level for the design of new AIs more specific and more potent to be introduced in breast cancer treatment.

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The authors are grateful to FCT (Fundação para a Ciência e Tecnologia) for grants SFRH/BD/48190/2008 (Cristina Amaral) and SFRH/BD/44872/2008 (C. L. Varela).

Antiproliferative and Cytotoxic Effects of Flavonoids in Human Cancer Cell Lines

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Phenolic compounds have important activities such as antioxidant, anti-viral, anti-allergic, anti-inflammatory and anti-tumoral capacity[1]. One group of phenolic compounds are flavonoids, which are natural constituents of foods and beverages (tea, fruit juices, beer, wine) consumed by humans.[2] The main purpose of this study was to evaluate the anti-proliferative effect of flavonoids present in beverages (naringenin, xanthohumol, gallic acid, catechin, rutin and quercetin) in a human thyroid cancer cell line (*TPC-1*) using the sulforhodamine B (SRB) colorimetric assay.

The obtained results show that xanthohumol (XN) at 100 μM is the most effective against the division of *TPC-1*, as well as quercetin at 10 μM. Rutin is less effective at the whole range of concentrations tested (0.1-100 μM), and catechin doesn't inhibit cell division and growth. The other polyphenolic compounds (naringenin and gallic acid) were effective at the maximum concentration tested. Data also revealed that quercetin has cytotoxic effect at 10 μM, and xanthohumol only at 100 μM. Naringenin, rutin and gallic acid inhibited cell growth by division inhibition (antiproliferative effect), with no cytotoxic effect. The differential activity of the phenolic compounds tested can be ascribed to the nature and position of substituents in the flavonoid molecule, as described by other authors.[3] A more detailed investigation on XN allowed us to conclude that this compound is not cytotoxic at concentrations bellow 20 μM. Growth inhibition of *TPC-1* cells by XN and naringenin was confirmed by cell counting with trypan blue.

Flow cytometry was applied as complementary technique to SRB assay, for XN and naringenin, in a cell line of leukaemia T lymphocytes (*Jurkat* E6.1). The results were very similar for both methods. Compounds showing cytotoxic effects were further investigated by analysis of DNA fragmentation using agarose gel electrophoresis and TUNEL technique in order to determine whether compounds induce apoptosis in *TPC-1*. XN was able to induce apoptosis in both methods. Quercetin, on the other hand, was not able to induce DNA fragmentation showing a low apoptotic effect by TUNEL.

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Methyl 3-[4-(3-arylureido)phenylamino]thieno[3,2-*b*]pyridine-2-carboxylates as potential inhibitors of VEGFR-2: synthesis and molecular modelling studies

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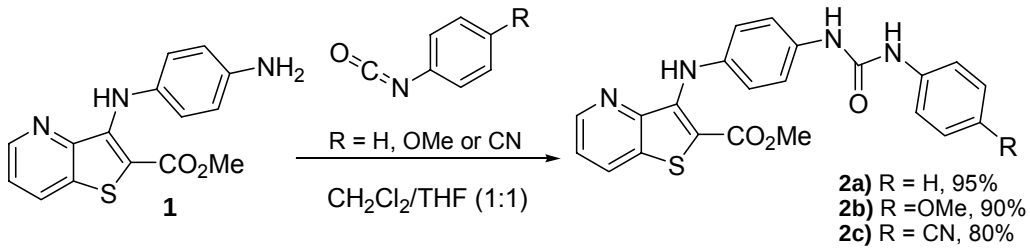
When overexpressed or mutated, protein tyrosine kinases become potent oncoproteins that cause deregulated cell growth angiogenesis and metastasis. Because of these characteristics, they are targets for small molecule inhibitors in the treatment of cancer. Recently some thieno[3,2-c]pyridine 1,3-diarylurea derivatives were prepared as VEGFR-2 (vascular endothelium growth factor receptor-2) inhibitors.[1] Here we present the synthesis of methyl 3-[4-(3-arylureido)phenylamino]thieno[3,2-*b*]pyridine-2-carboxylates **2** in excellent yields, by reaction of the methyl 3-(4-aminophenylamino)thieno[3,2-*b*]pyridine-2-carboxylate **1**, prepared also by us, with different arylisocyanates (Scheme).



Scheme

In this study we used AutoDock Vina to perform molecular docking in order to evaluate the capacity of compounds **2** to inhibit VEGFR-2, a protein related to tumour angiogenesis. The protein tyrosine kinase domain X-ray 3-D structure was obtained from the Protein Data Bank (PDB: 1YWN) and the estimated inhibition constants (Ki) of the compounds were obtained. In order to validate the molecular docking approach, the respective co-crystallized ligand (LIF) and Sorafenib, a known drug that inhibits VEGFR-2, were docked to the kinase domain. The difference between the X-ray conformation and the predicted docked conformations of both ligands as well as the difference between estimated Ki (Sorafenib: 109 nM; LIF: 7 nM) and experimental Ki (Sorafenib: 93 nM[2],LIF: 2 nM[3]) were negligible, validating the protein structure for virtual screening with the synthesised compounds. An initial drug-like analysis was performed by calculating several property parameters of the compounds and it was observed that all compounds **2** obey the Lipinski's Rule of Five. In this series compound **2c** is the most promising one presenting an estimated Ki value of 214 nM against 5276 nM for **2a** and 497nM for **2b**. The presence of the nitrile group lowers significantly the Ki value in this series.
Furthermore, the docking pose of the compound with the best docking score was analyzed in order to understand the key interactions between the compounds and the VEGFR-2 kinase domain structure.

Acknowledgments: FCT (Portugal) and COMPETE/QREN/EU for financial support through research project PTDC/QUI-QUI/111060/2009. RC Calhelha thanks to FCT, POPH-QREN and FSE for his grant (SFRH/BPD/68344/2010.

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Comparison of Accuracy, Precision and Linearity, Obtained by Different Methods for Analysis of Acethylcholinesterase Inhibitor Galanthamine Hydrobromide

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Galanthamine is specific, competitive and reversible acetylcholinesterase inhibitor and allosteric modulator at nicotinic cholinergic receptor sites potentiating cholinergic nicotinic neurotransmission. It is used in for the treatment of mild – to – moderate Alzheimer's disease, neuromuscular diseases such as myasthenia gravis, drug – induced respiratory depression, in anesthesiology for antagonism of skeletal neuromuscular blockade of non – depolarizing muscle relaxants, in ophthalmology, gastroenterology, cardiology, physiotherapy.

The aim of current study is to comprise the analytical parameters accuracy, precision, linearity, LOD and LOQ, obtained by different described methods for determination of Galanthamine hydrobromide alone and in combination with other drugs in biological materials (plasma, serum, liver tissues, bile, urine) and in dosage pharmaceutical preparations (tablets, capsules).

The used method is summarizing of analytical information from publications in scientific journals, sited from the following sources: Elsevier, Medline, Science Direct, Scopus, Springerlink. The study comprised comparison of techniques for identification and determination of: I) Galanthamine hydrobromide:1) UV spectrophotometry (SFM): a) followed for inhibition studies of enzyme acetylcholinesterase; b) zero order SFM at λ = 287 nm; c) first derivative SFM at λ = 277.4 nm; 2) spectrofluorimetric determination, through excitation – emission fluorescence matrices and second – order chemometric tools: parallel factor analysis (PARAFAC), unfolded partial least – coupled to residual bilinearization (U – PLS/RBL) and multidimensional partial least – squares coupled to residual bilinearization (N – PLS/RBL); 3) HPLC, after liquid – liquid or solid – phase extraction: a) NP/UV at λ = 235 nm (serum, urine and bile); b) RP/UV at λ = 289 nm (caps, plasma); c) HPLC – UV photodiode – array radiometric detection; d) isocratic HPLC/MS (human heparinised plasma; e) HPLC/MS/MS; 4) GC/MS; 5) thin layer chromatography (TLC); 6) high performance thin layer chromatography (HPTLC); 7) electrophoresis (E): a) paper E; b) capillary zone E (CZE) (pharmaceutical dosage formulations, serum and urine); 8) potentiometry (tablets); 9) enzyme immunoassay and radioimmunoassay (unpurified plant extracts from bulbs and leaves); II) Galantamine hydrobromide and it's metabolites: 1) RP HPLC with UV photodiode – array, fluorescence and MS detection (blood plasma, urine, liver tissues); III) Galantamine hydrobromide and Rivastigmine:1) micellar electrokinetic chromatography (MEKC) with UV detection at λ = 214 nm, after liquid – liquid extraction (plasma). The conclusion is, that the most used, accurate and precise method is HPLC.

Key words: Galanthamine hydrobromide, acethylcholinesterase inhibitors, accuracy, precision.

Screening for acetylcholinesterase inhibitors in essential oils from Azorean plants

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Alzheimer's disease (AD) is a neurodegenerative disorder characterized by progressive cognitive impairment in our ageing society. It has been observed that cholinergic pathways in the cerebral cortex and basal forebrain are compromised in AD and the resultant cholinergic deficit contributes to the cognitive impairment of these patients. Acetylcholinesterase (AChE) is directly involved in the regulation of the cholinergic system, and therefore it is of interest the search of natural active compounds that can serve as AChE inhibitors, in order to decrease the rate at which acetylcholine is cleaved, increasing the availability of this neurotransmitter in the brain. The choice of essential oils for this purpose relates to the fact that some of the drugs approved for therapeutic use show hepatotoxicity and gastrointestinal disorders, consequently there has been a continuous search for new drugs. To achieve this goal, four plants from the Azorean Flora were chosen. *Psidium cattleianum* Sabine, locally called "araçá", has few investigations and they suggest nutritional and functional potential. Although traditionally appreciated for its sensory attributes and expected functional properties, *P. cattleianum* is still poorly characterized. *Pittosporum undulatum* Vent. essential oil had good antithrombin activity and also antimicrobial activity. *Juniperus brevifolia* (Seub.) Antoine. and *Laurus azorica* (Seub.) Franco. are two endemic plants from Azorean archipelago. Leaves of *P. cattleianum*, *J. brevifolia*, L*aurus azorica* and flowers of *P. undulatum* were collected in São Miguel. The volatile components of the different samples were extracted for 3 hours by hydrodistillation in a Clevenger type apparatus. The AChE activity was tested using a modified method of Elmann[1]. All of the samples tested presented an IC_{50} lower than the standard (α-pinene) commonly used for essential oils (IC_{50} = 1.43 mg/mL). The results obtained demonstrate that they are good acetylcholinesterase inhibitors, presenting IC_{50} values ranging from 0.20 (*P. undulatum*) to 0.67 mg/mL (*J. brevifolia*). Nonetheless, the essential oil with the highest IC_{50} is still lower than the reference standard. As a preliminary study, it can be inferred that all the plants assayed can be used not only for the medicinal purposes already known, but also as a potential treatment for Alzheimer's disease through aromatherapy.

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In vitro toxicity and inhibition of acetylcholinesterase by *Hedychium gardnerianum* extracts

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Some species of Zingiberaceae have been the subject of a range of chemical and pharmacological investigations due to their significance in traditional medicine. The properties attributed to these plants are due to their richness in active compounds, such as terpenes and terpenoids. *Hedychium gardnerianum* Sheppard ex Ker-Gawl. is a rhizomatous perennial herb of the Zingiberaceae family and is typically named ''Conteira''. It also spreads rapidly wherever the native forest becomes degraded, as well as being scattered in the dense laurel forest of the island. Recently, studies have shown an antitumor, antithrombin and antibacterial activity of extracts, essential oils, and plant compounds. Cholinesterase inhibitors were introduced in the therapy of Alzheimer Disease in the 1990s. The hopes and interest raised by these drugs are well demonstrated by the 41,370 references listed by PubMed under '*Acetylcholinesterase inhibitors'*. In particular, the scientific community is searching for novel acetylcholinesterase inhibitors displaying less secondary effects. As part of a study whose main objective is the discovery of potential commercial uses of the Azorean invasive species, the acetylcholinesterase inhibition (Anti-AChE) properties of the methanol (MeOH) and dichloromethane (DCM) extracts from mature leaves collected on four different sites from S. Miguel Island (Azores) were determined. Anti-AChE inhibition was assayed using a modification of the Ellman method[1] and toxicity was assessed using *Artemia salina* [2.] All the DCM extracts were good acetylcholinesterase inhibitors, with IC_{50} between 0.28 and 0.41 mg/mL, lower than α-pinene, a known inhibitor of this enzyme (IC_{50} = 1.43 mg/mL). As for the MeOH extracts there was a lower inhibition of acetylcholinesterase. *A. salina* LC_{50} toxicity values are within the range of 756.42 and 835.92 µg/mL, with the DCM extract from Fogo presenting the highest toxic effect, and are therefore only moderately toxic when compared with standard compounds berberine chloride and phenol (4.12 and 124.45 µg/mL, respectively). As for the MeOH extracts there was a lower toxicity of *A. salina*, since only the extract from Furnas shows a value lower than 1.00 mg/mL. Finally, an attempt was made to characterize the inhibition type of the dichlomomethane extracts, since ideally reversible competitive inhibitors are preferable as therapeutic agents. As would be expected in mixtures, a mixed pattern of inhibition was detected in most of the cases, although the DCM extract from the Furnas was almost truly competitive. The obtained results show the feasibility of using *Hedychium gardnerianum* as an excellent source of acetylcholinesterase inhibitors, since it presents high activity and it isn't toxic at elevated doses.

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Study of inclusion of a 4-hydroxy-3-arylcoumarin with cyclodextrin derivatives: An antioxidant application

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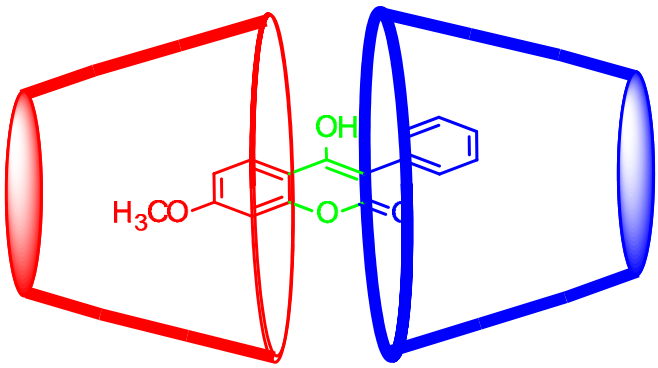
The 4-hydroxy-7-methoxy-3-phenylcoumarin (**4HC**) attracted considerable attention due to their extensively biological activities such as antibacterial.[1,2] Due to the physicochemical properties of the 3-arylcoumarin derivatives and the studies of β-cyclodextrins (βCDs) with several natural compounds with pharmacological activities, the inclusion complexes with three CD derivatives were investigated in order to improve the solubility and bioavailability in the living systems. Stability constant with 1:1 molar ratio was calculated from the phase solubility diagram and indicates the following trend: Dimethyl-βCD > Hydroxypropyl-βCD > βCD. Owing to the highest value of binding constant was for 4HC-Dimethyl-βCD, the binding association constant (Ka) for this complex was determined at different temperatures and the thermodynamic data indicate that 4HC-Dimethyl-βCD is mainly an entropically driven process. ^{1}H-NMR and ROESY were carried out, revealing that **4HC** is embedded in the apolar cavity of Dimethyl-βCD letting the 4OH group buried in the cyclodextrin cavity and the phenyl group outside by the primary rim. These results are in agreement with $ORAC_{FL}$ values, were the decrease in the antioxidant activity of 4HC-Dimethyl-βCD is explained by the effective protection of the hydroxyl group due to the complexation.



Acknowledgment
F. Pérez-Cruz and E. S.-S. thank Conicyt-Chile and “Isidro Parga-Pondal” program, respectively for the financial support.

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Accelerating Lead Optimization of MAO Inhibitors Throughout Microwave-assisted Organic Synthesis

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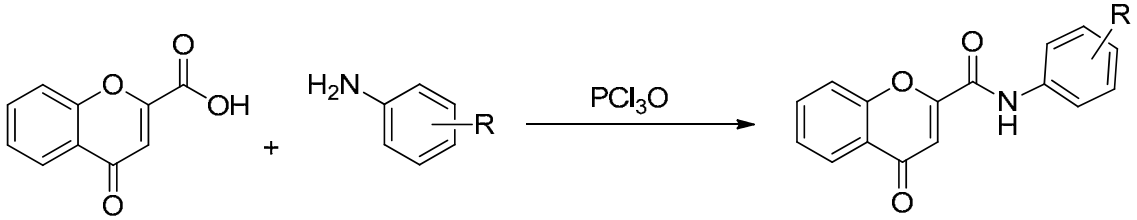
Microwave chemistry and microwave-assisted organic synthesis (MAOs) remain undeniably effective tools in medicinal chemistry. The availability of safe, single-mode dedicated microwave units has led to the incorporation of this new technology into most research laboratories intent on accelerating drug-discovery, hit-to-lead and lead optimisation programs. The development of more economical synthetic routes can ameliorate the overall process since drug discovery is a costly exercise with a high attrition rate. [1]

MAOS leads to increased productivity with the potential to reduce bottlenecks. In many instances, the use of microwave dielectric heating has been shown to dramatically reduce processing times, increase product yields, and to enhance product purities or material properties compared to conventionally processed experiments. Since several manufacturers of professional-grade equipment have arrived on the scene, and the further development of the technique has proceeded apace (e.g. from multi-mode to single-mode, and above all the use of synthesis robots), one can only conclude that this interest continues to grow.

Chromone scaffold ((4H)-1-benzopyran-4-one) has been nowadays recognized as a pharmacophore of a great number of bioactive molecules, either of natural or synthetic origin. At present, our team has a already demonstrated that chromone carboxamides can be an outstanding lead for the development of MAO inhibitors [2]. Chromone carboxamide derivatives have been synthesized straightforward by a one-pot condensation reaction that occurs, between the corresponding chromone carboxylic acid and aniline (phenylamine), or its ring-substituted derivatives. The coupling reagent selected for carboxylic acid activation was benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP) that while efficient is very toxic and expensive.



So, it was decided to study chromone carboxamide synthesis using a direct amidation process between chromone carboxylic acid and aromatic amines in the presence of PCl_3O and microwave irradiation[3]. This method has shown to present several advantages over conventional methods, including operational simplicity, good performance, and through significant reduction in reaction time. Microwave versus conventional synthesis data obtained so far will be presented in this communication.

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This work was supported by the Foundation for Science and Technology (FCT), Portugal (PTDC/QUI-QUI/113687/2009). A .Gaspar (SFRH/BD/43531/2008) and F. Cagide (SFRH/BPD/74491/2010) thank FCT grants.

