1 a 3 Dezembro 2015

Congresso

química orgânica e química terapêutica

PORTO · PORTUGAL

"Thinking Organic and Medicinal Chemistry in an inspiring atmosphere"

INDEX

WELCOME

“Thinking Organic and Medicinal Chemistry in an inspiring atmosphere”

The Organic and Medicinal Chemistry divisions of the Portuguese Chemical Society (SPQ) would like to express a warm welcome to the 11th ENQO, taking place from december 1-3 in Porto, Portugal. For the first time under the auspices of SPQ, the competences of these two important fields of Chemistry are brought together into a single event, to highlight complementarities and to promote new synergies.

The Scientific Committee has put high expectations on the excellence of the scientific program, which includes plenary/keynote lectures from renowned scientists whose work has been an inspiration for researchers in Organic and Medicinal Chemistry. Oral communications will focus on topics from the following main research fields:
Organic Synthesis
Spectroscopic Methods (in Organic Chemistry)
Organic Natural Compounds
Drug Metabolism and Disposition
Beyond Small Molecules
Computational Methods and Drug Design
Antitumor and Anti-infective Drugs
Industrial Applications
This meeting is expected to bring together researchers with different expertise and perspectives, from senior to young scientists, to discuss and share their latest achievements in a stimulating environment, taking advantage of the inspiring atmosphere of “Teatro Municipal Campo Alegre”, a theatre that is a cultural landmark of Porto.

We warmly invite you to come and enjoy the meeting and our beautiful city!

VICTOR DE FREITAS
Conference Chairman

11º ENCONTRO NACIONAL DE QUÍMICA ORGÂNICA
4º ENCONTRO DE QUÍMICA TERAPÊUTICA
1 A 3 DEZEMBRO DE 2015

Teatro Municipal Campo Alegre
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PROGRAM

	TUESDAY, **DEC. 1ST**		WEDNESDAY, **DEC. 2ND**		THURSDAY, **DEC. 3RD**	
08:30 - 09:00	**REGISTRATION**					
09:00 - 09:15			**QO PL1: Jonathan Clayden** Universidade de Manchester, UK *Chair persons: José Cavaleiro & Patricia Valentão*		**QO PL2: Thomas Carell** Universidade de Munique, Alemanha *Chair persons: Teresa Pinho e Melo & Maria João Araújo*	
09:15 - 09:30	**OPENING CEREMONY**					
09:30 - 09:45						
09:45 - 10:00	**QT PL1: Alessio Ciulli** Universidade de Dundee, UK *Chair persons: Artur Silva & Victor Freitas*		OC-9	OC-10	OC-20	OC-21
10:00 - 10:15			OC-11	OC-12	OC-22	OC-23
10:15 -10:30			OC-13	OC-14	OC-24	
10:30 - 11:00	Coffee break		Coffee break		Coffee break	
11:00 - 11:30	**QO KN1: Francesco Nicotra** Universidade de Milano-Bicocca, Itália *Chair persons: Fernanda Proença & Artur Silva*		**QT KN3: Rui Brito** Universidade de Coimbra, Portugal *Chair persons: Emília Sousa & Pedro Alexandrino*		**QT KN4: Matilde Marques** IST, Universidade de Lisboa, Portugal *Chair persons: Madalena Pinto & Amélia Rauter*	
11:30 - 11:45	OC-1	OC-2	MC-12	MC-13	MC-19	MC-20
11:45 - 12:00	OC-3	OC-4	MC-14	MC-15	MC-21	MC-22
12:00 - 12:15	OC-5	OC-6	MC-16	MC-17	MC-23	MC-24
12:15 - 12:30	OC-7	OC-8	MC-18		MC-25	MC-26
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14:00 - 14:30	**QT KN1: Hélder Santos** Universidade de Helsínquia, Finlândia *Chair persons: Luisa Sá e Melo & Paula Gomes*		**QO KN3: Yves Génisson** Universidade de Toulouse, França *Chair persons: Paula Branco & Diana Pinto*		**QT PL2: Marco Contelles** Universidade de Madrid, Espanha *Chair person: Paula Andrade*	
14:30 - 14:45	MC-1		OC-15	OC-16		
14:45 - 15:00	MC-3	MC-4	OC-17	OC-18	MC-27	
15:00 - 15:15	MC-5	MC-6	OC-19		MC-28	
15:15 - 15:45	**QT KN2: Tiago Rodrigues** ETH Zurich, Suiça *Chair persons: Carlos Afonso & Matilde Marques*		**QO KN4: Amparo Faustino** Universidade de Aveiro, Portugal		**QO KN2: Enrique Borges** Universidade do Porto, Portugal *Chair person: Luisa Vale*	
15:45 - 16:45	Coffee break and poster session 1		Coffee break and poster session 2		**CLOSING CERIMONY**	
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17:00 - 17:15	MC-9	MC-10				
17:15 - 17:30	MC-11					
17:30 - 18:30	MEETINGS OF THE SPQ'S ORG CHEM DIVISION AND MED CHEM GROUP		PORTUGUESE AWARD FOR BEST YOUNG ORGANIC CHEMIST 2015			
18:30 - 19:00	**WELLCOME RECEPTION**					
20:00			**CONFERENCE DINNER**			

SCIENTIFIC PROGRAM

OC: ORGANIC CHEMISTRY

Organic Synthesis
Spectroscopic methods (in OC)
Organic Natural Compounds
Industrial applications

PLENARY

JONATHAN CLAYDEN
9h45-10h30, Wednesday, Dec. 2nd
Conformational communication: design and synthesis of membrane-bound receptor mimics
School of Chemistry - Univ. of Bristol, Bristol, England, UK

THOMAS CARELL
9h00-9h45, Thursday, Dec. 3rd
DNA Bases Beyond Watson and Crick
Univ. Munich, Munich, Germany

KEYNOTE

FRANCESCO NICOTRA
11h00-11h30, Tuesday, Dec. 1st
Functionalized Nanoparticles for Alzheimer's Disease Treatment
Univ. Milano-Bicocca, Milan, Italy

YVES GÉNISSON
14h00-14h30, Wednesday, Dec. 2nd
New bio-inspired alkynylcarbinol pharmacophores for cytotoxicity
Univ. Toulouse, Toulouse, France

ORGANIC CHEMISTRY

KEYNOTE

AMPARO FAUSTINO

15h15-15h45, Wednesday, Dec. 2nd

Synthesis of porphyrin derivatives with antitumoral and antimicrobial activity

Univ. Aveiro, Aveiro, Portugal

ENRIQUE BORGES

15h15-15h45, Thursday, Dec. 3rd

Synthesis of secosteroidal VDR-Ligands with Alkylidene Groups at the D-ring

Univ. OPorto, Oporto, Portugal

SCIENTIFIC PROGRAM

MC: MEDICINAL CHEMISTRY

Drug Metabolism and Disposition
Beyond Small Molecules
Computational Methods and Drug Design
Antitumor and Anti-infective Drugs

PLENARY

ALESSIO CIULLI
Alessio Ciulli
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Univ. Dundee, Scotland, UK

JOSÉ LUÍS MARCO CONTELLES
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CSIC, Madrid, Spain

KEYNOTE

HÉLDER SANTOS
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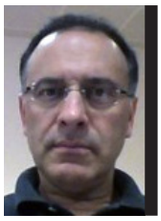
Univ. Helsinki, Finland

TIAGO RODRIGUES
15h15-15h45, Tuesday, Dec. 1st
Multi-objective de novo design of chemical probes and drug leads
ETH Zurich, Zurich, Switzerland

MEDICINAL CHEMISTRY

KEYNOTE



RUI BRITO

11h00-11h30, Wednesday, Dec. 2nd

Looking for a needle in a haystack with the right tools:
the discovery of potent transthyretin amyloid inhibitors

Univ. Coimbra, Coimbra, Portugal

MATILDE MARQUES

11h00-11h30, Thursday, Dec. 3rd

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Univ. Lisbon, Lisbon, Portugal

MEDICINAL CHEMISTRY + ORGANIC CHEMISTRY

KEYNOTE

PL. PHIL JONES

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European Lead Factory

European Lead Factory: Creating New Opportunities for Drug Discovery

ORAL COMMUNICATIONS - LIST

ORGANIC CHEMISTRY

OC-1 Margarida Gomes de Figueiredo
Faculdade de Ciências e Tecnologias da Universidade Nova de Lisboa
Synthesis and reactivity of 2-methyl-azolium derivatives

OC-2 Hélio Miguel Teixeira Albuquerque
University of Aveiro
Recent developments in the synthesis of novel xanthone-1,2,3-triazole dyads

OC-3 Luís Alexandre Almeida Fernandes Cobra Branco
LAQV-REQUIMTE
Organic Superbases as useful tools for synthesis and applications

OC-4 Marta Pineiro Gómez
Coimbra University

Sustainable synthesis of dihydropyrimidine-2(1H)-thiones under mechanical action

OC-5 Ana Lúcia Cabral Cardoso Lopes
University of Coimbra
Exploring the Reactivity of Novel Tetrazol-5-yl-Allenes for the Synthesis of Tetrazolyl-Heterocycles

OC-6 Luísa da Conceição Costa Rainho de Carvalho
LAQV@REQUIMTE
Chitobiose modification: a fast forward approach to attain relevant disaccharides

OC-7 Filipa Alexandra Delgado Siopa
iMed.ULisboa/FFUL
Ring-opening of alfa-hidroxy-cyclopentene-aziridines in water under mild conditions

OC-8 Elina Marinho, M. Fernanda Proença
Universidade do Minho
The search for new antipsychotic compounds incorporating the N-methyl piperazine nucleus

OC-9 Diana Cláudia Gouveia Alves Pinto
Universidade de Aveiro
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OC-10 João Guilherme Louçano Domingues Gomes
University of Minho
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OC-11 Joana Lia Cardoso de Sousa
University of Aveiro
3-Bromochromones as Building Blocks of Novel Furan and Cyclopropane Derivatives

OC-12 Daniela Andreia Dias Batista
Faculdade de Ciências, Universidade de Lisboa
Pyranosyl 6´-isonucleosides: synthesis and biological profile

OC-13 Jaime Alfredo da Silva Coelho
University of Lisbon
Synthesis of Symmetric Triarylmethanes Bearing Secondary Anilines

OC-14 Olga Yelenich
Chernivtsi National University
The mechanism for the electroanalytic action of triazolic derivatives of Conducting polymers and its mathematical representation

OC-15 Ashly Tania da Cruz Rocha
Universidade do Minho
New pyrimido[5,4-d]pyrimidines with enhanced anticancer activity on colorectal cancer cells: synthesis, SAR study and mechanism of action

OC-16 Carla Sofia da Palma Grosso
Universidade de Coimbra
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OC-17 Sara Martinho Almeida Pinto
University of Coimbra
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OC-18 João Filipe Seco Martins Marques Neves
Faculdade de Farmácia da Universidade do Porto
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OC-19 Saúl Alves Graça da Silva
ITQB-UNL
Enzymatic resolution of cyclic 4-hydroxy-acylaziridines

OC-20 Mariana Nunes Barbosa
REQUIMTE/LAQV
UHPLC-QqQ-MS/MS method for phytoprostane profiling in macroalgae

OC-21 Cristiany Barros Ludwig
Centro Paula Souza
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OC-22 Pedro Nuno da Costa Leão
University of Porto
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OC-23 Diogo Henrique Correia Matias
Universidade Lusófona de Humanidades e Técnologias
Bioactive abietane diterpenes from Plectranthus spp. extracts and its encapsulation into a novel phytosomal formulation

OC-24 Carlos Miguel Calisto Baleizão
Instituto Superior Técnico
Perylenediimides: ³a la carte² fluorescence

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IPATIMUP/i3S
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MC-2 Filipa Andreia Cardoso Carneiro
iMed.ULisboa
1,3,5-Triazole-Benzene Derivatives for Cancer Therapy: Synthesis and G-Quadruplex Stabilization

MC-3 Maria Manuel Duque Vieira Marques dos Santos
iMed.ULisboa, Faculdade de Farmácia, Universidade de Lisboa
Spirooxadiazoline oxindoles with promising in vitro antitumor activities

MC-4 Mariana Alves Reis
imed.ULisboa- Faculdade de Farmácia - Universidade de Lisboa
Surpassing multidrug resistance in cancer: a study on jolkinol D derivatives

MC-5 Alice Maria Esteves Dias
University of Minho
Synthesis and anticancer activity of N3-substituted-6,8-diaminopurines

MC-6 Abigail Filipe Ferreira
FCUP & REQUIMTE
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MC-7 Maria Manuel Cruz Silva
Faculty of Pharmacy
Cholesterol and Cancer. Exploring the Chemical Space of Oxysterols to Find New Drug Candidates

MC-8 Roberta Paterna
iMed
Synthesis and biological evaluation of 3-Hydroxyquinolin-2(1H)-ones derivatives as antitumor agents

MC-9 Miguel Maurício Machado dos Santos
LAQV - REQUIMTE
Pharmaceutical Ionic Liquids and Salts as antitumor agents

MC-10 Cláudia Marisa Fernandes Braga
Faculdade de Farmácia
Hybrid compounds for the treatment of glioma: a new approach

MC-11 Cláudia Sofia Santos Bessa
Faculdade de Farmácia da Universidade do Porto
Roy-Bz: the first small molecule selective activator of protein Kinase Cdelta

MC-12 Helder João Ferreira Vila-Real
IBET
Development of brain permeant peptidomimetic beta-secretase inhibitors forAlzheimer¹s disease

MC-13 Diogo Maria Trindade Fonseca Magalhaes e Silva
Faculdade de Ciencias da Universidade do Porto
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MC-14 Renato Joel Barros Pereira
REQUIMTE/LAQV
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MC-15 Filipa João Fernandes Ramilo Gomes
Centro de Química Estrutural - Instituto Superior Técnico - Universidade de Lisboa
Design, synthesis and biological evaluation of novel anti-bacterial agents

MC-16 Clara Grosso
REQUIMTE/LAQV
Herbal medicines: a source of phenolic monoamine oxidase A inhibitors

MC-17 Joana Oliveira Gama Soares
Faculdade de Farmácia Universidade do Porto
SLMP53-1: a new reactivator of mutant p53 with potent in vivo antitumor activity

MC-18 João Paulo Martins Ferreira Lavrado
Research Institute for Medicines (iMed.ULisboa)
G-quadruplex stabilisation by novel indolo[3,2-c]quinolines: a structural analysis of binging

MC-19 Catarina Alexandra Baptista Rodrigues
Faculadade de Farmácia - Universidade de Lisboa
Antimalarial activity of s-Triazine based hybrids in both erythrocytic and liver stages.

MC-20 José Fernando Xavier Soares
Faculty of Pharmacy, University of Porto
Lessons from the study of the binding mechanism of ³hit² compounds to albumin: fluorescence and in silico experiments applied to xanthone derivatives

MC-21 David Alexandre Micael Pereira
REQUIMTE/LAQV
ER stress and protein quality control pathways: Exploring the natural products chemical space

MC-22 Ricardo José Diogo Grácio Ferreira
Research Institute for Medicines (iMed.ULisboa)
Wrapping it all around: in silico approaches to improve the MDR-reversal properties of the macrocyclic diterpenic core

MC-23 Alexandra Maria Moita Antunes
Instituto Superior Técnico
The anti-HIV drug Rilpivirine: covalent adducts with amino acids and proteins

MC-24 José Carlos Ferraz Caetano
Faculdade de Ciências - Universidade do Porto
Interaction of Xanthone with Double Stranded DNA A Contribution for Xanthone Derivative Drugs

MC-25 Maria de Fátima Azevedo Brandão Amaral Paiva Marti
Faculty of Sciences, University of Porto
Synthesis of Phenolic Compounds Sulfate Metabolites

MC-26 Pedro Santos Gonçalves
iMed.ULisboa
Torin-based compounds as inhibitors against trypanosomatid parasites

MC-27 Daniela Sofia Almeida Ribeiro
ICETA/REQUIMTE/FFUP
Flavonoids[1] effects in proinflammatory signaling systems: in vitro structure/activity studies

MC-28 Diego Mendes Ferreira
Faculdade de Medicina da Universidade de São Paulo - FMUSP
Analysis of the Harmful Characteristics of the use of Mannitol ($C_6H_{14}O_6$) in Bags of Red Cell Concentrates

PORTUGUESE AWARD FOR BEST YOUNG ORGANIC CHEMIST 2015

Carlos Miguel Calisto Baleizão
FCT investigator, Instituto Superior Técnico, Universidade de Lisboa
The ORGANIC flavour in a materials CHEMISTRY journey

Maria Manuel D. V. Marques dos Santos
FCT investigator, Instituto Superior Técnico, Universidade de Lisboa
Organic chemistry: an important tool in drug discovery

Nuno Manuel Xavier
FCT investigator, Faculty of Sciences, University of Lisbon.
Efficient Synthetic Routes for New N-Glycosyl Compounds Containing Glucuronic Acid and Glucuronamide-based Moieties

LECTURES

Conformational communication: design and synthesis of membrane-bound receptor mimics

Jonathan Clayden

School of Chemistry, University of Bristol, Cantock's Close, Bristol BS8 1TS, UK

Email: j.clayden@bristol.ac.uk

Biology solves the problem of communicating information through cell membranes by means of conformationally switchable proteins, of which the most important are the G-protein coupled receptors (GPCRs).[1] We have explored the possibility of using synthetic foldamers as artificial mimics of GPCRs, with the ultimate aim of controlling function in the interior of an artificial vesicle. Inspired by the structures of the peptaibols—membrane-active fungal metabolites—we use foldamers built from 2-aminoisobutyric acid (Aib) as artificial mimics of GPCRs, showing that induced conformational preferences are reliably propagated in solution.[2] These global conformational preferences can relay stereochemical effects over extraordinarily long ranges, mediating for example 1,61 remote stereocontrol.[3]

Biological receptors adjust their conformation in response to non-covalent interactions with ligands. Using competitive boronate ester formation, ion pairing, hydrogen bonding, and metal-ligand interactions (Fig 1) it is possible to induce the communication of information through foldamer-based receptor mimics.[4,5] The construction an artificial GPCR requires molecules that will incorporate themselves into a membrane, and also necessitates the extension of solution state analytical tools[6] to the study of conformation in the membrane phase. Methods employing the tools of solid-state ^{19}F NMR and of fluorescence spectroscopy will be described, along with their application to the development of functioning membrane-bound switchable GPCR and photosensitive rhodopsin mimics.

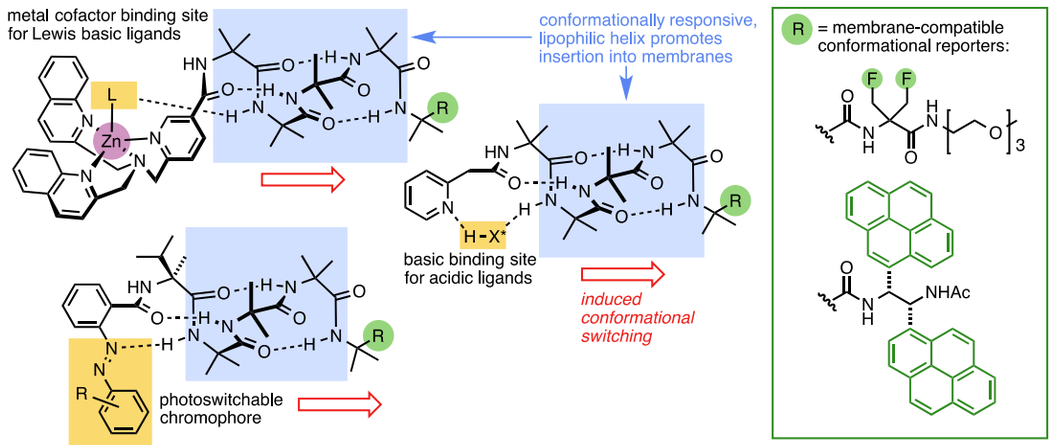

Figure 1: Alternative designs for functional mimics of membrane-bound receptors.

Acknowledgements: We thank the European Research Council (Advanced Grant ROCOCO), EPSRC and BBSRC for support

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DNA Bases Beyond Watson and Crick

Thomas Carell

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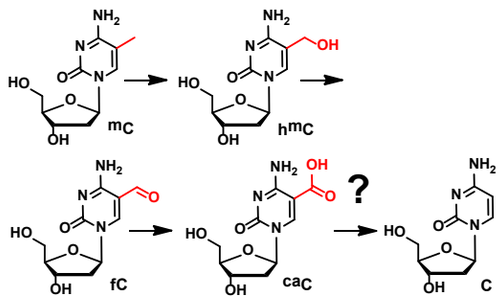
Epigenetic information is stored in the form of modified bases in the genome. The positions and the kind of the base modifications determines the identity of the corresponding cell. Setting and erasing of epigenetic imprints controls the complete development process starting from an omnipotent stem cells and ending with an adult specialized cell. I am going to discuss the latest results related to the function and distribution of the epigenetic marker bases 5-hydroxymethylcytosine (hmC), 5-formylcytosine (fC), 5-carboxycytosine (caC) and 5-hydroxymethyluracil (Scheme 1).[1] These nucleobases control epigenetic programming of stem cells and some of these bases are also detected at relatively high levels in brain tissues. Synthetic routes to these new bases will be discussed that enable today preparation of oligonucleotides containing the new bases. The second part of the lecture will cover mass spectroscopic approaches to decipher the biological functions of the epigenetic bases. In particular, quantitative mass spectrometry, new covalent-capture proteomics mass spectrometry and isotope tracing techniques will be discussed, which allow us to unravel the chemistry in stem cells and the protein networks that are controlled by epigenetic modifications.



Scheme 1: Depiction of the new epigenetic bases.

References:

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Functionalized Nanoparticles for Alzheimer's Disease Treatment

Francesco Nicotra*, Massimo Masserini, Francesca Re, Barbara La Ferla, Cristina Airoldi

Center of Nanomedicine, University of Milano-Bicocca, P.zza della Scienza 2 ,20126, Milano, Italia

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Alzheimer's disease is associated with the formation of fibrils and plaques in the neuronal network, resulting in widespread synapsis loss and neurodegeneration. The fibrils and plaques are formed by aggregation of the strongly fibrillogenic Aβ-peptides generated by abnormal cleavage of a protein defined amyloid precursor protein.

In a large integrated project, founded by the FP7 program, we generated nanoparticles properly functionalized at the surface, exploiting different chemoselective approaches. Ligands of Aβ peptides[1] and molecules favouring the transport through the blood brain barrier, have been selected or designed, synthesised and conjugated to the nanoparticles.[2,3]

The interaction with Aβ-peptides and the capacity to perform defibrillation of the ligands and the ligand-functionalized nanoparticles was studied with different methods including SPR and NMR.

In vivo studies showed the capacity of liposomes bi-functionalized with a peptide favouring the transport through the blood brain barrier and a ligand of Aβ-peptides, to ameliorate memory impairment in Alzheimer's disease mouse models.

Acknowledgements: The research leading to these results has received funding from the European Community's FP7/2007-2013 under grant agreement n° 212043 and will continue in the Horizon2020 MSCA-ITN-2014-ETN n° 642028.

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New bio-inspired alkynylcarbinol pharmacophores for cytotoxicity

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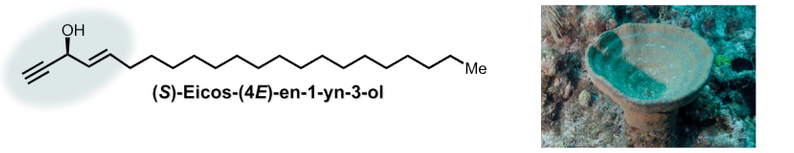
Chiral alkynylcarbinols have emerged as key structural fragments of numerous marine acetylenic lipids.[1] The (*S*)-configured asymmetric (4*E*)-ene-1-yn-3-ol unit is found at the termini of the carbon skeleton of the most cytotoxic metabolites. Many of those compounds display indeed significant antitumor activity, with IC_{50} down to the nanomolar range. Despite the isolation of more than 100 polyacetylenic metabolites from various sponge species, attempts at delineating structure-activity relationships has remained long time limited.



Cribrochalina vasculum marine sponge

Figure 1: Struture of an archetypical lipidic alkynylcarbinol derivative.

Thanks to synthetic chemistry methodological advances for the asymmetric alkynylation of ynals, we reported a precursory structural variation study based on an archetypical skeleton inspired from the cytotoxic metabolite (*S*)-eicos-(4*E*)-en-1-yn-3-ol.[2] Three pharmacophores were uncovered, among which the dialkynylcarbinol unit was the most synthetically accessible and the most potent. Moreover, a dramatic effect of the carbinol absolute configuration on the cytotoxicity was observed, the eutomers exhibiting an *R*-type configuration, opposite to the most abundant one in Nature. Extended structural modulations of these lipidic alkynylcarbinols further confirmed the relevance of the primary structure-activity trends.[3] We also reported the bi-directional syntheses of C_2-symmetrical lipids embedding two homochiral terminal alkynylcarbinol fragment allowing the first asymmetric synthesis of the marine double-headed acetylenic lipid (3*R*,4*E*,16*E*,18*R*)-icosa--4,16-diene-1,19-diyne-3,18-diol.[4] Two synthetic analogues embedding the newly identified (*S*)-dialkynylcarbinol pharmacophore were also secured using the Carreira's asymmetric alkynylation procedure adapted to ynals. The dramatic impact of the carbinol configuration on cytotoxicity was confirmed, with submicromolar IC_{50} values in the eutomeric series. Finally, the reactivity of the terminal alkyne moiety of an ω-functionalized analogue was exploited by means of "click chemistry" for cellular fluorescent labeling studies, opening prospects for the target identification of these antitumor acetylenic lipids.[5]

Acknowledgements: D. L. was supported by the French Embassy in Kiev, Ukraine, and his investigations were performed within the framework of the 'Groupement Franco-Ukrainien en Chimie Moléculaire' (GDRI) funded by the CNRS.

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Synthesis of porphyrin derivatives with antitumoral and antimicrobial activity

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Porphyrins and analogues due to their unique physico-chemical features are finding applications in different fields like artificial photosynthesis, catalysis, sensors, nanomaterials and medicine. In medicine, these compounds are being used with high success as photosensitizers (PS) in Photodynamic Therapy to treat oncological and non-oncological situations like infections caused by microorganisms.[1] In this therapy, the photoactivation of the PS by visible light in the presence of molecular oxygen affords highly cytotoxic reactive oxygen species (ROS) that are responsible by the death of target cells (*e.g.* tumoral or microbial). Although the ability of a PS to generate ROS, namely singlet oxygen ($^1O_2$) is important for an efficient PDT effect, the structural feature of PS is another crucial aspect that is dependent on the target. Herein will be discussed some recent synthetic strategies developed in the group to obtain PS with adequate solubility in physiological media, to improve their selectivity to target tumoral cells or to photoinactivate microorganisms, to have better penetration on the tissue and also to allow their immobilization in solid supports(Figure 1)[2].

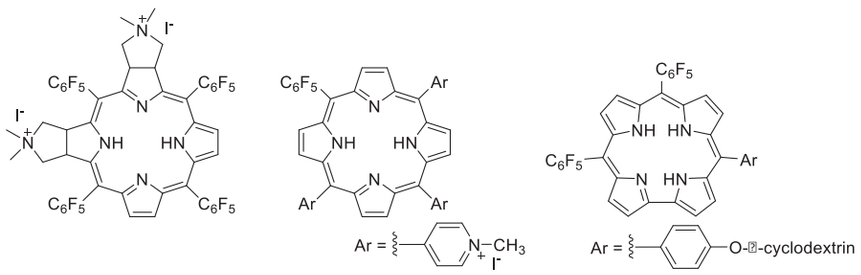

Figure 1: Tetrapyrrolic macrocycles with antitumoral or antimicrobial activity

Acknowledgements: Thanks are due to the University of Aveiro, Fundação para a Ciência e a Tecnologia (FCT, Portugal), European Union, QREN, COMPETE and FEDER for funding the QOPNA research unit (project PEst-C/QUI/UI0062/2013, FCOMP-01-0124-FEDER-037296).

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Synthesis of Secosteroidal VDR-Ligands with alkylidene groups at the D-ring

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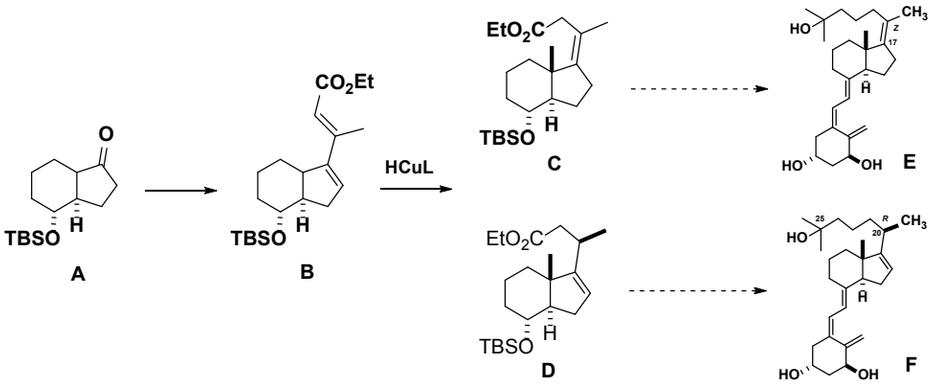
The hormone calcitriol (1,25D) through binding to vitamin D receptor (VDR) regulates a number of physiological and pathological processes, including cell proliferation and differentiation, which has led to the study of its effects in cancer. Significant antitumor activity (i.e. anti-proliferative effects, induction of apoptosis, stimulation of differentiation, and inhibition of invasion and metastasis) has been demonstrated for 1,25D in different types of cancers including breast, prostate, colon, and acute myeloid leukemia.[1] In fact, recent studies have established that VDR expression should be taken into account for breast cancer treatment.[1c]
In this study, we describe the design and the plan for the synthesis of two promising analogs of calcitriol, (17*Z)*-alkylidenic tetra-substituted analog (**E**) and (20*R)*-tri-substituted analog (**F**), modified at D-ring chain. The limiting step in this synthetic strategy is regio- and stereoselective reduction of chiral ester **B** to obtain the corresponding tetra-substituted olefin (**C**) and/or the tri-substituted olefin (**D**), from the same chiral ketone (**A**). (**scheme 1**)



Scheme 1: Synthetic strategy for the obtention of the promising analogs of calcitriol (**E** and **F**) from ketone **A**.

Acknowledgements: We thank the Fundação para a Ciência e Tecnolgia for financial support....

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Chemical biology approaches to target validation in the ubiquitin and chromatin systems

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This lecture will outline my laboratory's recent progress and current efforts at developing chemical tools to study and target complex molecular players in the ubiquitin-proteasome system (UPS) and epigenetics. These fundamental biological pathways are rich in potential drug targets identified by genetic or cell biology studies as important to human physiological and pathophysiology states, including cancer. However, most of these targets are still perceived as "undruggable" to conventional medicinal chemistry and remain to be fully validated chemically e.g. by means of high-quality and selective chemical probes, to truly enable the burgeoning opportunities for new therapeutics.

One such class of attractive yet challenging targets are cullin RING E3 ubiquitin ligase (CRL), multisubunit enzymes that impart substrate specificity to ubiquitination in the UPS.[1] CRL-targeting chemical tools can be used alone as E3 ligase inhibitors that modulate the pathway in which the specific CRL is involved. First, I will describe our discovery of potent inhibitors of the protein-protein interaction between the von Hippel-Lindau (VHL) CRL and its substrate protein HIF-1α using structure-guided design.[2] Mechanistic and cellular characterization of our VHL inhibitors provide the foundation for them to be widely used as new selective probes in the hypoxic signaling pathway. In addition, CRL-targeting ligands can be suitably conjugated with any protein ligands, yielding bifunctional proteolysis targeting chimeras (PROTACs) to hijack the UPS and induce the intracellular degradation of the target protein. I will illustrate how selective destruction of the epigenetic transcriptional regulator Brd4 was achieved by tethering the pan-selective BET bromodomain inhibitor JQ1 to a VHL ligand.[3] Our PROTAC molecule MZ1 induces rapid, time-dependent, long-lasting, and dose-dependent preferential removal of Brd4 over its homologous BET-family members Brd2 and Brd3 in cancer cells, leading to a more limited transcriptional response of MZ1 compared to JQ1, consistent with that of Brd4 RNAi.[3] This study provides proof-of-concept for inducing the selective degradation of any protein of interest using PROTACs.[4] In a separate study, we reported a general strategy to introduce controlled selectivity of BET bromodomain inhibitors via a bump-and-hole approach.[5] We developed compound ET, an ethyl derivative of JQ1, so that it can bind potently and with high selectivity (up to 540-fold) to a specifically-designed mutant bromodomain. We applied this method inside cancer cells to show that blockade of the first bromodomain alone is sufficient to displace Brd4 from chromatin.[5] Finally, I will outline how we are developing and optimizing this technology and applying it to address specific questions for BET-protein target validation.

Acknowledgements: I am grateful to many funding bodies for financial support, including the European Research Council (ERC), the UK Biotechnology and Biological Sciences Research Council (BBSRC), the European Commission Marie-Curie actions, the Wellcome Trust, and the Fundação para a Ciência e Tecnologia (FCT) amongst others.

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Multi-Target-Directed-Ligands for therapy of Alzheimer's Disease

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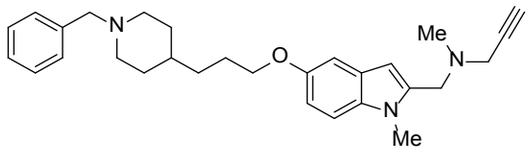
In this communication I will update our recent results on the synthesis and biological evaluation of new multipotent molecules derived from *N*-((5-(3-(1-benzylpiperidin-4-yl)propoxy)-1-methyl-1*H*-indol-2-yl)methyl)-*N*-methylprop-2-yn-1-amine (**ASS234**) (Figure 1),[1-6] able to inhibit ChE and MAO enzymes, and based on donepezil as reference molecule, for the potential treatment of Alzheimer's disease.



ASS234

Figure 1: Structure of MTDL **ASS234**.

Acknowledgements: JMC thanks MINECO (Government of Spain) for financial support (SAF2012-33304).

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Targeted Nanomedicines for Cancer Therapy

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Novel engineering technologies affords unprecedented advances toward long-elusive objectives of medical research. Personalized medicine responds to the basic but generally unattainable question of identifying the right therapy, reaching the right therapeutic target in the body at the right time, and securing immediate feedback as for its efficacy and undesired collateral effect. Nanotechnologies are of great interest in the context of the drive toward personalized medicine, and may prove to be the necessary catalyst for its large-scale implementation.

In this talk, I will present prominent biomaterials, such as porous silicon (pSi) as potential platforms for the individualization of medical intervention; these biomaterials are promising advanced drug delivery technologies for biomedical applications.[1-5] Emphasis will be given to the surface biofunctionalization of these biomaterials using advanced technologies, such as click-chemistry, etc. Examples on how these materials can be used to enhance the bioavailability of drug/peptide molecules, demonstrating their cytocompatibility, and *in vivo* biocompatibility and intracellular targeting (**Scheme 1**), will also be presented.

The recent cutting-edge advances on nanomaterials are anticipated to overcome some of the therapeutic window and clinical applicability of many drug/peptide molecules, and can also act as innovative theranostic platform and tool for the clinic, because they offer a less invasive alternative compared to the conventional therapeutic strategies and, thereby, enhancing the expectancy and quality of life of the patients.

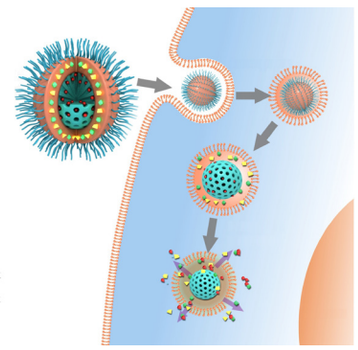

Figure 1: Intracellular targeting of chemical-modified nanoparticles for cancer therapy.

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Multi-objective de novo design of chemical probes and drug leads

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Computer-assisted de novo design is emerging as a disruptive technology in hit and lead finding programs.[1] De novo design methods have demonstrated their value for the tasks of scaffold-hopping, bioisosteric replacement, and fine-tuning of a candidate compound.[2] Herein I present ligand-based de novo design studies aiming at the discovery of innovative lead molecules for high value targets, using the state of the art software tools DOGS (Design Of Genuine Structures)[3] and MAntA (Molecular Ant Algorithm).[4] DOGS had been previously used for the discovery of new chemical entities targeting aurora A kinase,[5] human polo-like kinase-1,[6] and one of the most selective vascular endothelium growth factor receptor 2 (VEGFR-2) inhibitors known to date.[7] Furthermore, we designed new ligands for the adenosine, adrenergic and dopamine D4 receptors, presenting accurately predicted pKi values against a target panel, through multi-objective optimization.4, [8] Together with a brief overview over past efforts I present the automated synthesis of novel ligand efficient 5-HT2B receptor-selective ligands with predictable drug target panel affinities,[9] disclose novel Helicobacter pylori HtrA inhibitors and a tetrazole-based DAPK3 binding fragment.[10] The impact of these technologies in chemical biology and drug discovery will be discussed.

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Looking for a needle in a haystack with the right tools: the discovery of potent transthyretin amyloid inhibitors

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The aggregation of proteins into insoluble amyloid fibrils is the hallmark of many, highly debilitating, human pathologies such as Alzheimer's and Parkinson's diseases, or rare neurodegenerative diseases like Familial Amyloid Polyneuropathy (FAP). FAP is an amyloid disease caused by mutations in the protein transthyretin (TTR) and characterized by progressive peripheral and autonomic polyneuropathy, starting with loss of temperature and pain sensation on the lower limbs and evolving to severe autonomic dysfunction, usually resulting, if untreated, in the death of patients 10-15 years after the onset of the first symptoms.[1]

TTR is a homotetrameric plasma protein expressed in the liver. Thus, liver transplantation (LT) has been the standard treatment option for FAP for nearly two decades. LT halts progression of clinical symptoms by replacing the disease-associated mutant TTR gene by a wild-type gene. More recently, tafamidis meglumine (brand name Vyndaqel) reached the European and Japanese drug markets as the first drug therapy directed to the treatment of FAP. Tafamidis has shown that stabilization of the native tetrameric form of TTR by molecules endowed with chaperone-like activity is a viable approach to prevent (or at least stall) the formation of amyloid aggregates and fibrils, thus delaying disease progression. However, tafamidis demonstrated improvement of symptoms in only approximately 60% of the FAP patients.

Here, we report on successful efforts to discover new chemical entities (NCEs) with better activity profiles for TTR stabilization in human plasma, in particular in plasma of carriers of the most common amyloidogenic TTR mutations. Lead discovery and optimization was carried out *in silico* followed by experimental *in vitro*, *ex vivo* and *in vivo* validation.[2]

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Chemical mechanisms of drug toxicity – lessons from nevirapine

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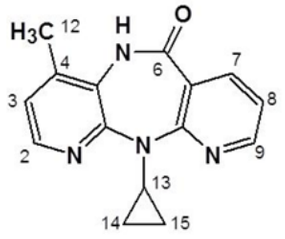
Nevirapine (**NVP**, **1**) is a non-nucleoside reverse transcriptase inhibitor (NNRTI) widely used in combined antiretroviral therapy and to prevent mother-to-child transmission of the human immunodeficiency virus type 1. NVP's clinical efficacy, along with low cost, favourable lipid profile, and suitability for use during pregnancy largely account for the widespread use of the drug.[1] Despite its benefits, NVP is associated with immune-mediated hepatotoxicity and skin rash, which can be fatal and are major causes of drug discontinuation. In addition, although direct correlation between NVP-based therapies and human cancer has yet to be demonstrated, long-term administration of the drug to rodent models resulted in increased incidences of hepatocellular adenomas and carcinomas;[2] moreover, epidemiological data indicated an association between the chronic use of NNRTIs and the occurrence of non-AIDS-defining cancers in HIV-positive patients.[3]



1. Nevirapine (NVP)

While the exact mechanisms underlying NVP-induced toxic events are still not fully understood, a considerable amount of work on the development of mass spectrometry-based tools to assess covalent NVP-protein and NVP-DNA adducts as biomarkers of metabolic activation has been conducted in recent years. The rationale for using these biomarkers is based on evidence that NVP is bioactivated to reactive metabolites able to modify biomacromolecules by forming covalent adducts that may trigger carcinogenic and immune-mediated processes.[4]

The current presentation will address our efforts to assess the contribution of benzylic (at C12) *versus* aromatic (at C2/C3) hydroxylation of NVP to the generation of electrophilic metabolites prone to bind biomacromolecules *in vitro* and *in vivo*. The ensuing implications to NVP's toxicity will be discussed.

Acknowledgements: Thanks are due to the Portuguese NMR and MS Networks (IST-UL nodes) for providing access to the facilities. This work was supported in part by Fundação para a Ciência e a Tecnologia (FCT), Portugal, through grants UID/QUI/00100/2013, RECI/QEQ-QIN/0189/2012 and RECI/QEQ-MED/0330/2012.

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The IMI European Lead Factory: New Opportunities For Drug Discovery

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The Innovative Medicines Initiative (IMI) European Lead Factory (www.europeanleadfactory.eu) is a major European project established with the aim of generating new lead structures for drug discovery projects in the public and private sectors. A Joint European Compound Library (JECL) has been established[1] by combining selections of compounds from participating pharma companies. This collection of approximately 300,000 compounds is being supplemented during the course of the project with upto 200,000 compounds designed and synthesised specifically for the purpose based on contributed ideas from around Europe.
Screening of the library and follow-up of resulting hits is carried out at the European Screening Centre based in Scotland and The Netherlands utilising state-of-the-art robotics for compound logistics and uHTS. Expert triaging and further characterisation of hits including medicinal chemistry is available to public programs (academic and SME based). The output is high quality hits that represent excellent starting points for drug discovery programs or tools for investigating and validating novel pharmacological approaches with the ultimate aim of creating benefit for patients.[2]
This talk will explain the background and current status of this project, describe key results and highlight opportunities to participate.

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ORAL PRESENTATIONS

Synthesis and reactivity of 2-methyl-azolium derivatives

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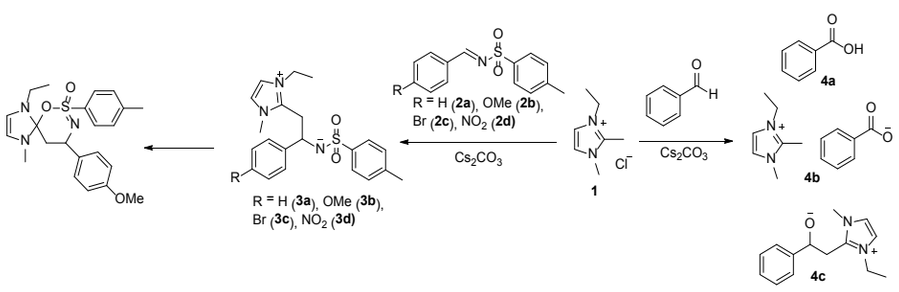
Azoles are a group of heterocyclic compounds with a high relevance in diverse areas of chemistry. Part of this group is composed by imidazolium derivatives that have been described as having biological and synthetic applications. Although the reactivity of 1,3-dialkyl-imidazolium salts has been thoroughly studied since derivatives have shown to have applications as ionic liquids[1] to *NHC* generating source[2], very little as been published concerning the reactivity of these imidazolium salts when functionalized at the C2 position[3]. This position has shown to be associated with some biological properties of azoles, and therefore is one of the most relevant substitution positions. The synthesis and reactivity of 2-methyl-imidazole derived salts was studied, in the presence of electrodeficient species, as imines and aldehydes.

When 1, 2-dimethyl-3-ethyl-imidazolium chloride (**1, Scheme 1**) was reacted with four different *N*-arylsulfonilimines (**2a-d**), in the presence of a base, arylethyl-2-imidazolium-1-tosilamides (**3a-d, Scheme 1**) were obtained. These compounds showed pronounced stability in acid and basic conditions. However, when heated it results, among other compounds, in a postulated isomer with a cyclic structure. The reaction with aromatic aldehydes showed a different reactivity. Although the transient identification of the addition product (**4c, Scheme 1**) was detected in some assays, the major product in the reaction with benzaldehyde (**4, Scheme 1**) was benzoic acid **4a**. This compound was identified in the acid form (**4a**) and under the form of a 1,2-dimethyl-3-ethyl benzoate salt (**4b**). No reduced product was until the moment possible to collect and identify. The mechanism behind this oxidation reaction is still under study.



Scheme 1: Schematic representation of the reactions involving 1,2-dimethyl-3-ethyl-imidazolium (**1**), with electrodeficient substracts, as imines (**2**) and aldehydes.

Acknowledgements: We thank to project UID/QUI/50006/2013. The NMR spectrometers are part of The National NMR Facility, supported by Fundação para a Ciência e a Tecnologia (RECI/BBB-BQB/0230/2012).

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Recent developments in the synthesis of novel xanthone-1,2,3-triazole dyads

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The development of multi-target drugs for treating complex multifactorial diseases constitutes an active research field. This kind of drugs has gained much importance as alternative strategy to combination therapy ("cocktail drugs").[1] A common way to design them brings together two different pharmacophores in one single molecule (so-called dyads). Following this idea and being aware that xanthones[2] and 1,2,3-triazoles[3] possess important pharmacological properties, we combined these two heterocycles in one molecule to create new dyads with improved therapeutic potential. In this work, new xanthone-1,2,3-triazole dyads were prepared from novel (E)-2-(4-arylbut-1-en-3-yn-1-yl)chromones by two different approaches to evaluate their efficiency and sustainability. Both methodologies involved Diels-Alder reactions to build the xanthone core, which were optimized using microwave irradiation as alternative heating method, and 1,3-dipolar cycloadditions to insert the 1,2,3-triazole moiety (**Figure 1**).[4] All final and intermediate compounds were fully characterized by 1D and 2D NMR techniques.

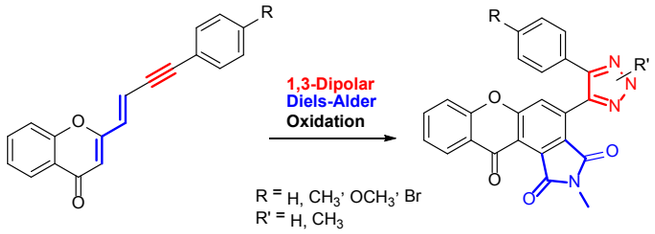

Figure 1: Synthesis of xanthone-1,2,3-triazole dyads.

Acknowledgements: Thanks are due to University of Aveiro, Polytechnic Institute of Bragança, FCT/MEC for the financial support to the QOPNA research Unit (FCT UID/QUI/00062/2013), through national founds and where applicable co-financed by the FEDER, within the PT2020 Partnership Agreement, and also to the Portuguese NMR Network. Hélio M. T. Albuquerque also thanks FCT for his PhD grant (SFRH/BD/86277/2012).

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Organic Superbases as useful tools for synthesis and applications

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Organic superbases[1] are relevant scaffolds that offer diverse perspectives in the field of organic synthesis, relatively low toxic/versatile, reagents, catalysts or even solvents. Nitrogen and Phosphorous are key elements in defining the pKa of such compounds. To illustrate such fact the order of basicity increases with the increment of conjugated nitrogen atoms in the molecule (amine < amidine < guanidine). There are examples in the literature where superbases such as the amidine (1,8-Diazabicycloundec-7-ene, DBU) and guanidine (1,1,3,3-tetramethylguanidine , TMG) have been used for different applications.

In our group, organic superbases such as DBU and tetramethylguanidine (TMG) have been applied for task-specific applications:

(i) Synthesis of protic organic salts or ionic liquids (PILs) based on aminoacids (L-cysteine; L-proline; L-alanine; L-phenylalanine; L-tryptophan; among others), carbohydrates (monosaccharides, disaccharides) and bile acids (cholic acid derivatives).[2] Additionally, characterization studies, physical-chemical properties and phase behaviour were evaluated for all the prepared PILs. These chiral PILs can be applied for asymmetric catalysis and chiral discrimination processes.

(ii) A second application of organic superbase related to an effective and comprehensive method of conversion of poorly water soluble Active Pharmaceutical Ingredients (API's) of different therapeutic families (e.g. anti-inflammatory, antibiotics) into highly soluble Ionic Liquid (ILs) and molten salts that form gels in the presence of water. The IL-APIs synthetic method is based on an acid-base reaction between the API and an organic superbase. This method will allow the increase of bioavailability of the parent compound drug and facilitate its local application/administration leading to the possibility of reduction of the therapeutical doses required as well as the side effects.[3]

(iii) For dissolution of different biomolecules and other biomaterials with the possibility to stabilize and further manipulation. A simple dissolution of biopolymers in organic superbases can create stable gels and homogeneous solutions for application in material science.

(iv) Effective systems for CO_2 capture based on a cheap organic superbase (TMG or DBU) and highly abundant natural aminoacids and saccharides were designed and tested. With D-mannose:DBU (ratio eq. = 0.625) was obtained an optimal wt% of CO_2 uptake of 13.9% corresponding to 3.3/5 alcohol groups converted into carbonates.[4]

Acknowledgements: We thank the Fundação para a Ciência e Tecnolgia for financial support, the National NMR Network and Solchemar.

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Sustainable synthesis of dihydropyrimidine-2(1*H*)-thiones under mechanical action

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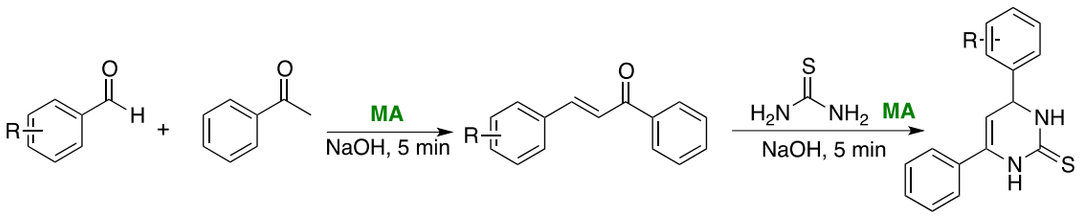
Chalcones and dihydropyrimidine-2(1*H*)-thiones are biologically active compounds that have shown rather diverse pharmacological properties, such as antiviral, antiparasitic and anticancer activities[1]. Beyond the classic Biginelli reaction, within the last decade, there are very few examples in the literature describing the synthesis of 3,4-dihydropyrimidin-2(1*H*)-ones or thiones carrying no substituents at the 5 or 6 positions of the heterocycle. Organic chemists have been looking for more sustainable methodologies and ways to improve de greenness of organic synthesis. Excluding solvent from the reaction medium, generally the main source of waste in a synthetic process, is probably the most efficient way to attain this objective. Mechanical activation (MA), which is normally carried out in the absence of, or with minimal use of solvents, could be and alternative to improve the sustainabiliaty of organic synthesis[2]. Herein we present the synthesis of chalcones and 4,6-diaryldihydropyrimidine-2(1*H*)-thiones in short reaction times and high yields using automatized mechanical action. The improvement of the sustainability of this method, compared with the use of conventional methodologies, was assessed by E-factor values under 10 and EcoScale values above 70.



Scheme 1: Two-step synthesis of 4,6-diaryldihydropyrimidine-2(1*H*)-thiones under automatized mechanical action.

Acknowledgements: The authors thank FCT (Coimbra Chemistry Centre, UID/QUI/00313/2013) for financial support and the UC-NMR facility for NMR spectroscopic data (www.nmrccc.uc.pt).

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Exploring the Reactivity of Novel Tetrazol-5-yl-Allenes for the Synthesis of Tetrazolyl-Heterocycles

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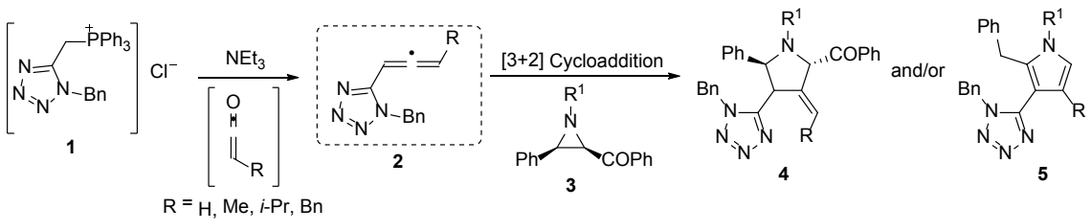
We have previously reported the reactivity of allenoates towards aziridines in organic solvents[1] and in supercritical carbon dioxide ($ScCO_2$).[2] This study gave an insight into the chemistry of allenes and provided a synthetic methodology to nitrogen-containing five-membered heterocycles. Recently, we become interested in developing new synthetic routes to 5-substituted-1*H*-tetrazoles which are used in medicinal chemistry as bioisosteres of carboxylic acids.[3] In this context, we envisaged that tetrazolyl allenes could be particularly interesting building blocks for the synthesis of new 5-substituted tetrazoles.

In this communication, we describe the synthesis of novel tetrazol-5-yl-allenes and their reactivity towards aziridines leading to the synthesis of functionalized methylenepyrrolidines and pyrroles (**Scheme 1**). The Wittig reaction between the ylide, formed from the phosphonium chloride **1**, and ketenes, generated *in situ* from the corresponding acyl chloride and triethylamine, gave the target allenes **2** in high yields. As previously observed for other allene derivatives,[1,2] tetrazol-5-yl-allenes can act as the 2π-component in [3+2] cycloadditions with azomethine ylides generated from aziridines, affording 4-methylenepyrrolidines **4** but they can also react with aziridines via formal [3+2] cycloadditions leading to functionalized pyrroles **5** Further details of this study will be disclosed.



Scheme 1: Synthesis and reactivity of allenes bearing a tetrazole substituent.

Acknowledgements: Thanks are due to FCT (Coimbra Chemistry Centre, UID/QUI/00313/2013 and BPD – CQC-QO-BPD-2015) for financial support. We acknowledge the UC-NMR facility for obtaining the NMR data (www.nmrccc.uc.pt).