Antioxidant Activity of Caffeic Acid and Analogues: A Theoretical Study

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Among naturally occurring phenolic compounds, phenolic acids (namely benzoic and cinnamic acids) are of special interest because of their potential biological properties, in particular their antioxidant properties. The antioxidant activity of these compounds was proposed to be dependent on their molecular structure[1]. Previous studies have shown the importance of the catechol group to the antiradical scavenging activity of cinnamic acids compounds, however, the role of the ethylenic side chain is still controversial. In the beginning of the century, Silva and co-workers based on experimental data have postulate that the ethylenic side chain plays an insignificant role in the radical scavenging activity of caffeic acid[2]. In the present work we expect to shed some light on their findings using first principles calculations.

Results show that both the esterification and the hydrogenation of the ethylenic side of caffeic acid chain bear little significance in the energetic differences between each neutral singlet molecule and its conjugated bases and free radicals. Nevertheless, solvent corrections using the Polarizable Continuum Medium (PCM) formalism allowed us to verify that the modifications in the side chain affect how these molecules interact with solvents. Such knowledge may allow the development of new anitoxidant compounds that operate through radical scavenging mechanism.

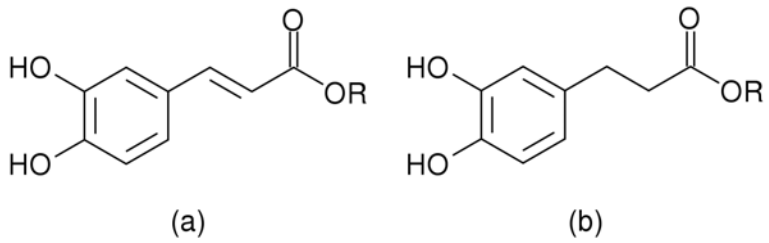

Figure 1: Generic structures of caffeic acid (a) and dihydrocaffeic acid (b) and their esters (R=H, Me, Et, *n*-Pr and *i*-Pr).

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Solvation Effects on the Theoretical Conformational Study of Chromone carboxamides as Potential MAO A and MAO B Inhibitors

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The chromone, in particular 3-carboxamide chromones, has been regarded as a promising scaffold for the development of MAO inhibitors. Several authors conjectured that the putative existence of an intramolecular hydrogen bond would play a major role in the biological activity outline of these compounds[1]. In a recent paper, Gaspar and co-workers reported the first data on the relationship between the biological activity of several of these compound with their structural and electronic features[2]. Nevertheless, solvent effects have never been taken into account in all these studies.

In this work, the results from the conformational analysis of 3-(*N*-phenylcarboxamide)chromone in vacuum are compared with those obtained when using the SCRF, PCM, COSMO and EFP solvation models. The data obtained so far make clear that in aqueous solution the target molecule has a lower energy barrier towards the torsion between the chromone ring and the amide group (dihedral A-B-C-D in Figure 1). Furthermore, the application of a solvent-explicit model (EFP) allowed the determination of sites in the molecule were solvent-solute interactions are most likely to take hold. In the case of aqueous solution, the carbonyl group at C4 is far more likely to take part in a hydrogen bond or dipole-dipole interaction with the solvent than the amide group, even in situations where the amide group is electronic and sterically uninhibited. This data may provide new clues on the mechanism of action of this type of molecules, namely the type of interactions with the enzyme, and therefore push forward the optimization of this lead compound.

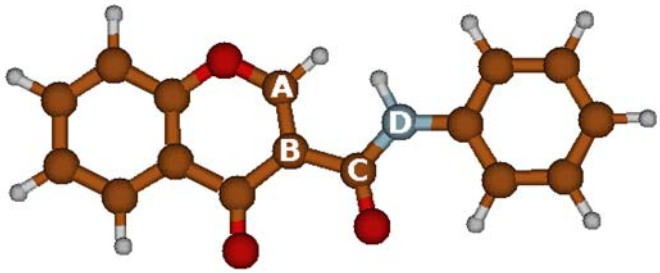

Figure 1: Structure of 3-(*N*-phenylcarboxamide)chromone showing the dihedral angle A-B-C-D.

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Force field parameterization of nickel coordination spheres for metalloproteins

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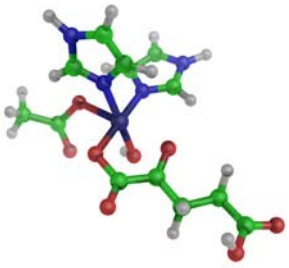
Metalloproteins are a very important group of proteins in biological organisms. There are a few transition metals that one can most commonly find in metalloproteins – Manganese, Iron, Cobalt, Nickel, Copper and Zinc[1]. The inexpression or over expression effects range from pathogenic infections to cancer. The presence of iron in the haem group in haemoglobin, the cobalt in cobalamin aka vitamin B12 and the two nickels in Urease, all have an important role in our human organism.
For this work it was decided that we would begin with nickel spheres from metalloproteins because zinc[2] was already extensively studied and copper lacks the constancy of oxidation state in metalloproteins that nickel offers. The fact that Urease[3] plays a major historical role among proteins helped the choice as well.
Besides Urease we are parameterizing also JMJD2A[4], Methyl-CoM, and CODH (Carbon Monoxide Dehydrogenase).
In our present work, we are parameterizing nickel spheres from metalloproteins and we intend in the future to create an open access parameter library that complements the currently available AMBER protein force fields. In particular, we are parameterizing the bonds, the angles and the electrostatic interactions. The van der Walls interactions are being considered as transferable and the dihedrals are neglected.
We are using B3LYP density functional and 6-31g (d, p) as basis set for the second group atoms and for the metal we are using the SDD basis set.

Fig1.- Ni sphere from JMJD2A



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Synthesis of 2-phenylbenzofurans. Structures with rigid *trans*-resveratrol's core

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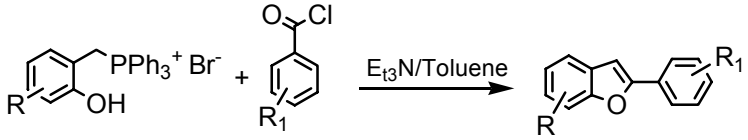
Benzo[b]furan derivatives are an important class of organic compounds that occur in many compounds and natural products because of their biological activities, including antitumor properties. They can be used as inhibitors of 5-lipoxygenase, antagonists of the angiotensin II receptor, blood coagulation factor Xa inhibitors, ligands of adenosine A1 receptor and so forth.[1]
In this work we describe the synthesis of a new series of 2-phenylbenzofurans suitably modified. These compounds included in their structure the resveratrol's nucleus, always in their *trans* configuration. Resveratrol is a natural substance produced by several plants, as vines. A number of beneficial health effects are associated to this compound. Anticancer, antiviral, anticoagulant, neuroprotective, antiaging, antiinflammatory and life-prolonging effects have been reported.[2] Recently 3-phenylcoumarins (also with the *trans*-resveratrol's core in their structure) have showed a antioxidative, anticancer, and enzymatic inhibition properties. Some coumarins proved to be monoamine oxidase inhibitors (MAOI).[3]
With the aim to find out the structural features for the MAO inhibitory activity and selectivity, in the present work we report the synthesis and pharmacological evaluation of a new series of 2-phenylbenzofurans.
The synthesis of benzofurans was successfully achieved by a Wittig reaction between the appropriate triphenylphosphonium salt and the corresponding benzoyl chloride. The desired Wittig reagent was readily prepared from the conveniently substituted 2-hydroxy-benzyl alcohol and triphenylphosphine hydrobromide. [4]



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Docking-based MAO investigaton on new coumarin derivatives

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Monoamine oxidase (MAO) are a class of flavin adenine dinucleotide (FAD) containing enzymes involved in the metabolism of different neurotransmitters. Several studies have proved that the human MAO (hMAO) acts as a target for diverse classes of drugs, such as coumarins, which show a high inhibitory activity toward both isoforms MAO-A and MAO-B.[1,2]
Molecular docking studies represent helpful tools to predict the binding free energy interaction for a ligand-receptor complex, and to deeply investigate the preferred orientation adopted by a ligand into the binding site. Using different crystal structures available in the Protein Data Bank (PDB) docking experiments were performed in order to better analyze how active molecules interact with the two MAO isoforms.[3,4]
In this study we focused the attention on 3-phenylcoumarins and 3-benzoylcoumarins derivatives recently synthesized and pharmacologically evaluated by our group.[5] Interestingly, these compounds show diverse inhibitor activity against hMAO-A and hMAO-B. We compare the binding pose for this class of compounds and carried out several docking calculations based on hMAO-A and hMAO-B crystal structures, with the aim of rationalizing the experimental data. The present results demonstrate that the existing differences in the binding cleft of hMAO-A and hMAO-B (including water molecules) play a significant role in governing the selectivity of these coumarin derivatives. They seem to be useful for the rational drug design of new coumarins with improved inhibitory activity and/or better selectivity towards this class of enzymes.

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Alignment-free Models Based On the TI2BioP methodology to Identify Rnase III Protein Members

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Alignment-free classifiers are especially useful in the functional classification of protein classes with variable homology and different domain structures. Thus, the **TI2BioP** (**T**opological **I**ndices **to BioP**olymers) methodology[1] inspired in both the **TOPS-MODE** and the **MARCH-INSIDE** methodologies allows the calculation of simple topological indices (TIs) as alignment-free classifiers. These indices were derived from the clustering of the amino acids into four classes of hydrophobicity and polarity revealing higher sequence-order information beyond the amino acid composition level. The predictability power of such TIs was evaluated for the first time on the RNase III family due to the high diversity of its members (primary sequence and domain organization) as well as its relevance in drug design since they are involved in several biological processes[2]. Three non-linear models were developed for RNase III class prediction: Decision Tree Model (DTM), Artificial Neural Networks (ANN)-model and Hidden Markov Model (HMM). The first two are alignment-free approaches using TIs as input predictors. Their performances were compared with a non-classical HMM, modified according to our amino acid clustering strategy. The alignment-free models showed similar performances on the training and the test sets reaching values above 90% in the overall classification. The non-classical HMM showed the highest rate in the classification with values above 95% in training and 100% in test. Although the higher accuracy of the HMM, the DTM showed simplicity for the RNase III classification with low computational cost. Such simplicity was evaluated in respect to HMM and ANN models for the functional annotation of a new bacterial RNAse III class member isolated and annotated by our group.

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N-myristoyltransferase: computational studies of its catalytic mechanism as a path to a new *Candida albicans* antifungal

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Candidiasis is a fungal infection caused by *Candida* species, of which *Candida albicans* is the most common. This pathology acquires relevance in mouth or genitals infections. *Candida* species are yeasts that take advantage of organism weakness or immunodeficiency to multiply and spread beyond normal levels, since they are part of the normal gut microbiota and certain mucous membranes such as the vagina and throat. The inhibition of the enzyme N-myristoyltransferase (NMT) is extremely important because this enzyme is essential for the growth and survival of the fungus. NMT catalyzes the addition of myristate (fatty acid C14) from the myristoyl-CoA cofactor (m-CoA) and the N-terminal glycine of protein substrates[1,2]. The fungal NMT has binding properties clearly different from the human NMT, so it is possible it selective inhibition.
The current antifungal, such as Fluconazol, present limitations, namely relative toxicity and several interferences in human metabolism[3], so the main objective of our work is create a compound that serves as a model to a new inhibitor. In order to create a new inhibitor, we have started to unravel the N-myristoyltransferase catalytic mechanism at atomic level, performing computational calculations using the Density Functional Theory (DFT) with B3LYP functional and the 6-31G(d) basis set in a model with 131 atoms (Fig.1).
In the literature, a three step mechanism is proposed, but considering the results obtained so far, we are aware that there is the possibility of a different catalytic pathway than the proposed experimentally from the second step, but this results still lack evidence of maturation.

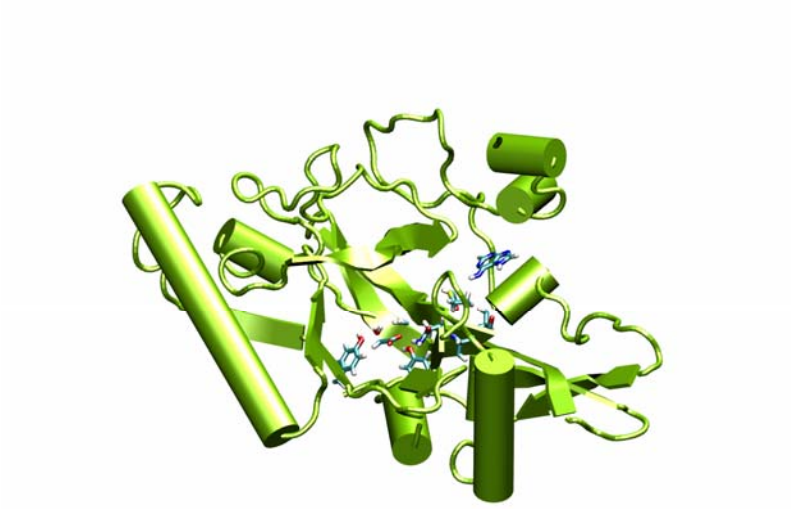

Fig.1 – N-myristoyltransferase with relevance to the 131 atoms model.

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VEGFR2 selective residue flexibility enriches AutoDock Vina docking results

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VEGFR2 (Vascular Endothelial Growth Factor Receptor 2) is a recognized anti-cancer protein target with already one known inhibitor, soranefib, in the market for therapeutic use.[1] Protein-ligand docking tools can be used in order to find potential new VEGFR2 inhibitors. However, the quality of docking results can be affected by the simplification of treating protein structures as rigid entities. Selective residue flexibility is a recent option available on several molecular docking tools, including AutoDock Vina used in this work,[2] where only selected residues are allowed to be flexible. This approach is promising as it attempts to provide a more realistic protein environment while preventing an escalation of the computer power need. In this study four residues were selected from the catalytic site of the VEGFR2 structure (PDB: 1YWN): GLU883, LYS866, CYS917 and ASP1044. Each residue was individually made flexible and, for benchmarking, docking experiments were performed using the DUD (Directory of Useful Decoys) dataset. The DUD dataset is composed of 88 VEGFR2 ligands and 2906 decoys compounds, and the virtual screening of all compounds was performed using MOLA software[3] for parallel computing, in a cluster of 14 computer nodes, and AutoDock Vina for molecular docking. The best overall docking result was obtained by flexibilizing the GLU883 residue, with a marked increase in distinguishing VEGFR2 ligands from decoys, when compared with the rigid docking results. The pay-off was a manageable 51% increase on the computer processing time needed. This study proves that carefully flexibilization of key aminoacid residues can improve the predictive power of docking without an unmanageable increase in computer processing time.

Acknowledgments: FCT (Portugal) and COMPETE/QREN/EU for financial support through research project PTDC/QUI-QUI/111060/2009. R.M.V. Abreu thanks FCT, POPH-QREN and FSE for SFRH/PROTEC/49450/2009 grant.

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Mdm2 as a potential target for mushrooms LMW compounds

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In some human cancer cases, the activity of p53 is inhibited by the overexpressed Mdm2 (E3 ubiquitin-protein ligase Mdm2) oncoprotein.[1] Mdm2 acts as an ubiquitin ligase, resulting in p53 ubiquitination and subsequent p53 proteasomal degradation. The disruption of the Mdm2-p53 interaction using small-molecule inhibitors is recognized as a promising strategy for anticancer drug design.[2] Mushrooms are a vast and yet largely untapped source of powerful new pharmaceutical products. In particular, and most importantly for modern medicine, they represent an unlimited source of compounds with antitumor and immunostimulating properties.[3] In this study, a total of 85 LMW (low molecular weight) compounds present in mushrooms were used in a protein-ligand docking experiment using a Mdm2 protein structure (PDB:1T4E) as receptor protein target.
The 1T4E X-ray structure presents Mdm2 co-crystallized with a known inhibitor, benzodiazepinedione, located in the Mdm2-p53 interaction site with an experimental Ki value of 80 nM.[4] AutoDock Vina, the docking tool used in this study, predicted a Ki value of 79 nM and RMSD (Root Mean Standard Deviation) of 0.033 Å for benzodiazepinedione, when compared to the co-crystallized benzodiazepinedione, thus validating the 1T4E structure for docking subsequent compounds. Virtual screening of the 85 LMW compounds was then performed. Ergosterol (Ki=824 nM) and ergosta-4,6,8(14),22-tetraen-3-one (Ki=824 nM) stand out as the top ranked potential inhibitors for Mdm2. Both compounds were then manually inspected in order to investigate its specific binding mode. The information provided shows several interesting starting points for further development of Mdm2 inhibitors. Furthermore, this study contributes to the valorisation of mushrooms as functional foods.

Acknowledgments: FCT (Portugal) and COMPETE/QREN/EU for financial support through research project PTDC/AGR-ALI/110062/2009.

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Ionotropic $P2x_7$ Receptors Favours Osteogenic Differentiation and Membrane Cell Blebbing of Human Bone Marrow Stromal Cells

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Osteoblasts play an essential role in bone development and remodelling. Nucleotides (*e.g.* ATP) are released from bone cells in response to mechanical stress (shear forces) and signal in an autocrine / paracrine manner via multiple cell surface P2 receptors.[1] Among these, the $P2X_7$ receptor exhibits unique properties. It responds to ATP as a ligand-gated cation channel and can induce formation of large non-selective membrane "pores". While triggering reversible morphological changes (*e.g.* blebbing) and ATP release, these "pores" are usually involved in cell apoptosis. Given the putative pathophysiological relevance of the molecular pathways sustaining propagated ATP/Ca^{2+} waves on mechanically-stimulated osteoblast primary cultures, we aim at investigating purinergic signalling measuring cytosolic free calcium $[Ca^{2+}]_i$ and monitoring membrane cell protrusions. Pharmacological characterization of human bone marrow stromal cells in culture was performed to clarify the role of $P2X_7$ receptors in proliferation and osteogenic differentiation.

Human bone marrow specimens were obtained from postmenopausal female patients (68±5 years old, *n*=16) undergoing total hip arthroplasty. Ethical approval and informed consent to use the biological material that would be otherwise discarded, was obtained. Primary osteoblast cell cultures were characterized for proliferation (MTT assay), total protein content (method of Lowry) and alkaline phosphatase (ALP) activity during 30 days. For monitoring $[Ca^{2+}]_i$, cells were loaded with Fluo-4NW (2.5 μM, 45 min at 37ºC) and observed under the confocal microscope (Fluoview 1000, Olympus, Japan) in the time-lapse mode. To examine cell membrane dynamics, osteoblast cultures were superfused with FM4-64 dye (1 μM, 1 mL/min at 37ºC).