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Chitobiose modification: a fast forward approach to attain relevant disaccharides

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The biological importance of glycostructures made them popular targets in modern synthetic chemistry, in particular those incorporating N-acetyl-D-glucosamine (NAG) units. The urgent need of these compounds in pure form and in significant amount has implied vast synthetic efforts. Usually oligosaccharides are constructed through sugar monomers manipulation, which implies time-consuming protection/deprotection steps and wild glycosylation reactions. Thus, in the last years, several approaches have been developed to attain complex glycostructures.[1] However, it has been demonstrated that glycosylation using NAG derivatives as glycosyl donors is still a difficult task.[2] Our group has been also involved in the synthesis of glycostructures based on NAG units,[3] more specifically on the assembly of small fragments of bacterial peptidoglycan which will allow the identification of key interactions that determine their recognition by the host. Since these structures are composed of NAG and N-acetylmuramic acid (NAM) units connected via a β-1,4 linkage, it was envisaged the direct modification of chitobiose to attain the desired compounds. Taking advantage of our preliminar work, we report our recent advances on the modification of chitobiose towards relevant disaccharides.

Acknowledgements: The authors acknowledge to Fundação para a Ciência e Tecnologia (FCT) for funding the project PTDC/QEQ-QOR/2132/2012. The NMR spectrometers are part of The National NMR Facility, supported by Fundação para a Ciência e a Tecnologia (RECI/BBB-BQB/0230/2012).

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Ring-opening of α-hidroxy-cyclopentene-aziridines in water under mild conditions

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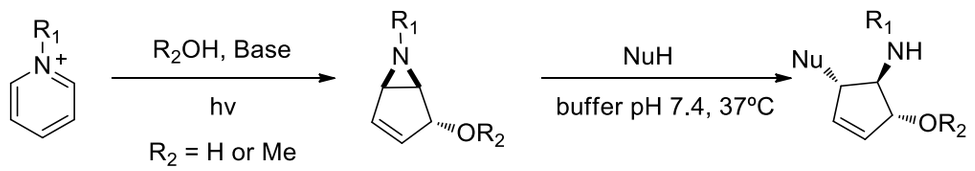
Aziridines are reactive three-membered heterocycles, used as intermediates in the synthesis of carbocycles with significant biological activity, such as aminocyclopentitols and beta-lactams.[1] An easy and useful procedure for the synthesis of aziridines was reported in 1972 by Kaplan *et al.*, describing the photochemical transformation of pyridinium salts to bicyclic vinyl azridines under basic conditions (Figure 1).[2] The scope of the pyridinium ion photohydration reaction has been investigated, [3] and the aziridine product studied in several reactions, such as, nucleophilic ring-opening[4,5] and as a precursor in the total synthesis of important natural products.[3,6]

Herein is reported the ring-opening of α-hidroxy-cyclopentene-aziridines was explored in water and at physiological pH (pH=7.4) at 37 ºC by carbon, oxygen, nitrogen and sulphur nucleophiles allowing the exclusive reaction for azide, anilines, thiols (aryl, alkyl, cysteine and derivatives) providing high to moderate isolated yields. This reactivity was also demonstrated for the bioconjugation with the peptide hormone salmon calcitonin (sCT).



Scheme 1: Photohydration of pyridinium salt followed by nucleophilic ring-opening of the bicyclic vinyl aziridine under physiological conditions.

Acknowledgements: We thank the Fundação para a Ciência e Tecnolgia for financial support of PTDC/QUI-QUI/119210/2010 and SFRH/BPD/88666/2012.

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The search for new antipsychotic compounds incorporating the N-methyl piperazine nucleus

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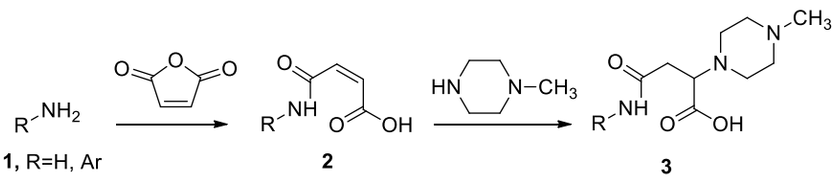
The treatment of psychiatric disorders is still an unsolved problem of our society and the search for new antipsychotic drugs with reduced side effects, resulted in a multitude of compounds, mainly acting as dopamine antagonists. The 5-HT_{2A} serotonin receptor, the most abundant serotonin receptor subtype in the cortex, has been associated with several psychiatric disorders and was used as a target for several antipsychotic, anxiolytics and antidepressants.[1] Clozapine, a molecule incorporating the *N*-methyl piperazine nucleus, is a gold standard among antipsychotic medications for schizophrenia. Its therapeutic use is restricted by agranulocytosis, a fatal blood disorder associated with the use of the drug.[2] It is crucial to develop new molecules inspired in the clozapine scaffold, with potential antipsychotic activity, and reduced side effects, capable of interacting with different receptors associated to this type of disorders, namely the 5-HT serotonine receptors.[3]

In this work we describe the synthesis of a selection of compounds incorporating the piperazine unit in an amide linker associated with the aromatic nucleus. Different arylamines were reacted with maleic anhydride to generate compounds **2**. The alkene moiety selectively incorporated *N*-methyl piperazine, 5-bonds away from the aromatic fragment (compound **3**, Scheme 1). Details on the synthesis of these compounds will be presented and also the biological activity at serotonine 5-HT_{2A} receptors. All compounds were fully characterized by elemental analysis and spectroscopic techniques (IR spectroscopy, ^{1}H and ^{13}C NMR including HMQC and HMBC).



Scheme 1: Synthesis of compounds **3** incorporating the piperazine nucleus.

Acknowledgements: We gratefully acknowledge the financial support by the University of Minho and FCT through the Portuguese NMR network (RNRMN), the Project F-COMP-01-00124-FEDER-022716 (ref. FCT PEst-C/QUI/UI0686/2011) FEDER- COMPETE, and a PhD grant awarded to Elina Marinho (SFRH/BD/73659/2010).

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Strategies towards the synthesis of new (*E*)-2-aryl-3-styryl-4*H*-chromen-4-ones and (*E*)-1-methyl-2-aryl-3-styrylquinolin-4(1*H*)-ones

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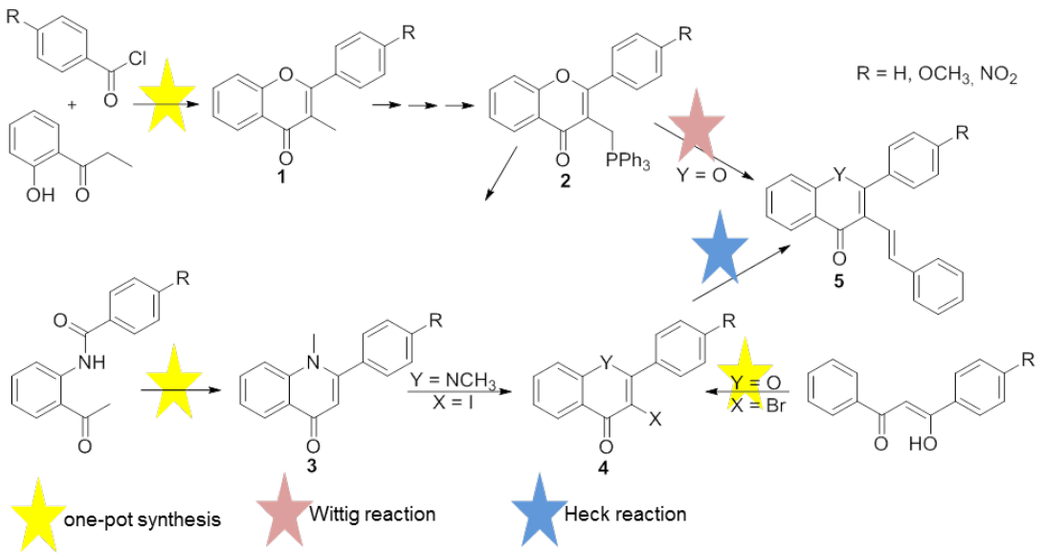
Flavones (2-aryl-4*H*-chromen-4-ones) and quinolones [2-arylquinolin-4(1*H*)-ones] are important classes of bioactive drug targets in the pharmaceutical industry, as they are the core structure of numerous biologically active compounds.[1] On the other hand the presence of a styryl group attached to a chromone core also seems to enhance the biological activity.[2] Taking these important aspects into consideration we have developed new and efficient routes towards the synthesis of 2-aryl-3-styryl-4*H*-chromen-4-ones and 1-methyl-2-aryl-3-styrylquinolin-4(1*H*)-ones **5** (Scheme). These routes include efficient one-pot methods and the use of Wittig and Heck type reactions.



Scheme

Acknowledgements: We would like to thank University of Aveiro and FCT/MEC for the financial support to the QOPNA research Unit (FCT UID/QUI/00062/2013), through national founds and where applicable co-financed by the FEDER, within the PT2020 Partnership Agreement, and also to the Portuguese NMR Network. D.H.A.R. also thanks FCT for her PhD grant (SFRH/BD/68991/2010).

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1-(2-Oxo-2*H*-chromen-3-yl)pyridinium Chloride: Subtleties in the Reaction with Nucleophiles

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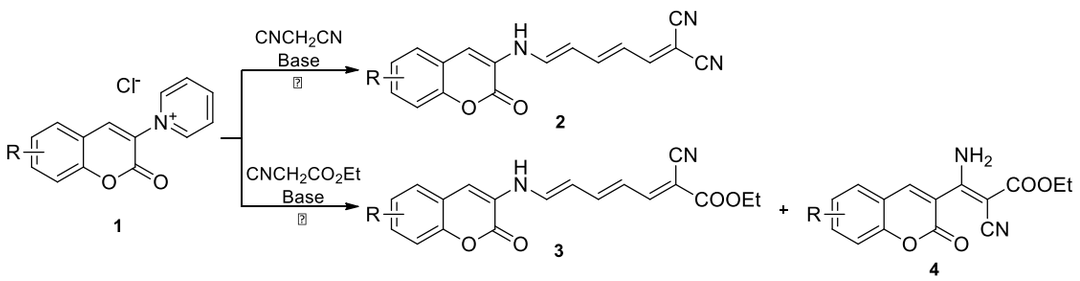
Chromenes are privileged scaffolds because they comprise natural and synthetic derivatives presenting a diverse array of pharmacological activities[1]. Substituted 1-(2-oxo-2*H*-chromen-3-yl)pyridinium chloride salts **1** were recently prepared from the reaction of an appropriate salicylaldehyde and 1-(cyanomethyl) pyridin-1-ium chloride, in two steps.[2]

In previous work, chromene **1** (**Scheme 1**) was found to react with nitrogen nucleophiles as described in the Zincke reaction, yielding 3-aminochromenes[3]. In this study, the use of active methylene compounds (malononitrile and ethyl cyanoacetate) allowed the isolation of the postulated intermediate of the Zincke reaction (chromenes **2** and **3**) for the first time. Chromenes **2** and **3** incorporate a side chain bearing a push-pull conjugated system that can be further functionalized. The reaction of chromene **1** with ethyl cyanoacetate led also to the formation of trace amounts of chromene **4** with a 3-amino-2-cyanoacrylate moiety in the C_3-position. Following a retrosynthetic approach, chromene **4** was prepared in good to quantitative yields, by a one pot reaction, from the corresponding salicylaldehyde and ethyl cyanoacetate. Despite the simplicity of the synthesis, this is the first time that chromene derivatives **4** were isolated. A detailed discussion of these methodologies, scope and reaction mechanisms will be presented.



Scheme 1: Reaction of oxochromene **1** with active methylene compounds.

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3-Bromochromones as Building Blocks of Novel Furan and Cyclopropane Derivatives

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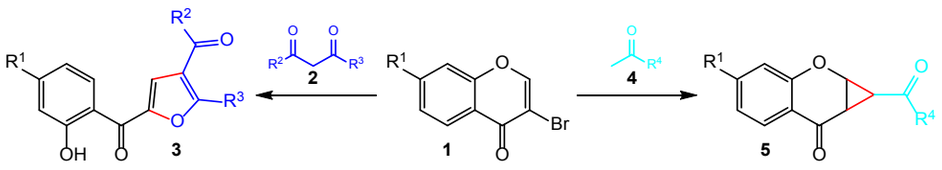
The furan and cyclopropane rings can be found in a variety of biologically active synthetic and natural compounds.[1] Due to their importance as potential pharmaceutical agents, the synthesis of novel furan- and cyclopropane-containing compounds is a highly active research field. For this purpose, 3-bromochromones arise as desirable and versatile starting materials.[2] Exploring the singular chemical features of such chromone derivatives, herein we present the synthesis of two different families of oxygen-containing heterocycles – furan-based polyphenolics and fused chromanone-cyclopropanes – starting from the same 3-bromochromones under alkali catalytic conditions. Our methodologies relay on tandem reactions of 3-bromochromones **1** with 1,3-dicarbonyl compounds **2** to afford a series of furan-based polyphenolic derivatives **3**, and ketone compounds **4** to prepare a library of fused chromanone-cyclopropane derivatives **5** in the presence of organic and inorganic bases, respectively (**Scheme 1**). The reaction conditions and reaction mechanisms will be presented and discussed.



R^1 = H, OMe; R^2 = Me, 2-OH-Ph, 2-OH-4-OMe-Ph, 2-OH-4,6-$(OMe)_2$Ph;
R^3 = H, Me, 4-Me-Ph, 4-Cl-Ph, 4-OMe-Styryl, 4-Cl-Styryl, 3,4-$(OMe)_2$Styryl; R^4 = Me, Ph, *p*-OMe-Ph, *p*-Cl-Ph, *p*-NO_2-Ph

Scheme 1: Synthetic pathway for the synthesis of furan **3** and cyclopropane **5** derivatives from 3-bromochromones **1**.

Acknowledgements: Thanks are due to FCT/MEC for the financial support of the QOPNA research Unit (FCT UID/QUI/00062/2013) and CICECO–Aveiro Institute of Materials (FCT UID/CTM/50011/2013), through national founds and, where applicable, co-financed by the FEDER, within the PT2020 Partnership Agreement, and to the Portuguese NMR Network. We would like to thank the General Directorate for Scientific Research and Technological Development–DGRSDT of Algeria for the financial support. Further thanks are due to CICECO for funding the purchase of the single-crystal X-ray diffractometer. J.L.C.S. is grateful to FCT for her PhD grant (SFRH/BD/76407/2011).

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Pyranosyl 6´-isonucleosides: synthesis and biological profile

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Isonucleosides are regioisomers of nucleosides in which the nucleobase is linked to the carbohydrate unit at a non-anomeric position. These nucleoside analogs have attracted some interest as synthetic targets, due to their propensity to display antiviral and antitumor activities.[1] This bioactivity profile arise from their ability to interfere in biological processes in which natural nucleosides are involved, such as nucleic acid synthesis and cell division, which are deregulated in diseases such as cancer or viral infections.[2] In addition, such molecules present better stability towards enzymatic hydrolysis that their physiological counterparts.

The reported isonucleosides mostly encompass furanosyl derivatives comprising the nucleobase at C-2 or C-3. Hence, we were motivated to explore other positions of a sugar moiety for the coupling of a nucleobase, namely the C-6 position, towards new types of isonucleosides based on hexopyranosyl units.

In this communication, the synthesis of 6´-isonucleosides (**Figure 1**) embodying purine or pyrimidine motifs is presented. The synthetic approach was based on the Mitsunobu coupling of partially protected glycosides containing a free OH-6 with a nucleobase. Variations on the substituents at the sugar moiety were made, extending the panel of compounds for further bioactivity screening. The outcome of the Mitsunobu reaction using pyrimidines was shown to be dependent on the substitution pattern of the sugar ring, leading to pyrimidine-linked disaccharides or to products of mono-coupling, *i.e.* the N^1- or N^3-linked pyrimidine 6´-isonucleosides

The compounds were subsequently subjected to biological assays, which focused the evaluation of their inhibitory abilities towards cholinesterases and their cytotoxic effects on tumor and on healthy cells. The synthetic work and the results of the biological assessment will be revealed and discussed.

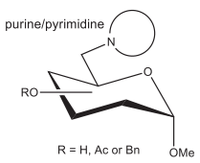

Figure 1: General structure of the synthesized 6´-isonucleosides.

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Synthesis of Symmetric Triarylmethanes Bearing Secondary Anilines

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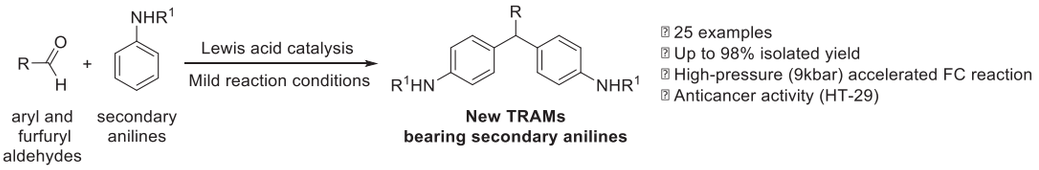
Triarylmethanes (TRAMs) are an important class of compound in materials science and medicinal chemistry.[1] Symmetric TRAMs are commonly synthesized by Friedel-Crafts reaction between aromatic aldehydes and electron-rich arenes in acidic media at high temperatures.[1] In addition to the lack of general methods for the synthesis of aniline-based TRAMs, the scope of anilines as the electron-rich arenes is limited to tertiary anilines.[2] Moreover, the use of the reported harsh reaction conditions are incompatible with acid-labile functional groups. Motivated by our desire to access new TRAMs, a direct, general and mild protocol via Lewis acid-catalyzed Friedel-Crafts reaction between secondary anilines and aryl or furfuryl aldehydes (**Scheme 1**) was developed.[3] Herein we present the results on the reaction conditions optimization, effect of the reaction pressure[4], reaction substrates scope including the important biorenewable chemical platform 5hydroxymethylfurfural (HMF)[5], proposed mechanism based on experimental results and DFT calculations, and the anticancer activity of the new synthesized TRAMs.[3]



Scheme 1: Synthesis of Symmetric Bis(*N*-alkylaniline)triarylmethanes via Friedel−Crafts-Catalyzed Reaction between Secondary Anilines and Aldehydes

Acknowledgements: We thank the Fundação para a Ciência e a Tecnologia (SFRH/BD/73971/2010 and PTDC/QUI-QUI/119823/2010), and Fundação Calouste Gulbenkian (Prémio de Estimulo à Investigação 2012) for financial support and REDE/1518/REM/2005 and the Portuguese NMR Network (IST-UTL Center) for providing access to the MS and NMR facilities.

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The mechanism for the electroanalytic action of triazolic derivatives of conducting polymers and its mathematical representation

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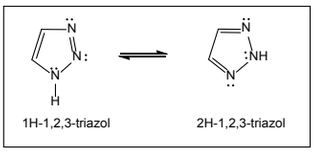
Conducting polymers are one of the most studied class of materials during the last five decades, because they are capable to combine the properties of plastics (versatility in shaping, corrosion resistence, flexibilty, *etc.)* with the conductivity of metal [1 – 3], reason why they are called “synthetic metals”. Being also easy to modify, they have a vast and rich spectrum of use, beginning in the corrosion protecting coating and finishing with sensors and biosensors.
At the other hand, 1,2,3-triazole is a heterocyclic aromatic compound, whose behavior also attracts interest [4]. While not substituted on pyrrolic nitrogen atom, represents 2 tautomery forms:



As it possesses the nitrogen heteroatoms of different configurations (the atom 1 is pyrrolic and the atoms 2 and 3 are pyridinic), its behavior tends to amphoterity, so, triazole is either an acid, stronger than pyrrole (and its acidity is close to phenolic), or a base, which makes its behavior pH-dependent, which makes its presence definitive for the electroanalytic behavior of the material.
In the work [5] was described a sensor of different drugs, based on graphene oxide, doped with a ferrocenic derivative, containing triazole moiety. The sensibility of sensor was comparable with that of other sensors of the class. Nevertheless, by describing its function the attention to the presence of triazole ring and its impact to the properties of the material wasn´t given, despite of being the important part on the detection of its mechanism. In this work the working mechanism of the sensor, based on a conducting polymer, modified by triazole ring in the presence of interfering compounds in different media. The mechanism will be accompanied by corresponding mathematic model (developed and analyzed by means of linear stability theory and bifurcation analysis).
In the case of an acid compound as an analyte or interfering compound, the reaction is hold by one of pyridinic nitrogen atoms and the function of triazolic ring in the sensor will be described as:

$$TriAz\ (\text{less conducting}) + HA \rightarrow [TriAzH^+][A^-]\ (\text{more conducting}) \quad (1)$$

$$[A^-] - ze^- \rightarrow [A^-]Ox\ \ (\text{conducting or not an electric current}) \quad (2)$$

The formed compound may be not only a neutral substance, analogic to the anion, but also another anion (especially in the case of carboxylic acids or their derivatives).
In the case of a basic compound, the triazole forms salts, and the reaction is realized by pyrrolic nitrogen atom:

$$TriAz + B^- \rightarrow [´Triaz^-] + HB \quad (3)$$

Strong inorganic bases in this system are seen only as reaction media, modifying substance or binding substance and not as na analyte.
In the case of halogenic derivative as na analyte (which succedes in the case of different drugs and especially pesticides), the quaternary salt is formed and its fragment, literarily stimmed to the electrode surface is oxidized more easily.

$$TriAz + RHal \rightarrow [TriazR][Hal] \quad (4)$$

$$[TriAzR+] - ne^- \rightarrow [TriAzR´+] \quad (5)$$

To describe mathematically the work of electrochemical sensor, based on a ferrocenic compound, or conducting polymer, modified by triazole moiety, we use three variables.
c – the analyte concentration;
θ - the surface concentration of modified triazolic compound, expressed in the form of coverage degree;
e – the active concentration of an interfering compound.
To simplify the modeling, we suppose that the reactor is intensively stirred (so it is possible to neglect the convecting flow), that the background electrolyte is in excess (and it´s possible to neglect the migration flow). Also we suppose that the pre-surface layer has the linear concentrational profile and the constant thickness, equal to δ.
It´s possible to prove, that the system may be described by the following equation set:

$$\begin{cases} \frac{dc}{dt} = \frac{2}{\delta}\left(\frac{\Delta}{\delta}(c_0 - c) - r_1 - r_2\right) \\ \frac{d\theta}{dt} = \frac{1}{\Gamma}(r_1 + r_3 - r_4) \\ \frac{de}{dt} = \frac{2}{\delta}\left(\frac{D}{\delta}(e_0 - e) + r_4 - r_3 - r_2\right) \end{cases} \quad (6),$$

In which Δ is the analyte diffusion coefficient, c_0 its concentration in the bulk, r_1 the rate of the reaction with triazolic compound, r_2, with interfering compound, Γ the maximal concentration of the triazolic compound, r_3 the reaction rate with the initial triazolic compound and interfering substance r_4 is the oxidation reaction rate, D is the diffusion coefficient of interfering compound and e_0 its bulk concentration.
From the analysis of the differential equation set(6) by means of linear stability theory and bifurcation analysis it may be concluded that:
1. The behavior of triazolic moiety plays na important part in the work of the sensors, based on ferrocenic compounds and conducting polymers, modified by triazolic ring. Besides of contributing on the conductivity it has the capacity of analyte retention (alone, or by means of binding compounds) and the retention of interfering compounds.
2. As triazole is a compound with flexible behavior, the behavior of the compounds, containing the triazole moiety is equally complicated (from the point of view of computational chemistry) either in acid media, or in basic one, which isn´t a case for the typic sensors, based in ferrocene and conducting polymers.
3. In the absence of the interfering compound or in the presence of its minimal concentrations, the steady-state stability and the sensing function is controlled by diffusion. But in the strong influences of interfering compound it will depend on them too. The curve of the dependence between pH and electrochemic parameter has to have a center and a peak on pH=7, but closer to symmetry, than in the case of ferrocene and conducting polymers.
4. The oscillatory and monotonic instabilities in the system are possible, being caused by influences of oxidative electrochemical process in the double electric layer (DEL) and also by autocatalytic reaction (if it is present).

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New pyrimido[5,4-*d*]pyrimidines with enhanced anticancer activity on colorectal cancer cells: synthesis, SAR study and mechanism of action

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Colorectal cancer (CRC) is amongst the most common types of cancer in developed countries due to common sedentary lifestyles and unhealthy diets.[1] Chemotherapy has a key role in combating this disease but the current approved drugs are not effective for all patients due to the tumours' different genetic profiles. The p53 is frequently mutated in several types of cancer causing treatment resistance.[2,3,4] Other genetic alterations frequently present in CRC include KRas or BRaf activating mutations which increase proliferative behavior. These mutations are known to be responsible for resistance to targeted therapy with growth factor receptor inhibitor mediated agents. Therefore, the need to increase treatment efficacy and to overcome resistance continues to stimulate anticancer drug discovery.

Our research group has established an efficient synthetic approach to generate new 4,8-disubstituted pyrimido[5,4-*d*]pyrimidines.[5,6,7] Furthermore, this new class of heterocyclic compounds have been identified as active against HCT116 (p-53 wt) colon cancer cells.[8]

Recently new derivatives of pyrimidopyrimidines were synthesized and evaluated against HCT116 (p-53 wt) colon cancer cells. The SAR analysis suggests that the activity is dependent on the substituents present on C4 and C8 of the pyrimido[5,4-*d*]pyrimidine core. The most active compound was selected to assess its activity in p53 knockout and mutant BRaf cell lines (HCT116 (p53-null), CO115). The compound showed higher activity in the studied cell lines than the reference drug 5-FU. Moreover, the capacity of the compound to induce apoptosis and its effect on the cell cycle was also assessed. The compound showed low induction of apoptosis, an accentuated arrest of cell cycle in the G2/M and a slight increase of cells in the sub-G1 phases.

The synthetic approach to obtain these compounds and all the biological results will be presented.

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8. Unpublished results.

Synthesis of new bis(indolyl)methanes with anti-cancer properties

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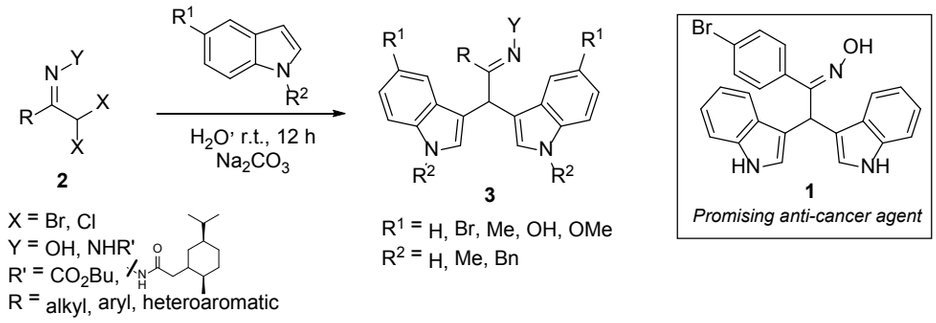
We have recently disclosed an approach to novel bis(indolyl)methane oximes (BIM Oximes) *via* Hetero-Diels-Alder reactions of nitrosoalkenes with indoles. This class of compounds showed very interesting anti-cancer activity, in particular against leukaemia and lymphoma cell lines (*e.g.* **1**). In this communication, the tuning of the structure of scaffold **1** which led to the preparation of a range of new BIM oximes will be described.

Moreover, the one-pot synthetic strategy to BIMs was extended to novel bis(indolyl)methane hydrazones via bis-hetero-Diels-Alder reaction of azoalkenes with indoles. Particularly interesting was the diastereoselectivity observed in these transformations, when mono-halogenated hydrazones bearing a chiral substituent were used. The biological evaluation of these new BIMs as anti-cancer agents will also be disclosed.



Scheme 1: Synthesis of new bis(indolyl)methanes.

Acknowledgements: Thanks are due to FCT (Coimbra Chemistry Centre, UID/QUI/00313/2013) for financial support. We acknowledge the UC-NMR facility for obtaining the NMR data (www.nmrccc.uc.pt).

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Mn(III) biocompatible phthalocyanines for Molecular Imaging

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Phthalocyanine derivatives present as a main drawback for biomedical applications their low solubility in water,[1] which stems from their planar structure that induces aggregation by π-π stacking. Many strategies have been developed to overcome this disadvantage, including the use of dendrimers as substituents on the phthalocyanine macrocycle, and the introduction of hydrophilic moieties, such as sulfonate, quaternized amines and PEG [1]. Nowadays, the contrast agents (CA) most used clinically for MRI imaging involve water soluble complexes of Gd^{3+}. Because the release of free Gd^{3+} from some Gd-based MRI CAs has recently been linked with a medical condition known as nephrogenic systemic fibrosis (NSF), much emphasis has been placed on alternative approaches based on non-lanthanide metals, in particular manganese. So far, only one Mn(II)-phthalocyanine (tetrasulfonated manganese(II) phthalocyanine, $[Mn(II)PcS_4]^{4-}$) has been studied as MRI-CA.[2] With the objective of developing new water soluble phthalocyanines and metallophthalocyanines with potential for molecular imaging applications, we report herein the synthesis of water soluble phthalocyanines, bearing either four PEG500 or four choline substituents in the macrocyclic structure, together with their Zn(II) and Mn(III) complexes. We also describe the photophysical characterization, proton relaxivity study, and cytotoxicity evaluation, using epithelial HeLa cells, of the Mn(III)-phthalocyanine complexes.[3]

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Unveiling the Chemistry of the Homemade Drug "Krokodil"

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Homemade drugs are not a new problem, but its use is growing and affecting seriously our society**.** "Krokodil", which psychoactive substance is thought to be desomorphine, is one of these homemade drugs[1]. Used as a cheap alternative to heroin, "Krokodil" has ignited a great interest by the media and scientific community due to its high toxicity, characterized by a wide range of clinical signs, including gangrene and open ulcers in the skin of the addicts.[2] "Krokodil" is prepared using easily commercially available materials and the manufacture is performed "at home" under uncontrolled conditions. These facts are responsible for an extremely complex product, which composition is, in fact, poorly understood.[3]

In order to study the chemical composition of "Krokodil" we mimicked a street synthesis resorting to the same materials and conditions used by street manufacturers. The chemical profile of a representative sample was outlined and the chromatographic behavior in TLC, GC-MS and HPLC-DAD revealed the extreme complexity of "Krokodil", which is composed by dozens of substances with wide structural diversity. Several analytical spectroscopic techniques, namely IR, UV/Vis, ^{1}H-NMR, ^{13}C-NMR, ^{31}P-NMR and solid state NMR were also employed in order to study the inorganic and organic composition of the drug. Desomorphine and some other morphinans were identified and we propose a mechanism for the formation of desomorphine. With this work we intent to eventually aid the competent authorities in dealing with "Krokodil", in terms of identification and characterization, and to shed some light upon the chemistry behind its harmful effects.

Acknowledgements: Research developed under the project PEst-OE/SAU/UI4040/2014 and partially supported by the European Regional Development Fund (ERDF) through the COMPETE - Operational Competitiveness Programme and national funds through FCT - Foundation for Science and Technology, under the project PEst-C/MAR/LA0015/2013. To FCT for the research grant (SFRH/BD/98105/2013). To Brazilian agency Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) (process 245844/2012-0) for research grants and scholarship.

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Enzymatic resolution of cyclic 4-hydroxy-acylaziridines

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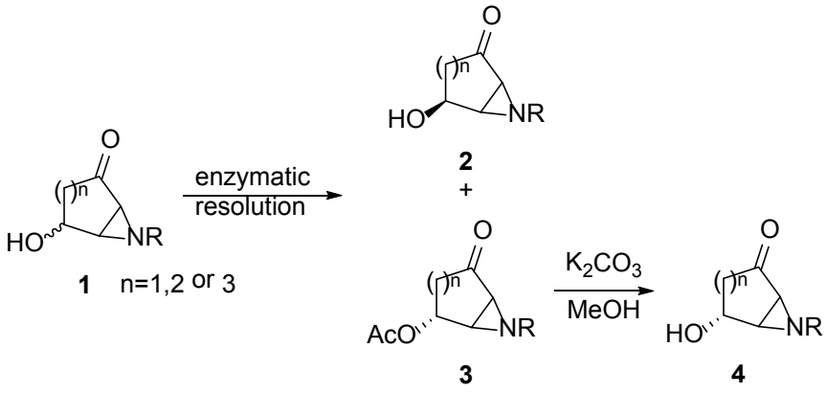
The stereoselective synthesis of aziridines is still an important topic, since they are useful building blocks and synthetic targets. In particular, cyclic 4-hydroxy-acylyaziridines ((x+1)-hydroxyazabicyclo-[x.1.0]-alkane-2-ones) **1** have been used as synthetic intermediates for natural bioactive compounds[1] and more recently for the synthetic drug Oseltamivir[2] (Tamiflu®). They can also be used in the production of cyclic chiral amino alcohols, important compounds for asymmetric synthesis.[3] Stanley Roberts[4] developed a very efficient enzymatic resolution method for 3-thiobenzyl-4-hydroxycycloketones. In the present work this method was applied in the production of the cyclic 4-hydroxy-acylyaziridines almost optically pure.



Scheme 1: Enzymatic resolution of cyclic 4-hydroxyaziridines **1**.

The cyclic 4-hydroxy-acylaziridines **1** (n=1-3, R= e.g. Bn or Ts) were produced stereoselectively (*cis* or *trans* depending on the R-group) as racemates using a method previously reported[3]. Resolution with Novozyme 435® and vinyl acetate provided both enantiomers (**2** and **4**) in excellent yields (46-50%) and enantiomeric excesses (91-99%). The enantiomeric excess of the products was determined by chiral HPLC and it was found that analysis of the acetates **3** gave false values of the enantiomeric excess. Hydrolysis of these acetates and further analysis of the produced alcohols **4** resulted in reproducible results. This procedure provides an easy and reliable method for the production of important chiral building blocks in both enantiomeric forms.

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UHPLC-QqQ-MS/MS method for phytoprostane profiling in macroalgae

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The analysis of phytoprostanes in natural matrices is extremely challenging, requiring highly sensitive and specific tools for their profiling and characterization. Moreover, the great diversity granted by the presence of racemic mixtures of phytoprostanes increases the complexity of those analyses.[1]

Our work aimed at determining naturally occurring classes of free phytoprostanes in 24 macroalgae species belonging to Chlorophyta, Phaeophyta and Rhodophyta, collected along the western coast of Portugal and from integrated multitrophic aquaculture (IMTA) systems. For this, a fast, selective, and robust ultrahigh-performance liquid chromatography coupled to triple-quadrupole mass spectrometry (UHPLC-QqQ-MS/MS) method was employed. Three classes of phytoprostanes were identified and quantitated for the first time in the analyzed species (**Figure 1**). The total phytoprostane content ranged between ca. 6 and 1381 ng/100 g of dry algae, F_{1t}-phytoprostanes and L_1-phytoprostanes constituting the major and minor classes, respectively.

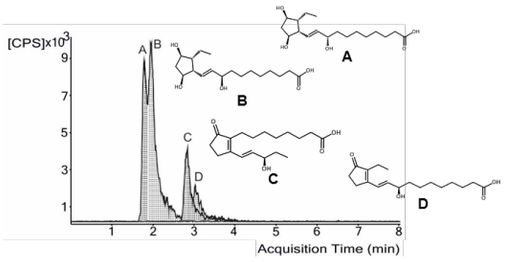

Figure 1: Representative UHPLC-QqQ-MS/MS chromatogram of detected phytoprostanes in *Codium tomentosum* Stackhouse. 9-F_{1t}-phytoprostane (**A**), 9-*epi*-9-F_{1t}-phytoprostane (**B**), 16-B_1-phytoprostane (**C**), and L_1-phytoprostane (**D**).

Currently, the interest in phytoprostanes comprises two general areas: as biomarkers of oxidative stress in plant-derived foodstuffs and as bioactive mediators with potential benefits in human health. Therefore, the determination of phytoprostane levels in macroalgae is of extreme importance, encouraging the exploitation and characterization of new natural dietary sources of these compounds.

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Change in Cognitive Effects Caused for Consumption of Caffeine - $C_8H_{10}N_4O_2$

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There are times that humanity is daily consumption of substances ha basis of caffeine - $C_8H_{10}N_4O_2$ (teas, coffee and drugs) in their various forms of preparation. Studies indicate that daily consumption of caffeine causes improvements in memory capacity, however, ha indication of adverse consequences, such as increased anxiety, among various other cognitive effects in humans and animals. After the analysis in two databases - Science Library Online (Scielo) and ... (PubMed) - using the criteria search “free full text” and “last five years”, we obtained the following data from the terms:

Term	Full Articles	Articles indicating improvements	Articles indicating worsening	Articles that don’t point difference
Memory	13	12	0	1
Cognition	13	5	3	5
Learning	9	3	4	2
Anxiety	11	1	9	1
Depression	9	0	8	1
Stress	5	4	1	0

Table 1. Data collected from the banks of SciELO and PubMed data.

Although caffeine is consumed daily by a significant portion of the world’s population, yet there are few clinical studies that demonstrate the actual effects of it in humans and animals, which indicates a great need for experimental research in humans and animals based on consumption caffeine.

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Discovery, structure elucidation and biosynthesis of the bartolosides, a new family of glycolipids from cyanobacteria

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Cyanobacteria are a chemically-rich group of photosynthetic microorganisms. Owing to their unique ecophysiology, a large number of cyanobacterial secondary metabolites are structurally unparalleled. Here, we report the discovery of a new family of glycolipids - the bartolosides - from filamentous (*Nodosilinea* sp.) and unicellular (*Synechocystis salina*) cyanobacteria, that are mildly cytotoxic against human cancer cell lines. These natural products consist of a dialkylresorcinol (DAR) core decorated with halogenations and glycosylations. Bartolosides can build up to over 0.5% of the cyanobacterial biomass (d.w.) in laboratory cultures and are also released into the culture medium by exponentially-growing cells.

The highly similar alkyl moieties featured in the bartolosides posed several challenges for traditional NMR and MS-based structural elucidation. To overcome this, and motivated also by the unusual structural features of these molecules, we studied their biosynthesis. Using genome mining, we identified the bartoloside biosynthetic gene cluster (*brt*) and reconstituted the enzymatic steps involved in the formation of the DAR moiety from fatty acid derivatives. This information enabled the elucidation of the full planar structures of the bartolosides, by placing constrains on the structural possibilities and allowing for further information to be extracted from HSQC-TOCSY experiments. Overall, our approach exemplifies the usefulness of incorporating contemporary biosynthesis investigations into structural elucidation efforts.

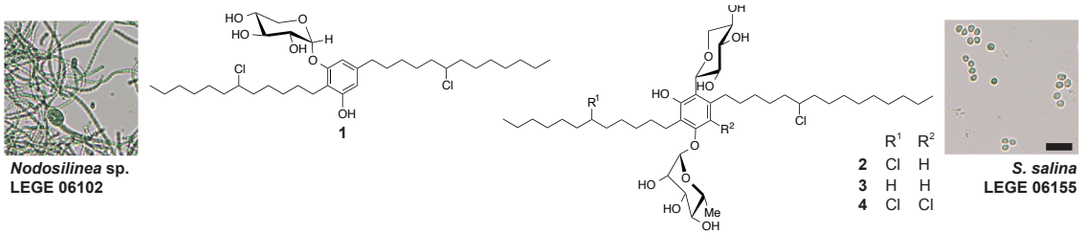

Figure 1: Structures and biological sources of bartolosides A-D (**1**-**4**).

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Bioactive abietane diterpenes from *Plectranthus* spp. extracts and its encapsulation into a novel phytosomal formulation

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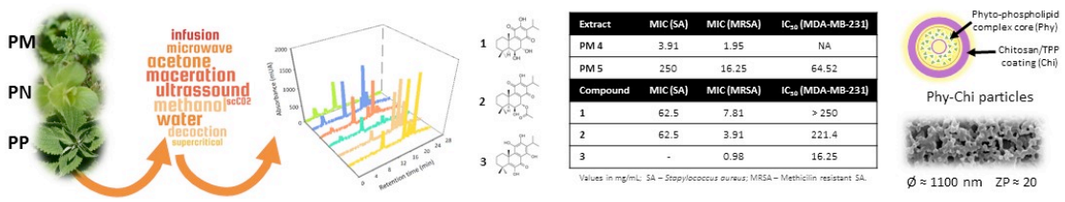
Medicinal plants have been one of the most valuable sources of new drug leads. Plants of the *Plectranthus* genus (Lamiaceae) have diverse ethnopharmacological applications including gastro-intestinal, infections and skin conditions.[1] These uses may be related to the presence of bioactive diterpenes, namely abietanes.[2] The intrinsic instability of these bioactive abietanes, as well as their unfavorable partition coefficient, affects the bioavailability and therefore their bioactivity. New drug delivery systems as the Phytosome®, have proven to be effective in surpassing those limitations.[3]

In this study, three plants from the *Plectranthus* genus (*P. madagascariensis* [PM], *P. neochilus* [PN] and *P. porcatus* [PP]) were screened for their antibacterial and antiproliferative activities. Extracts with different polarities were prepared using combinations of different extraction methods and solvents. PM extracts showed antibacterial (*Staphylococcus aureus* and MRSA strains) and cytotoxic activities (MDA-MB-231 cells). 7α,6β-Dihydroxyroyleanone (**1**), 7α-acetoxy-6β-hydroxyroyleanone (**2**) and coleon U (**3**), which were identified by HPLC-DAD as the major abietanes in the PM bioactive extracts, also showed antibacterial and cytotoxic activities (**Scheme 1**). The most potent antibacterial PM extract (PM 4) was encapsulated into phytosomes which were then microencapsulated with chitosan/TPP complexes. The median size of the microspheres was 1118.8 ± 177.5 nm and they showed a very low polydispersitivity index (0.224) and a zeta potential of 20.59 mV. The encapsulation efficiency ranged between 88 and 96%. Preliminary stability studies during 1 month suggested high stability of this phytosomal formulation based on morphologic and physico-chemical analyses. The results confirm the presence of antibacterial and cytotoxic compounds and the usefulness of the encapsulation technology applied to improve the stability of bioactive extracts of *P. madagascariensis*.



Scheme 1: Summary of the study main steps: phytochemical, antibacterial, cytotoxicity and phytosome development results.

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Perylenediimides: "*a la carte*" fluorescence

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Perylenediimides (PDIs, Figure 1) show interesting properties, such as near-unity fluorescence quantum yield, excitation in the visible region, strong and reversible electron-accepting character, and high electron mobility. PDIs start to be used as industrial pigments for tissues and paints (1910s-1980s), but recently they have been extensively studied as organic field-effect transistors, as fluorescent solar collectors, in organic photovoltaics, or as imaging agents. The synthesis of PDIs derivatives, starting from the commercially available perylene-3,4,9,10-tetracarboxylic acid dianhydride, allows the selective introduction of substituents in the imide group or in the bay region (Figure 1).

This communication will shows how bay substituents induce different torsion angles to the perylene core [1] or tune the photophysical properties (visible to NIR fluorescent emission)[2,3], and how PDIs can be introduced in different nanostructures through specific imide substituents.[4] Additionally, our recent efforts to developed new water soluble PDIs for imaging and 3D optical nanoscopy will be reported.

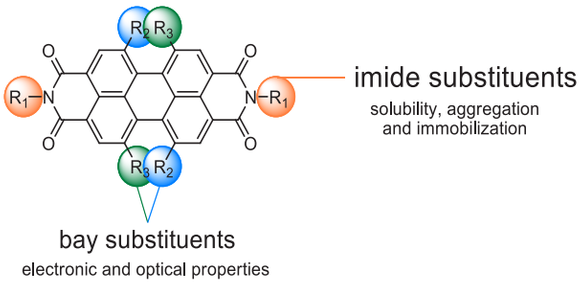

Figure 1: PDIs general structure and influence of the different substituents position.

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Antitumor activity of TXA1, an autophagy inducer which affects cholesterol localization

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Some of us have previously identified TXA1 as a hit thioxanthone with antitumor potential. The present study aimed to investigate its mechanism of action *in vitro* and in human non-small cell lung cancer (NSCLC) cells xenografted in nude mice.

The effect of TXA1 and its salt (TXA1.HCl) was studied in melanoma and NSCLC cell lines, regarding: i) cell growth and viability (sulforhodamine B and Trypan blue exclusion assays), ii) cell cycle profile and proliferation (flow cytometry with propidium iodide (PI) and BrdU incorporation assay); iii) apoptosis (TUNEL assay and flow cytometry with Annexin V/PI labeling); iv) autophagy (Western blot, fluorescence microscopy and electron microscopy). Additionally, cDNA microarrays analysis was carried out in NSCLC cells treated with TXA1.HCl. Finally, the toxicity of TXA1.HCl was analysed in nude mice as well as its antitumor activity in NSCLC xenografts.

TXA1 presented antitumor activity associated with the induction of autophagy and apoptosis, in both cell lines. Interestingly, this molecule (soluble salt) affected lipid biosynthesis and resulted in an abnormal cellular cholesterol localization in NSCLC cells. The soluble salt of TXA1 was not toxic to nude mice, significantly reducing the growth of human NSCLC cells xenografts. Overall this study provides new insights into the mechanism of action of a novel small molecule, which may be relevant for the development of anticancer strategies.

Acknowledgements: Fundação para a Ciência e Tecnologia -FCT for post-doc grant: SFRH/BPD/68787/2010. This research was partially supported by the Strategic Funding UID/Multi/04423/2013 through national funds provided by FCT – Foundation for Science and Technology and European Regional Development Fund (ERDF), in the framework of the programme PT2020.

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1,3,5-Triazole-Benzene Derivatives for Cancer Therapy: Synthesis and G-Quadruplex Stabilization

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G-quadruplex nucleic acids have attracted intense interest as an important target for drug design and development due to their biological significance and anticancer potential.

G-quadruplexes (G4) are formed from guanine-rich nucleic acid sequences that can fold into four-stranded DNA structures. The building blocks of these structures are G-quartets, almost planar arrangements of four guanine bases bonded by Hoogsteen base pairing and stabilized by a monovalent cation (e.g. K^+, Na^+) localized in the centre of the structure. It has been shown that the formation of G4 inhibits the telomere extension by the telomerase enzyme, which is up-regulated in cancer cells, as well as negatively regulates oncogenes transcription.

In the last decade, an intensive search for small-molecules as G4 ligands/stabilizers, led to the development of a variety of chemical classes, of which many are selective for G4 structures over ds-DNA. However, the design of inter-G4 selective ligands remains challenging. We have recently shown that selectivity of indoloquinolines to G4 can be modulated by the number and relative position of basic side side chains. [1]

Herein, we present the synthesis of 1,3,5-tris-triazol-benzene derivatives, the thermal stabilization of G4 strutures of the human KRAS proto-oncogene promoter (KRas21R) and of the telomeric sequence (F21T) by these compounds and compare with the efficiency and selectivity of bis-triazol-benzene analogues and di- and tri-substituted indoloquinolines.[2,3] These compounds are weaker G4 ligands compared with indoloquinolines but more selective to G4 structures vs. duplex DNA.

Acknowledgements: The authors acknowledge FCT for project grant EXPL/QEQ-MED/0502/2012 and Post-Doctoral grant SFRH/BPD/72903/2010.

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Spirooxadiazoline oxindoles with promising in vitro antitumor activities

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Tumor suppressor p53 is a transcription factor widely regarded as the "guardian of the genome" that plays an important role in the regulation of several biological processes. So, it is not surprising that the p53-signaling pathway is inactivated in all types of cancers and that restoring p53 function in cancer cells represents a valuable anticancer approach.[1] In tumors that retain wild-type p53 but have defects in p53 regulatory pathways, the main goal is to inhibit the function of its negative regulators, such as MDM2. Generally, p53-MDM2 interaction inhibitors contain three lipophilic groups attached to a rigid heterocyclic scaffold to mimic the three most important p53 amino acids (Phe19, Trp23 and Leu26) that interact with MDM2. Furthermore, all the interactions are primarily hydrophobic, with potency increasing essentially by introduction of halide-substituted aromatic groups. Based on this information, we developed several novel chemical scaffolds with potential anticancer activity.[2] Herein, we report the synthesis of a library of spirooxadiazoline oxindoles and the biological evaluation as p53-MDM2 interaction inhibitors (Figure 1). The most active compound showed a GI_{50} value of 1.7 μM in HCT 116 *p53*$^{(+/+)}$ cell line, representing a 15.4-fold increase in potency when compared to the most active spiroisoxazoline oxindole obtained previously.[2a] Together, our results indicate that spirooxadiazoline oxindoles reduce the p53 inhibition by MDM2, subsequently increasing the expression levels of p53 target genes, representing a promising scaffold for the development of novel anticancer agents.[3]

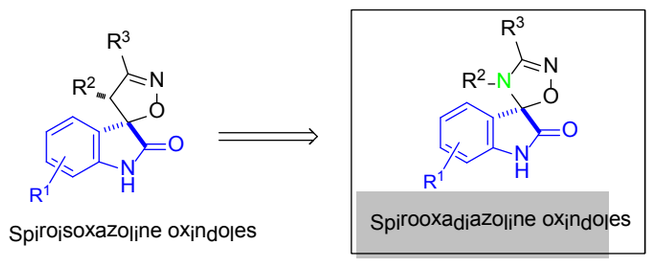

Figure 1: Spirooxindoles with *in vitro* antitumor activity developed in our group.

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Surpassing multidrug resistance in cancer: a study on jolkinol D derivatives

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The emergence of multidrug resistance (MDR) in cancer accounts for 90% of the chemotherapy failure. In such cases the administration of drugs that are structurally and functionally unrelated, no longer produce the desired effect. The main mechanisms attributed to the cancer MDR phenotype include increased drug efflux due to overexpression of the membrane transporter P-glycoprotein (Pgp), evasion of apoptosis, alterations in drug targets and drug inactivation. The most promising strategies to surpass MDR lay on the development of molecules that are able to modulate Pgp mediated efflux and on the collateral sensitivity effect. This effect is verified when some compounds selectively exert a hyper-cytotoxicity on MDR cells, but not in the parental ones.[1] Macrocyclic diterpenes with the lathyrane scaffold have been shown to have an anti-MDR potential, however the challenge to optimize these molecules is still in progress.[2] Hence, our main goal is to attempt to optimize macrocyclic lathyranes as new leads for MDR reversion, as Pgp modulators and/or collateral sensitivity agents.

Aiming to stablish structure-activity relationships (SAR) with Pgp modulation, a small library of 27 macrocyclic lathyrane diterpenes, was achieved by molecular derivatization of jolkinol D, isolated from *Euphorbia piscatoria*. This process was accomplished mainly by using several alkanoyl and aroyl anhydrides/chlorides as acylating agents. The MDR reversal activity of these compounds was evaluated through combination of transport and chemosensitivity assays, using a mouse lymphoma MDR1-transfected cell model. The most relevant SAR results highlighted the importance of the lipophilicity and the presence of an aroyl moiety for Pgp efflux modulation. The effects on the Pgp ATPase function were evaluated for the most potent derivative that showed an inhibitory profile. Drug combination experiments also corroborated the anti-MDR potential of these diterpenes due to their synergistic interaction with doxorubicin. The potential collateral sensitivity effect of the derivatives was evaluated against gastric (EPG85-257) and pancreatic (EPP85-181) human cancer cells and their drug-selected counterparts, resistant to novantrone (RN) and to daunorubicin (RDB), using a proliferation assay. The most promising activity, in EPG85-257RDB cells, was obtained for two derivatives that were able to decrease the level of resistance in 65%, in comparison with the parental cell line, showing an IC_{50} of 3 μM. In reference to the pancreatic cells, the best results were obtained in EPP85-181RDB cells with reduction the levels of resistance in about 32 - 65%. The cytotoxic drug-induced mechanisms of cell death were investigated using annexin V/PI and active caspase-3 assays. It was verified that cell death occurred through caspase-dependent apoptosis.

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Synthesis and anticancer activity of N^3-substituted-6,8-diaminopurines

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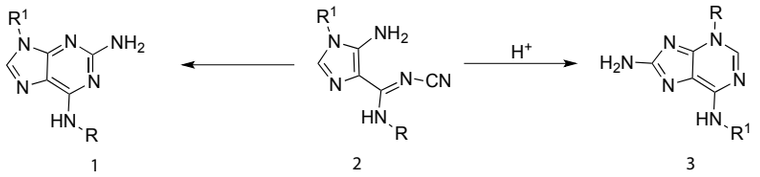
Cancer is the second most common cause of death in developed countries appearing just after cardiovascular *diseases.* The treatment of cancer remains a major medical challenge and the development of new anticancer drugs is an emerging topic for the scientific community. During the past three decades several chemical classes of anticancer drugs have been identified. In particular, 2,6-diamino purines proved to be important candidates as new anti-cancer agents.[1,2]

In a previous work, a set of 2,6,9-substituted adenines was screened as anti-cancer agents in colorectal cancer cell lines HCT116. Hit structures of 2-methyladenines could be identified leading to the synthesis of the bioequivalent 2,6-diaminopurines **1**. A synthetic strategy involving key intermediates **2** was developed on the basis of previous results,[3] starting from 5-amino-4-cianoformimidoyl imidazoldes and cyanamide. Intramolecular cyclization of **2** led to a series of the target products isolated in very good yield and identified as structures **1** by IR and NMR techniques. Attempts to improve the rate of reaction in the presence of acid led to a different product. Structural elucidation by NMR spectroscopy (^{1}H, ^{13}C and 2D NMR) revealed that a novel 8-aminopurine **3** was obtained through an unexpected rearrangement (**Scheme 1**). Structures **3** could be definitively assigned by X-Ray crystallography.



Scheme 1: Synthesis of 2,6-diaminopurines **1** and N^3-substituted-6,8-diaminopurines **3** from imidazole precursors **2**.

The effects of compounds 3 in colorectal cancer cell lines HCT116 were evaluated and a subset of a dozen of compounds emerged as important inhibitors of the cell proliferation with IC50 ~ 1-5 μM.