Application of ATP (100 μM) and BzATP (100 μM) (which can activate P2Y and/or P2X receptors) induced transient elevations of $[Ca^{2+}]_i$ as well as dramatic changes in osteoblast cells morphology, including initial cell retraction followed by the formation of dynamic plasma membrane blebs (zeiosis) with a latency of onset of 4-6 min. BzATP (100 μM) and ATP (100 μM) treatment induced dynamic membrane blebbing in 70±1 % (*n*=6) and in 38±1 % (*n*=3) of the cells, respectively. Similarly, UTP (100 μM) and UDP (100 μM) (which activate P2Y receptors) elicited elevations of $[Ca^{2+}]_i$, but failed to induce blebbing. $[Ca^{2+}]_i$ rises induced by BzATP was partially inhibited by the selective $P2X_7$ antagonist, A438079 (3 μM), which also prevented formation of plasma membrane blebs. Membrane blebbing induced by BzATP (100 μM) was observed independently of extracellular Ca^{2+} concentrations, but prevention of cytoskeletal rearrangements with H1152 (3 μM, a Rho-kinase inhibitor) effectively blocked membrane zeiosis. These results indicate that Ca^{2+} signals and membrane blebbing are independent phenomena. Supplementation of the culture medium with BzATP (100 μM) anticipated osteogenic differentiation (increase in ALP activity) of bone marrow stromal cells from culture day 7-14 to day 4 (*n*=3; this effect was partially prevented upon blocking the $P2X_7$ receptor with A438079 (3 μM). The expression of $P2X_7$ receptors in human bone marrow stromal cells positive to osteoblast cell-markers (e.g. type I collagen, osteocalcin) was confirmed by immunofluorescence confocal microscopy.

Data suggest that the $P2X_7$ receptor is an important regulator of osteogenic differentiation of bone marrow stromal cells from postmenopausal women. Activation of $P2X_7$ receptors on osteoprogenitor cells triggers intracellular $[Ca^{2+}]_i$ transients and cell membrane blebbing; the latter phenomenon is independent of intracellular calcium but involves activation of a Rho-kinase-dependent pathway. Our results provide a rationale supporting data from epidemiological studies indicating that single nucleotide polymorphisms of the $P2X_7$ receptor gene may increase the loss bone mineral density and the risk of fracture in postmenopausal women.[2]

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This work was supported by FCT (FEDER funding, PTDC/SAU-OSM/73576/2006 and UMIB-215/94).

Contribution to the Synthesis of Peptide Analogues of GPE and PLG from 3,5-bis(substituted) Proline Derivatives

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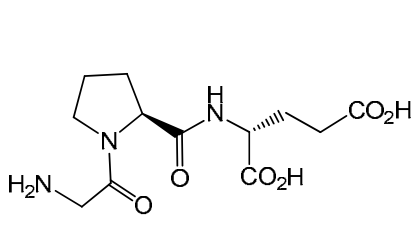
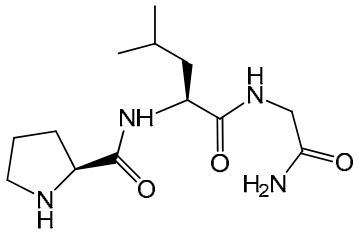
Small molecules are key to understand the modulation of protein functions, as well as to develop new therapies and diagnosis methods.[1] In recent years,[2] small heterocyclic molecules have attracted considerable attention; among them, proline-derived structures constitute a particularly interesting class of molecules.[3]

The fact that some of the most bioactive peptides have proline in their sequence, besides the important roles that neuropeptides such as GPE (glycyl-L-prolyl-L-glutamate)[4] and PLG (L-prolyl- L-leucyl-glycinamide)[5] play in the central nervous system prompted our interest to explore the possibility of using these species and/or their analogues to treat neurodegenerative pathologies such as Alzheimer's disease, Parkinson's disease and Huntington's chorea.

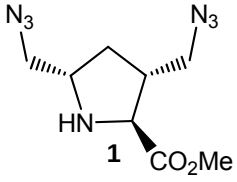


GPE



PGL

We recently reported the synthesis of 3,5-disubstituted proline derivative **1**.[6] In this communication, the preparation of **2** and its utility as scaffolds in the synthesis of peptide analogues of GPE or PGL (**3**, **4**) is described.



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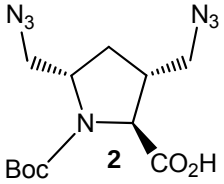

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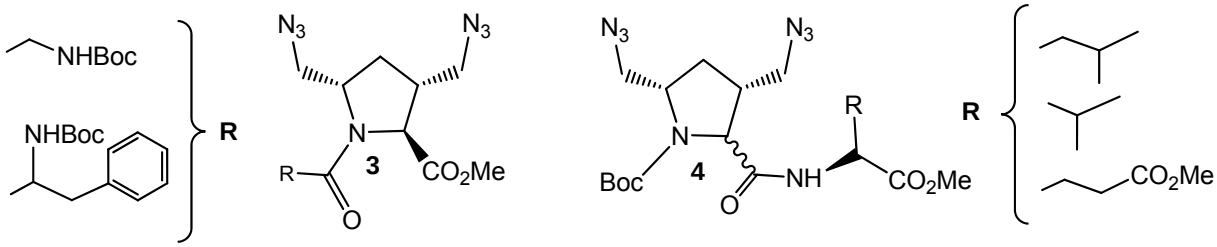

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Theoretical Study of GSK-3*α* Using 2D-descriptors

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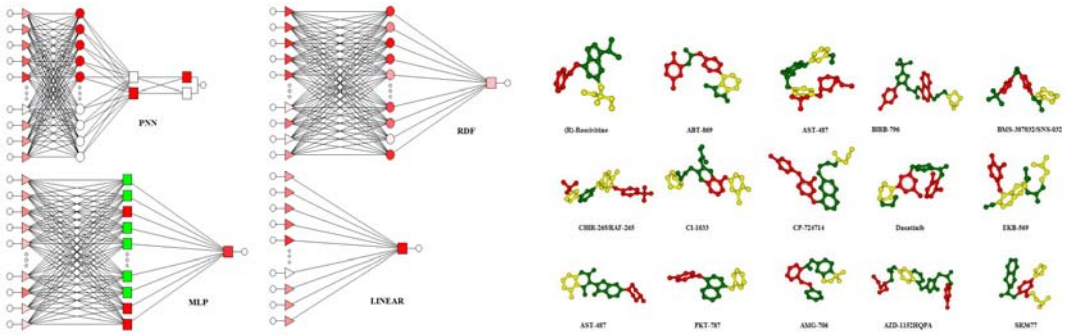
GSK-3 targets encompass proteins implicated in Alzheimer disease (AD),[1] neurological disorders. The functions of GSK-3 and its implication in various human diseases have triggered an active search for potent and selective GSK-3 inhibitors. In this sense, Quantitative Structure-Activity Relationship (QSAR) could play an important role in studying these GSK-3 inhibitors. For this reason we developed QSAR models for GSK-3*α*,[2] Linear Discriminant Analysis (LDA)[3] and Artificial Neural Networks (ANNs)[4] from nearly 50000 cases with more than 700 different GSK-3*α* inhibitors obtained from ChEMBL database server; in total we used more than 20000 different molecules to develop the QSAR models. The model correctly classified 237 out of 275 active compounds (86.2%) and 14870 out of 15970 non-active compounds (93.2%) in the training series. The overall training performance was 93.0%. Validation of the model was carried out using an external predicting series. In these series the model classified correctly 458 out of 549 (83.4%) compounds and 29637 out of 31927 non-active compounds (83.4%). The overall predictability performance was 92.7%. In this work, we propose three types of non Linear ANN and we show that it is another alternative model to the already existing ones in the literature, such as LDA. The best model obtained was Linear Neural Network: LNN: 236:236-1-1:1 which had an overall training performance of 96%. In addition, we did a study of different fragments that exist in the molecules of the database in order to see which fragments had more influence in the activity. All this can help to design new inhibitors of GSK-3*α*. In this communication we report the attempts to calculate within a unified framework probabilities of GSK-3*α* inhibitors against different molecules found in the literature.

$$GSKI - 3\alpha_{score} = -34.3 \cdot ATS1m + 19.3 \cdot ATS3m - 14.3 \cdot ATS4e - 2.9 \cdot ATS6e + 37.0 \cdot ATS1p + 39.1 \cdot MATS3v - 7.3 \cdot MATS3e$$
$$- 43.3 \cdot MATS3p - 3.7 \cdot GATS5m - 4.0 \cdot GATS2e - 46.5 \cdot BELm3 - 34.6 \cdot BELm4 + 6.8 \cdot BELe8 + 41.0 \cdot BELp3$$
$$+ 36.0 \cdot BELp4 + 1.1 \cdot GGI3 - 227.2 \cdot JGI4 + 163.4 \cdot VEm2 + 40.7$$
$$N = 48{,}721 \qquad \chi^2 = 3127.3 \qquad p - level < 0.001$$



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Design and Development of a Novel Chemical Library, Based on a Privileged Scaffold, Suitable to Speed up the Drug Discovery Process of IMAO-B

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Monoamine oxidases (MAOs) are widely distributed enzymes that contain a flavin adenine dinucleotide (FAD) unit covalently bounded to a cysteine residue[1]. Many living organisms possess MAOs and in mammals two isoforms are present, MAO-A and MAO-B, which are located in the outer membrane of the mitochondria. The MAO-B isoform has a crucial role in neurotransmitters metabolism, representing an attractive drug target for neurodegenerative diseases therapy, such as Parkinson's. Parkinson's disease (PD) is a neurodegenerative disorder characterized by a myriad of symptoms that gradually decrease the life quality of the patient. The first line of PD treatment is dopamine replacement therapy with Levodopa[2]. At present monoamine oxidase inhibitors (IMAO), specifically of MAO-B type, are considered also to be beneficial therapeutic drugs. The inadequacy of the current pharmacotherapy and the lack of drugs that can be effective in PD, mainly declined by side-effects, are the reasons why the discovery of novel chemical entities (NCE) is still a demand.

Chromones (benzo-γ-pyrone) are one of the most abundant groups of naturally occurring heterocyclic compounds. Because of their structural features they are important building blocks in the natural product and synthetic organic chemistry areas. In addition, remarkable antioxidant, anticancer and enzymatic inhibition activities were ascribed to these benzopyrone compounds.

The present project consists on the design and development of a versatile library incorporating a privileged structure based on the benzo-γ-pyrone scaffold as a putative shortcut for the early drug-development stage on the discovery of new NCE for IMAO-B. Accordingly, a diversity-oriented synthesis methodology was adopted, by means of modular syntheses that involve few steps, to obtain structurally varied drug-like compounds. Efforts were done to cover as much chemical space as possible to maximize the likelihood of discovering a novel and patentable lead class of active compounds. The results obtained so far will be presented supported by synthetic, biologic and docking studies, pointed out a crucial and undisclosed role of the presence of a carboxamide group in C3 of the pyrone ring that is able to establish hydrogen bond interactions with the active site of the MAO-B enzyme[3].

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This work was supported by the Foundation for Science and Technology (FCT), Portugal (PTDC/QUI-QUI/113687/2009). A .Gaspar (SFRH/BD/43531/2008) and F. Cagide (SFRH/BPD/74491/2010) thank FCT grants.

Hydroxyphenylcoumarins as a potential antioxidant moiety: Electrochemical study

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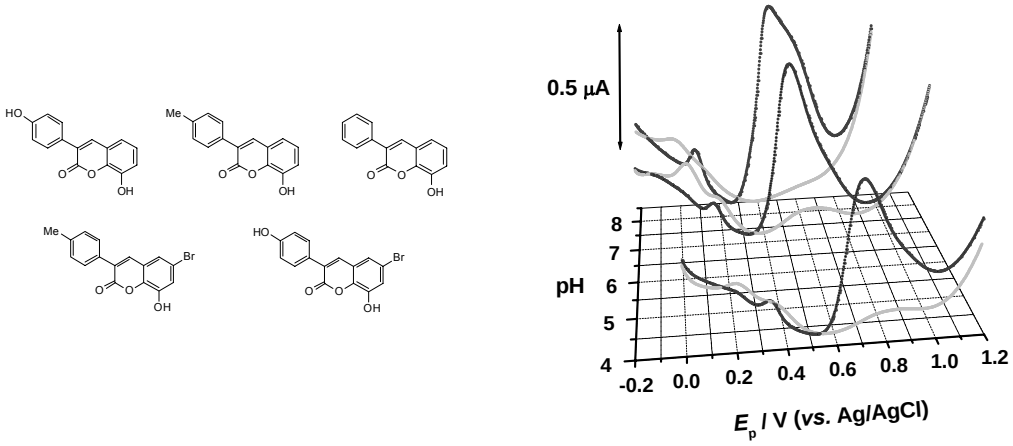
Coumarins are a large family of compounds, of natural and synthetic origin, that show numerous biological activities.[1] Therefore, they occupy an important place in the realm of natural products and synthetic organic chemistry. Recent studies pay special attention to their antioxidative, anticarcinogenic and enzymatic inhibition properties.[2] In the last years they have been used as medicines, additives in food and cosmetics and in the preparation of insecticides.
On the other hand, in the last few years have been reported that resveratrol show a number of biological activities including anti-inflammatory and anticancer properties.[3] Several studies have demonstrated that may protect against coronary heart disease as a result of different effects, including significant antioxidant activity.[4]



Scheme 1 Chemical Structures of coumarin-resveratrol hybrids and a 3D plot of first and second differential pulse voltammograms of *compound 3,* 0.5mM as a function of pH.

Due to some coincident properties of resveratrol and coumarins, new hybrid compounds that incorporate their skeleton have been designed and synthesized. The investigation of the properties of these compounds, the study of the structural pattern and the elucidation of their biological role is of great interest for further development of coumarin-like antioxidant drugs.
The electrochemical behaviour of a group of hydroxycoumarins was investigated using cyclic, differential pulse and square wave voltammetry, in aqueous media at a glassy carbon electrode over the whole pH range. The oxidation is an irreversible process and the reaction products adsorb strongly on the electrode surface and are reversibly oxidised. An oxidation mechanism of these coumarins is proposed.

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New Architectures to Fight Infectious Diseases under a Computational Themed Perspective

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Iron is an essential micronutrient for both vertebrates and microbial pathogens. Its accumulation has been suggested to potentiate the susceptibility to mycobracterial infections such as tuberculosis (TB).[1] Diseases like TB are very problematic as outbreaks of strains resistant to the ongoing used pharmacological therapy have been reported. Therapies based on the deplection of iron have been growing as good potential routes for treatment of diversified diseases and recently a new potential pharmacological agent has been proposed (figure 1).[1,2] This agent is an iron hexadentate chelator of the 3-hydroxy-4-piridinone family. Different *in vivo* and *in vitro* studies performed by our collaborators at FCUP and IBMC in an infection model of *Mycobacterium avium* support the drug as a potential new pharmacological agent. In our project we aim to evaluate the interaction of the potential drug and analogues with biomembrane models developed in our group. These biomembrane models are composed of DMPC and DMPG, often used experimentally as eukaryotic and prokaryotic membrane models, respectively. This could help to rationalize the experimental results obtained so far by our collaborators at FCUP and IBMC and hopefully drive the rational improvement of the lead compounds. For this purpose classical molecular dynamics simulations were performed. This methodology is the most common used method for biomolecular simulations and has shown great progress in membrane simulations.[3,4] The parameterization of the different molecules using quantum mechanical procedures was also performed. The bilayer computational models created so far have been subjected to validation, through the comparison with experimental available data to which the values showed to be in good agreement.[5] The structural parameters compared so far include the area per lipid (A_L), volume per lipid (V_L) and the bilayer thickness. We have also some preliminary results regarding the evaluation of drug-membrane interaction. This study is a good example of the possibilities regarding theoretical and computational techniques applied to biological systems in order to evaluate different potential pharmacological compounds.

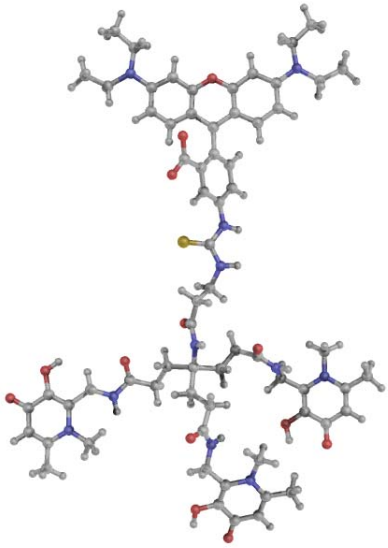

Figure 1: Structure of hexadentate chelator

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Neighbouring-Group Participation as a Source for Anticancer Drugs

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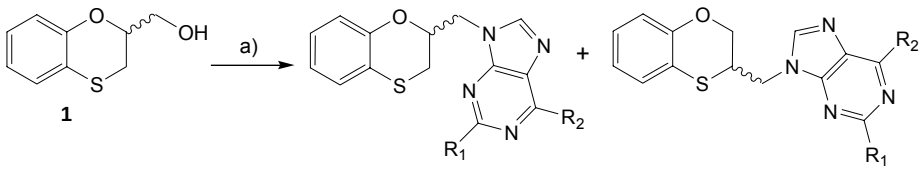
The chemotherapeutic agent 5-fluorouracil (5-FU) was first introduced in cancer therapy in 1957, but even today this pyrimidine analogue still remains an essential part of the treatment of a wide range of solid tumours. Like the mayority of the anticancer agents, 5-FU leads to several toxicities.[1] That explains the need for more effective and less toxic 5-FU derivatives. We have reported the evolution from 5-FU acyclonucleosides to benzo-fused six- and seven-membered rings linked to pyrimidine and purine bases.[2]
Substituted 9-(2,3-dihydro-1,4-benzoxathiin-2- or 3-ylmethyl)-9*H*-purines **2-7** were synthesized by the Mitsunobu reaction.



Scheme 1 : Reagents and conditions: a) Substituted purines, Ph_3P, DIAD, anhydrous THF, microwave irradiation, 140 °C, 5 min.

When the Mitsunobu reaction was carried out between 2,3-dihydro-1,4-benzoxathiin-3-methanol and the heterocyclic bases 6-chloro-, 2,6-dichloro, and 6-bromo-purines under microwave-assisted conditions, a formal 1,4-sulfur migration takes place through two consecutive oxyranium and episulfonium rings, giving rise to the corresponding 9-(2,3-dihydro-1,4-benzodioxin-3-ylmethyl)-9*H*-purine derivatives. The most active compound 2,6-dichloro-9-(2,3-dihydro-1,4-benzoxathiin-2-ylmethyl)-9*H*-purine **4** shows an IC_{50}= 2.75 ± 0.02 μM. Treatment of MCF-7 human breast cancer cell line with **4** shows a significant increase of apoptotic cells (70.08 ± 0.33%) was obtained in relation to the control ones.
At present, studies are being carried out to determine the mechanism of action at the molecular level of the most active compounds.