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Development of New Peptide-Drug Conjugates for Cancer Therapy

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In developed countries, cancer is one of the major causes of death. Gemcitabine (**Figure 1**) is a nucleoside analogue which has been proven efficient against a wide range of solid tumors.[1] Gemcitabine is activated *in vivo* via phosphorylation of its 5'-monophosphate by deoxycytidine kinase, and is subsequently phosphorylated by intracellular kinases to the triphosphate form.[2] However, gemcitabine may be deaminated to its inactive uridine metabolite, 2',2'-difluorodeoxyuridine, by cytidine deaminase, which is present at high levels in both human plasma and liver.[3]

This communication aims at showing the chemical modification of gemcitabine and subsequent conjugation to Cell Penetrating Peptides (CPP), in an effort to facilitate delivery of that drug into cancer cells, taking advantage of the fact that CPP are able to efficiently pass through cell membranes while being non-cytotoxic and carrying a wide variety of cargos inside cells.[4] Two different CPP were synthesized by Solid Phase Peptide Synthesis (SPPS), purified and characterized chromatographically (HPLC and LC-MS/MS). Gemcitabine was successfully modified and conjugated to both CPP. The stability of the hydrolysable bonds was studied in PBS buffer at physiological pH and temperature and the results show a different time-dependent kinetics of gemcitabine release. The effect of the conjugates on the growth of the three cell lines was also evaluated by SRB assays.

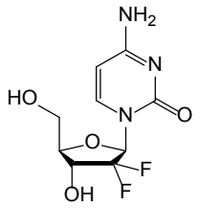

Figure 1: Chemical structure of Gemcitabine or 2′,2′-difluorodeoxycytidine.

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Cholesterol and Cancer. Exploring the Chemical Space of Oxysterols to Find New Drug Candidates.

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Oxygenated derivatives of cholesterol, known as oxysterols, have gained increased attention in medicinal chemistry, due to their wide range of biological effects. Depending on the concentration, these cholesterol derivatives exert beneficial or detrimental effects at the cellular level. In several diseases, like cardiovascular and neurodegenerative diseases, osteoporosis and some cancers, oxysterols are often found at increased concentrations in the tissues and in various biological fluids.[1]

In the last years, natural and synthetic oxysterols have shown cytotoxic activity against cancer cell lines.[2,3] Diverse oxysterols and derivatives were synthesized by selective chemical and enzymatic methods. The cytotoxicity of the new compounds was evaluated in vitro in a panel of cancer and non-cancer cell lines and structure-activity relationships were obtained.[4-6]

The oxysterol derivatives synthesized were, in general, selective for cancer cells, although some modifications reversed the selectivity. Combination of an endogenous oxysterol with the anticancer drug doxorubicin improved its therapeutic profile, while combination with cisplatin led to an opposite effect. Apoptosis induction studies were performed in HT29 cells for selected oxysterol derivatives.

Taken together, our data show the potential of oxysterols as anticancer drugs and the need for further exploitation of the mechanisms underlying their cellular effects.

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Synthesis and biological evaluation of 3-Hydroxyquinolin-2(1H)-ones derivatives as antitumor agents

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The development of new methodologies for the synthesis of 3-Hydroxyquinoline-2(1*H*)-ones derivatives has been stimulated by a wide spectrum of pharmacological activity attributed to these molecules. Recently reported as nonclassical bioisosteres of α-glycine, these derivatives are potent inhibitors of the Human D-amino acid oxidase (DAAO).[1,2] 3-Hydroxyquinoline-2(1*H*)-one derivatives were synthetized in our laboratory with an innovative methodology that enable the introduction of different amide groups in position 4 on the main core. In an effort to investigate this scaffold as a potential anticancer lead molecule, we synthesized several 4-substituted 3-hydroxyquinoline-2(1H)-ones (**2**) to elucidate the structure−activity relationships (SAR). Our previously established protocol consisting on aldol type condensation of α-diazo carbonyl compounds to isatin derivatives and $Rh_2(OAc)_4$ catalyzed ring expansion[3] was applied to the preparation of **1** in up to 90% yield, *via* an efficient NHS-diazoacetate addition. Therefore, simple substitution with primary, secondary amines, and amino acids gave different carboxamide derivatives with excellent yield, leading this methodology a useful tool for coupling peptide moieties to the α-glycine bioisostere. Additionally, all derivatives were evaluated for the first time in different cancer cell lines, wherein carboxamide derivatives derived from leucine exhibited promising anticancer activity up to 2 μM in NCI-460 cancer cell line, thus leading to a potential candidate for further investigations.

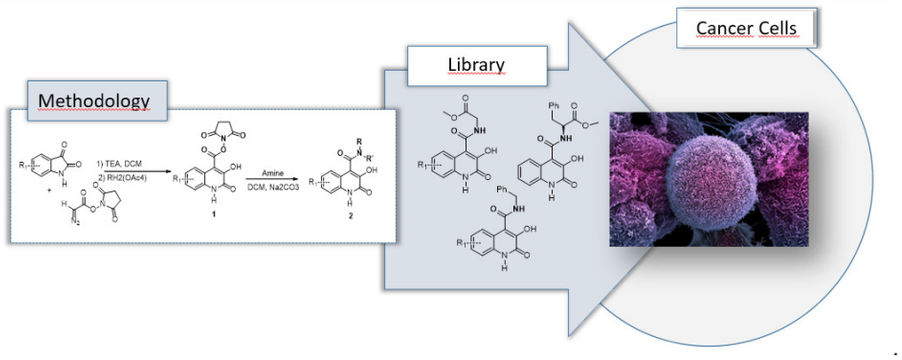

Figure 1. Methodology development for the 3-Hydroxyquinoline-2(1H)-ones carboxamide derivatives (**2**).

Acknowledgements: We thank the Fundação para a Ciência e Tecnolgia for financial support (PEst-OE/SAU/UI4013/2011; PTDC/QUIQUI/099389/2008; SFRH/BD/78301/2011).

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Pharmaceutical Ionic Liquids and Salts as antitumor agents

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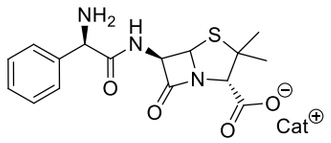
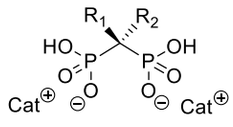
Synthesis of Ionic Liquids from Active Principle Ingredients (API-ILs) has been the main focus of our group for the last years. The combination of APIs as anions or cations with appropriate organic counter ions can be an innovative solution to the polymorphism behavior of several drugs as well as to improve their water solubility, permeability and corresponding bioavailability and biological activity.[1] Within this context, novel ionic liquids with anti-cancer properties and decreased toxicity have recently been investigated.[1]

In this communication we present the anti-proliferative effect against diverse tumor cell lines of novel Ionic Liquids based on anionic Ampicillin and Bisphosphonates combined with appropriate biocompatible organic cations (e.g. choline, cetylpyridinium and alkylimidazolium) (**Figure 1**).[2] This approach has conferred antitumor activity against five different human cancer cell lines with IC50 values in the low micromolar/nanomolar ranges to Ampicillin, a well-known antibiotic against many Gram-positive and Gram-negative bacteria, while showing enhanced antibacterial properties against sensitive and Gram-negative resistant bacteria.[2] On the other hand, by taking advantage of the high affinity of bisphosphonates towards bone tissue,[3] the new API-ILs based on these compounds have been also tested against related cancer cell lines. The discussion will be complemented with the study of different physicochemical properties.



Ampicillin



bisphosphonates

Figure 1: Novel API-ILs based on anionic Ampicillin and bisphosphonates combined with biocompatible organic cations.

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Hybrid compounds for the treatment of glioma: a new approach

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Glioma is a type of primary brain tumour that arises from glial cells. The most common of malignant gliomas, glioblastoma multiforme, has a median survival of approximately 14 months after diagnosis.[1]
Temozolomide is the major anticancer agent for gliomas. It is a triazene alkylating agent and, owing to its lipophilicity, has two features that make it an antitumor of choice: can be given orally and is able to cross the blood-brain barrier. However, therapeutic effectiveness is often disappointing, largely in consequence of the lack of selectivity for tumor cells, insufficient drug concentration in the tumor and notorious resistance.[2] Effective new strategies are desperately needed.
Valproic acid is an anticonvulsant used in the treatment of epilepsy. Also, valproic acid inhibits a subset of histone deacetylases (HDAC) and affects gene transcription. Furthermore, it inhibits DNA repair, thereby potentiating cytotoxic treatments such as chemotherapy or radiation therapy.[3]
Our team has been involved in the design of novel HYBRID COMPOUNDS (HYBCOM, HYBBUT, HYBISO, HYBETHYL and HYBGLINIB) with two units, a triazene and one HDAC inhibitor. Preliminary results towards glioma cell line (GL261), demonstrated that this hybrid compound has efficacy with lower concentrations, when compared to Temozolomide.
In this research work we report the evaluation of hybrid compounds with an aryltriazene as an alkylating agent and various carboxylic acids with known HDAC inhibitory activity. The stability of the compounds in phosphate buffer pH 7.4 and human plasma (80% v/v) were evaluated by HPLC. Our first results demonstrated a high stability in physiological conditions, with half-lives >72h. Moreover, the determination of lipophilicity in order to acess compounds permeability through the blood-brain barrier is described. We also assessed the stability towards nucleophiles, such as NaOH and N-acetylcysteine. Additionally, hybrid compounds were screened for their inhibitory activity against HDAC , showing that HYBCOM2 and HYBBUT presented substantial activity. Further investigation and optimization of ^{1}H-NMR titration experiments for the evaluation of complexation with metals are still ongoing.

Acknowledgements: We thank the Fundação para a Ciência e Tecnologia for financial support, by iMed.ULisboa (UID/DTP/04138/2013)

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Roy-Bz: the first small molecule selective activator of protein Kinase Cδ

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The protein Kinase C (PKC) is a family of serine/threonine kinases with at least ten isoforms grouped into three major subfamilies: classical (cPKC: α, βI, βII and γ), novel (nPKC: δ, ε, η and θ) and atypical (aPKC: ζ and λ\ι). PKCδ has been one of the most studied PKC isoforms in cancer due to its well-known tumor suppressor function. Based on this, PKCδ has been widely recognized as a promising therapeutic target in anticancer drug development. Despite this, due to the complexity of this family of structurally-related kinases, the search for isoform-selective PKC modulators, particularly of PKCδ, has been a great pharmacological challenge. Actually, for the particular case of PKCδ, to date, selective activators of this isoform remain unknown.[1]

During the last years, the use of yeast *Saccharomyces cerevisiae* individually expressing PKC isoforms to search for isoform-selective PKC modulators led us to the identification of an abietane diterpene compound (Coleon U) as activator of nPKCδ and ε.[2] In this work, this yeast-based screening approach led us to the identification of Roy-Bz, a semi-synthetic derivative of a natural diterpenoid royleanone isolated from *Plectranthus grandidentatus*, as potential PKCδ-selective activator. The selectivity and direct binding of Roy-Bz to PKCδ was confirmed using an *in vitro* PKC binding assay and recombinant PKC isoforms of the three subfamilies. In human colon carcinoma cell lines (HCT116), Roy-Bz exhibited a potent growth inhibitory effect (GI_{50} value of 0.63 ± 0.05 μM), through activation of a p53-dependent mitochondrial apoptotic pathway involving p53 phosphorylation at Ser46. All these effects showed to be dependent on PKCδ activation, since either the PKCδ inhibition, using the PKCδ selective inhibitor rottlerin, or its knock-down abolished the Roy-Bz-induced apoptotic effects in HCT116 cells. Supporting its *in vitro* antitumor potential, Roy-Bz inhibited cell migration of HCT116 tumor cells. Additionally, it had no genotoxic effects, and exhibited a low cytotoxicity against human normal cells. Finally, in xenograft mice models, Roy-Bz exhibited a potent antitumor activity, without apparent toxicity.

Collectively, several evidences are here provided confirming the identification of the first selective small molecule activator of PKCδ.

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Development of brain permeant peptidomimetic β-secretase inhibitors for Alzheimer's disease

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β-secretase (BACE-1) inhibitors are potential useful drugs for the management of Alzheimer's disease (AD), but their incapacity to cross the blood-brain barrier and reach the Central Nervous System (CNS) is still a major reason for failure.[1] In this work we have tested the hypothesis of whether the conjugation of a peptidomimetic inhibitor, OM00-3, with a β-amyloid peptide sequence, Aβ18-23, (**Figure 1**) facilitates its delivery into the brain.

Inhibitors were synthesized by Solid Phase Peptide Synthesis. Their potency against BACE-1/2, cytotoxicity in Caco-2 cells, metabolization in serum and mice brain were determined. A pharmacokinetic assay was performed in mice.

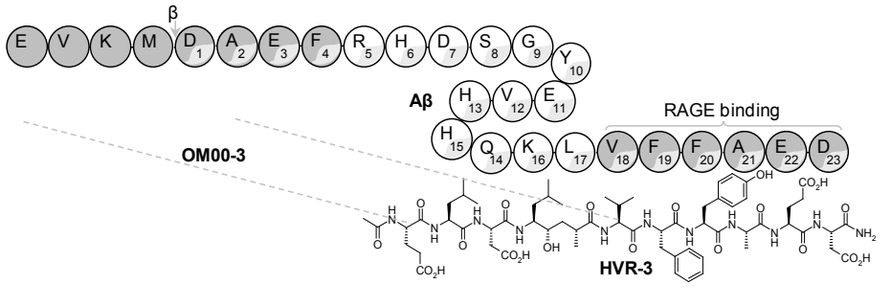

Figure 1: Rational drug design of HVR-3, starting from OM00-3 and Aβ18−23.

HVR-3 was found to be as potent as OM00-3 but 4-fold more selective toward BACE-1 in relation to BACE-2 and also 3-fold more stable against *in vitro* metabolization in Human serum. Intravenous administration to mice generated an active metabolite recovered from the rodent's brain. The success of this conjugation strategy to target the CNS corroborates the potential of HVR-3 as new anti-AD drug.[2]

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Development of neurotrophic agents based on hydroxycinnamic acid scaffold

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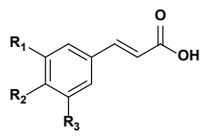
Parkinson's disease (PD) is the second most common neurodegenerative disease and affects 1-2% of the population above the age of 65 worldwide. L-DOPA, dopamine agonists, MAO-B inhibitors and NMDA receptor antagonists are used to ameliorate the motor symptoms in PD patients. However, none of the current therapies is able to halt disease progression.[1] On the other hand, neurogenesis is often impaired in aging brains, PD and other neurodegenerative disorders due to the insufficient production of neurotrophic factors. Nerve growth factor (NGF), a neurotrophic factor of the nervous system, has an important role in neuronal differentiation and survival and to be critical for the development and maintenance of the nervous system.[2] In accordance, NGF based therapeutic approaches have been developed to enhance neurogenesis. However, the clinical applications of NGF and several other neurotrophic factors have been dampened by side effects and bioavailability issues.[3,4] Significant efforts have been made to obtain small molecules able to mimic or enhance the pharmacological effects of neurotrophic factors. In this context, the present study reports the design and synthesis of a bioinspired small library based on *p*-coumaric, caffeic, ferulic and sinapic acids (**Figure 1**) and the evaluation of their neuroprotective activity in a PC12 cell line.



1, *p*-Coumaric acid, R_1 = H, R_2 = OH, R_3 = H
2, Caffeic acid, R_1 = R_2 = OH, R_3 = H
3, Ferulic acid, R_1 = OCH_3, R_2 = OH, R_3 = H
4, Sinapic acid, R_1 = OCH_3, R_2 = OH, R_3 = OCH_3

Figure 1: Chemical structure of the parent cinnamic acids (**1**-**4**).

The derivatives based on *p*-coumaric, ferulic and sinapic acids did not have any significant effect on cell viability. However, caffeic acid derivatives were able to significantly promote the survival of PC12 cells. In particular, caffeic acid ester derivatives successfully promote NGF-induced neurite outgrowth. The data obtained so far can be used in the rational design of pharmacological agents able to protect the architecture of cells in the central nervous system.

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Fatty acids from edible sea hares: Anti-inflammatory capacity in LPS-stimulated RAW 264.7 cells involves iNOS modulation

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In recent years, marine macroinvertebrates gained great importance by their fatty acid composition. Sea hares of *Aplysia* genus are known to be consumed, in oriental countries;[1] however, their nutritional composition and potential health effects are nearly unknown. In the present study we intended to characterize the fatty acids composition and evaluate the anti-inflammatory potential of lipophilic extracts of two sea hares, *Aplysia fasciata* Poiret and *Aplysia punctata* Cuvier.

Twenty-five fatty acids were identified, nine of them not yet reported in these species. Both extracts revealed similar anti-inflammatory properties in the culture medium of LPS-stimulated macrophages, as proved by the decreased •NO levels. A similar decrease was also observed in L-citrulline levels, indicating a possible modulation of inducible nitric oxide synthase (iNOS) by the action of the compounds found in the extracts (**Figure 1 A.**). Regarding lipoxygenase (LOX) inhibition, *A. punctata* extract was more effective (**Figure 1 B.**), probably because it contains more polyunsaturated fatty acids (PUFA) that can compete with linoleic acid for the active site.

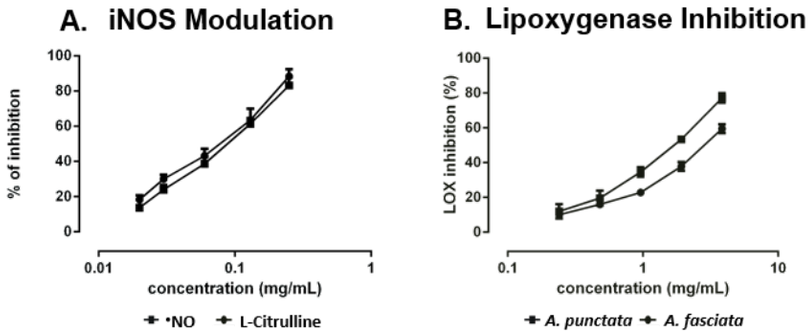

Figure 1: Effect in •NO and L-citrulline levels of RAW 264.7 cells pre-treated with *Aplysia fasciata* extract (**A**) and inhibition of soybean lipoxygenase by extracts of *Aplysia* spp. (**B**).

Overall, the results indicate that, in addition to their direct ingestion, *A. fasciata* and *A. punctata* may be good sources of nutraceuticals providing beneficial health effects, by reducing the levels of inflammatory mediators involved in the genesis of several diseases.

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Design, synthesis and biological evaluation of novel anti-bacterial agents

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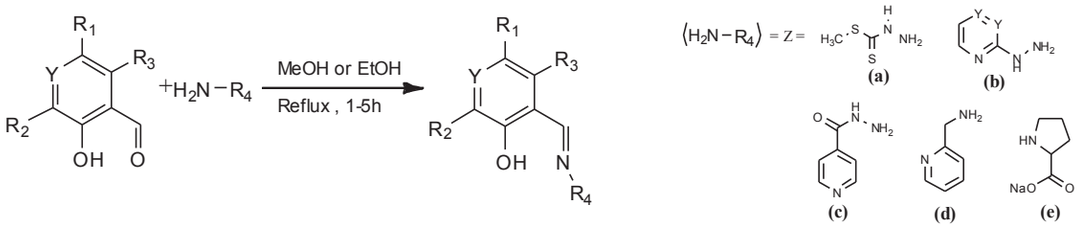
Aromatic Schiff bases represent versatile pharmacophores with potential antimicrobial properties. As part of a program aimed at identifying new drug candidates with multi-target antibacterial activity, we have been exploring several classes of bis-hydrazone compounds and their iron complexes (**Scheme**).



Scheme: General synthetic procedure for the ligands used in this study

The antimicrobial activities of the synthesized compounds against the Gram-positive *S.aureus* Newman and the Gram-negative *P.aeruginosa* 477 were assessed using the Disk Diffusion Test. The Minimum Inhibition Concentrations (MICs) were also determined. Although the results are still preliminary, moderate activities were observed for compounds **1** and **7**. The results obtained will be discussed on the basis of structure-activity relationships.

Table: Representative test compounds used in this study

Compound	1	2	3	4	5	6	7	8	9	10	11
R_1	CH_3	$C(CH_3)_3$	CH_3		CH_3	H	H	H	CH_3	H	CH_3
R_2	CHO	CHO	CHO		CHO	H	OCH_3	CH_3	CHO	H	CHO
R_3	H	H	H		H	H	H	CH_2OH	H	H	H
Y	C	C	C		C	C	C	N	C	C	C
Z	(b)	(e)	(b)	(d)	(a)	(a)	(a)	(a)	(c)	(c)	(c)
complex	iron	iron	-	-	-	-	-	-	-	-	-

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Herbal medicines: a source of phenolic monoamine oxidase A inhibitors

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The use of herbal drugs for improving health and for treating several disorders, including depression, is becoming increasingly popular in western societies. The enzyme monoamine oxidase A (MAO-A) is one of the targets of the antidepressant drugs available in the market. Since almost 30% of the patients do not respond to the current treatments it is urgent to find new antidepressants.[1] Herbal teas prepared from several plant species with claimed antidepressant properties, as well as from other species non-related with depression treatment, have been screened for the first time against MAO-A by our group, with promising results. The following IC_{50} values were obtained: 699.8 μg/mL (*Jasminum officinalis* L.),[2] 19.3 μg/mL (*Annona muricata* L.),[3] 40.5 μg/mL (*Hyssopus officinalis* L.),[3] 428.1 μg/mL (*Cereus grandiflorum* L.),[3] 55.2 μg/mL (*Turnera diffusa* Willd. ex Schult.), 38.5 μg/mL (*Cochlospermum angolensis* Welw.),[4] 17.4 μg/mL (*Jacaranda caroba* (Vell.) A. DC.)[5] and 99.5 μg/mL (*Grindelia robusta* Nutt.).[6]

The herbal teas were also analyzed by HPLC-DAD-ESI-MS^n and HPLC-DAD, revealing the presence of different hydroxybenzoic acids, hydroxycinnamic acids and flavonoids.

Since *A. muricata* displayed a strong anti-MAO-A activity and is traditionally used against depression, its herbal tea was selected to be further incorporated in liposomes functionalized with ApoE. From the chemical point of view, the extract contains 14.1 mg of phenolic compounds/g of dried extract and is composed by 5-*O*-caffeoylquinic acid, quercetin-3-*O*-galactoside, quercetin-3-*O*-glucoside, quercetin-3-*O*-rutinoside and kaempferol-3-*O*-rutinoside. Afterwards, the same approach will be carried out with the *J. caroba* herbal tea. Further studies will include the assessment of the effect of these herbal teas on serotonin and/or noradrenaline reuptake transporters, which are also extremely important targets for antidepressant drug design.

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SLMP53-1: a new reactivator of mutant p53 with potent *in vivo* antitumor activity

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The p53 tumor suppressor protein is a transcription factor inactivated in all human cancers due to mutations in the p53 protein (about 50%) or to the overexpression of endogenous negative regulators of wild-type (wt) p53. The high prevalence, and the often observed increased drug resistance of mutant p53-expressing tumors, make mutant p53 a highly appealing target for novel cancer therapies.[1] In the present work, yeast assays consisting of *Saccharomyces cerevisiae* cells expressing human wt p53 or the most prevalent human mutant p53 forms were used to screen for reactivators of these inactive forms of p53. Using this approach, a chemical library of enantiopure tryptophanol-derived oxazoloisoindolinones (from Santos' research group) was tested, and SLMP53-1 was selected as a potential activator of wt p53 and reactivator of mutant p53R280K.[2] The molecular mechanism of SLMP53-1 was further validated in human colon carcinoma tumor cells with wt p53 (HCT116 $p53^{+/+}$) and in its p53-null isogenic derivative (HCT116 $p53^{-/-}$), as well as in breast adenocarcinoma MDA-MB-231 cells expressing the mutant p53 R280K. In these cells, SLMP53-1 exhibited a p53-dependent growth inhibitory effect associated with a G1-phase cell cycle arrest (in HCT116 $p53^{+/+}$ cells) and apoptosis (in HCT116 $p53^{+/+}$ and MDA-MB-231 cells), and increased the expression levels of several p53 target genes in HCT116 $p53^{+/+}$ and MDA-MB-231 cells, but not in HCT116 $p53^{-/-}$ cells. In MDA-MB-231 cells, SLMP53-1 reestablished the wt-like DNA binding ability to mutant p53R280K. Additionally, SLMP53-1 potently triggered a mitochondrial apoptotic pathway in HCT116 $p53^{+/+}$ and MDA-MB-231 cells, involving Bax and wt/mutant p53 translocation to mitochondria. Besides this, SLMP53-1 sensitized HCT116 $p53^{+/+}$ and MDA-MB-231 cells to the effects of conventional chemotherapeutic agents and inhibited cell migration. Contrary to the majority of known p53 activators, no genotoxicity and *in vivo* toxicity were observed with SLMP53-1. Finally, the p53-dependent antitumor activity of SLMP53-1 were validated *in vivo* using xenograft mouse models.

Collectively, besides its potential as anticancer drug, SLMP53-1 belongs to a new chemical family, and its scaffold is a starting point for the development of effective drugs targeting mutant p53 forms.

Acknowledgements: This work was funded by FEDER funds through the Operational Programme for Competitiveness Factors - COMPETE and by National Funds through FCT - Foundation for Science and Technology – under the UID/Multi/04378/2013 (UCIBIO/REQUIMTE), UID/DTP/04138/2013 (iMed.ULisboa), PTDC/QUI-QUI/111664/2009 and PTDC/DTP-FTO/1981/2014. J. Soares (SFRH/BD/78971/2011), C. Bessa (SFRH/BD/87109/2012), S. Gomes (SFRH/BD/96189/2013) and A. S. Gomes (PD/BI/105912/2014) are recipients of FCT fellowships.

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G-quadruplex stabilisation by novel indolo[3,2-*c*]quinolines: a structural analysis of binging

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G-quadruplexes (G4) are four-stranded nucleic acid tertiary structures formed by guanine (G) bases from short tracts of G-rich sequences. These DNA structures play an important role in cell growth and gene expression and its formation in eukaryotic telomeric and oncogenic DNA is well established.[1, 2] G4 formation can effectively inhibit the activity of telomerase, a critical enzyme to the proliferation of cancer cells, as well as to inhibit the transcription of several oncogenes, thus leading to the development of novel strategies for improved anticancer therapy.[3, 4] Therefore, it is of great interest the development of small molecules that can bind and stabilize selectively G4 structures and as such act as anticancer agents.

A library of 5-methyl-indolo[3,2-*c*]quinolines (IQc), with different substitution patterns of alkyldiamine side chains, was synthetized and evaluated for G4 binding.[5] FRET melting assay showed that positively charged IQc with two weak basic side chains are potent and selective human telomeric (HT) and oncogenic G4 stabilisers. Spectroscopic studies with HT G4 as model, showed that IQc stabilising complex involves the binding of two IQc per G4 unit, in two non-independent but equivalent binding sites. Molecular dynamic studies suggest that end-stacking of IQc induces a conformational rearrangement in the G4 structure, driving the binding of a second ligand to a G4 groove. Modelling studies also suggest that IQc with two three-carbon side chains have the appropriate geometry to participate in direct or water-mediated H-bonding, assisted by the side chains terminal nitrogen atoms, to phosphate backbone and/or G4 loops.

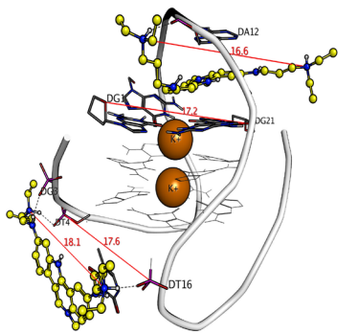

Figure 1: Binding interactions between IQc and the human telomeric G4. Values in Å.

Acknowledgements: Authors would like to thank to Fundação para a Ciência e Tecnologia (FCT) for financial support through project grants PEst-OE/SAU/UI4013/2014 and EXPL/QEQ-MED/0502/2012. J. Lavrado also acknowledges FCT for the Post-Doctoral grant SFRH/BPD/72903/2010.

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Antimalarial activity of *s*-Triazine based hybrids in both erythrocytic and liver stages.

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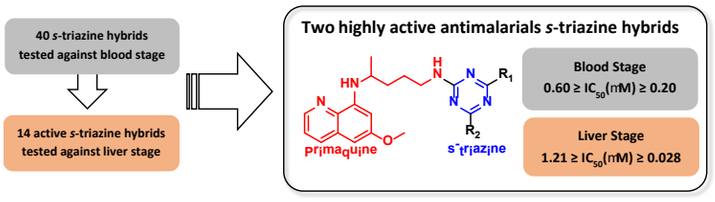
Malaria is a deadly disease that, despite being preventable and curable, is threatening the world wide health. It is caused by *Plasmodium* parasites that are transmitted to people through the bites of infected female *Anopheles* mosquitoes[1]. A considerable range of structurally diverse antimalarial drugs is under research but an efficient molecule in both liver and blood stages was not yet established. The major problem in finding an effective molecule against malaria is that *Plasmodium* parasite gains a fast resistance to the new drugs[2]. Combination of structures known to have antimalarial activity – hybrids - is a very used strategy to circumvent this inefficiency drawback once they can reach several biological targets by attaching different structures that act by different mechanisms in one single molecule[3]. Among them, *s*-triazine is a versatile core widely applied in the synthesis of hybrids with antimalarial activity, namely 4-aminoquinoline-*s*-triazine[4]. Primaquine is an 8-aminoquinoline compound which presents the highest activity in liver stage. It is also the only registered drug for radical cure of blood and liver stages malaria caused by *P. vivax* and *P.ovale* infection[5]. Herein we study the combination of the liver stage active primaquine with *s*-triazine core, aiming to find a hybrid molecule active in both liver and blood stage of malaria disease. In vitro tests in blood stage against *P. Falciparum W2* strain have shown encouraging results, for *s*-triazine hybrids carrying one or two primaquine moieties were obtained IC_{50} ranging from 0.2 to 8.3 microM. One primaquine-*s*-triazine hybrid also showed promising results in in vitro human hepatoma Huh-7 cells infected with a firefly luciferase-expressing *P. berghei* line at a 1 microM dose.



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Lessons from the study of the binding mechanism of "hit" compounds to albumin: fluorescence and *in silico* experiments applied to xanthone derivatives

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In the pipeline that drives "hit" compounds to drug candidates, special attention must be given to both pharmacodynamic and pharmacokinetic behaviors. Regarding pharmacokinetic behavior, the binding of drugs to plasma proteins is an important issue, since it affects dramatically ADMET properties. Human serum albumin (HSA) is the most abundant and important plasma protein. Therefore, its potential to interact with a series of *in-house* xanthone derivatives[1] has been studied using fluorescence and *in silico* techniques.
The binding of the studied xanthone derivatives to HSA was evaluated by fluorescence quenching technique and UV–Vis absorption spectroscopy at four different temperatures[2]. The number of binding sites and the apparent binding constant were calculated using nonlinear fitting model. The results of this experiment revealed that the evaluated analogues bind tightly to HSA. The binding to HSA was also investigated by Förster resonance energy transfer technique[2]. This experiment showed that the bound compounds were 30Å apart from tryptophan-214 in the subdomain IIA[2]. In order to clarify how xanthones bind to HSA, an *in silico* study was also performed[3]. Using AutoDock Vina program, a molecular docking to a HSA crystal structure (pdb code: 2VUE) was conducted. The results revealed that all xanthone derivatives bind in a hydrophobic pocket located in subdomain IB, which is not a common HSA binding site for drugs. Ibuprofen and warfarin were used as markers to validate these results[3].
The obtained results allowed to enhance our knowledge about the binding of xanthones to HSA and to guide the multidimensional optimization of these "hit" compounds.

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ER stress and protein quality control pathways: Exploring the natural products chemical space

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The endoplasmic reticulum (ER) is an organelle comprising a network of branching tubules and sacs that is present in all eukaryotic cells. ER stress has been identified as a hallmark, and sometimes trigger, of several pathologies, notably cancer, inflammation and neurodegenerative diseases like Alzheimer's and Parkinson's.

One of the factors that can trigger ER stress is the presence of unfolded or misfolded proteins, which can be a consequence of the inhibition of the proteasome, an eukaryotic protein complex that is involved in proteolysis of undesired proteins.

Among the molecules described in literature known to affect ER and proteasome function, the majority are natural products, suggesting that natural molecules may constitute a significant arsenal of chemical entities for modulating this cellular target.

In this presentation, we will start present briefly the current knowledge of ER biology and the hallmarks of ER stress, thus paving the way for presenting the natural products that have been described as being ER modulators, either stress inducers or ER protectors.

The chemistry, distribution and mechanism of action of these compounds will be presented and discussed, including examples both from the literature and from our Laboratory.

Wrapping it all around: *in silico* approaches to improve the MDR-reversal properties of the macrocyclic diterpenic core

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The increasing number of chemotherapy failures reported worldwide identifies multidrug resistance (MDR) to anticancer drugs as a serious health concern. As the over-expression of ABC transporters in cancer cell lines is one of the most reported MDR mechanisms, the inhibition of MDR-related efflux pumps as P-glycoprotein (Pgp) remains a promising approach for overcoming MDR.[1]

Macrocyclic diterpenes isolated from *Euphorbia* species have been characterized as potent Pgp efflux modulators. However, their potency can be further improved by molecular manipulation of the diterpenic core. Thus, *in silico* approaches can be valuable tools for the identification of the most suitable modifications for MDR modulation.[2]

Several computational approaches were applied to a small diterpenic library (n= 25) obtained by chemical derivatization of compounds isolated from *Euphorbia boetica*.[3] A virtual screening procedure involving pharmacophoric identification and molecular docking was used to assess the accuracy of structure-based drug discovery techniques in the prediction of the experimental MDR-reversal activities. Simultaneously, ligand-based drug discovery techniques were used to characterize the relationship between chemical modifications and the respective modulation capabilities.[4] From these procedures, a thorough characterization of the groups involved in the MDR-reversal activity was obtained, which can be further used to guide chemical derivatization, avoiding the synthesis of low-activity compounds.

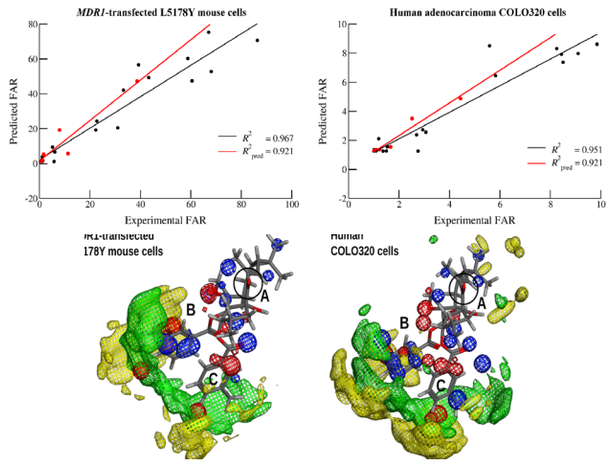

Figure 1: Regression curves for the best model (left); pharmacophoric hypothesis for the diterpenic core (right).

Acknowledgements: Fundação para a Ciência e a Tecnologia (*FCT, Portugal*) is acknowledged for financial support (project PTDC/QEQ-MED/0905/2012 and PhD scholarship SFRH/BD/84285/2012).

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The anti-HIV drug Rilpivirine: covalent adducts with amino acids and proteins

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Rilpivirine (4-[[4-[[4-[(*E*)-2-cyanoethenyl]-2,6-dimethylphenyl]amino]-2-pyrimidinyl]amino]benzonitrile, RPV) is a 2nd-generation non-nucleoside reverse transcriptase inhibitor (NNRTI) that was added to the set of treatment possibilities with the aim to overcome the most common adverse effects of the 1st-generation NNRTIs, nevirapine and efavirenz, as well as their viral cross-resistance. However, post-marketing reports of RPV-associated depressive disorders[1] are a cause for concern when contemplating chronic administration regimens. It should be emphasized that patients who start 2nd generation NNRTI-based combined antiretroviral therapy (cART) have a history of virus resistance and typically experienced several drug-induced side effects. Hence, the administration of a potentially toxic drug to these already debilitated patients must be considered carefully and the development of reliable prognostic tools for early risk/benefit estimations is urgent.

Covalent adduct formation with cysteine residues of synaptic proteins is considered a major mechanism of neurotoxicity induced by chemical toxicants such as acrylamide.[2] The identification of urinary Phase II conjugates stemming from initial 1,4 Michael addition of glutathione (GSH) to the α,β-unsaturated system of RPV supports the likelihood of reaction with proteins *in vivo*.[3]

With the ultimate goals of disclosing the mechanisms underlying RPV-induced neurotoxicity and developing suitable biomarkers of toxicity, the reactivity of RPV towards amino acids (*N*-acetyl-lysine and *N*-acetyl-cysteine) and model proteins such as Human Serum Albumin (HSA) was investigated by liquid chromatography – mass spectrometry (LC-MS) methodologies. The results obtained support the role of protein modification by RPV in the onset of the toxic effects elicited by this anti HIV drug.

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Interaction of Xanthone with Double Stranded DNA – A Contribution for Xanthone Derivative Drugs

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Xanthones are an important group of oxygenated heterocyclic compounds, which are known to exhibit interesting pharmacological properties, such as anti-tumoral activity.[1] For this reason xanthone and xanthone derivatives have received considerable attention in recent years. Especially, several studies have emphasized the high potential of xanthones as promising building blocks for the development of a new class of anti-cancer agents.

Numerous studies have revealed that DNA is one of the primary intracellular targets of anticancer drugs, due to the interaction between small molecules and DNA. Therefore nucleic acids represent a major target in drug development strategies. For this reason, characterization of the interaction of small molecular ligands with DNA can be a vital contribution for understanding the biological mechanisms of their bioactivities, as well as providing useful guidance in drug development studies.

In this communication we report the study of the interaction of xanthone with calf thymus DNA, using UV-vis spectroscopy, including DNA melting experiments, and viscosity measurements. Absorption spectra, as well as UV melting curves, were recorded for solutions with constant DNA concentration and different concentrations of xanthone. DNA melting experiments were carried out by recording the change in absorbance at 260 nm for DNA in the absence and presence of xanthone. The denaturation temperature and the thermodynamic parameters of DNA thermal denaturation were determined from the curves of melted base pairs as a function of temperature. The hyperchromicity at 260 nm was obtained for each concentration of xanthone, at the denaturation temperature and at a higher temperature, at which it is assumed that the strands of DNA have been totally separated. The binding constant, at 293 K, of the xanthone–DNA complex was also calculated. The results indicate a strong binding affinity of xanthone with DNA, showing that xanthone interacts with DNA, affecting the stability of the double helix. The results suggest the binding of xanthone to DNA mainly by intercalation. This study is expected to provide new insights into the mechanism of interaction between xanthone and DNA.

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Synthesis of Phenolic Compounds Sulfate Metabolites

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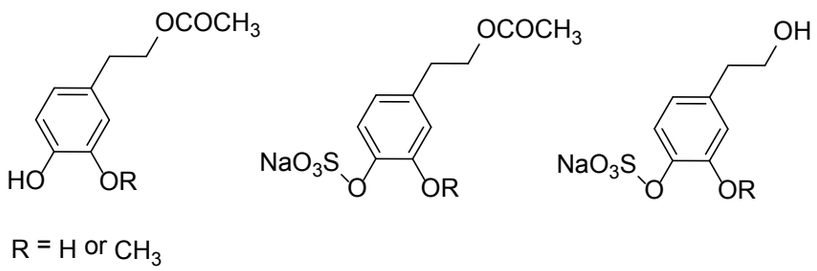
Although many studies have investigated the in vitro antioxidant properties of virgin olive oil phenolics as well as their protective effects against cell injury[1], the biological properties of these phenols in vivo depends on the extent to which they are absorbed and metabolized. In a recent work, the metabolites hydroxytyrosol (3,4-dihydroxyphenylethanol) sulfate and hydroxytyrosol acetate sulfate were found to be the most useful metabolites for monitoring the intake compliance of extra virgin olive oil, showing a significant post-treatment increase both in plasma and urine[2].

The growing interest in the bioactivity of natural polyphenols and of their metabolites requires metabolites to be used in bioassays and as standards in research protocols. Therefore, we report here the synthesis of several hydroxytyrosol and hydroxytyrosol metabolite sulfates achieved using a simplified protocol with improved yields. A synthetic solution based on avoidance of high temperature conditions during the synthesis and of low pressure conditions during purification has been established. Sulfates of several phenolic compounds (Figure 1), namely hydroxytyrosol, hydroxytyrosol acetate, homovanillyl alcohol, homovanillyl alcohol acetate, homovanillilic acid, ferulic acid, and 3,4-dihydroxyphenylethanoic acid, were efficiently synthesized in 1-2 steps in a good yield and purified form using simple procedures. The proposed protocol was shown to be relatively fast, efficient, cheap and widely applicable to a number of catechol scaffolds.



Scheme 1: Hydroxytyrosol sulfate formation

Acknowledgements: This work was supported by FCT (UID/QUI/50006/2013 and UID/Multi/04378/2013)

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Torin-based compounds as inhibitors against trypanosomatid parasites

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American Trypanosomiasis (AT) and Human African Trypanosomiasis (HAT) are neglected tropical diseases (NTDs) caused by the vector-borne infection with parasitic protozoa of the genus *Trypanosoma*. According to the World Health Organization (WHO), these diseases affect millions of people and are responsible for thousands of deaths every year. Despite of the high prevalence in developing countries, the available drugs are outdated, present several side effects, lack efficacy and/or are hard to administer [1]. Therefore new drugs are urgently needed.

One powerful approach to fight the dearth of drugs for NTDs has been directed at repurposing established knowledge about classes of molecular targets that the pathogen holds in common with humans (target repurposing) [2]. We have recently disclosed Torin2, an ATP-competitive mTOR kinase inhibitor [3], as a potent antimalarial with *in vivo* activity against both liver and blood stages and a distinct mode of action compared with currently used antimalarials [4].

In order to access the applicability of this new chemotype to other human parasitic protozoan diseases, we have synthesized a small library of Torin2 analogues and tested it against *Trypanosoma cruzi* and *Trypanosoma brucei*, the agents of AT and HAT respectively. Identifying the target of the Torin-based compounds is also a major interest, thus adequate chemical tools are currently being developed to allow live-cell imaging and proteome profiling based on affinity probes.

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Flavonoids' effects in proinflammatory signaling systems: *in vitro* structure/activity studies

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Flavonoids have been associated with various health benefits, in which their anti-inflammatory effects play an important role. These properties associated with their ubiquitous distribution in nature, and their presence in the great majority of foods, as part of our daily diet, confer flavonoids great value-added molecules. Taking in account the potential anti-inflammatory properties, flavonoids started to be considered a valuable alternative to modulate and prevent inflammatory processes; and moreover, to be the base for the synthesis of more potent and efficient anti-inflammatory drugs.[1] The work herein developed intended to extend and rationalize the current knowledge on the alleged anti-inflammatory properties of flavonoids by elucidating the mechanism of action related with their structure (structure-activity relationship). For this purpose, a group of 24 flavonoids were selected, belonging to three flavonoid classes, flavones, flavanones and flavonols (**Figure 1**). Their ability to modulate various proinflammatory signaling systems was assessed using various approaches: modulation of human neutrophils' oxidative burst; inhibition of leukotriene B_4, via 5-lipoxygenase, and prostaglandin E_2, via cyclooxygenases -1 and -2, production; and apoptosis induction. The experimental studies performed in the scope of this work allowed the conclusion that among the tested flavonoids, the ones with a catechol group in B-ring revealed overall best anti-inflammatory activities. Indeed, flavonoids 3',4'-dihydroxyflavone, 5,3',4'-trihydroxyflavone, 7,3',4'-trihydroxyflavone, and luteolin are undoubtedly good modulators of all the pro-inflammatory mediators evaluated, constituting promising alternatives for the resolution of inflammatory processes.

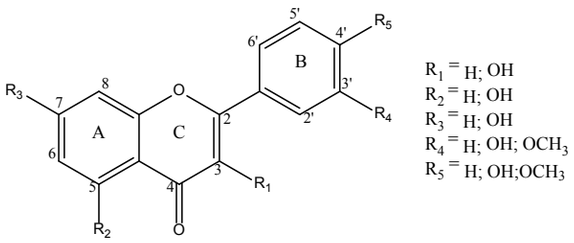

Figure 1: Representative chemical structure of the studied flavonoids.

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Analysis of Characteristics Mannitol ($C_6H_{14}O_6$) used in Bags of Red Cell Concentrates

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The oxygen supply to the cells need meet the metabolic demand, and have a critical effect in energy requirement in the cardiorespiratory system, this offer can be seriously affected in the case a person suffering a injury because of a surgery trauma or car accident causing blood loss, in the cases it is normal doctors recommend the transfusion of concentrates of red blood cells to help O2 supply maintenance in the human organism. The red blood cells (CH) have a complex system[1], having three different stages of C = 3 substances (plasma, proteins and oxygen) being multiphase P + 3 (solid, liquid and gas). For -CH be stored is necessary that it be carried out at temperatures 2° C to 6° C, depending on how this occurs can lead to formation of ice crystals, causing damage to the red blood cells. Depending on the severity of the damage caused during the cryopreservation process can lead to cell death. To reduce effect caused because formation of ice crystals in the blood component, in the storage process is added cryoprotectant solutions such as mannitol ($C^6H^{14}O^6$), in the bags blood derived bags[2]. Besides the natural toxicity caused by the use of cryoprotectants agents in the cryopreservation process, also observed the formation of reactive oxygen species (ROS), and in the case of mannitol ($C^6H^{14}O^6$) subject to the following reaction:

$$2\ C_6H_{14}O_6 + 13\ O_2 \rightleftharpoons 12\ CO_2 + 14\ H_2O$$

The use of mannitol (C6H14O6), leads to the consumption of oxygen released by molecules of hemoglobin contained in the concentrated bags of red cell - CH, leading to decreased efficacy of oxygen transport, since only 50% of Hemogloina-Hb to P50 is covers carry molecules of O2, also taking into account that the human body at room temperature is covers to extract only 30% of O_2 Hb, and this forms an efficiency of only 15% extraction of the total of all a bag CH hemacia- concentrate[3,4]. Thus the addition of mannitol ($C_6H_{14}O_6$) as cryoprotectants ultimately further reduce the effectiveness of blood transfusion and its blood products.

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POSTER PRESENTATIONS

CotA-laccase as biocatalyst in the “green” synthesis of phenazines and acridines cores

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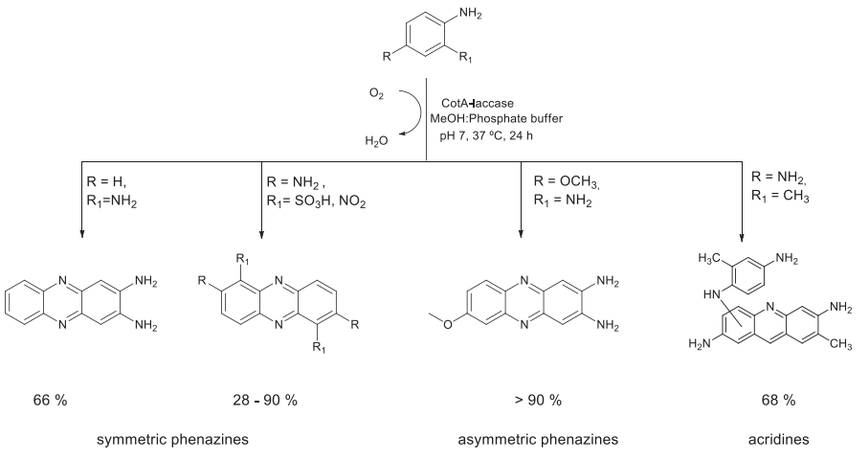
Phenazines and acridines are an important class of benzoheterocyclic compounds which exhibit a broad spectrum of biological activities and number of derivatives are widely used as antibacterial, antifungal, antiviral and anticancer drugs.[1] There are several chemical methods for the synthesis of these important cores starting from substituted *o*-aromatic amines that are also good substrates for laccases. Based on this property, and searching for a cleaner synthetic method we present a “green” approach for the formation of symmetric and asymmetric phenazines and acridines using CotA-laccase, a multicopper oxidase, as biocatalyst (**Scheme 1**). Laccase promote the oxidation of the precursor molecules to the *o*-quinone-diimine intermediate which undergo a Michael addition leading to the final heterocycle core.[2] Reactions were performed in aqueous media and with mild reaction conditions of pH and temperature.



Scheme 1: Formation of phenazines and acridines catalysed by CotA-laccase

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Impact of Citrus Pectin on Oenin Copigmentation Mechanisms

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Anthocyanins present a high potential as natural colorants due to their attractive colors. Additionally they are non-toxic, non-mutagenic [1] and their pharmacological properties are also well known and account for their therapeutic use. [2] However, the use of these colorants may face some problems due to their instability. For instance, the color of anthocyanins is highly dependent on pH due to changes in the concentration of the four species present in acidic and neutral aqueous solutions. [3] Copigmentation mechanism is regarded as one factor of structure stabilization, i.e., coloration of anthocyanins. [4,5] This mechanism consists of hydrogen-bind interaction and hydrophobic interactions (✓-✓ stacking) between the colored forms of the anthocyanins (i.e., flavylium cation ion and quinoidal forms) with other colorless organic molecules (i.e., copigments). [6] Copigmentation complexes adopt a sandwich configuration (vertically stacked) that protects the flavylium chromophore from the nucleophilic attack of water, thus anthocyanin hydration equilibrium toward the pigmented forms and color enhancement. [7] Anthocyanins – polysaccharides interaction is likewise a very important topic to be considered on anthocyanin's stabilization, as these pigments are bio-synthetized in a polysaccharide rich environment where extensive contact may occur. [8,9] The association between macromolecules resulting from disrupted cell walls (e.g. pectins) and anthocyanins may affect pigments chemical stability and color properties thus playing an important role in the final color of food products. The anthocyanins-polysaccharide association could also be important for anthocyanin's copigmentation. Bering this, the main goal of this work is to evaluate the impact of pectin on anthocyanin's copigmentation mechanisms.

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Morella faya (Aiton) Wilbur leaves and bark: bioactivities and isolated compounds

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Myrica and *Morella* species have traditional medicine uses (*e.g.* treat diarrhea, digestive problems, headache, burns and skin diseases) and metabolites that exhibit promising bioactivities (*e.g.* cytotoxic, antibacterial, antioxidant, anti-inflammatory, phytotoxic).[1] *Morella faya* (Aiton) Wilbur (syn. *Myrica faya* (Aiton)) (figure 1), is an evergreen nitrogen-fixing subdiodecious tree or bush from the Myricaceae family and native to Macaronesia.[2]The dark red to purple fruit can be used as an additive in the production of wine or brandy and elderly people from S. Miguel remember the fruits as edible. However, few studies have been published research the pharmacological potential of *Morella faya*. Here we present the anticholigenic and anti-xanthine oxidase effect of bark and leaves extracts of *M. faya* and some compounds isolated and structurally characterized from these extracts.

Dry leaves and bark were sequentially extracted with dichloromethane and acetone and the resulting acetone and dichloromethane solutions were filtered and concentrated under reduced pressure to yield crude extracts. Extracts were assayed for anticholinesterase, anti-xanthine oxidase and cytotoxic activities. Acute toxicity was also evaluated. Dichloromethane extracts were inactive across all activities. Acetone extracts present good reversible xanthine oxidase inhibition [leaf (IC_{50}=50.55 ± 0.04 µg/mL); bark (IC_{50}=48.46 ± 1.22 µg/mL)] compared to allopurinol, used as standard inhibitor (IC_{50}=48.46 ± 1.22 µg/mL). But the highlight is the uncompetitive anticholinesterase activity found in the bark extract (IC_{50}= 63.316 ± 2.83µg/mL) that rivals the activity of medicinally relevant galanthamine (IC_{50} =78µg/mL). Several compounds were isolated from the most active extracts by preparative chromatographic techniques and their structures elucidated by spectroscopic methods (1D and 2D NMR and MS). These compounds were identified as fatty alcohol, pentacyclic triterpenes with lupane and oleanane skeleton, cyclic diarylheptanoids and a phthalate not reported before. All these compounds belong to organic families well known by the broad spectrum of biological activities exhibited by its members. These results show the potential of *M. faya* as medicinal plant and source of pharmacologically active compounds.

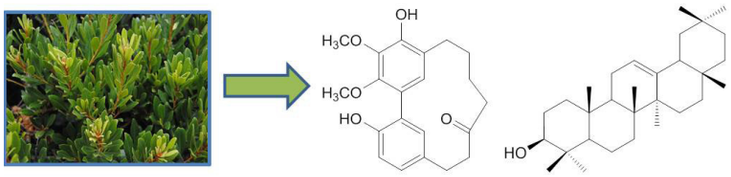

Figure 1:*Morella faya*(Aiton) Wilbur on left and some isolated compounds, on the right.

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Synthesis of Novel *N*-Glycosylsulfonamides: Conformational Preferences, Exo-anomeric Effect and Furanose/Pyranose Isomerization

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Mimetics of *O*-glycosides have attracted considerable interest in medicinal chemistry due to their relative stability to enzymatic hydrolysis and ability to inhibit enzymes such as glycosidases.[1] Such analogs include thioglycosides, *C*-glycosyl or *N*-glycosyl derivatives. *N*-Glycosylsulfonamides constitute rather stable *N*-glycosyl analogs when compared with the majority of glycosylamines, whose stability is normally low. Moreover, sugar derivatives incorporating sulfonamide moieties have shown interesting biological properties, such as antitumor effects.[2,3]

Hence, we were motivated to explore the synthesis of novel anomeric sulfonamides (Figure 1) derived from glucose, glucuronamide and ribose and to study particularly the stereochemical and conformational outcome of the reactions involved in their synthesis. Their access involved the N-glycosylation of sulfonamides, including heteroaromatic derivatives, with 1-*O*-acetyl glycosyl donors. In the case of the obtained acetylated *N*-ribofuranosyl methanesulfonamide, its subsequent deacetylation occurred with isomerization to the pyranose form. Moreover, all the deprotected derivatives were in the β-anomeric configuration, most likely arising from the exo-anomeric effect.

The synthetic details and results will be presented and discussed.

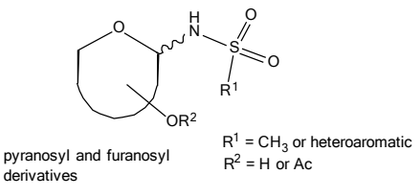

Figure 1: General structure of the target molecules.

Acknowledgements: 'Fundação para a Ciência e Tecnologia' (FCT) is acknowledged for funding (IF/01488/2013 and CQB strategic project UID/MULTI/00612/2013).