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Kinetic Characterization of New Bisquaternary Derivatives as Choline Kinase Inhibitors with Antiproliferative Activity

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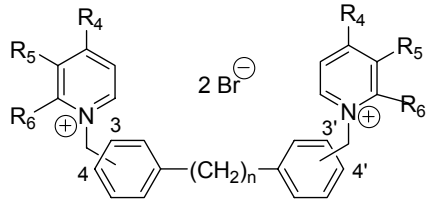
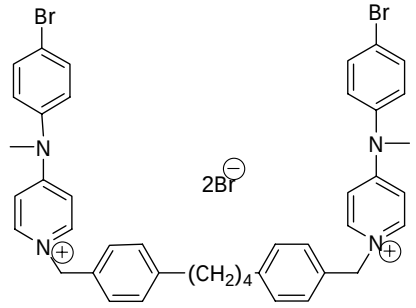
Choline kinase is the first enzyme in the CDP-choline pathway that synthesizes phosphatidylcholine, the major phospolipid in eukaryotic cell membranes. In humans, choline kinase exists as three isoforms (ChoKα1, α2, and β).[1] Specific inhibition of ChoKα has been reported to selectively kill tumour cells.[2] Ten new symmetrical bis-pyridinium and bis- quinolinium derivatives (1) were synthesized and tested for their ability to inhibit human choline kinaseα2. These compounds have electron-releasing groups at position 4 of the pyridinium or quinolinium rings. 1,1'-[(Butane-1,3-diylbis(benzene-1,4-diylmethylene)]bis[4-(4-bromo-N-methylanilino)-pyridinium)] dibromide (2) was identified as highly potent choline kinase inhibitor with an IC50 value of 0.09 μM. Kinetic enzymatic assays indicated a mixed, predominantly competitive, inhibition mechanism for this compound. These novel compounds showed strong antiproliferative activity on the human breast cancer SKBR-3 cell line.



1



2

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

Acknowledgements: This work was supported by MICINN (HD2008-0028)

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Alkyl esters of hydroxycinnamic acids have antioxidant activity and protect PC12 cells against oxidative damage

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Hydroxycinnamic acids (HCAs) are prevalent phenolic compounds in dietary plants that have been ascribed to possess antioxidant activity. In order to increase the lipophilicity of HCAs and improve their ability to cross cell membranes, we synthesized methyl, ethyl, propyl and butyl esters of the naturally-occurring caffeic and ferulic acids. Antioxidant activity, redox potential and partition coefficients of the compounds were measured and their ability in protecting against hydrogen peroxide induced oxidative damage was assessed in neuronal-like PC12 cells. All caffeate esters had a slightly lower DPPH IC_{50} (3.5-14.5 μM) and higher FRAP values (1490-1588 mM equivalent of quercetin/mole (mEqQ/mole)) compared to caffeic acid (16.6 μM and 1397.7 mEqQ/mole, respectively) in antioxidant assays. In contrast, ferulate esters were less active compared to ferulic acid, with DPPH IC_{50} and FRAP value of 44.6 μM and 324 mEqQ/mole, respectively. Redox potentials were in agreement with the antioxidant data. All 4 esters of caffeic acid significantly inhibited the oxidative damage in PC12 cells at the concentrations of 5 and 25 μM. These data suggest that HCA alkyl esters based on caffeic moiety possess high antioxidant activity and superior lipophilicity and can efficiently protect cells against oxidative damage. In summary, these caffeic derivatives represent outstanding molecules to be applied for management of oxidative stress-related diseases.

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New mitochondria-targeted antioxidants based on cinnamic scaffold for neurodegenerative disease therapy

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Neurodegenerative diseases (ND) have becoming a health concern to societies worldwide affecting millions of people. Neurodegenerative diseases, namely Parkinson and Alzheimer diseases normally ensue in middle and late life but their specific cause remains unknown; however, increased production of ROS is described to be a hallmark in early steps of these disorders. The matrix space in mitochondria is the intracellular compartment that is potentially the most active unit in production of reactive species (ROS and RNS) and in turn is the most vulnerable to oxidative damaging effects. For this reason, modulating mitochondrial functions appears to be a desirable target for ND. It could be an effective way to slow the neurodegenerative progression and consequently the ageing processes in the brain. In this context, the control of mitochondrial redox processes is an attractive perspective and the development of mitochondriotropic compounds is of great importance in such an effort. Lipophilic cations, composed to antioxidant unit, a linker and a penetrating cation (e.g. triphenyl phosphonium-TPP), have been described as potential drugs as they can pass directly through phospholipid bilayers, without requiring a specific uptake mechanism, and can accumulate significantly within mitochondria owing to the large membrane potential.

Cinnamic acids are phenolic compounds present in diet that have been often used as templates for the design and development of new antioxidants. Until the date, the majority of natural antioxidants studied have limited success a fact that could be related with their limited distribution throughout the body and with the inherent difficulties to attain the target sites (e.g. the mitochondria). Therefore, novel antioxidants were designed, and synthesised, based on hydroxycinnamic antioxidant derivatives containing the positive charges of TPP cations at physiological pH (hence capable of mitochondrial accumulation). These antioxidants (TPP-OH and TPP-OCH_3) could be used as potent and selective agents throughout specific targeting the mitochondria. The biological data obtained so far show that the presence of a triphenylphosphonium (TPP^+) group in the conjugated side chain led to an increase of the partition coefficients (octanol-PBS) of the newly synthesized compounds when compared with lead compounds. These new TPP compounds are rapidly taken up for mitochondria when the respiratory substrate succinate was added to generate a membrane potential ($\Delta\psi m$). The accumulated TPP-OH and TPP-OCH_3 are released once $\Delta\psi m$ is abolished by the uncoupler FCCP. The lipophilicity improvement might validate the data so far obtained In addition, the abilities of these compounds to prevent lipid peroxidation in mitochondria exposed to ferrous chloride, hydrogen peroxide and ascorbate, were evaluated and only TPP-OH shows any effect to prevent TBARS accumulation. The preliminary toxicity screening using zebrafish as a model reveal that TPP-OCH_3 could be toxic (promotes bradycardia), while TPP-OH present no toxic effect.

Microwave-Assisted Synthesis of New Triazole Derivatives by Click Chemistry, as Potential D_3 Receptor Ligands

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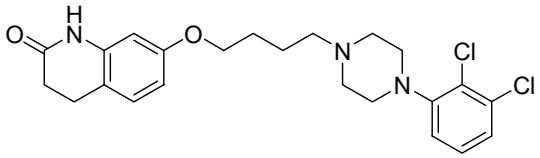
Schizophrenia is a chronic, severe, and disabling mental disorder that affects about 1% of the world population. Recent studies[1] have suggested that the dopamine D_3 receptor subtype, since it is selectively located in the limbic brain areas known to be associated with cognitive and emotional functions, might be a promising rational target for development of new drugs for the treatment of this pathology. Thus, aripiprazole (Abilify®) is the first dopamine D_2/D_3 receptor partial agonist approved for use in the treatment of psychiatric disorders.

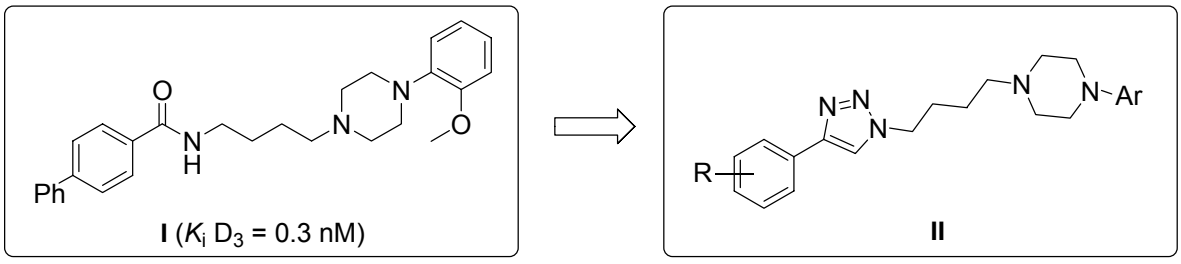


Aripiprazole

Major progress in D_3 receptor drug discovery would lead to a better understanding of this illness as well as an improvement of its treatment.[2] Therefore, compounds with dopamine D_3 antagonist profile may give rise to beneficial antipsychotic activity avoiding extra-pyramidal side effects of the currently available therapies. So far, numerous ligands have been developed with a number of lead compounds showing high selectivity for the D_3 receptor.[3] However, there is still a need for more selective molecular tools to facilitate a relevant differentiation between D_3 actions and those mediated by the D_2 receptors, in order to elucidate the function and potential therapeutic advantages of targeting D_3 receptors.

Taking advantage of previous studies[4] indicating that the carboxamide function in some D_3 ligands can be successfully replaced by five-membered heterocycles, new 1,2,3-triazolyl analogues could be approached via *click chemistry*[5] by copper(I) catalyzed [3+2] azide-alkyne cycloaddition reactions (Huisgen reaction) (**Figure 1**).

Using this approach, in this communication we will report the microwave-assisted synthesis[6] and binding affinity of a new 1,2,3-triazole derivative as a prototype of a new series of potential D_3 ligands where the carboxamide moiety of compound **I**[4] (**Figure 1**) has been replaced by a triazole ring (**II, Figure 1**).



I (K_i D_3 = 0.3 nM) **II**

Figure 1

Acknowledgments:
This work was supported by a grant from the *Xunta de Galicia* (Spain, **INCITE09 203 184 PR**).

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Antifungal activity of *Hedychium gardnerianum* essential oils on *Candida albicans*

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Candidiasis or thrush is a fungal infection (mycosis) of any of the *Candida* species (all yeasts), of which *Candida albicans* is the most common. Also commonly referred to as a yeast infection, candidiasis is also technically known as candidosis, moniliasis, and oidiomycosis. Candidiasis encompasses infections that range from superficial, such as oral thrush and vaginitis, to systemic and potentially life-threatening diseases. *Candida* infections of the latter category are also referred to as candidemia and are usually confined to severely immunocompromised persons, such as cancer, transplant, and AIDS patients as well as non-trauma emergency surgery patients. Superficial infections of skin and mucosal membranes by *Candida* causing local inflammation and discomfort are common in many human populations. While clearly attributable to the presence of the opportunistic pathogens of the genus *Candida*, candidiasis describes a number of different disease syndromes that often differ in their causes and outcomes. Many volatile oils are known to possess antifungal properties and are potentially applicable as antimycotic agents. *Hedychium gardnerianum* Sheppard ex Ker-Gawl. is a plant native to the Himalayas that grows to 2.4 m tall with long, bright green leaves clasping the tall stems. This is the most widely cultivated *Hedychium* species preferring a warm climate although it will tolerate temperate areas that have light, infrequent frosts. The fragrant, red and pale yellow flowers held in dense spikes, appear towards the end of summer. It is known as 'wild kahili ginger' and is listed as a weed of concern on conservation land in New Zealand, Hawaii and the Azores. It has been recognized as one of "The World's 100 Worst Invasive Alien Species" by the IUCN Invasive Species Specialist Group (ISSG). As part of a study whose main objective is the discovery of potential commercial uses of the Azorean invasive species. Since, essential oils of leafs of *Hedychium gardnerianum* collected on five different sites from S. Miguel Island (Azores) were extracted by hydrodistillation. The trial was conducted by a modification of the diffusion Bauer's technique[1] on solid medium using filter paper discs. From a culture of *Candida albicans*, originally isolated from a hospital, kept at a temperature of 10 ºC, the inoculum was prepared in saline (sodium chloride) 0.85% sterile. The suspension was compared, using qualitative methods, with a suspension of barium sulphate Tube 0.5 McFarland Scale (bioMerieux®, France). Results were recorded at 24 and at 48 h. All the fractions assayed showed a highly effective antifungal activity, although in different extents. The effectiveness values at 24 h are between 1.03 and 1.33 cm inhibition hales and at 48 h are between 1.13 cm and 0.8 cm, which are higher than the value found for NIPAGIN®, a known effective antifungal (0.87 cm at 24 h and 0.80 cm at 48 h). These results provide experimental evidence suggesting the potential value of *Hedychium gardnerianum* essential oil for the treatment of oral or vaginal candidiasis.

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Desirability-based Multi-criteria Quantitative Structure-Selectivity Relationships of Antimicrobial Peptidomimetics

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[c] Applied Chemistry Research Center - Faculty of Chemistry and Pharmacy, & Molecular Simulation and Drug Design Group - Chemical Bioactive Center, Central University of "Las Villas", Santa Clara, 54830, Cuba.

Microbial drug resistance has achieved a global dimension and an alarming magnitude[1,2]. Antimicrobial peptides possess unique action mechanisms making peptide antibiotics an attractive therapeutic option against resistant bacteria. However, their high haemolytic activity lack the selectivity required for a human antibiotic[3]. Therefore, additional efforts are needed to develop new antimicrobial peptides that possess greater selectivity for bacterial cells over erythrocytes. We introduce in this work a practical approach to simultaneously deal with these two conflicting properties[4]. The convergence of machine learning techniques[5] and desirability theory[6] allowed us to derive a simple, predictive and interpretable multi-criteria classification rule (m-cCR) for simultaneously handling the antibacterial and haemolytic properties of a set of cyclic β-hairpin cationic peptidomimetics (Cβ-HCPs)[7]. The m-cCR exhibited a prediction accuracy of about 80% on training and external validation sets. Results from a further concordance test showed an excellent 86% of agreement between m-cCR predictions and predictions from independent classifiers for complementary antibacterial and haemolytic activities, respectively; evidencing the reliability of the m-cCR. The m-cCR was also consistent with the general mode of action of cationic peptides that indicates their biological and biophysical relevance. A multi-criteria virtual screening strategy based on the joint use of the m-cCR, desirability, similarity and chemometrics concepts is also proposed. The ability of such virtual screening strategy to prioritize selective (non haemolytic) antibacterial Cβ-HCPs was challenged on training, validation, and overall data. Overall the results suggest that the method is able to rank a selective antibacterial Cβ-HCP earlier than a biologically inactive or non selective antibacterial Cβ-HCP with a probability of *ca.* 0.9. These results allow considering both the m-cCR and the multi-criteria virtual screening strategy as promising chemoinformatics tools suitable for the discovery and development of potent and selective antimicrobial peptides.

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Natural products and pharmaceutical industry

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The aim of this communication is to present a study about rosemary as example of the usage of plants with therapeutic interest at the different levels of manufacturing.
One of these plants that appears wildly due to the excellent growing conditions that take place in the island of Sardinia. This plant by itself, as well as its extracts, shows a wide range of pharmacological properties, such as antibacterial, anti-rheumatic or antipasmodic [1]. Recent studies pay special attention to the application of the rosemary alcoholic extract in the antineoplasic field. Particularly, rosmarinic acid and rosmanol have shown that it is possible to induce apoptosis in colon cancer cells [2]. However, deeper studies must be done in order to evaluate the importance of these extracts at pharmacological level.
Referring to the rosemary in its application in industry, it is needed to evaluate the economical viability at big scale, taking into account the main steps and stages of the industrial process:

a)Plant collection, dryness and accurately storage
b) Extraction, or different types of extraction
c) Purification and extraction of the active principles, paying special attention to camphor, 1-8 cineole, α- and β-pinene, carvone, flavonoids, rosmarinic acid and rosmanol

A study of cost of production and marketing viability at the different stages of the industrial process will be presented, showing if the ecological, technological and economical features make all this process is worth it. In addition, it will be evaluated the interest of the extraction of the main active compounds comparing to its production from other plants or by synthetic methodologies.

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An alternative scaffold on neurodegenerative diseases: Differently substituted coumarins as MAO-B selective and potent inhibitors

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With the increase of the life expectancy of the population in the actual society, the importance of drug discovery focused in the neurodegenerative diseases emerges. Therefore, neurodegenerative diseases are an important topic in Medicinal Chemistry. However the drug discovery is considered a complex and slow process, new approaches and methods have been developed, with the intention of finding new chemical entities in a more efficient way.
Coumarins are a large family of compounds of natural and/or synthetic origin that proved to have numerous pharmacological properties.[1] In our group, we already synthesized and evaluated new potent MAO-A and MAO-B inhibitors.[2-4]

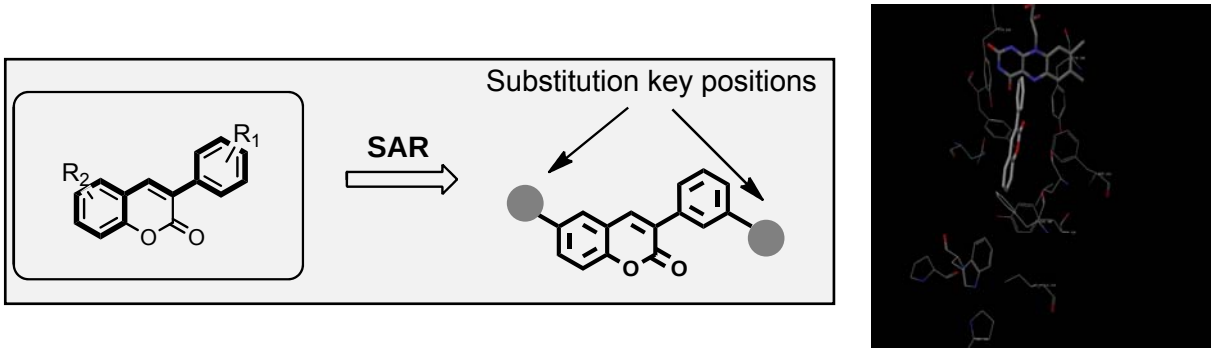

Figure 1. Structural studied coumarin scaffold and a molecular docking image.

In this work we developed synthetic methodologies that led us to new series of differently substituted 3-arylcoumarins (figure 1). The synthesis of these new compounds was carried out using a modified Perkin reaction as key step. The experimental data of the pharmacological study using human recombinant MAO-A and MAO-B isoenzymes indicated that most of the synthesized compounds are powerful and selective MAO-B inhibitors with IC_{50} values in the low nanomolar range. Different modifications on the coumarin scaffold, which we are studying more deeply, led us to improve their pharmacological profile on the potential treatment of Parkinson's disease.

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Arylcoumarins as new tyrosinase inhibitors

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Tyrosinase (EC 1.14.18.1) is a multifunctional dinuclear copper centre enzyme widely distributed in nature and mainly involved in the formation of pigments such as melanins and other polyphenolic compounds.[1,2] Coumarins (figure 1), the moiety which we are deeply exploring, are a large family of compounds of natural and/or synthetic origin that proved to have a large variety of pharmacological properties,[3] such us different enzymatic inhibition.