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Natural products: tools for inflammation management

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Inflammation is one of the body's natural defenses. Nevertheless, when the anti-inflammatory defenses are not enough to antagonize the deleterious activities of the overproduced pro-inflammatory mediators, the inflammatory response may contribute to the development of several diseases. The recurrent use of anti-inflammatory drugs and their side effects led to a growing demand for viable and safer alternatives. In this context, natural products arise, playing an important role in the treatment of this pathology.

Amongst natural compounds with anti-inflammatory properties, flavonoids can be highlighted. In this work, the ability of *Grindelia robusta* Nutt aqueous extract and of some of its flavonoids (representative of different classes and with distinct substitution patterns), to reduce nitric oxide (NO) levels in RAW 264.7 cells was assessed.

Results revealed that the extract of *G. robusta* reduced cells' viability (Figure 1). In addition, a tendency to reduce NO levels, in a dose-dependent way and at non-toxic concentrations, was also observed. All flavonoids were able to decrease NO levels in a concentration-dependent manner, quercetin being the most effective one (IC_{50} values of 7.47 μM). The presence of quercetin, apigenin and luteolin derivatives in the extract of *G. robusta* can partially explain its capacity to decrease NO levels. In a general way, aglycones revealed to be more active than the respective glycosides. In addition, the catechol group on ring B and the hydroxyl group in C3 seem to be essential for the anti-inflammatory activity of these compounds.

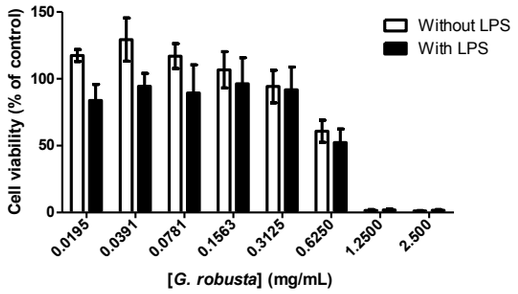

Figure 1: Cell viability of RAW 264.7 cells pre-treated for 2 h with *G. robusta* aqueous extract, followed by 22h co-treatment with LPS (1 μg/mL) with LPS or vehicle (culture medium). Results represent the mean ± standard deviation of four independent experiments performed in triplicate.

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Antitumor Activities of Invasive Alien Species from the Azores

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Numerous marine species are moved beyond their natural ranges as a result of activities such as shipping, aquaculture, ornamental trade and canal construction. This artificial re-distribution of species has resulted in bio-invasions that upset the balance of biological systems, with adverse ecological and economic consequences [1]. Non-native species with invasive behavior, because of their abundance and of the need to control their population, are an attractive source to be explored as potential producers of natural therapeutic agents. In this context, several marine alien species from the Azores were selected to be investigated as a source of antitumor agents, considering the potential found in marine organisms as sources of pharmacologically active compounds [2].

The macroalgae *Asparagopsis armata* Harvey, *Asparagopsis taxiformis* (Delile) Trévisan Saint-Léon, *Codium fragile* subsp. *fragile* (Suringar) Hariot, and the invertebrates *Microcosmus squamiger* Michaelsen, 1927, *Bugula neritina* (Linnaeus, 1758), *Tricellaria inopinata* d'Hondt & Occhipinti Ambrogi, 1985 and *Zoobotryon verticillatum* (Delle Chiaje, 1822) were collected in marinas from Santa Maria (SMA) and of S. Miguel (SMI) islands (Azores). Extracts were prepared by sequentially extracting the lyophilized material with dichloromethane and methanol at room temperature. The in vitro antitumor activity of the extracts against HeLa (cervix tumor), A549 (lung), MCF-7 (breast) and Vero (control) cell lines was determined by the MTT method [3].

None of the extracts presented antitumor activity against A549 cell line or against any cells lines in log phase (cytotoxic) up to 200 µg/mL, and since growth inhibition (lag phase) is determined by the exposure of the cells to the extract in the initial growth phase, it can be concluded that the process of cell adhesion to the substrate was the most affected. In lag phase, the dichloromethane extract from *Z. verticillatum* presented the best activity against MCF-7 cells, slightly higher than the value of 30 µg/mL considered promising for crude extracts by the NCI, and was the only extract active against HeLa cells. This extract also showed pronounced selectivity against these cell lines, with a SI (selectivity Index) of 1.94 and 4.64, respectively.The results obtained show the potential of *M. squamiger*, *B. neritina* and *Z. verticillatum* as sources of antitumor agents, and the interest to carry out the chemical characterization of the active extracts and the identification of the molecules responsible for these activities.

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Following the removal of fluoroquinolones of the environment: an HPLC-FD method

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Antibiotic residues have been detected in various environmental matrices, such as surface water and even drinking water. Although present at low levels (µg/L, ng/L), many antibiotics are bioaccumulative, pseudo-persistent and can promote resistance/alterations in bacterial populations.[1]

In this work we present the biosorption of three widely used fluoroquinolones (FQ) - ofloxacin (OFL), norfloxacin (NOR) and ciprofloxacin (CPF) to activated sludge (AS) and aerobic granular sludge (AGS). A High Performance Liquid Chromatography with Fluorescence Detection (HPLC-FD) method was validated and optimized to follow the biosorption of the targeted FQ.[2]

The HPLC-FD method was validated according to Q2B ICH guideline.[3]The validated method demonstrated good selectivity, linearity ($r^2 > 0.999$), intra-day and inter-day precisions (RSD < 3%) and accuracy. The detection limits were 0.6 ng/mL for NOR and CPF and 0.7 ng/mL for OFL and quantification limits were 1 ng/mL for three FQs.

Several parameters that can affect FQ biossorption kinetics, namely contact time, pH, biosortion mass were studied. At pH 7 AS showed better performance to biosorb OFL, NOR and CPF than AGS. The higher biosortion capacity of AS was probably due to the negative charge on its surface, evaluated by a zeta potential of -25.65 mV, at pH 7. OFL was the less biosorbed antibiotic, both onto AS and AGS, because at pH 7 this FQ is mainly present in its anionic form. The equilibrium data for AS showed a better fit to the Langmuir model, while the model that presented better fit for AGS was the Freundlich model. The FQ could be desorbed from AGS at pH 3, pH 8 and pH 9 whereas at pH 4 the biosorption process was promoted.

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In vitro antioxidant activity of novel 3-hydroxy-2-styrylchromones as compared to 3-hydroxyflavones

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Flavonoids are chemically based on a fifteen-carbon skeleton consisting of two benzene rings (ring A and ring B) joined by a linear three carbon chain (C6-C3-C6) forming an oxygenated heterocycle pyran ring (ring C). 2-styrylchromones (2-SC), a small group of compounds characterized by the attachment of a styryl group to the 2-position of the chromone skeleton, have structural similarities with flavonoids, particularly those belonging to the class of flavones. Flavonoids possess many biological activities, from which the antioxidant properties are the best described. Considering the structural similarities of 2-SC and the fact that their styryl moiety may greatly contribute to their molecular stabilization under redox challenges, some of its biological activities are likely to be similar or even enhanced in comparison to flavonoids, although it needs to be experimentally confirmed. Thus, the purpose of the present study was to evaluate and compare the putative scavenging of reactive oxygen (ROS) and nitrogen (RNS) species by synthetic 3-hydroxyflavones and 3-hydroxy-2-styrylchromones (Figure 1), using in vitro non-cellular systems. The obtained results show that both groups of compounds have high capacity to scavenge ROS and RNS. Interestingly, the efficacy of 3-hydroxy-2-styrylchromones and 3-hydroxyflavones vary among the tested reactive species, constituting a good option as antioxidant agents.

(A1) R_1=OH; R_2= OH, R_3=OH; R_4= OH
(A2) R_1=OMe; R_2= OMe, R_3=OH; R_4= OH
(A3) R_1=OH; R_2= OH, R_3=OMe; R_4= OMe

(B1) R_1=OH; R_2= OH, R_3=OH; R_4= OH
(B2) R_1=OMe; R_2= OMe, R_3=OH; R_4= OH
(B3) R_1=OH; R_2= OH, R_3=OMe; R_4= OMe

Figure 1: Chemical structures of the tested 3-hydroxy-2-flavones (A) and 3-hydroxy-2-styrylchromones (B).

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Synthesis and biological evaluation of bimodal *meso*-sulfonamide porphyrins

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The development of new molecular entities capable of promoting the inactivation of bacteria without developing drug resistance depends on finding alternative mechanisms of action for antibiotics. Photodynamic Inactivation of microorganisms (PDI), is emerging as an alternative to classical antibiotics because PDI is not associated with the development of microorganism resistance after treatment. This work presents new methods of synthesis of bimodal molecules that incorporate sulfonamides and 5,10,15,20-tetrakis(pentafluorophenyl)porphyrin in their structure, in order to attain new chemical entities that can potentially target bacteria and act both as bacteriostatic and photosensitizing agents (**Figure 1**). Additionally, this work presents the fundamental photophysical assessment of the new photosensitizers, namely in terms of their electronic absorptions, singlet oxygen quantum yields and reactive oxygen species generation. The cytotoxicity of selected photosensitizers against A549, CT26, 2H11 and B16F10 cells showed that investigated photosensitizers had no effect on the viability with concentrations ranging from 0 – 20 μM. On the other hand, after the their excitation with the visible light, significant photodynamic effect against studied cell lines is observed.[1]

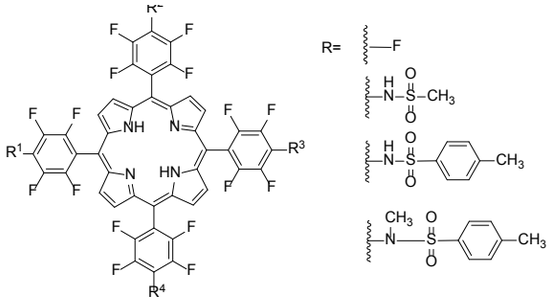

Figure 1: *meso*-sulfonamide porphyrins

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An example of the key role of HPLC in Medicinal Chemistry: determination of lipophilicity of a xanthone derivatives library

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For the last several years, searching of new xanthone derivatives (XD) with potential pharmacological properties has remained in the area of interest of our group. In addition, the assessment of the drug-likeness of the bioactive XD synthesized is crucial in an early stage of the drug discovery pipeline considering that problems related to physicochemical properties are one of the main reasons of failure during pre-clinical trials.
Lipophilicity, commonly expressed by the logarithm of the partition coefficient (log P), is one of the most important physicochemical properties having a great impact both in pharmacokinetics (absorption, distribution, metabolism, excretion, and toxicity - ADMET) and pharmacodynamic processes.
In this work, we describe the lipophilicity of a series of XD, previously synthesized in our group[1], using two different methods: RP-HPLC[2] and vortex-assisted liquid-liquid microextraction coupled with liquid chromatography (VALLME-HPLC)[3]. Both methods were validated accordingly with OCDE guidelines.
In the RP-HPLC method retention factors (log k) were determined and correlated to log P. The analyses were carried out under optimized isocratic conditions, using two different hydrophobic silica-based stationary phases (C8 and C18) and different water/methanol ratios as mobile phases. Linear correlations were found between log k values and the volume fraction of methanol in the mobile phase (R^2 higher than 0.99) for both stationary phases. The log k values were extrapolated for pure aqueous eluent to correlate with the values obtained using VALLME method. Low sample consumption, low sensitivity to impurities, good accuracy and excellent reproducibility were observed.
The VALLME-HPLC method demonstrated to be a successful technique for log P evaluation. Linear correlations were obtained, with R^2 >0.99 in all cases and, in most cases, low variation coefficients (%) were observed for all the XD tested. Comparing the results obtained for each compound by the two used methods, different log P values were observed and the justification for this behaviour is presented.
HPLC technique was crucial for the determination of log P values, showing its importance in the evaluation of physicochemical parameters that can be useful for pharmacokinetics prevision.

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Investigation of the interaction of vancomycin with synthetic bacterial muropeptides

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Vancomycin is a member of the glycopeptides family antibiotics considered a last-resort agent in the treatment of infections caused by Gram-positive bacteria.[1] In order to find new agents to combat bacteria resistance it is important to understand the details of the mechanism of action of glycopeptides antibiotics.[2] Those antibiotics prevent the formation of peptidoglycan (PGN), the major component of the bacterial cell wall which is constituted by a glycan chain of alternating β(1→4)-linked *N*-acetylglucosamine (GlcNAc) and *N*-acetylmuramic acid (MurNAc), cross-linked by short peptide bridges.[3]

It is well established that the replacement of the last amino acid in the peptide chain linked to the MurNAc moiety changes the interaction with the glycopeptides antibiotics. So far the interactions studies have been limited to the use of *N*-protected dipeptides and tripeptides.[4] In order to clarify how the different compositions of the bacterial peptide chain and carbohydrate unit affect the recognition by vancomycin we have developed a study involving synthesis and screening of small bacterial muropeptides.

We have been dedicated to the synthesis of glucosamine disaccharides and GlcNAc-MurNAc moieties.[5] Herein we will present our studies on the interaction between vancomycin and the synthesized muropeptides.

Acknowledgements: We thank the Fundação para a Ciência e Tecnologia for financial support for the projects PTDC/SAU-IMU/111806/2009 and PTDC/QEQ-QOR/2132/2012 and the NMR spectrometers are part of The National NMR Facility, supported by Fundação para a Ciência e a Tecnologia RECI/BBB-BQB/0230/2012.

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Synthesis and biological evaluation of mono and bis naphthalimides derivatives against SH-SY5Y, human brain cancer cells

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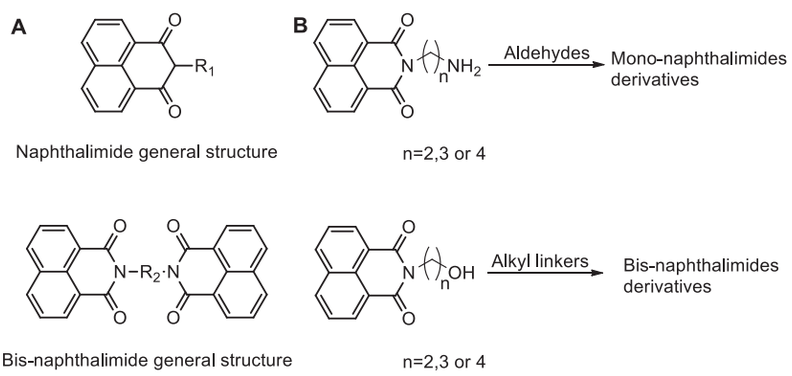
Naphthalimides (1H-benzo[*de*]isoquinoline-1,3-(2*H*)-diones) consists of a flat, generally π-deficient aromatic or heteroaromatic system and show strong hydrophobicity.[1] These types of compounds with this moiety showed fluorescence and biological properties such as anticancer, antimicrobial, antitrypanosomal, analgesic, antioxidative and antiviral properties[1-2] The naphthalimides compounds are also known to be very good DNA intercalators[3], since the planar naphthalimido moiety binds by perpendicular insertion between the base pairs of the double helix of DNA.[4] Previous work had already shown that mono and bis naphthalimido derivatives to exhibit strong activity against different cancer cell lines.[5-6] Here in this work we would like to demonstrate that the alkyl chain i.e. the linker between the naphthalimido groups or the substituent attached at the end of the linker chain, do have an impact on the biological and DNA binding properties. The synthesis of new mono-naphthalimides derivatives involved the reaction with different aldehydes and with different length of alkyl chain. For the new bis-naphthalimides the reactions consist of an N-alkylation reaction between with different linkers and the corresponding O-tosyl alkylnaphthalimides. The biological activities of the newly synthesized compounds includes their ability to bind DNA, their toxicity against SH-SY5Y human brain cancer cells *in vitro*, cell morphology and cellular uptake will also be presented.



Scheme: A) General structure of mono and bis naphthalimides derivatives; B) General scheme of the synthesis

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Identification and characterization of small molecule interactions with transthyretin amyloid fibrils

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Transthyretin (TTR) is one of several proteins that are known to undergo conformational changes and form aggregates and insoluble highly stable amyloid fibrils, which accumulate in extracellular tissues, causing diseases. Senile systemic amyloidosis (SSA), familial amyloid polyneuropathy (FAP), familial amyloid cardiomyopathy (FAC), and the rare central nervous system selective amyloidosis (CNSA) are the main pathologies in which TTR is implicated[1].

Although liver transplant and more recently the medicine Tafamidis are in use as therapeutic approaches, currently there is no cure or completely effective treatment for TTR amyloidosis and several therapeutic strategies targeting different steps of the fibril formation pathway are under development[2]. The disruption of amyloid aggregates and fibrils is one of those strategies. To properly select compounds for that purpose a specific screening protocol that can identify and characterize the interaction of the compounds with the fibrils must be developed.

With this purpose three wild typeTTR (WTTTR) fibril formation protocols were studied by dynamic light scattering (DLS), circular dichroism (CD), turbidimetry, transmission electron microscopy (TEM) and fluorescence spectroscopy to select and characterize the most relevant and appropriate fibril formation protocol. Additionally saturation transfer difference nuclear magnetic resonance (STD NMR) was selected and used to study the interaction of doxycycline, a known TTR fibril disrupter, with WTTTR fibrils, and a DLS assay was developed and performed to characterize the effect of this compound on fibril disruption.

The results show that the heat-induced fibril formation protocol has some advantages over the other fibril formation protocols but requires further characterization.^{1}H STD NMR was successfully applied in probing the interaction of doxycycline with WTTTR fibrils, with the results indicating that doxycycline interacts diversely with WT-TTR amyloid fibrils formed in different experimental conditions. The DLS assay allowed the characterization of the effect of doxycycline on WTTTR fibrils over time and demonstrated that doxycycline disassembles preformed WTTTR fibrils.

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Xanthone as a valuable scaffold to identify new promising antitumor agents via inhibition of p53/p73:MDM2 interaction

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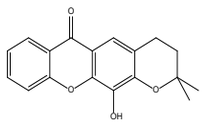
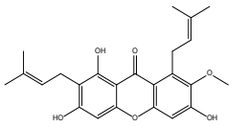
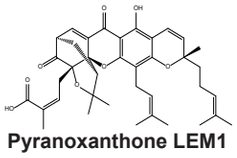
The p53 family proteins (p53, p63, p73) are transcription factors which play important roles in the control of cell proliferation, differentiation and cell death. At cellular level, the growth-suppressive activity of p53 is tightly regulated by physical interaction with two negative modulators, murine double minute 2 (MDM2) and X (MDMX), which are overexpressed in about half of all human tumors. Disruption of p53:MDM2/MDMX interaction may reactivate p53-dependent pathway and may offer an attractive strategy for cancer therapy. The knowledge of crystallographic structure of p53:MDM2 and p53:MDMX complexes enabled the identification of several classes of small-molecule inhibitors of p53:MDM2/MDMX interaction Xanthones have been reported as potential anticancer agents with the ability to target p53:MDM2 interaction. In fact, the natural xanthones, α-mangostin and gambogic acid, and the synthetic derivative pyranoxanthone LEM1 (**Figure 1**) exhibited potent cytotoxic activity against several human tumor cell lines accompanied by an inhibitory effect on p53:MDM2 interaction.[1]



Gambogic acid



α-Mangostin



Pyranoxanthone LEM1

Figure 1. Xanthones inhibitors of p53:MDM2 interaction.

Recently, the carbaldehydic xanthone LEM2 was identified as a potent antitumor agent via inhibition of p73:MDM2 interaction. Based on the pattern of substitution of LEM2 and on amine moiety of known p53:MDM2 inhibitors, a molecular hybridization strategy was followed to obtain new potential p53/p73:MDM2 disruptors with drug-like properties. By a reductive amination procedure, a library of LEM2-aminated derivatives was successfully synthesized. The optimization of the reaction conditions was accomplished by using sodium triacetoxyborohydride and tetrahydrofuran as a reducing agent and solvent, respectively. Eleven new LEM2-aminated derivatives were obtained and their structures were elucidated by spectroscopic techniques, including NMR (^{1}H and ^{13}C), IR and mass spectrometry. Docking studies carried out in MDM2 have shown a higher binding affinity of these derivatives against MDM2 when compared to the respective precursors and a similar binding affinity with known small-molecule disruptors of p53:MDM2 interaction. Using yeast-screening assays, the inhibitory activity of the LEM2 derivatives on p53:MDM2 interaction was investigated. No significant effects were observed on the growth of yeast cells co-expressing p53 and MDM2 proteins after the treatment with compounds. Further studies on p73:MDM2 may disclose potential inhibitors of p73:MDM2 interaction.
Overall, the xanthone scaffold has been a source of inspiration for the identification of new disruptors of p53:MDM2 interaction. These studies will contribute to the knowledge of structural requirements for the construction of novel potent and selective small-molecule p53/p73 activators with antitumor activity.

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Synthesis of novel dipyrrolic compounds with potential application in fluoride detection

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Over the past decades a great attention has been devoted to the synthesis and development of new analytical methods for the reliable detection of target species such anions. The detection, differentiation and visualization of these entities are crucial challenges for the design of selective optical chemosensors.[1] Nonetheless, the synthesis of materials that behave as selective optical chemical sensors is still a challenge in organic chemistry. In this area, pyrrole units are particularly attractive since the N–H protons remain in place over a wide pKa range making possible their use as a hydrogen bond donor group within a large pH window. Also, the reasonably easy functionalization and incorporation into elaborate cyclic and acyclic systems are reasons to have into account when synthesizing this kind of receptors.[2] More recently, attention has turned towards acyclic receptors using a push-pull chromophore approach on the β-position of pyrrolic moieties giving rise to a colorimetric effect towards different anions.[3]

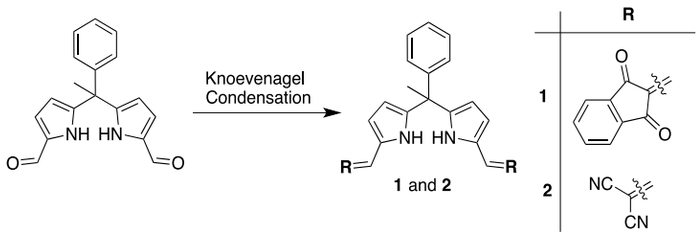

Figure 1

Herein we report a methodology to functionalize the α-positions of dipyrromethane compounds through Knoevenagel reactions with indane-1,3-dione and malononitrile. The corresponding products (**1** and **2**) were evaluated as optical anion chemosensors and revealed high selectivity for fluoride (Figure 1).

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Promising Chalcone Derivatives as New Inhibitors of p53-MDM2 Interaction

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The p53 tumor suppressor protein is one of the major regulators of cell proliferation and death, its activity and stability being regulated by the endogenous negative regulator, MDM2. Hence, the discovery of inhibitors of p53-MDM2 interaction is considered a promising strategy for cancer treatment [1].

The virtual screening of a library of chalcone derivatives led us to the identification of novel potential MDM2 ligands. The chalcones with the best docking scores which respect the Lipinski rule of five were subsequently prepared by base-catalyzed aldol reactions. The activity of these compounds as inhibitors of p53–MDM2 interaction was investigated using a yeast-based assay. Using this approach, two chalcones were identified as putative small-molecule inhibitors of p53–MDM2 interaction. The activity of both chalcones was further investigated in a panel of human tumor cell lines. Both chalcones revealed a pronounced tumor cell growth inhibitory effect on tumor cell lines. Additionally, one of these chalcones caused alterations in the cell cycle profile, induced apoptosis and increased the levels of p53, p21 and PUMA proteins in NCI-H460 cells.

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Xanthone and Flavone Derivatives as Potential Dual Agents for Alzheimer's Disease

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Alzheimer's disease (AD) is the most prevalent form of dementia observed in elderly. It is known that the malfunctions of different, but interconnected, biochemical complex pathways are related to the pathogenesis of AD. Inhibition of acetylcholinesterase (AChE) is one of the most accepted therapy strategies for AD. Although several AChE inhibitors have been approved for commercial use, these drugs have lack of selectivity and AD-patients suffer from side-effects, suggesting that there is a considerable need for development of new clinical tools.[1] Among the multipotent therapeutic strategies, the association between cholinesterase inhibition and antioxidant activity has been considered as an attractive approach.[1,2] Considering that concept, this work aims to obtain new xanthone and flavone derivatives as dual agents (AChE inhibitors and antioxidant) with potential in treatment for AD.

Here in, we describe the synthesis of a hydroxylated xanthone (**ICX1**) and the methylated (**ICX2**) and Mannich base (**ICX2a**) derivatives, as well as the synthesis of a Mannich base analogue of baicalein (**ICB1**). The evaluation of their antioxidant and AChE inhibitory activities and molecular docking studies of the most active compound (**ICB1**) with AChE were also performed. From this study, compounds **ICX2a** and **ICB1** emerged as dual agents with antioxidant and AChE inhibitory activities. In conclusion, these amino derivatives will serve as models for the design and synthesis of new xanthone and flavone derivatives as promising multipotent compounds for treating AD.

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Antimicrobial activity and biofilm formation inhibition of alginate microspheres of dehydroabietic acid.

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Nowadays, common and nosocomial infections are associated with increasing therapeutic difficulties, requiring 2nd and 3rd line treatments and also contributing to multidrug resistance (MDR) development. This antimicrobial resistance (AMR) is due to the increase of resistant pathogens to the prevailing therapy, presenting therefore an emergent worldwide threat to public health [1]. Natural products, widely used in traditional medicine, are a common source for new bioactive molecules and drug prototypes for the treatment of bacterial infections.

A wide spectrum of biological activities including antitumor, anti-inflammatory and antimicrobial are displayed by the abietane dehydroabietic acid (DHA) [2]. In a recent study, DHA demonstrated the ability to act against *Staphylococcus aureus* biofilm, which not only prevents bacterial colonization, but also inhibits biofilms formation [3]. Its antimicrobial properties have recently been studied, specifically against methicillin-resistant *Staphylococcus aureus* strains [3]. This aromatic abietane and its derivatives have also demonstrated an activity against Gram-positive organisms (i.e. *Salmonella* species). Taking into account these promising results, the aim of this study was to investigate the efficacy of DHA for inhibiting the biofilm formation by a diversified panel of Gram-positive and -negative bacteria as well as its efficiency against standard and isolate strains. Minimum Inhibitory Concentration (MIC) and Minimum Biofilm Inhibitory Concentration (MBIC) were determined by two-fold serial broth microdilution assay, as well as crystal violet staining methods, as described previously [4]. Experimentally, DHA presented MIC values ranging between 15.63–500 µg/mL and with a range of 32–90% of biofilm inhibition, therefore demonstrating efficacy against resistant bacteria and their biofilms. After MIC and MBIC screening, DHA was encapsulated in alginate microspheres through the emulsification-internal gelation method using calcium carbonate insoluble salt, and tested against the reference strain of *S aureus* ATCC 25925 (lower MIC value). DHA-loaded alginate microspheres demonstrated also an antimicrobial activity and further tests will be performed.

Although DHA demonstrates to be a promising natural antimicrobial agent, its efficacy and spectrum augmentation must be enhanced. Future studies will focus on a synergistic effect between an antimicrobial synthetic molecule and an encapsulation polymer, namely a new abietane cationic amphiphile (ACA) derived from dehydroabietic acid.

Acknowledgements: The authors thank Professor Aida Duarte from Faculty of Pharmacy (University of Lisbon) for providing the bacterial strains.