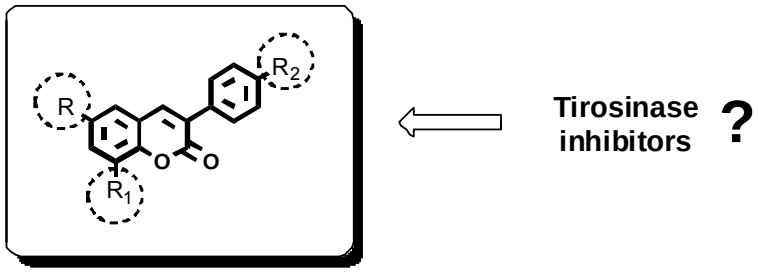

Figure 1. Structural studied coumarin moiety.

With the aim to find out structural features for the tyrosinase inhibitory activity, in the present communication we report the synthesis and pharmacological evaluation of a new series of 3-arylcoumarin derivatives with different number of hydroxyl or ether groups and bromo substituent in the scaffold. The synthesized compounds were evaluated as mushroom tyrosinase inhibitors showing, two of them, lower IC_{50} than the umbelliferone, used as reference compound. Posterior studies suggested us that these compounds are non-competitive tyrosinase inhibitors. The bromo and hydroxyl substitutions, which we are studying more deeply, proved to be one of the important modifications to improve coumarin´s biological profile.

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Methyl 3-amino-6-[4 or 3 or 2-(3-arylureido)phenyl]thieno[3,2-b]pyridine-2-carboxylates: synthesis and molecular modelling studies using VEGFR-2

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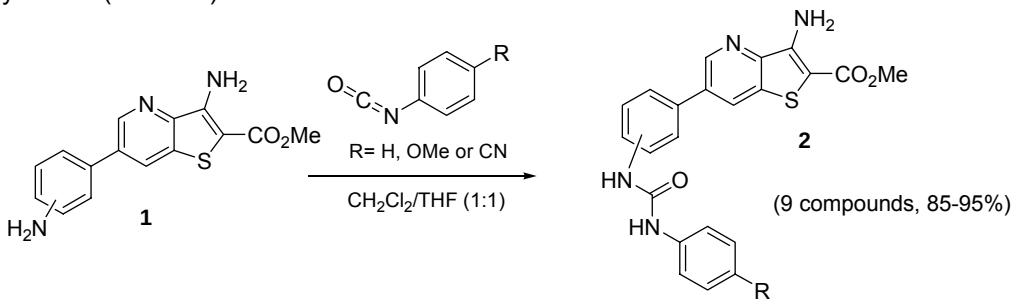
The membrane receptor tyrosine kinases (RTKs), possess both extracellular and intracellular domains and selectively catalyze the phosphorylation of tyrosine hydroxyl groups in response to binding of certain extracellular growth factors. RTK signalling pathways are normally highly regulated, yet their over-activation has been shown to promote the growth, survival, and metastasis of cancer cells, and has been associated with the progression of various human cancers. Recently some thieno[3,2-c]pyridine 1,3-diarylurea derivatives were prepared as VEGFR-2 (vascular endothelium growth factor receptor-2) inhibitors.[1] Here we present the synthesis of methyl 3-amino-6-[4 or 3 or 2-(3-arylureido)phenyl]thieno[3,2-*b*]pyridine-2-carboxylates **2** by reaction of the methyl 3-amino-6-(4 or 3 or 2-aminophenyl)thieno[3,2-*b*]pyridine-2-carboxylates **1**, synthesized earlier by our group,[2] with arylisocyanates (Scheme).



Scheme

Compounds **2** were evaluated as potential VEGFR-2 inhibitors using AutoDock Vina as molecular docking software. The enzyme X-ray 3-D structure was obtained from the Protein Data Bank: VEGFR2 (PDB: 1YWN) and the estimated inhibition constants (Ki) of the synthesised compounds were obtained. In order to validate the molecular docking approach, the respective co-crystallized ligand (LIF) and Sorafenib, a known drug that inhibit VEGFR2, were docked to the kinase domain. The difference between the X-ray conformation and the predicted docked conformations of both ligands as well as the difference between estimated Ki (Sorafenib: 109 nM; LIF: 7 nM) and experimental Ki (Sorafenib: 93 nM[3], LIF: 2 nM[4]) were negligible, validating the protein structures for virtual screening with the synthesised compounds. The potential use of the compounds as future drugs was studied by applying the Lipinski's Rule of Five analysis and it was observed that all compounds respected this rule.Comparing the three compounds of the three different substituted positions, the best results for Ki were observed for the compounds **2** with R = H when the amino group was in the 2 or 3-position in compounds **1** (214 and 180 nM, respectively) while when the amino group was in the 4-position of compounds **1** the best compound **2** was the one with R = OMe (253 nM).
Moreover, the docking pose of the compounds with the best docking score was analyzed in order to understand the key interactions between the compounds and the VEGFR-2 kinase domain structure.

Acknowledgments: FCT (Portugal) and COMPETE/QREN/EU for financial support through research project PTDC/QUI-QUI/111060/2009.

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CombiQSAR to study the agonist effect at the human A_3 adenosine receptor

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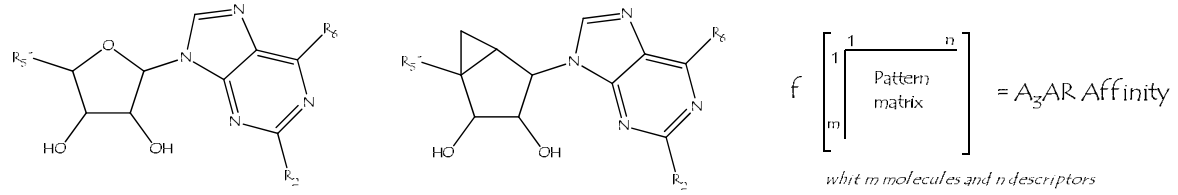
The A_3 Adenosine Receptor (A_3AR) is involved in a variety of physio-pathological processes; therefore its agonists are potential agents for treatment in different therapeutic fields like ischemia, inflammation and cancer.[1]
In order to provide simple, useful and key structural information for understanding the behaviour of A_3AR agonist ligands, we have combined methodologies of Quantitative Structure-Activity Relationship (**CombiQSAR**) -through classification and regression linear analysis- using one-dimensional descriptors with structural fragments and groups information.



Dataset of 124 molecules: 2, 6 or 5´adenosine substituted derivatives

The final model accuracy of linear discriminant analysis was 88.12%. The predictive ability of the model was assessed by a prediction set of 23 A_3AR ligands (82.61% of prediction accuracy) and the prediction affinity for A_3AR ligands of external data set of 43 patented adenosine derivatives (76.50% of good prediction).
A linear regression model was also developed using a genetic algorithm for variable selection. The final model explained 83% of the variance and the predictive ability was assessed by a *leave-one-out* cross-validation (Q^2_{LOO} = 79.50), the prediction set of the same 23 A_3AR ligands (Q^2_{EXT} = 82.20 and *s*=0.36) and the prediction affinity for A_3AR ligands of external data set of patented adenosine derivatives.
All molecular descriptors involved in both models are interpreted and directly related to structural features necessary for the agonist activity of adenosine derivates against the A_3AR. The results of this study suggest that with the present methodology it is possible to obtain models with strong predictive ability which can be prospectively used in the molecular design of novel potent A_3AR ligands and in the virtual screening of potential candidates in large datasets.
Both linear models were easily calculated and suitable to identify those regions of the adenine ring (R_2 and R_6) and the ribose moiety ($R_{5'}$) as well as those substituents (sub-structural fragments or atoms) with a highly positive contribution to the affinity for A_3AR.

Ackowledgements
We are grateful to the Universidade de Vigo and Xunta de Galicia (IN845B-2010/020) for the financial support. P.B. thanks the Xunta de Galicia for an Isidro Parga Pondal contract.

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Selection of Endemic Plants of Macaronesia with anticholinesterase activity for the treatment of Alzheimer's Disease

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Alzheimer's disease (AD) is a progressive and irreversible neurodegenerative affection of insidious onset, which causes memory loss and various cognitive disorders in adults over 60 years. It is associated with a decreased amount of some neurotransmitters such as acetylcholine, noradrenaline and serotonin in certain brain areas. Cholinesterase inhibitors were introduced in the therapy of AD in the 1990s. The hopes and interest raised by these drugs are well demonstrated by the 41,370 references listed by PubMed under *'Acetylcholinesterase inhibitors'*. In particular, the scientific community is searching for novel acetylcholinesterase inhibitors displaying less secondary effects than those presently used in therapy. In the present work, three plants endemic to Macaronesia, *Laurus azorica* (Seub.) Franco, *Juniperus brevifolia* (Seub.) Antoine and *Persea indica* (L.) Spreng., were studied for their anticholinesterasic action. The leaves of all plant material were collected in the vicinity of Lagoa do Fogo (island of São Miguel (Azores)). Extracts were prepared by extraction with methanol (CH_3OH) and dichloromethane (CH_2Cl_2) for 10 hours, using a ratio of 1g / 4mL, and essential oils were extracted by hydrodistillation for 3 hours in a Clevenger type apparatus. Anti-AChE inhibition was assayed using a modification of the Ellman method [1]. *Juniperus brevifolia* was an excellent source of acetylcholinesterase inhibitors in both the methanol extract (IC_{50} = 0.95 ± 0.10 mg / mL) and the essential oil (IC_{50} = 0.67 ± 0.07 mg / mL). As for *Laurus azorica*, it also proved to be an excellent source of acetylcholinesterase inhibitors in the case of the essential oil (IC_{50} = 0.48 ± 0.01 mg / mL). Conversely, *Persea indica* showed no anti-AChE activity in all the extracts analyzed. Several researchers consider a good a IC_{50} value less than 1.00 mg / mL, in the case of plant extracts [2,3,4]. The results obtained show the feasibility of using *Laurus azorica* and *Juniperus brevifolia* as excellent sources of acetylcholinesterase inhibitors. The active compounds responsible for this effect appear to be nonpolar in nature, as evidenced in essentials oils. In addition, they may contain a new active compound different than those already used in the treatment of Alzheimer's disease, which are mainly alkaloids. As they are nonpolar and lipophilic, they will easily cross the blood brain barrier, thus allowing a better bioavailability of compounds in the target area for the treatment of Alzheimer's disease, the synaptic clefts of brain neurons.

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Synthesis of 2,6,9-trissubstituted adenine scaffold for adenosine receptors

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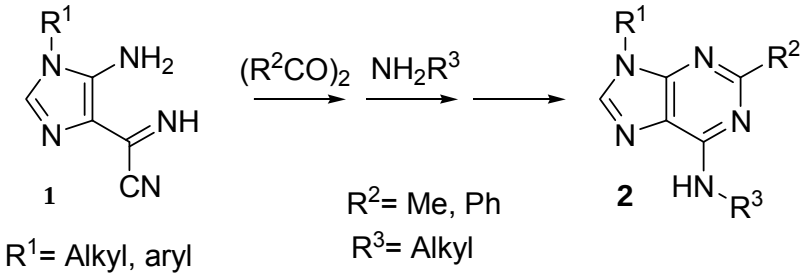
Adenosine is a neuromodulator on the central and peripheral nervous system and activates the G protein-coupled membrane receptors (GCPRs) classified as A_1, A_{2A}, A_{2B} and A_3. Adenosine regulates several physiological effects including bronchoconstriction, inhibition of platelet aggregation, inhibition of lipolysis, induction of sedation, vasodilation, suppression of the cardiac rate and contractility and stimulation of gluconeogenesis. Thus, adenosine receptor antagonists have emerged as a promising strategy for the treatment of neurological disorders, asthma, heart and renal failure, cardiovascular disorders, PD, glaucoma, cancer, chronic pulmonary diseases and asthma, or as potential antidiabetic, antidiarrheal and antiatherosclerotic drugs.[1]
The adenine scaffold is well represented in reported adenosine agonists and antagonists.[2] Our interest in synthetic 2,6,9-trissubstituted adenines as adenosine agonists/antagonists led us to developed a simple method to obtain 2-alkyl and 2-aryl N^6-substituted adenines. This method starts with the condensation of imidazole **1** with acetic or benzoic anhydride to generate the desired acylated imidazole.When the acylated imidazole is combined with primary amines, a slow replacement of the cyano group occurs leading to the corresponding 4-amidino imidazole. This compound evolves to the appropriate 6-substituted adenine **2** under reflux conditions. The products were isolated in good to very good yields in one-pot three steps reactions.



Some of the prepared adenines were submitted to a pharmacological screening on the four subtypes of adenosine receptors and the results of the biological assays will also be presented.

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Thanks are due to Universidade do Minho, Centro de Química and Fundação para a Ciência e Tecnologia (SFRH/BD/73721/2010) for financial support for a PdD grant to Nádia Senhorães.

Synthesis and Neuroprotective Activity of New Rasagiline Derivates

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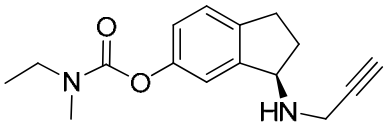
Current therapeutic approaches suggest that drugs acting at a single target may be insufficient for the treatment of multifactorial neurodegenerative diseases such as Parkinson's disease (PD), Alzheimer's disease (AD), Huntington's disease (HD) and amyotrophic lateral sclerosis (ALS), as multiple etiopathologies[1] coexist. These include, among others, oxidative stress and reactive oxygen species formation, protein misfolding and aggregation, mitochondrial dysfunction, inflammation, metal dyshomeostasis and accumulation at the sites of neurodegeneration. [2]

Therefore, a novel and much promising approach is the use of dual-action drugs. Among them, there is a class of compounds known as dual inhibitors characterized by having dual inhibitory activity.[3]

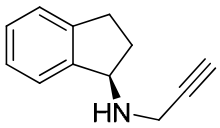
Ladostigil, **I**, (TV3326) [(N-propargyl-(3R) aminoindano-5-yl)-ethyl methyl carbamate][4] is one of the most common examples of dual inhibitors. This compound combines in a single molecule the neuroprotective/neurorestorative effects of a novel anti-Parkinsonian drug (a selective monoamine oxidase (MAO-B) inhibitor) called **Rasagiline**, **II**, with the cholinesterase (ChE) inhibitory activity of **Rivastigmine**.

As part of a programme to investigate the synthesis and biological activity of new rasagiline derivatives,[5] we herein report the preparation and pharmacological evaluation of a new class of new derivatives and/or analogues of rasagiline, carrying one or two propargyl groups on the nitrogen atom of the rasagiline with a N,N-dimethyl or a N,N-diethyl carbamoyloxy substituent on the position 3 of the indan ring.

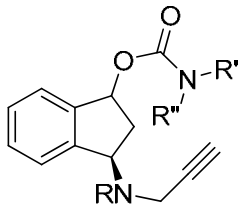
The synthetic processes of preparation of different rasagiline derivatives type **III** are further described in this communication.



I
Ladostigil



II
Rasagiline



III

R= H, $CH_2-C\equiv CH$
R'= $-CH_3$, $-CH_2CH_3$
R''= $-CH_3$, $-CH_2CH_3$

Acknowledgments

The authors acknowledge the Project 07CSA008203PR. Nerea Alonso acknowledges a research position grant at the University of Santiago de Compostela from the FPU program, Spanish Ministry of Education.

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Entropy Multi-Target QSAR Model for Predition of Antiviral Drugs Complex Networks

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The antiviral QSAR models today have an important limitation. Only they predict the biological activity of drugs against only one viral species. This is determined due the fact that most of the current reported molecular descriptors encode only information about the molecular structure. As a result, predicting the probability with which a drug is active against different viral species only with a single unifying model is a goal of major importance. In this presentation we use the Markov Chain theory to calculate new multi-target entropy to fit a QSAR model that predict by the first time a mt-QSAR model for 500 drugs tested in the literature against 40 viral species. We used Linear Discriminant Analysis (LDA) to classify drugs into two classes as active or non-active against the different tested viral species whose data we processed. The best model found was:

$$actv = -1.75 \cdot \theta_2(s)_{het} + 0.99 \cdot \theta_1(s)_{total} + 0.66 \cdot \theta_4(s)_{total} - 3.19 \cdot \theta_3(s)_{C_{sat}} - 12.39 \cdot \theta_0(s)_{Cunst}$$
$$- 2 - 23 \cdot \theta_1(s)_{Het} + 11.79 \cdot \theta_4(s)_{h-het} - 2.86$$
$$N = 2639 \qquad \lambda = 0.248239 \qquad \chi^2 = 2463.469 \qquad p < 0.001$$

In the model the coefficient λ is the Wilk's statistics, statistic for the overall discrimination, χ^2 is the Chi-square, and p the error level.[1-3] The model correctly classifies 1424 out of 1445 non-active compounds (98.55%) and 281 out of 333 active compounds (84.38%). Overall training predictability was 95.89%. Validation of the model was carried out by means of external predicting series,[4] the model classifying, thus, 698 out of 704 non-active compounds and 143 out of 157 active compounds. Overall validation predictability was 97.68%.The present communication report the first attempts to calculate within a unify framework probabilities of antiviral drugs against different virus species based on entropy analysis. We assembled for the first time a drug-virus complex network, for observed possible mechanism of action for the different drugs against viruses.

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Hypericum foliosum Aiton presents higher anti-fungal activity on *Candida albicans* compared with Nipagin

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Renewed interest in plant antimicrobials has emerged during the last 20 years probably due to the small number of drugs available for the treatment, the increasing development of drug resistance to human pathogenic organisms, as well as the appearance of undesirable side effects of certain antibiotics and the emergence of previously uncommon infections[1]. Therefore, research on new low cost and low toxicity antimicrobial compounds based on traditional therapies and natural products, is increasing worldwide in the attempt to find out new formulas to treat resistant strains[2]. There are countless examples of plants which are used topically and systemically to treat bacterial infections, one of which is *Hypericum perforatum* (St. John's Wort) which has been used in herbal medicine, externally for the treatment of skin wounds, eczema and burns[3]. In fact, the *Hypericum* (Guttiferae) genus is a well known plant in herbal medicine due to the therapeutic efficacy of its different species. One study showed than from 34 species collected at the Royal Botanical Garden Kew at Wakehurst Place (United Kingdom), 32 presented significant anti-staphylococcal activity on multi-resistant strains of *Staphylococcus aureus*. One of the most promising was *Hypericum foliosum* Aiton[2]. Recently, this endemic plant from the Azores Islands (Portugal) was shown to present significant antioxidant activity and acetylcholinesterase inhibition properties on methanolic extracts of its morphological constituents[4,5]. Since, the attempt to search for new biologically active compounds from *H. foliosum*, several specimens were collected from six different Islands of the Azores and assayed for anti-fungic activity on *Candida albicans*, an opportunistic diploid fungus responsible for several oral and genital infections in humans which have become a cause of major health concern in hospital-acquired infections. The extracts were prepared by extracting dry powder plant material successively with hexane, dichloromethane and hot water.

The anti-*Candida* assay was performed by a modification of the diffusion Bauer's technique[6] on solid medium using filter paper discs of 6 mm. From a culture of *Candida albicans*, originally isolated from a hospital and maintained at 10 ºC, the inoculums were prepared in saline (sodium chloride) 0.85% sterile. The suspension was compared, using qualitative methods, with a suspension of barium sulphate Tube 0.5 McFarland Scale (bioMerieux®, France). The diameter of inhibition was recorded at 24 and at 48h after the addition of 15 µL of the each extract at 5% (w/m) concentration. Nipagin® was used as positive control at the same concentrations of the extracts.

From all the crude aqueous, dichloromethane and hexane extracts only the extract of dichloromethane of *H. foliosum* from Santa Maria and all the aqueous extracts didn't not present any inhibition of the fungus. The inhibition zone of the six hexane extracts varied from 0.90 cm to 1.23 cm at 24 h. The dichloromethane extracts seem to be more potent, since the inhibition zones were between 1.10 and 1.43 cm. The results seem to indicate that crude dichloromethane and hexane extracts may be more effective than Nipagin® since the inhibition zone of this compound, a methylparaben used as food preservative, on pharmaceutical and cosmetics industries, was 0.87 cm at 24 hours and 0.80 cm at 48 hours.

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Synthesis of several N-substituted phthalazinones as potential dual AChE/MAO inhibitory agents

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In recent years, it has been proposed the multitargeting strategies for treatment of diseases with multiple pathogenic factors for instance, neurodegenerative disorders such as Parkinson's and Alzheimer's diseases.[1] In addition, it has been described that cholinesterase (AChE and BuChE) and monoamine oxidase (MAO-A and MAO-B) are enzymes which modulate biochemical changes related to these kind of disorders.[2] Therefore, the design, synthesis and the pharmacological study of potential dual AChE and MAO inhibitors may be an interesting approach to their therapy.[3]
The potential mixed AChEI/MAOI proposed are based on the 2H-phthalazin-1-one scaffold including N-benzyl piperidine fragments on the hydrazido functionality, combining pharmacophoric features of both donepezil, a potent AchEI, and isocarboxazid a nonselective MAOI, respectively (Figure 1).

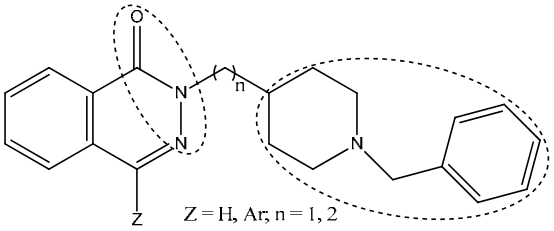

Figure 1. General structure of AChEI/MAOI proposed

The designed compounds were synthesized in four steps using as starting materials the adequate 2H-phthalazin-1-ones and two commercially available N-Boc protected 4-hydroxyalkylpiperidines. First, the hydroxyalkyl derivatives were selectively transformed into the corresponding 4-bromoalkylpiperidines following standard procedures; then, the phthalazinones were treated with sodium hydride and the appropriate bromoalkyl derivative in DMF to give in good yields the corresponding 2-(N-Boc-4-piperidinylalkyl)phthalazin-1-ones, which, after acid hydrolysis (HCl) of protecting group, were converted into the desired compounds by reaction with benzyl bromide in the presence of sodium hydride.
The synthesized compounds were evaluated as cholinesterase (hAChE and hBuChE) and monoamine oxidase (hMAO-A and hMAO-B) inhibitors. The results of this biological study will be reported.

Ackowledgements
We are grateful to the Xunta de Galicia (IN845B-2010/020) for the financial support. P.B. thanks the Xunta de Galicia for an Isidro Parga Pondal contract and T.C. thanks the University of Vigo for a pre-doctoral contract.

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Antimicrobial peptides – new antibiotic paradigm? SAXD studies of cecropin A-melittin hybrid peptides

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Every year new emergent strains of infectious agents resistant to known antibiotics appear. This reality is alarming as the current situation is reaching a point when the rate of discovery of effective antibiotics will be surpassed by the rate of emergence of new resistant strains.
In recent years, antimicrobial peptides have emerged as a possible new paradigm in antibiotic therapy. These biomolecules are characterized by their amphipaticity and high content of positively charged amino acids, a fundamental feature since the cellular membranes of bacteria comprise a significant percentage of lipids with negative electric charge. This characteristic enables strong electrostatic interactions as at least the first step in action. On the other hand, mammalian cell membranes are zwitterionic (establishing thus weak interactions) and this fundamental difference is implicated in the selectivity mechanism. Many antimicrobial peptides have been studied and found to be potent, broad spectrum antibacterial drugs, as well as promising as immunomodulators. They are thus seen as new therapy agents, against Gram negative and Gram positive bacteria and even fungus.
A biophysical approach in this area is to make use of simple systems that mimic the characteristics of the membranes under study, the so called “model membranes”. These studies contribute to the discrimination of main factors responsible for action, and are thus a very valuable tool to use in parallel with Microbiology research on the same agents, to fully unravel their mechanism of action.
The accepted mechanisms of action of antimicrobial peptides rely mainly on their interaction with the pathogen membrane, and thus depend strongly on their electrostatic interactions with the outer and inner membranes. The peptide/membrane interaction implies for the peptide the adoption of a proper folding and induces modelling of the topology of the lipidic membrane surface. The changes may be so pronounced that induce pore formation or even micellization of the membrane, increasing therefore the permeability of bacteria cell membranes. This permeabilization alone may not only be enough to eliminate the pathogenic agent, but can *e.g.* enable the bacteria-fighting agents to enter directly into the cytoplasm, providing immediate access to the cellular machinery.
The occurrence of changes in membrane topology validates the use of spectroscopic diffraction techniques to study the influence of peptides and exogenous conditions on the formed structures (1). The technique used in this study is Small Angle X-Ray Diffraction (SAXD), which allowed us to identify the different phases present for the membranes alone and when mixed with peptide at pre-defined critical ratios.
Results will be presented for model membranes of Phosphatidilethanolamine (PE), a lipid that is a majour component of Gram negative inner membrane, and its mixture with CA(1-7)M(2-9), a hybrid peptide of Cecropin A – Melittin (2). The influence of temperature on the observed phase behaviour was established by performing experiments within the temperature range 10-90 ^{o}C, both at chosen temperatures and in temperature scans at controlled scan rate. Various phases were indentified, lamellar (gel phase and liquid crystalline phase), hexagonal and cubic phases, and their characteristic parameters obtained from data analysis of the obtained spectra.

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1-Aryl-3-[4-(thieno[3,2-*d*]pyrimidin-4-yloxy)phenyl]ureas as potential inhibitors of VEGFR-2: synthesis and molecular modelling studies

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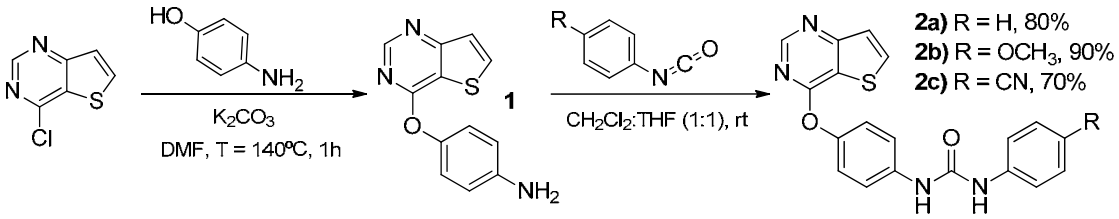
Angiogenesis is a requirement for tumor growth and metastasis and occurs through several signalling pathways. One key pathway that initiates proliferation and migration of endothelial cells is signalling through the vascular endothelial growth factor receptor-2 (VEGFR-2).[1] Therefore, small molecules that block this signalling pathway through inhibition of the VEGFR tyrosine kinase activity could potentially inhibit angiogenesis and tumour growth. Recently works describing thienopyrimidines[2] and thienopyridine ureas[3] as inhibitors of VEGFR-2 have appeared in the literature. Here we present the synthesis of new 1,3-diarylureas **2** starting by regioselective nucleophilic substitution of the 4-chlorothieno[3,2-*d*]pyrimidine with 4-aminophenol to obtain 4-(thieno[3,2-*d*]pyridin-4-yloxy)aniline **1** which reacts with different arylisocyanates (Scheme).



Scheme

The synthesized compounds **2** were evaluated as potential VEGFR-2 tyrosine kinase inhibitors using AutoDock Vina as molecular docking software. The receptor X-ray 3-D structure was obtained from the Protein Data Bank: VEGFR2 (PDB: 1YWN) and the estimated inhibition constants (Ki) of the synthesised compounds were obtained. In order to validate the molecular docking approach, the respective co-crystallized ligand (LIF) and Sorafenib, a known drug that inhibit VEGFR-2, were docked to the kinase domain. The difference between the X-ray conformation and the predicted docked conformations of both ligands as well as the difference between estimated Ki (Sorafenib: 109 nM; LIF: 7 nM) and experimental Ki (Sorafenib: 93 nM[4], LIF: 2 nM[5]) were negligible, validating the protein structures for virtual screening with the synthesised compounds. Their potential use as future drugs was studied by applying the Lipinski's Rule of Five analysis and it was observed that all compounds respected this rule. The estimated values of Ki were 180 nM for **2a**, 214 nM for **2b** and 66 nM for **2c**, showing that the presence of the nitrile group significantly lowers the Ki value when compared to the methoxy group of **2b**. In this series compound **2c** is the most promising one.
Moreover, the docking pose of the compounds with the best docking score was analyzed in order to understand the key interactions between the compounds and the VEGFR-2 kinase domain structure.

Acknowledgments: FCT (Portugal) and COMPETE/QREN/EU for financial support through research project PTDC/QUI-QUI/111060/2009.

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1-aryl-3-(4-(7-methylthieno[3,2-*d*]pyrimidin-4-yloxy)phenyl)ureas: synthesis and molecular modelling studies using VEGFR-2

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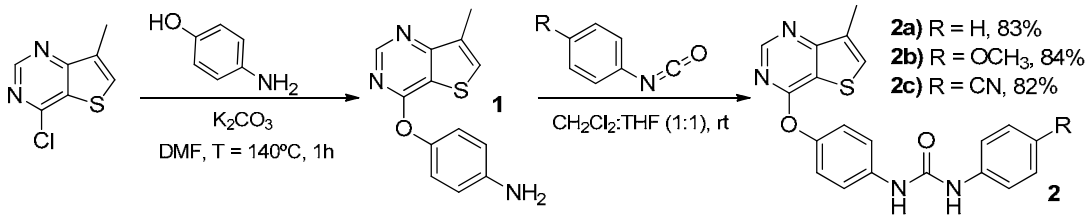
The development of anticancer drugs inhibiting angiogenesis has been an area of extensive research in the past decade. Angiogenesis is a requirement for tumor growth and metastasis and occurs through several signalling pathways. One key pathway that initiates proliferation and migration of endothelial cells is signalling through the vascular endothelial growth factor receptor-2 (VEGFR-2).[1] Therefore, small molecules that block this signalling pathway through inhibition of VEGFR-2 tyrosine kinase activity could potentially inhibit angiogenesis and tumor growth. Recently works describing thienopyrimidines[2] and thienopyridine 1,3-diarylureas[3] as VEGFR-2 inhibitors have emerged in the literature. Here we present the synthesis of new 1-aryl-3-(4-(7-methylthieno[3,2-*d*]pyrimidin-4-yloxy)phenyl)ureas **2** in high yields by reaction of 4-[(7-methylthieno[3,2-*d*]pyridin-4-yl)oxy]aniline **1** with arylisocyanates. The former was prepared by regioselective nucleophilic substitution of 4-chloro-7-methylthieno[3,2-*d*]pyrimidine with 4-aminophenol (Scheme).



Scheme

Compounds **2** were evaluated as potential VEGFR-2 tyrosine kinase inhibitors using AutoDock Vina as molecular docking software. The receptor X-ray 3-D structure was obtained from the Protein Data Bank: VEGFR2 (PDB: 1YWN) and the estimated inhibition constants (Ki) of the synthesised compounds were obtained. In order to validate the molecular docking approach, the respective co-crystallized ligand (LIF) and Sorafenib, a known drug that inhibit VEGFR-2, were docked to the kinase domain. The difference between the X-ray conformation and the predicted docked conformations of both ligands as well as the difference between estimated Ki (Sorafenib: 109 nM; LIF: 7 nM) and experimental Ki (Sorafenib: 93 nM[4], LIF: 2 nM[5]) were negligible, validating the protein structures for virtual screening with the synthesised compounds.
The potential use of compounds **2** as future drugs was studied by applying the Lipinski's Rule of Five analysis and it was observed that all the compounds respected this rule. The estimated values of Ki were 109 nM for **2a**, 354 nM for **2b** and 47 nM for **2c**, showing that the presence of the nitrile group makes this compound the most promising of this series.
Moreover, the docking pose of the compounds with the best docking score was analyzed in order to understand the key interactions between the compounds and the VEGFR-2 kinase domain structure.

Acknowledgments: FCT (Portugal) and COMPETE/QREN/EU for financial support through research project PTDC/QUI-QUI/111060/2009.

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Synthesis, Characterization and Antiproliferative Activity on Cancer Cell Lines of New Ionic Liquids from Ampicillin

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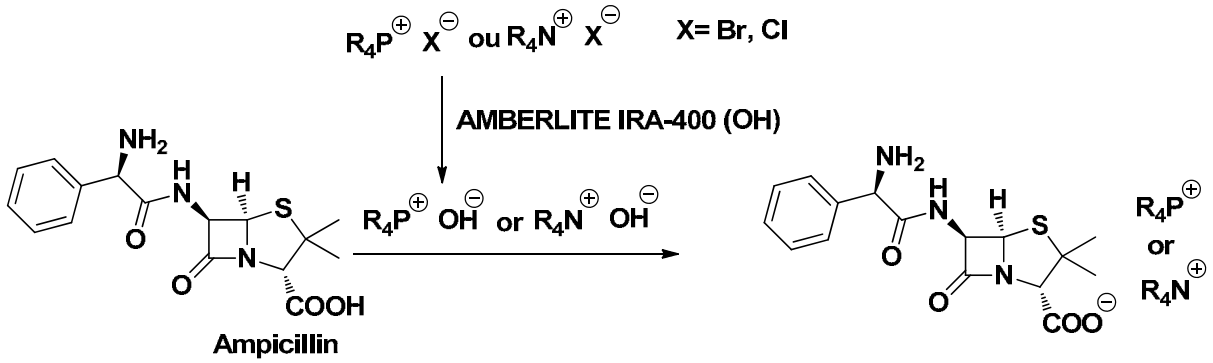
Ionic Liquids (ILs) are ionic compounds that possess melting temperature below 100ºC and they have been a topic of great interest since the mid-1990s due to their unique properties. The range of IL uses has been broadened, due to a significant increase in the variety of physical, chemical and biological ILs properties. They are now used as Active Pharmaceutical Ingredients (APIs) and recent interests are focused on their application as innovative solutions in new medical treatment and delivery options.[1]

In this work, our principal objective was the synthesis and investigation of physicochemical and medical properties of ionic liquids (ILs) and organic salts from ampicillin. This approach is of huge interest in pharmaceutical industry as cation and anion composition of ILs and organic salts can greatly alter their desired properties, namely the melting temperature and even synergistic effects can be obtained.[2,3] For the synthesis of these compounds we used a recently developed method proposed by Ohno *et al*.[4] for the preparation of quaternary ammonium and phosphonium hydroxides, that were neutralized by ampicillin. After purification we obtained pure ILs and salts in good yields.

These ILs shows good antimicrobial and antifungal activities. As it is well known that some ionic liquids containing phosphonium and ammonium cation also shows anti-cancer activity[1,5] we also decided to study these compounds against some cancer cell lines.



Scheme 1. Synthesis of ampicillin salts and ILs.

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Linear analysis vs. Artificial Neural Network to predict new enzyme target drugs

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Predicting new enzymes as targets for new drugs is a major goal in the medicinal chemistry in order to optimize the synthesis and the . In recent years the development of computational techniques aimed to the identification of new targets has increased substantially, either molecular dynamics or docking and Quantitative Structure Activity Relationship (QSAR). In this context our group has always been at the forefront of providing more advanced and reliable QSAR models, based on a Markov Chain approach, to predict new drugs and drugs targets[1-3]. The main aim of this study is compare the Linear Discriminant Analysis (LDA) with the Artificial Neural Network (ANN) models to predict new drugs targets using a Markov Chain approach as source for the molecular descriptors. With our experience we found that models that use neural networks have better performances especially when the database is very large; we compare the results using three different dataset: the first one with 1300 entries, the second one 4500 and the last one with 65000.

DATASET	LDA acc	LDA nv	ANN acc	ANN top	Ref.
1371	74.18	2	75	MLP: 5:5.29	[4]
4755	74.86	3	98.9	MLP 4:4-9-8-1:1	[5]
65000	75	6	92	MLP	

*Dataset= number of inputs in the dataset; LDA acc= accuracy for the LDA model; LDA nv= number of protein features used by the model; ANN acc= accuracy of the ANN models; ANN top= ANN topology.

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Mediated G-protein photosensory responses in melanopsins photoreceptors – an evolutionary approach

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Melanopsins were found in 1998 by Dr. Ignacio Provencio and their colleagues. Melanopsins are related with conopsins and rhodopsins in the image forming functions of the ganglion cells in the human eye, specifically in the regulation of circadian rhythms and pupillary light reflex[1]. Melanopsins have several particular characteristics: they resemble the visual opsins of invertebrates and their photosensory response is sensible to low-light wavelength[2].

The photosensory response of all photoreceptors is characterized by a g-protein mediated response. Melanopsins have an unknown G-protein complex, and because of that is difficult to define their molecular response in the presence of light, namely the biochemical response that regulates the circadian rhythms in vertebrates. In this study, we used bioinformatics tools in a comparative genomic/proteomic approach to assess this question.

We found some important biochemical patterns in melanopsins: (1) their amino acid sequence is very similar with invertebrate rhodopsins which allowed the prediction of tridimensional structure; (2) the third intracellular loop shows an unexpected variability when compare with other photoreceptors; and (3) we determined three potential G-couple proteins that are responsible for their photosensory response, with very different cellular responses. We found that melanopsins have changed their amino acid properties during vertebrate evolution, mainly expressed by changes in the isoelectric point. Those results suggest that melanopsins could mediate different responses to light and consequently a high physiological plasticity with relevant importance on different photic environments.

The study of melanopsins proteins could provide answers about how photoreceptors work and which are the molecular mechanisms involved in their photoresponse. G-protein-coupled receptors are key elements in the vertebrate signal transduction system, and constitute the majority of drug targets to prevent cancer disorders[3]. The assessed structural information on melanopsins could provide important contributes for drug screening and structure-based drug design.

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Decreased adenosine A_1 receptor expression in hippocampus and neocortex of patients with mesial temporal lobe epilepsy (MTLE): influence on neuronal $[Ca^{2+}]_i$ signalling

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Adenosine (ADO) is an endogenous neuromodulator acting through G-protein-coupled A_1, A_{2A}, A_{2B} and A_3 receptors. It controls predominantly excitatory glutamatergic synapses through its action on inhibitory A_1 receptors, which are highly abundant in the CNS. ADO is a ubiquitous homeostatic substance that acts as an "endogenous anticonvulsant". Unbalanced ADO modulation is implicated in pathological situations resulting from excessive function of glutamatergic pathways, such as epilepsy. Controversy exists, however, on the role of ADO on epileptic hippocampi perhaps because ADO neuromodulation in this region might result from the equilibrium between inhibitory A_1 and excitatory A_{2A} receptor responses.

Here, we investigated whether adenosine receptor protein expression and function is altered in drug-resistant MTLE patients submitted to surgery as compared to control tissues obtained from non-MTLE patients or cadaveric organ donors. Procedures were approved by the Ethics Committees of CHP-HGSA and ICBAS-UP. Protein expression was evaluated by western blot using an anti-A_1 antibody (1:500) from Abcam, followed by the incubation with horseradish peroxidase-conjugated anti-rabbit secondary antibody (1:30.000). The membranes were developed with chemiluminescent reagent ECL Plus and analyzed by ImageJ software. The influence of inhibitory A_1 and excitatory A_{2A} adenosine receptor-mediated responses in the hippocampus and adjacent neocortex was assessed in synaptosomes incubated with the calcium fluorescent indicator, Fluo4-NW.

Western blot analysis of adenosine A_1 receptor expression in whole membrane lysates of the neocortex and hippocampus suggests that the A_1 protein levels are decreased in MTLE when compared to cadaveric tissue. Interestingly, neocortex A_1 receptor protein expression was decreased by 15%, whereas in the hippocampus it was further reduced to 50% of control tissue normalized to β-tubulin protein levels. Functional results showed that veratridine (VT, 10μM)-induced calcium influx ($[Ca^{2+}]_i$) increased by 32% in synaptosomes from cadaveric hippocampus upon eliminating endogenous adenosine with ADA (0.5U/ml). Conversely, this enzyme inhibited VT-induced $[Ca^{2+}]_i$ in synaptosomes from both cortex (23%) and hippocampus (13%) of MTLE patients. The adenosine A_{2A} antagonist, ZM241385 (50nM) mimicked the effect of ADA, thus indicating that endogenous adenosine exerts a preferential tonic excitatory effect on $[Ca^{2+}]_i$ influx in MTLE patients. R-PIA (100nM, an A_1 receptor agonist), decreased (7-30%) $[Ca^{2+}]_i$ influx by synaptosomes of cadaveric hippocampus and non-MTLE neocortex depolarized with VT(10μM), but it was devoid of effect in the hippocampus of MTLE patients (7.6%). Blockade of A_1 receptors with DPCPX (10nM) prevented inhibition caused by R-PIA (100nM). Activation of A_{2A} receptor with CGS21680C (10nM) decrease roughly by 24% VT-induced $[Ca^{2+}]_i$ in the hippocampus and neocortex control tissues. Interestingly, our results suggest that A_{2A} receptor-mediated facilitation of $[Ca^{2+}]_i$ influx predominates in the hippocampus and neocortex of MTLE patients. This was shown since CGS21680C increased VT-induced $[Ca^{2+}]_i$ and ZM241385 prevented this effect.

Data suggest that the adenosine A_1/A_{2A} receptor activation balance in the hippocampus and neocortex of MTLE patients is shifted towards a predominant activation of excitatory A_{2A} receptors, most probably due to decreased expression of inhibitory A_1 receptors compared to control tissues.

Supported by FCT (FEDER funding, PIC/IC/83297/2007, UMIB-215/94 and FSE-POPH-QREN).

The Ca_v1 inhibitor, verapamil, predisposes atria to the negative inotropic action of adenosine by uncoupling A_1 receptor-mediated $K_{Ca}2$/SK channel closure from its effector

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Adenosine (ADO) exerts a critical role in modulating cardiac function. Intravenous ADO and its agonists are used for the prompt conversion of paroxysmal supraventricular tachycardia to sinus rhythm and also as a ventricular rate controlling agent in atrial fibrillation.[1] Activation of A_1 receptors (A_1R) mediates negative chronotropic, dromotropic and inotropic responses in the heart and promotes anti-β-adrenergic and anti-arrhythmic effects. Like atrial muscarinic M_2 receptors (M_2R), the negative inotropic effect of adenosine A_1R results from K^+ efflux via G protein-coupled inwardly rectifying K^+ channels (GIRK or KIR 3.1/3.4) operated by βγ subunits. Up-regulation of GIRK currents reduces action potential duration thereby decreasing the time available for Ca^{2+} influx and, thus, heart rate and the amplitude of muscle contraction. However, in contrast to acetylcholine (ACh) acting via M_2R, the negative chronotropism operated by A_1R is evidenced at much lower concentrations of ADO than its negative inotropic action (unpublished observations). In this study, we explored the rationale for "ADO chronoselectivity" testing its effect in the absence and presence of specific Ca^{2+} and K^+ channel blockers on spontaneously beating or electrically-paced rat atria. For comparison purposes, the modulation of M_2R activation by these channel blockers was also evaluated.
The experiments were performed in isolated atria from Wistar rats (*Rattus norvegicus*; 250–300 g) of either sex, continuously superfused with gassed (95% O_2/5% CO_2) Tyrode's solution, at 37ºC. Isometric muscle tension of the samples was continuously monitored on a computer screen via a PowerLab data acquisition system (Chart 5, v.4.2 software; AD Instruments, USA). In some of the experiments, atria were paced at a constant rate (4 Hz; 2 ms, 60 V). The parameters reported on the rat spontaneously beating atria include rate and force of contraction.

The effects of increasing concentrations of R-PIA (a selective A_1R agonist; 0.001-1 μM; *n*=40-47) and oxotremorine (Oxo; a M_2R agonist; 0.01-3 μM; *n*=17-31) were evaluated in the absence and presence of DPCPX (A_1R antagonist; 100 nM), AF-DX 116 (M_2R antagonist; 10 μM), apamin ($K_{Ca}2$/SK channel blocker; 30 nM), 4-aminopiridine (4-AP; K_v channel blocker; 10 μM), glibenclamide (K_{ATP} channel blocker; 10 μM), tertiapin Q (GIRK/KIR 3.1/3.4 channel blocker, 300 nM), nifedipine or verapamil (Ca_v1 (L-type) channel blockers; 1 μM). Blockade of $K_{Ca}2$/SK channels with apamin and of Ca_v1 (L-type) channels with nifedipine or verapamil sensitized rat spontaneously beating atria to the negative inotropic action of R-PIA, without affecting the nucleoside chronotropic effect; these compounds failed to affect the cardiodepressant effects of OXO. Tertiapin Q prevented the negative chronotropic effects of both R-PIA and OXO. Both apamin and tertiapin Q failed to modify verapamil (0.01-10 μM)-induced negative chronotropism. Verapamil (1 μM) reversed the mild positive inotropic effect of apamin (0.001-1 μM, max. increase ~20% at 0.1 μM), leading to a moderate negative inotropic action (max. decrease ~10% at 0.1 μM). During atrial pacing, verapamil (1 μM) was still capable of sensitizing rat electrically-driven atria to the negative inotropic effect of R-PIA. This suggests that the negative chronotropism resulting from combined A_1R activation and Ca_v1 (L-type) channel blockade cannot account for the predisposition to cardiac depression triggered when one associates verapamil plus ADO.

Data indicate that ADO acting via A_1R is a "chronoselective" atrial depressant as compared to the M_2R agonist, Oxo. While both A_1R and M_2R promote the opening of tertiapin Q-sensitive GIRK/KIR 3.1/3.4 channels modulating SA node automatism, adenosine A_1R (via G protein α subunit) may prevent $K_{Ca}2$/SK activation increasing the time available for Ca^{2+} influx through Ca_v1 (L-type) channels, which might partially override the nucleoside tendency to produce cardiac depression. Thus, blockade of Ca_v1 (L-type) channels by verapamil (or nifedipine) predisposes atria to the negative inotropic action of adenosine by uncoupling A_1 receptor-mediated $K_{Ca}2$/SK channel closure from its effector system.

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Work supported by FCT (FEDER funding, UMIB-215/94). B. Bragança was in receipt of a Young Investigator Fellowship from FCT (BII/UMIB-ICBAS/2009-2).

New Scaffolds as Potential Antioxidants: Coumarin-Chalcone Hybrids

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Many hydroxylated coumarin and chalcone derivatives have special ability to scavenger reactive oxygen species (ROS).[1-4] Nevertheless, only a few methodologies have been employed to assess the antioxidant ability of these compounds and none of these studies allowed to give an index of capacity and antioxidant reactivity.
In the present work and with the aim of developing new antioxidant compounds, we have synthesized a new series of coumarin-chalcone hybrids (**Figure 1**) and we have studied its antioxidant capacity and reactivity.

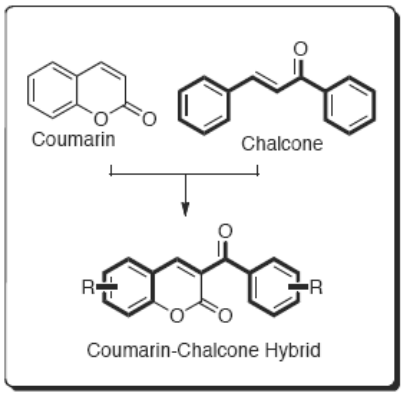

Figure 1. Coumarin-chalcone hybrids containing hydroxy groups.

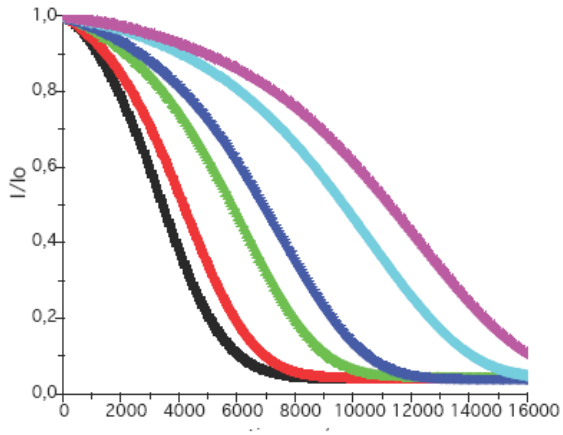

Figure 2. $ORAC_{FL}$ method for one of the synthesized compounds. The antioxidant capacity is reflected in the protection of fluorescence through time at different concentrations of derivatives

In order to study the antioxidant capacity, we have employed the oxygen radical absorbance capacity method including the use of fluorescein as the probe (ORAC-FL) (**Figure 2**) which measures antioxidant capacities using standard TROLOX, an hydrophilic vitamin E simile. All coumarin derivatives showed ORAC values better than TROLOX control and results will be further presented. We have also used the Electron Spin Resonance spectroscopy (ESR) in order to characterize the radical species generated and be able to compare these results with the antioxidant activities obtained by other techniques (ORAC methods). ESR results were concordant with ORAC methodologies using DMPO as spin trap.

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In Situ Electrochemical and Gel-Electrophoresis Evaluation of DNA-Hydroxyl Radical Interaction

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Oxidants, particularly reactive oxygen species (ROS), play an important role in double stranded DNA (dsDNA) damage which is strongly related to mutagenesis, carcinogenesis, auto-immune inflammatory and neurodegenerative diseases. The hydroxyl radical is considered the main contributing ROS to endogenous oxidation of cellular DNA. However, consensus over the interaction between DNA and hydroxyl radicals is still needed. The DNA-electrochemical biosensor incorporates immobilised dsDNA as molecular recognition element on the electrode surface, and measures *in situ* specific binding processes with DNA, being a complementary tool for the study of biomolecular interaction mechanisms of compounds binding to DNA and enabling the screening and evaluation of the effect caused to DNA by radicals and health hazardous compounds[1,2]. The DNA-electrochemical biosensor was used to study the interaction between dsDNA immobilized on a boron doped diamond electrode (BDDE) surface[3] and electrochemically generate hydroxyl radicals. The results showed, by the detection of purinic bases and 8-oxoGua oxidation peaks, that the electrochemically generate hydroxyl radicals on the BDDE surface damaged *in situ* the immobilised dsDNA. The voltammetric data was confirmed by nondenaturing agarose gel-electrophoresis obtained for the dsDNA film exposed *in situ* on the electrode surface to the electrochemically generate hydroxyl radicals. The importance of DNA-electrochemical biosensor in the evaluation of the DNA-hydroxyl radical interaction is clearly demonstrated.

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A new series of 4-hydroxy-3-phenylcoumarins as MAO inhibitors

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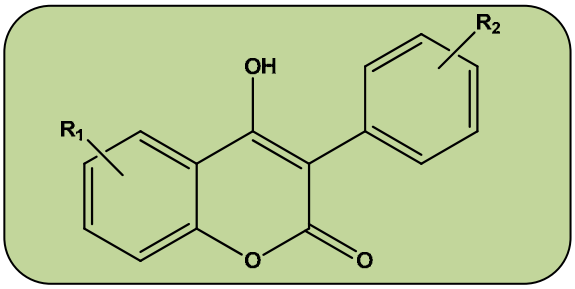
Coumarins are an important group of organic compounds, of natural and synthetic origin, that present a broad range of biological activities like antioxidant,[1] anti-inflammatory,[2] antibacterial,[3] cardioprotective, vasorelaxant,[4] anti-HIV and anticarcinogenic.[5] Recent study pay special attention to their monoamine oxidase (MAO) inhibitor property. MAO is an important FAD-containing enzyme present in the mitochondrial outer membrane of glial, neuronal and other cells. It exists in two isoforms MAO-A and MAO-B and plays a vital role in the monoamines degradation and in the inactivation of neurotransmitters. MAO-A preferentially deaminates serotonin and noradrenaline, while MAO-B preferentially deaminates phenylethylamine and benzylamine. These properties determine the interest of MAO inhibitors. Related to that, first in the last years our research group synthesized and investigated the MAO inhibitory activity of 7-substituted coumarin derivatives.[6] Then we studied the MAO-inhibitor activity of coumarin-resveratrol hybrids incorporating an aryl group in the 3 position of the coumarin skeleton.[7] Based on the SAR studies we can concluded that substituents at the 7-position are not essential for the MAO activity, when an aryl group is present at the 3-position of the coumarin. Then, in order to evaluate the effect of the introduction of π-excedent heteroaryl groups in the biological activity, we synthesized series of 3-indolyl and 3-thiophenylcoumarins. The good MAO inhibitor activity found for these compounds[8] encouraged us to synthesize a new series of 3-phenyl coumarins introducing two modifications: substituents with different electronic and/or steric properties and incorporation of an additional hydroxyl group at the 4 position of the coumarin scaffold.



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Antimicrobial activity of essential oil of *Cymbopogon citratus* (DC) Stapf. *Poaceae-Gramineae* from Angola in ATCC and multidrug resistant strains

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Cymbopogon citratus (DC) Stapf. Poaceae-Gramineae (C. citratus) is an herb from India, which grows in several countries in tropical and sub-tropical, as the Republic of Angola, where tea is known as caxinde. This tea is consumed, as aromatic drink, as well as in traditional cuisine due to its lemon flavor. Cymbopogon citratus (DC) is widelly used in folk medicine, where infusions and decoctions, feature anti-spasmatic, carminatives and anti-hypertension properties. The essential oil of C. citratus is often applied, in pharmaceutical industry, as flavor and fragrance, and is also used as a source of new phytochemical molecules for the development of new pharmaceutical products. The aim of this study is to evaluate the antioxidant activity of different extracts (ethanolic, methanolic and aqueous) of caxinde's leaves and assay the antimicrobial activity of essential oil of C. citratus against Staphylococcus aureus (ATCC 25923), Staphylococcus epidermidis (ATCC 12228), Enterococcus faecalis (ATCC 29212), Escherichia coli (ATCC 25922), Klebsiella pneumoniae (ATCC 13883), Proteus mirabilis ATCC 25933, Pseudomonas aeruginosa (ATCC 27853), Acinetobacter baumanni (ATCC 19606), Candida albicans (ATCC 10231), C. parapsilosis (ATCC 2219) and C. tropicalis (ATCC 750) and multi-drug hospital resistant strains . The aqueous extract showed high antioxidant action compared to a standard synthetic antioxidant (BHT). This result suggests that the aqueous extract could be used in human, as scavenger of free radicals. The essential oil was obtained by hydro-distillation of dried plants. Anti-microbial activity of essential oil and majority compound, the citral, was evaluated by the NCCLS/CLSI with injunction on growth of all the strains tested, dependent of concentration. The essential oil as high antimicrobial activity against Gram-positive, meticillin-resistant strains or not, and yeasts.

Keywords: Cymbopogon citratus, essential oil, antibacterial and antifungal activity, multi-resistant clinical strains

Acknowledgements: CITS-IPSN-CESPU

Antifungal activity of endemic *Origanum virens* from Portugal

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Origanum virens is widely used in Portuguese, and Mediterranem, cuizin. Althout it is use as food condiment, it is also used in traditional medicin as antiseptic.
The antifungal activity of Origanum virens essential oil on Candida albicans ATCC 10231 and physic-chemical characterization of several extracts were evaluated. The essential oil were obtained from the aerial parts of the plant by hydro-distillation and minimal inhibitory concentration (MIC) as well as the minimal lethal concentration (MLC) were used in order to assay the antifungal activity against Candida albicans. MIC and MLC values were 0,005% and 0,040% respectively, ranging from 0,005% to 0,080% of essential oil. Concentrations, lower than MIC values strongly prevent fungal growing (fig.1). The antifungal effect is time and concentration dependent, it is observed after 6 hours of incubation for lower concentration (0,040%) and after 2 hours of incubation of essential oil with Candida albicans for higher concentrations. It is difficult to attribute the activity of a complex mixture to particular constituents. Nevertheless, it is reasonable to speculate that the activity of this oil can be related to the presence of carvacrol and thymol.
The antioxidant activity of several extracts (methanol, ethanol and water) was also evaluated. Very promissing results were obtained, indicating a high antioxidant activity of the extracts.

Keywords Origanum virens; antifungal activity, physic-chemical characterization, antioxidant activity

Acknowledgements: CITS-IPSN-CESPU

Targeting Phenolic Antioxidants to Mitochondria: Synthesis and Characterization of Novel Triphenylphosphonium-conjugated Lipophilic Cinnamic Derivatives

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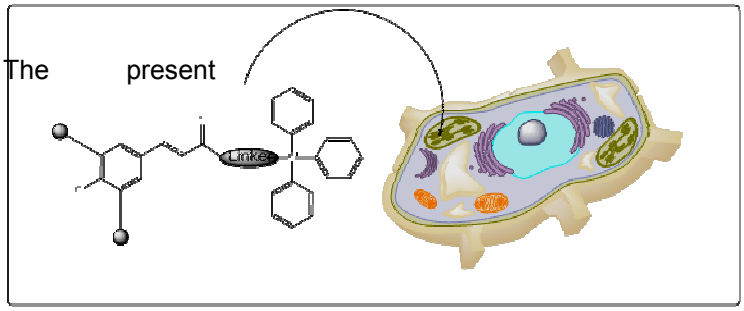
Mitochondrial oxidative damage contributes to a wide range of pathologies, including cardiovascular disorders and neurodegenerative diseases. Therefore, protecting mitochondria from oxidative damage should be an effective therapeutic strategy. However, conventional antioxidants have limited efficacy due to the difficulty of delivering them to mitochondria *in situ*.
Lipophilic monocations can pass through phospholipid bilayers and accumulate in negatively-charged compartments such as the mitochondrial matrix, driven by the membrane potential. This property is used to visualize mitochondria, to deliver therapeutic molecules to mitochondria and to measure the membrane potential. Such a principle was recently successfully employed by Murphy and coworkers to target antioxidants like CoQ or vitamin E to mitochondria. The conjugation of triphenylphosphonium cations with antioxidants and other probes to direct them selectively to mitochondria within cells and in vivo is a promising strategies and one such compound is now in phase II trials for Parkinson's disease. As well as promising potential therapeutic agents, these compounds are useful tools to explore mitochondrial oxidative damage.



The present project is related to the design and development of suitable delivery systems for hydroxycinnamic derivatives, harbouring positively charged functional groups at physiological pH, hence capable of mitochondrial accumulation. The natural models used for structural modification are present in the diet, a fact that validated the benefit of the chosen system. These compounds could be used as potent and selective agents in the treatment of neurodegenerative disorders, through specific targeting to mitochondria. The results obtained so far on the design, synthesis and characterization of a novel series of antioxidants with different linkage units will be presented in this communication.

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Borges, F., *et al.*, *National Patent 104560 E.*

Asymmetric Synthesis of Pyrroloisoquinolones from L-DOPA Derivatives

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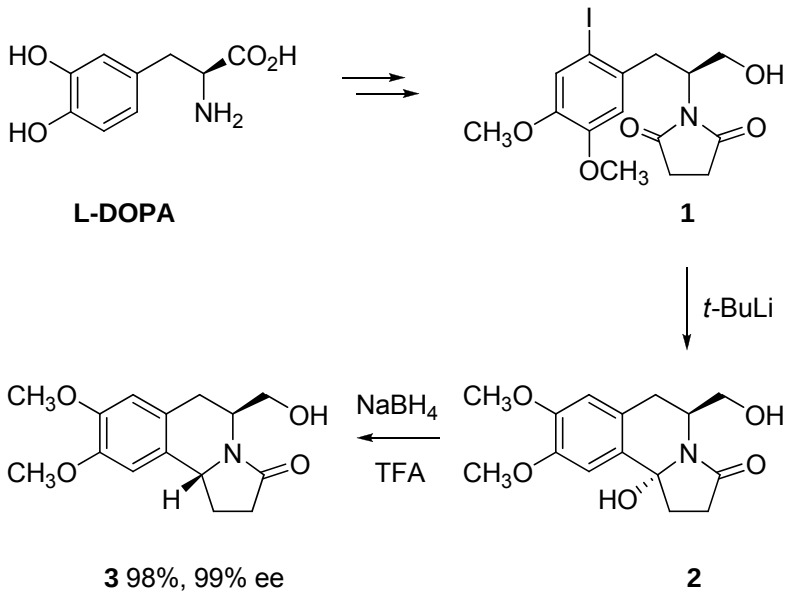
Pyrrolo[2,1-a]isoquinolines[1] are extremely interesting compounds due to their potential role in therapeutics for the central nervous system related disorders. This is attributed to their dual H_3 antagonist/serotonin transporters inhibitor activity.[2] Because of their biological activity, these alkaloids are also regarded as promising muscarinic agonist, antiplatelet, and antitumor agents. The importance of these nitrogen heterocycles is further enhanced by their utility as advanced key intermediates for the synthesis of more complex alkaloids.[3] For several years we have investigated new methods for the synthesis of nitrogen heterocycles, based on diastereoselective Parham cyclization, and intramolecular a-amidoalkylation reactions. Through this effort we were able to achieve a stereodivergent approach for synthesis of the enantiopure C-10b substituted 5,6-dihydropyrroloisoquinolones.[4]

Herein we report the asymmetric synthesis of pyrrolo[2,1-a]isoquinolines *via* a Parham-type cyclization of the corresponding iodinated *N*-phenethylsuccinimide followed by reduction of the so-obtained fused isoquinolones, which are immediate precursors of bicyclic *N*-acyliminium ions. Iodinated *N*-phenethylsuccinimide was prepared after several transformations on L-DOPA which is widely used in the clinical treatment of Parkinson's disease and dopamine-responsive dystonia. Thus, pyrroloisoquinoline (5*S*,10b*R*)-**3**[5] was obtained as a single diastereomer in good yield and high enantiomeric purity, determined by CSP HPLC.



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Dipeptidyl-peptidase IV (DPPIV/CD26)-activated prodrugs of hydroxy-containing compounds: a tripartate approach

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The lymphocyte surface glycoprotein dipeptidyl-peptidase IV enzyme (DPP-IV), also known as CD26, belongs to a group of atypical serine proteases preferentially cleaving X-Pro (or X-Ala) dipeptides from the N-terminus of a variety of natural peptides. We have developed an entirely novel enzyme-based prodrug approach that designs conjugates of therapeutic agents with a di- (or oligo) peptide moiety as a carrier, wherein the conjugate [peptide]-[drug] is specifically cleavable by the endogenous DPP-IV/CD26.[7] The approach was applied to a variety of drugs containing a free amino group that was directly coupled with the carboxyl group of amino acids via an amide bond (*bipartate prodrug*).[2] With these conjugates it was possible to modulate the hydrolysis rate (half-life) and the physicochemical properties of the compounds by modifying the nature and length of the peptide (di- or tetrapeptides) moiety.

Recently, we expanded, for the first time, our prodrug strategy to an hydroxy-containing compound such as the highly potent and selective bicyclic nucleoside analogue (BCNA) inhibitor of Varicella Zoster Virus (VZV), named Cf1743,[3] which exhibit a very low water solubility and poor oral bioavailability. Several of the synthesized prodrugs that contain a dipeptide moiety (cleavable by DPPIV/CD26), an heterobifunctional connector [released by chemical or enzymatic (esterase-catalysed) hydrolysis of the ester bond] and the drug (*tripartate prodrugs*) showed a high improvement in water solubility together with an efficient conversion to the biologically active parent drug in the presence of DPPIV/CD26 and in bovine, murine and human serum.[4]

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

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Synthesis of novel pyridazinone analogues as antiplatelet and vasorelaxant agents

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Pyridazinone derivatives show important pharmacological properties, particularly on cardiovascular system. Thus, several pyridazinone analogues have been reported as selective antiplatelet agents and with vasorelaxant activity and cardiotonic properties.[1] Previously, we have developed a series of 6- and 2,6-substituted pyridazin-3(2*H*)-ones with vasorelaxant and selective platelet antiaggregatory activities.[2] The promising pharmacological results obtained have prompted us to design new derivatives increasing the structural variability of the pyridazinone core in order to carry out more exhaustive structure activity relationship studies.

Thus, we describe the synthesis of new 5,6-disubstituted and 2,5,6-trisubstituted pyridazin-3(2*H*)-ones (Figure 1). The synthetic strategy developed to build the pyridazinone nucleus is based on the oxidation of alkyl furans with singlet oxygen to give 4-methoxy or 4-hydroxy butenolides, which are suitable for reaction with hydrazine or methyl hydrazine. Similarly, bicyclic pyridazinones are synthesized using the appropriate lactone which is obtained via an intramolecular Michael addition on the corresponding 4-methoxibutenolide.[3] Finally, standard procedures allow us to obtain the pyridazinone derivatives with the desired functionality. All compounds synthesized were tested as antiplatelet and vasorelaxant agents and their pharmacological data will be discussed.

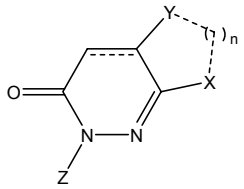

Figure 1. Pyridazinone derivatives synthesized

Ackowledgements
We acknowledge the Xunta de Galicia (IN845B-2010/020) for the financial support. P.B. thanks the Xunta de Galicia for an Isidro Parga Pondal contract and T.C. thanks the Universidade de Vigo for a predoctoral contract.

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Synergistic effect of lactoferricin (17-30) and ethambutol against *Mycobacterium avium* growing inside macrophages

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Once thought to be a solved problem, the treatment of bacterial infections is currently a major human health concern. The increasing levels of bacterial resistance to the existent antibiotics, together with the lack of any new effective antibacterial compounds in several decades, poses a great challenge on the development of alternative therapies.

Antimicrobial peptides (AMPs) are present in almost all living organisms, as part of their immune system. A great variety of AMPs have been discovered from different sources, varying in length, sequence and structure. The mode of action of AMPs is still under debate, although in most cases they are thought to act primarily by membrane disruption, by a variety of mechanisms, although the concomitant presence of internal targets has also been proposed. AMPs have the capacity to directly kill bacteria, and other microorganisms, but the possibility of their use together with conventional antibiotics, acting as adjunct agents, leading to a synergistic effect deserves detailed study.

Mycobacterium avium causes disseminated disease in immunocompromised hosts, especially in AIDS patients. The fact that these bacteria are intracellular, residing mainly in macrophages, and have an extremely complex and highly impermeable cell wall, contributes to the poor action of the antibiotics in use. Current therapy consists of a combination of antibiotics taken for at least six months, with no more than 60% of overall clinical success. Furthermore, mycobacterial antibiotic resistance is increasing worldwide, urging the need to develop novel classes of antimicrobial drugs.

In this context, we have been studying the effect of lactoferrin-derived antimicrobial peptides against *M. avium,* both alone and in combination with conventional antibiotics. We studied one AMP derived from bovine lactoferrin, (LFcin 17-30), which significantly inhibits the axenic growth of *M. avium,* although it is not effective against *M. avium* growing inside macrophages. However, when this peptide is combined with ethambutol (ETH), an antibiotic which is also not effective alone against *M. avium* growing inside macrophages, there is a synergistic effect, suggesting that the AMP increases the access of the antibiotic to the mycobacteria, when they are inside its host cell.

We have also studied the effect of LFcin 17-30 in model membranes of POPE/POPG (3:1) by Differential Scanning Calorimetry (DSC), and the results showed that the peptide significantly perturbs the gel-to-liquid crystalline transition of the liposomes.

Altogether, our data indicate that LFcin 17-30 acts as an antimicrobial peptide alone in axenic cultures, probably by attacking the bacterial membrane, as our DSC studies indicate. This effect can also contribute to the observed combined efficacy with ethambutol inside macrophages, by facilitating its membrane crossing.

The exploration of the route of synergistic effects between AMPs and conventional antibiotics seems to be a very promising road to increase the efficacy of antimicrobial chemotherapy against mycobacteria.

Development of Novel Lipophilic Antioxidants Based on Ferulic Acid

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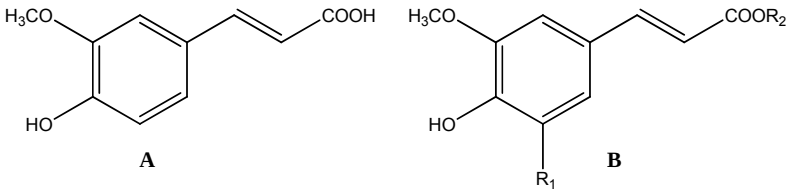
The development of new antioxidant entities is an increasingly important research area in the field of Medicinal Chemistry. Oxidative damage induced by reactive oxygen and nitrogen species (ROS and RNS, respectively) is inherent to inflammation processes, which in turn play a key role on a wide range of pathologies, from cancer to neurodegenerative diseases (ND). [1]

Phenolic acids are naturally occurring compounds that exhibit potent antioxidant activity by different mechanisms such as scavenging ROS and RNS, binding to pro-oxidant transition metals (mainly Cu and Fe) and inhibiting ROS/RNS-generating enzymatic systems.[2] The combination of these mechanisms hinders both the initiation and progression of free radical formation blocking or minimizing the oxidative damage cascade. Actually, epidemiological studies suggest an inverse relationship between dietary intake of phenolic antioxidants and the occurrence of diseases such as cancer and ND.[3]

Hydroxycinnamic acids like ferulic acid (Fig. 1A) are ubiquitary phenolic compounds, accounting for approximately one third of the phenolic compounds in our diet. Until the date, the majority of natural antioxidants studied have limited therapeutic success a fact that could be related with their limited distribution throughout the body and with the inherent difficulties to attain the target sites. So, if conditions are met to overpass the mentioned drawbacks these compounds can efficiently operate as potent exogenous antioxidants and in that way supplement the body's endogenous antioxidant defense systems.

As antioxidant activity is known to be strongly dependent on the compound's structural characteristics[4] a project was designed related to the development of novel ferulic acid derivatives (Fig 1B) with the aim to increase the lipophilicity, and the efficacy, of the natural compound. The overall structural modifications would enable a better diffusion across the membrane and, ultimately, better antioxidant activity. The results obtained so far will be presented in this communication.



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This work was supported by the Foundation for Science and Technology (FCT), Portugal (PTDC/QUI-QUI/113687/2009). A .Gaspar (SFRH/BD/43531/2008) thanks FCT grant.

Phosphotyrosine Electrochemical Oxidation Mechanism at a Glassy Carbon Electrode

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A protein tyrosine kinase (TK) is a kinase enzyme that modifies other proteins by chemically removing a phosphate group from adenosine triphosphate (ATP) and covalently attaching it to a tyrosine (Tyr) residue of the other protein [1]. This process named phosphorylation usually results in a functional change of the target protein. Any deregulation in this mechanism is considered a frequent cause of disease, particularly cancer.

Considering the importance and the need for the detection of protein phosphorylation process an electrochemical study of phosphotyrosine (PO_4-Tyr) has been carried out using cyclic, differential pulse and square wave voltammetry at a glassy carbon electrode. The oxidation of PO_4-Tyr occurs in a single irreversible process that involves the transfer of one electron and no proton, Fig. 1.

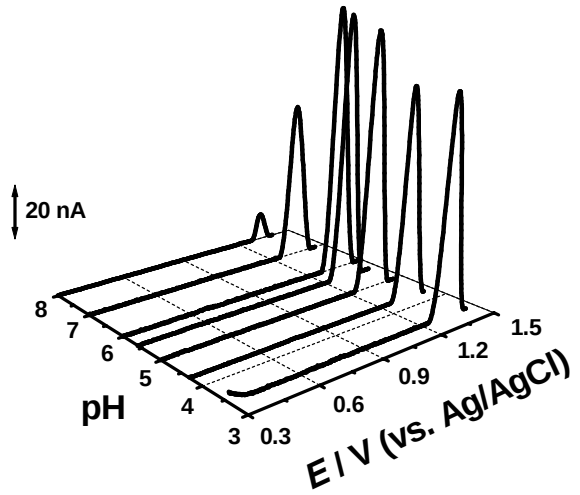

Fig. 1 – 3-D graph of differential pulse voltammograms in solutions of 100 μM PO_4-Tyr vs. pH of the supporting electrolyte

For acid and neutral electrolytes, the oxidation of PO_4-Tyr is at high positive potentials, close to oxygen evolution, and involves the hydroxylation reactions [2] of the aromatic ring of the Tyr moiety. These reactions occur at different positions on the aromatic ring. The formation of several oxidation products, that undergo reversible redox reactions in acid electrolytes, was observed. For electrolytes with pH > 7.0, the compound was chemically degraded in solution. Two consecutive charge transfer reactions of the chemically degraded compound were observed. For stronger alkaline electrolytes, pH > 10.0 the oxidation of PO_4-Tyr was not detected. A mechanism was proposed to explain the electrochemical oxidation of phosphotyrosine.

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New Approach for the Synthesis of Enantiomerically Enriched Alkaloids

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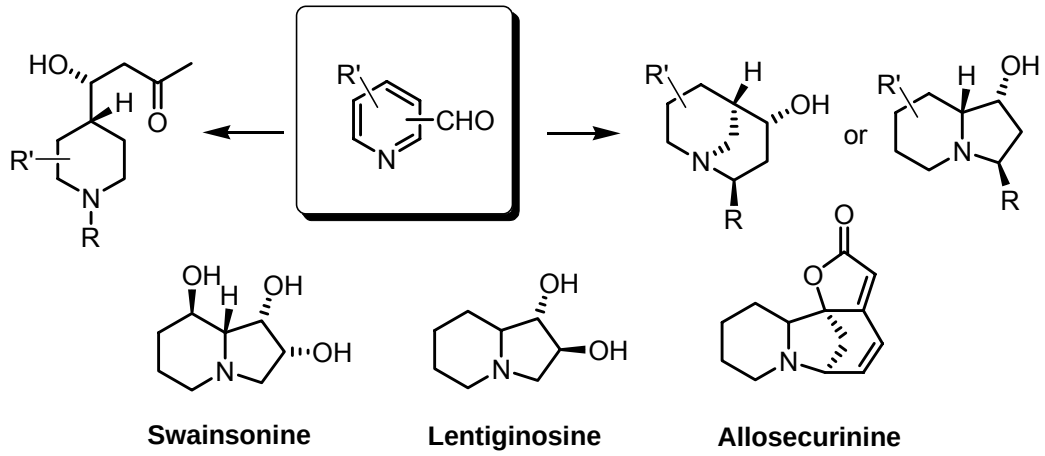
Cyclic aromatic and aliphatic amines are ubiquitary in nature. Most of the old and new pharmaceutically active compounds posses in their architecture at list one nitrogen atom which often is related with the biological activity of the molecule.[1] On the other hand nitrogen containing compounds are key molecules used as catalysts in a large number of organometallic chemistry processes and in organocatalysis. Due to their importance we have focused our attention on aromatic N-heterocycles and their use in medicinal chemisty as suitable starting material for the preparation of enantiomerically enriched alkaloids such as swainsonine[2] derivatives and allosecurinine precursors,[3] reducing the use of toxic solvents, complex procedures and limiting the use of transition metals.



Scheme 1

As a matter of fact we developed a two step synthesis that combine organocatalized-aldol reactions and enantio- diastereoselective hydrogenation processes characterized by high synthetic flexibility that allowed us to access to different molecular scaffolds which can be easily modified to our needs as described in the scheme 1.

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