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Identification and quantification of blackberry anthocyanin metabolites in human plasma and urine

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The health effects associated with the consumption of anthocyanins-rich foods is unquestionable. However, only small amounts (<1%) of the ingested dose is recovered from human samples [1].

There are many doubts on which compounds are really involved and which are the molecular mechanisms underlying all those biological events. Another particularity of anthocyanins regards their rapidly detection in plasma in their parent forms, which may suggest their absorption at the gastric level [2]. Our *in vitro* preliminary results suggests that anthocyanins are absorbed through the gastric epithelium [3].

The aim of this work was to perform a human study in which volunteers ingested a blackberry fruit juice as an acute dose, containing mainly cyanidin-3-glucoside (Cy3glc) with or without 12% ethanol. A thorough screening analysis for anthocyanins and/or metabolites was performed in plasma and urine samples by mass spectrometry. Cy3glc in its parent form was detected in plasma and urine samples. The identification and quantification of anthocyanin conjugates was also performed yielding several sulphate, glucuronyl and methyl conjugates of Cy3glc and Cy.

Anthocyanins can be further metabolized by colon microbiota and excreted in the urine, yielding several phenolic acid and their metabolized forms. Some protocatechuic, vanilic, benzoic and hippuric acid conjugates were identified in urine samples, mainly with sulfate groups.

So far, it has been quite difficult to clearly assess both native and metabolized forms and also catabolites *in vivo* and to distinguish their different biological roles.

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Reactivity between cork extracts and major wine components

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Cork is a suberized cellular tissue that is continuously produced by the phellogen of the cork oak tree (*Quercus suber L*.) native species of the Mediterranean region. The unique properties of this material allowed its use in a wide range of applications. Wine cork stoppers are still its most common and valued use[1,2]

Different phenolic compounds have been found to migrate from different cork stoppers into bottled wine model solutions[3]. Some these compounds were found in larger quantities including gallic acid, protocatechuic acid, protocatechuic aldehyde, caffeic acid, vanillin, sinapic acid, ferrulic acid and ellagic acid and trace amounts of more complex polyphenols such as mongolicain A/B, valoneic acid, valoneic acid dilactone, ellagic acid-pentose, castalagin/vascalagin, HHDP-galloyl-glucose, di-HHDP-galloyl-glucose were also present.

These compounds interfere in some color and taste features of wines, namely in bitterness and astringency[4]. On the other hand, some of these compounds participate in polimerization reactions with some wine components, changing their sensorial properties and redox status[5,6]. In addition, it is also acknowledged that phenolic acids and aldehydes can react resulting in more complex structures found in aged wines[7,8,9] that affecting some chromatic qualities.

Bearing all this, the aim of this study is to evaluate the reactivity between cork extracts and major wine components and understand the impact of this reactivity in wine properties.

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Synthesis of dipeptides with β,β-disubstituted dehydroalanine and *C*$^{\alpha,\alpha}$-dimethylglycine residues

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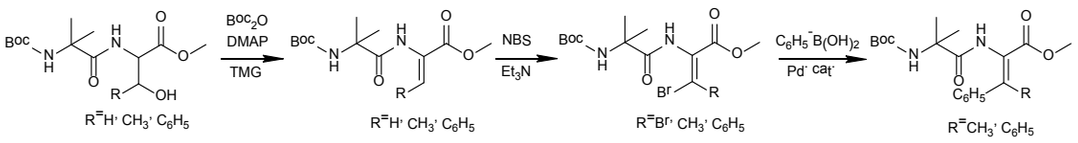
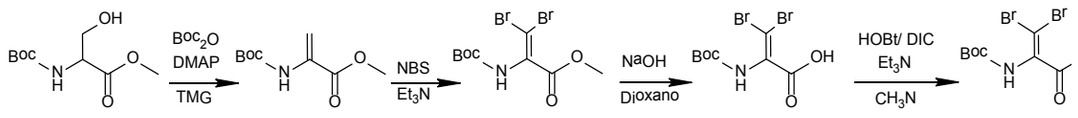
Peptides are known to have important pharmacological properties [1,2]. Non-proteinogenic amino acids are an important class of organic compounds, either because they have an intrinsic biological activity, or by being part of peptides with antiviral, anti-tumor, anti-inflammatory or immunosuppressive activities. The incorporation of non-proteinogenic amino acids into peptides can change their structural, chemical and pharmacological properties [1]. Non-proteinogenic amino acids, including dehydroamino acids, *C*$^{\alpha,\alpha}$-dialkylglycines and β-substituted alanines have a wide application in medicinal chemistry [3]. The introduction of these non-natural amino acids into peptides causes conformational constraints, thus favouring a particular conformation of the chain and can also increase resistance to proteases [4]which exhibits both immunological activity (induction of the colony stimulating factor (CSF. Recently, Monteiro et al. prepared dehydrodipeptide derivatives with a *C*$^{\alpha,\alpha}$-dimethylglycine residue that could additionally be *N*-alkylated at the amino terminal [5].
Dipeptides containing *N*-(*tert*-butyloxycarbonyl)-*C*$^{\alpha,\alpha}$-dimethylglycine as amine terminal residue and the methyl ester of β-hydroxyamino acids as carboxyl residue were prepared. These *N*-(*tert*-butyloxycarbonyl)-dipeptide derivatives were subject to dehydration using the *tert*-butylpyrocarbonate [$(Boc)_2O$]/4-dimethylaminopyridine (Dmap) methodology developed by Ferreira et al. [6]N-diacyldehy- droamino acid derivatives to prepare N-monoprotected dehy- droamino acids and dehydrodipeptides. Thus, several dehy- droalanine, dehydroaminobutyric acid and dehydrophenyl- alanine derivatives have been prepared by treating the cor- responding L-serine, L-threonine and D,L-3-phenylserine (threo-type, to give *N*-(*tert*-butyloxycarbonyl)-dehydrodipeptide derivatives. These were treated with *N*-bromossuccinimide yielding a *N*-(*tert*-butyloxycarbonyl)-β,β-dibromodehydrodipeptide and *N*-(*tert*-butyloxycarbonyl)-β-bromo-β-substituted dehydrodipeptide derivatives. The brominated dehydrodipeptide derivatives were used in Suzuki-Miyaura cross-couplings reactions (**Scheme 1**).



Scheme 1: Synthesis of dipeptides with *C*$^{\alpha,\alpha}$-dimethylglycine as amine terminal residue and β,β-disubsituted dehdydroalanine as carboxyl residue.

The synthesis of a dehydrodipeptide derivative containing β-brominated dehydroalanine as amine terminal residue and *C*$^{\alpha,\alpha}$-dimethylglycine as carboxyl residue was also attempted. Initially, the methyl ester of *N*-(*tert*-butyloxycarbonyl)-serine was subject to dehydration to give the methyl ester of *N*-(*tert*-butyloxycarbonyl)dehydroalanine. This compound was treated with *N*-bromossuccinimide (NBS) and triethylamine (Et_3N) to give the methyl ester of *N*-(*tert*-butyloxycarbonyl)-β,β-dibromodehydroalanine. The methyl ester was removed and the derivative obtained coupled with the methyl ester of *C*$^{\alpha,\alpha}$-dimethylglycine, yielding the *N*-(*tert*-butyloxycarbonyl)-β,β-dibromodehydrodipeptide required (**Scheme 2**).



Scheme 2: Synthesis of a dipeptide with β,β-dibromodehydroalanine as amine terminal residue and *C*$^{\alpha,\alpha}$-dimethylglycine as carboxyl residue.

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Phenolic compounds of *Bactris setosa* extracts are highly effective scavengers of reactive nitrogen species

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Brazil has a large diversity of native fruits, and most of them are still unknown and studies addressing the phytochemical and antioxidant potential of these fruits are currently nonexistent, such as *Bactris setosa* fruits (Brazilian name: "tucum" or "uva-do-mato"), which are typical from Brazilian Atlantic Forest. In this study, the phenolic compounds composition, including anthocyanins, of seed, pulp and peel freeze-dried extracts of *B. setosa* fruits were identified and quantified (dry matter) by high performance liquid chromatography coupled to diode array and mass spectrometer detectors (HPLC-DAD-MS/MS). Additionally, the scavenging capacity of these extracts against the most physiologically relevant reactive nitrogen species (RNS) was evaluated, namely $^{\bullet}$NO (nitric oxide) and $ONOO^-$ (peroxynitrite). In general, (-)-epicatechin (*m/z* 289), the mixture of proanthocyanidin trimer (*m/z* 865) and chrysoeriol deoxyhexose hexoside (*m/z* 607) and (+)-catechin (*m/z* 289) were the major non-anthocyanin phenolic compounds identified in the peel extract, while L-tryptophan (*m/z* 203) and *trans*-piceatannol (*m/z* 243) were the major compounds in the seed extracts and (epi)catechin hexoside (*m/z* 451) and (epi)catechin deoxyhexose hexoside (*m/z* 597) were the major ones in the pulp extract. The highest content of non-anthocyanin phenolic compounds were found in peel extract (21730 µg/g extract), followed by pulp (4062 µg/g extract) and seed (3384 µg/g extract) extracts. Regarding the anthocyanins composition, cyanidin deoxyhexose hexoside (*m/z* 595) and cyanidin hexoside (*m/z* 449) were the major anthocyanins detected in the peel and pulp extracts of *B. setosa* extracts, and no anthocyanin was detected in the seed extract. The total anthocyanin contents of pulp and peel extracts were 90 and 17447 µg/g extract, respectively. All *B. setosa* extracts were able to scavenge all the tested RNS, the peel extract being the highest efficient scavenger, probably due to the highest phenolic compounds contents, with IC_{50} values at 6.68 ($^{\bullet}$NO) 1.01 ($ONOO^-$) and 1.49 µg/mL ($ONOO^-$ with $NaHCO_3$). This study clearly suggests that *B. setosa* extracts could be a potential source of natural antioxidants with high scavenging capacity, the peel of *B. setosa* fruits being the most promising part for future studies and application at pharmaceutical and food industries.

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Synthesis of 3-Oxo-β-Sultam activity-based probes for biomarker discovery in Chronic Obstructive Pulmonary Disease

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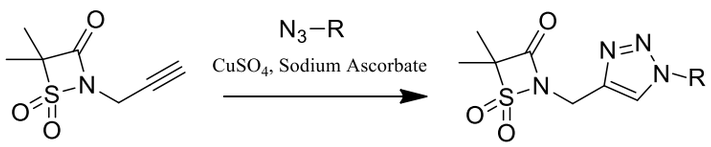
Chronic Obstructive Pulmonary Disease (COPD) refers to a condition of inflammation with progressive weakening of the structure of the lung and irreversible narrowing of the airways which is reported by the World Health Organization as the third leading cause of death worldwide[1]. Novel biomarkers to predict the activity of this disease are urgently needed. The central hypothesis of this project is that COPD induced inflammation causes modulation of proteolytic enzymes in the lungs, like Human Neutrophil Elastase (HNE), which are clinically relevant for the diagnosis and prognosis of COPD. Adequately targeted probes can be designed to quantify the active catalytic state of these enzymes[2]. Following the extensive work of our group with HNE inhibitor synthesis[3] we developed a library of HNE inhibitors and activity-based probes (ABPs) based on the 3-oxo-β-sultam[4] scaffold using the copper-assisted azide-alkyne Huisgen cycloaddition[5] (**Scheme 1**). These ABPs target only the active form of HNE and will be used to covalently tether a reporter tag to the enzyme, providing protein activity quantification on patient derived biospecimens by proteomics-based analysis. We envisage the study and validation of HNE as a potential biomarker in COPD. The outcome of this project will be a breakthrough in the field that ultimately will lead to important advances for COPD biomarker discovery.



Scheme 1: Copper-assisted azide-alkyne Huisgen cycloaddition in the synthesis of a 3-oxo-β-sultam based library of HNE inhibitors.

Acknowledgements: Fundação para a Ciência e a Tecnologia (Portugal) (SFRH/BD/100400/2014, PEst-OE/SAU/UI4013/2014, IF/00472/2014).

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Chemical acylation vs enzymatic esterification of malvidin-3-glucoside-rich wine extract with oleic acid

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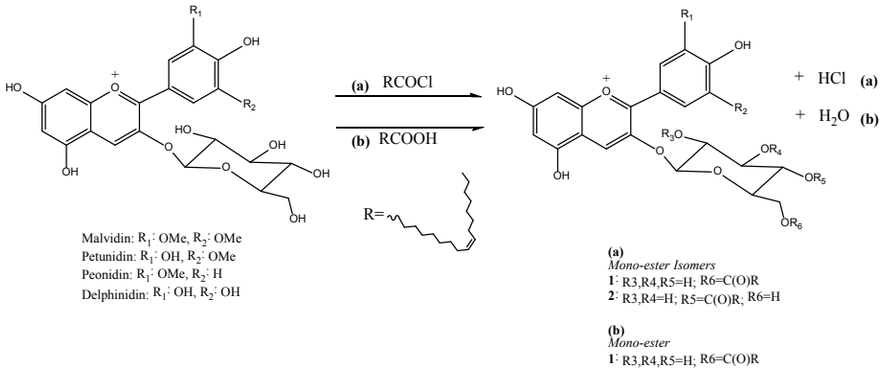
The structural modification of anthocyanins (water soluble pigments) into more lipophilic compounds is very important to expand their application in the food, medicinal and cosmetic industries.[1, 2] In this work, the synthesis of anthocyanin oleic acid ester derivatives was achieved by chemical and enzymatic approaches (Scheme 1). Enzymatic approach allowed to synthesize mv3glc-oleic acid ester which was structurally characterized by mass spectrometry and for the first time by NMR spectroscopy. Enzymatic reaction revealed to be more efficient and regioselective in the conversion of native anthocyanins into their ester product rather than the chemical reaction. Antioxidant features of the obtained products by means of DPPH and FRAP assays confirms that their antioxidant potential was not compromised, which is an important insight for future technological applications.



Scheme 1: The reaction scheme of **(a)** chemical acylation and **(b)** enzymatic esterification of the malvidin-3-glucoside-rich wine extract.

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New peptide-primaquine conjugates for malaria therapy

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Despite all efforts against malaria, this infection remains a worldwide threat. A cost-effective reduced-risk strategy for developing new antimalarials (AM) is recycling classical drugs, a suitable approach for AC, as this is mostly addressed to low-income countries: most of the latest efforts are focused on known drugs, either to find more potent derivatives, or to develop dual-action hybrids by linking two antimalarials together [1]. However, these approaches seldom produce truly valuable candidates to replace available drugs, so it may be wiser to invest on targeted delivery (TD) of known drugs, as it may increase drug's concentration at site of action, reducing side effects associated to high doses. Moreover, drug masking with a suitable carrier may elude parasite resistance.

Cell-penetrating peptides (CPP) are becoming prominent shuttles for intracellular drug delivery as a consequence of their low toxicity, ability to be uptaken by diverse cell types, and compatibility with different cargo sizes or types [2]. Progress in this field has been mainly focused in cancer and gene therapy, but there are still no reports on CPP application into intracellular delivery of AM, which constitutes a relevant void in AC. CPP uptake into Plasmodium-infected erythrocytes (PiRBC) is usually higher than healthy erythrocytes (hRBC), as RBC undergo significant changes upon infection, including acquired adhesion properties, improving their permeability towards cationic amphipathic CPP [3]. Moreover, amphipathic peptides specifically targeting PiRBC have been identified. Carefully chosen peptides may act as selective shuttles for intracellular delivery of antimalarials into PiRBC.

In this work, several CPP were conjugated with primaquine, a well-known antimalarial, and evaluated for their activity against liver-stage *Plasmodium berghei*, with interesting results that will be presented in this communication.

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Synthesis of Innovative Anticoagulant Hybrids

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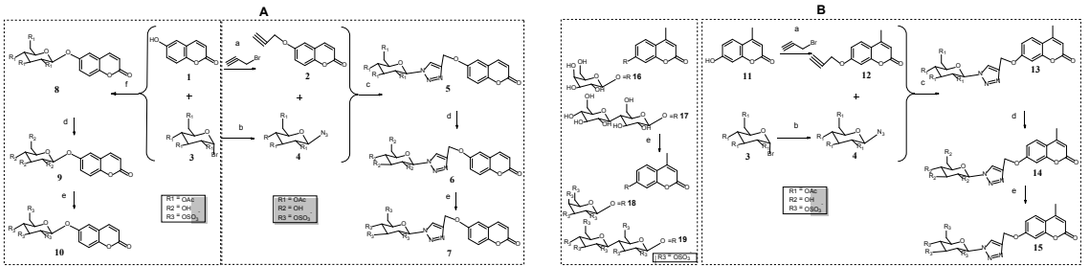
In this work, a hybridization strategy was planned in order to develop new anticoagulants joining a coumarin scaffold with a heparin-like sugar sulfated moiety. With this approach we expected to mimetize the sulfated polysaccharide anticoagulants, while adding some hydrophobic character to the resulting molecule. Due to the evidence that 6-substituted coumarins with heteroaromatic rings can act as FXa inhibitors,[1,2] the 6-hydroxycoumarin (**1, Scheme 1A**) was selected for molecular modification and triazole was used as a linker between the coumarin scaffold and the glucosidic moiety (compounds **5**, **6**, and **7**). For structure-activity relationships purposes, the syntheses of 7-triazole-linked coumarin glucosides (compounds **13**, **14**, and **15**, **Scheme 1B**) as well as of sulfates of non-triazole linked 6- and 7- coumarin glucosides (compounds **10**, **18**, and **19**) were performed.



Scheme 1: (a) TBAHS, $CsCO_3$, 65ºC; (b) NaN_3, r.t.; (c) Sodium ascorbate, $Cu_2SO_4.5H_2O$, THF/H_2O, MW, 70ºC; (d) NaOMe, MeOH, r.t.; (e) TEA:SO_3, DMA, MW, 100ºC; (f) TBAB, K_2CO_3, r.t.

In conclusion, thirteen new coumarin derivatives were obtained, i.e., two propynyl, three acetyl glucosides, three glucosides, and five sulfated derivatives, and the structure elucidation of the synthesized compounds was stablished by IR, NMR, and HRMS for the first time. This small library of compounds will allow the study of the effect of the presence of the triazole moiety on the anticoagulant activity and mode of action of these new anticoagulant hybrids.

Acknowledgements: This research was partially supported by Strategic Funding UID/Multi/04423/2013 through FCT and ERDF.

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Identification of complexes formed between salivary proteins and procyanidin B3 by mass spectrometry. Effect of profile saliva and tannin concentration

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Phenolic compounds are in a widespread of vegetables resulting of their secundary metabolism. They have a strong focus of research interest because of their health effects in the prevention and/or treatment of several chronic diseases[1]. Tannins were often described in the past as antinutritional factors because they can impact negatively on animal production since tannin could bind to and precipitates proteins and various other organic compounds[2]. Tannins can also interact with salivary proteins, and this interaction is supposed to be at the origin of the astringency sensation in foods and beverages. Astringency is a mouthfeel of dryness, roughness and puckery promoted by different foods containing tannins. Currently, because of the complexity of astringency and the likelyhood of multiple mechanisms occurring simultaneously, there is still a gap in knowledge regarding how these mechanisms affect each other and how these lead to altered mouthfeel after consumption of astringent stimuli. Major related astringency published works were focused on studies on the insoluble protein-tannin aggregates[3]. However, soluble tannin-protein aggregates could be very relevant. In order to contribute to astringency knowledge, a synthetic tannin commonly found in food and beverages (procyannidin B3) was used to mimic the *in vivo* aggregation process with salivary proteins in mouth. Several factors could influence the tannin-protein interaction such as the human salivary protein profile, the tannin tested and the tannin/protein ratio. Hence, the goal of this study aims to study the effect of different salivas (A, B and C) and different tannin concentration (0.5 and 1 mg/mL) in the interaction process as well as the complexes stability over time. Procyannidin B3 was synthetized according to[4] and different volunteers was used as saliva donors. After reaction, the complexes formed were truly characterized by mass spectrometry (MALDI-TOF and ESI-MS). Forty eight major B3-human salivary protein aggregates were identified regardless of the saliva and tannin concentration tested. Higher number of aggregates was found at lower tannin concentration. Moreover, the number of protein moieties involved in the aggregation process was higher when the tannin concentration was also higher. The selectivity of the different groups of proteins to bind tannin was also confirmed.

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Determination and comparison of the chemical composition of *Calendula* L. species growing in continental Portugal

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The flora of Continental Portugal includes three species of *Calendula* L. (*Calendula officinalis* L., *C. arvensis* L., *C. suffruticosa* Vahl), one of which comprises three subspecies (*C. suffruticosa* subsp. *algarbiensis* (Boiss.) Nyman, *C. suffruticosa* subsp. *lusitanica* (Boiss.) Ohle and *C. suffruticosa* subsp. *cinerea* (Ohle) P.Silveira & A.C.Gonç.). *C. officinalis* is recognised for its medical properties and its chemical composition has been widely studied.[1] Nevertheless, little is known about the chemical composition of *C. arvensis* and even less regarding the different subspecies of *C. suffruticosa*. Therefore, the present study aims were the elucidation of these plants' chemical composition and to compare and identify differences and/or similarities among their taxa.

To accomplish this, one sample of each *taxon*, *C arvensis*, *C. officinalis*, and *C suffruticosa* subsp. *lusitanica*, and two samples, from two different populations, of *C. suffruticosa* subsp. *algarbiensis* were collected in the field, washed with running water, and dried in a woven at 40ºC until stabilization of weight was reached. The hexane extract of each *taxon* was obtained from dried and powdered plant and completely characterized by GC-MS after silylation, which allowed the identification and quantification of their constituents.

The achieved data showed the presence of mono- and disaccharides, terpenoids, fatty acids, sterols, alkanes, long chain alcohols and some amino acids. It was found that the monosaccharides and fatty acids are the most abundant families in *C. officinalis* being the palmitic acid and α-linoleic acid the most abundant compounds. The last one was also found in higher quantities in *C. arvensis*. Fatty acids like α-linoleic acid, palmitic acid and linoleic acid are also the most abundant in *C. suffruticosa* subsp. *lusitanica*. Lastly, the two samples of *C. suffruticosa* subsp. *algarbiensis* showed in major quantities a branched alkane and one compound from the ursano family. Some carbohydrates as well as lignoceric acid and linoleic acid were described for the first time in the *Calendula* L. genus.

Through the accomplished findings, including a preliminary Principal Component Analysis (PCA), a taxonomic differentiation among the taxa can be made. Irrelevant variations were also found in the two samples of the subsp. *algarbiensis*. The compounds detected for the first time improved our knowledge of the chemical profile of this genus. Additionally, some of the reported compounds have a major importance on a nutritional level.

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α-Glucosidase and α-amylase inhibitors from *Myrcia* spp.: a stronger alternative to acarbose?

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Traditional medicines have long employed the use of plant and herbal extracts as anti-diabetic agents. These plants are typically rich in polyphenolic compounds, which are known to inhibit intestinal α-glucosidase and pancreatic α-amylase *in vitro*. [1] "Pedra hume caá" is the common name of five species of *Myrcia* genus used as traditional medicine for the treatment of diabetes mellitus. Different extracts (aqueous, methanol, water-methanol (1:1) and boiling water) from *M. salicifolia*, *M. sphaerocarpa* and *M. speciosa* were investigated for the first time, to determine their phenolic composition and *in vitro* inhibitory potential against α-glucosidase and α-amylase, both enzymes involved in hyperglycemia. [2]

An HPLC-DAD method was validated and used to establish the phenolic compounds profile. The total amounts varied from 25.45 to 76.56 mg g^{-1} of dry extract. Eight flavonols were identified, including derivatives of myricetin, quercetin and isorhamnetin, being 3-*O*-rhamnoside derivatives of myricetin and quercetin the major ones. Regarding phenolic acids, the only compound identified was gallic acid. In addition, all extracts of the three analyzed species of *Myrcia* genus contained the same four flavan-3-ols, though quantitative differences were found in their profile.

The bioactivity assays revealed that the extracts inhibited 90-500 times more α-glucosidase (IC_{50} = 0.7 to 4.1 μg mL^{-1}) than acarbose and displayed a mild inhibition against α-amylase (IC_{50} = 6.1-29 μg mL^{-1}).

Our findings suggest that these phenolic-rich species may be used as specific glucosidase and amylase inhibitors in pharmaceutical formulations to suppress hyperglycemia, supporting their traditional use as anti-diabetic herbal extracts.

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Development of antitumoral monastrol analogues: synthesis, cytotoxicity evaluation and SAR studies

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Background: Despite the intensive research efforts to improve the anticancer therapy through the years, cancer persists as one of the major global public health concern with a large incidence and mortality.[1] Thus, the search for novel anticancer agents with higher selectivity and lower toxicity, remains a priority for the scientists.
Objectives: This work aims to find novel anticancer drug candidates based on the structure of monastrol and to perform the *in vitro* evaluation of their cytotoxicity in different cell lines.
Methods: Forty five dihydropyrimidinones/thiones were synthesized *via* the Biginelli reaction by condensation of an aldehyde (1 mmol), a β-ketoester (1 mmol) and urea/thiourea (1.3 mmol). The first screening of the cytotoxicity of the compounds (at 30 μM) was evaluated on MCF-7, T47D, LNCaP, HepaRG, Caco-2 and NHDF cell lines by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The concentration inducing 50% inhibition of cell growth (IC_{50}) was assessed for the most toxic compounds using different concentrations (0.01, 0.1, 1, 10, 50 and 100 μM).
Results and discussion: After the compounds had been successfully synthesized and characterized, the evaluation of their *in vitro* toxicity was then performed. Generally, the results revealed that the compounds did not show significant toxicity in normal dermal cells, in the prostatic cancer cell line and in the breast cancer cell line T47D (relative cell proliferation higher than 50% at 30 μM). On the other hand, the compounds incorporating chloro atoms in their structure expressed considerable toxicity in the remaining cell lines. Interestingly, the chloro-containing compounds belonging to the urea series showed selective hepatic toxicity (5.28 μM ≤ IC_{50} ≤ 15.9 μM for HepaRG cells) whereas their thiourea analogs demonstrated to have lower selectivity, being significantly toxic for hepatic, colon and breast cancer cell lines (0.749 μM ≤ IC_{50} ≤ 31.9 μM for HepaRG; 5.51 μM ≤ IC_{50} ≤ 13.7 μM for Caco-2; and 2.95 μM ≤ IC_{50} ≤ 10.9 μM for MCF-7).
Conclusion: In this study it was found that the molecules containing chloro atoms in their structure, particularly, belonging to urea series, demonstrated selective toxicity for hepatic cancer cells. Additional studies are ongoing to understand what mechanisms of action are involved in the toxicity of these molecules as well as the existence of differences between the two series.

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Antifungal action of three (*Z*)-5-amino-*N'*-aryl-1-methyl-1*H*-imidazole-4-carbohydrazonamides

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The incidence of fungal infections has greatly increased in patients under sustained immunosuppression with considerable risk associated. Difficulties regarding prompt diagnosis and the limited therapeutic options dictate high mortality rates. Available antifungals display substantial toxicity, a predictable consequence of the cellular structure of the organisms involved, reduced spectrum of activity, and drug interactions. Our group had previously identified three (*Z*)-5-amino-*N'*-aryl-1-methyl-1*H*-imidazole-4-carbohydrazonamides 1 [aryl= phenyl (1a), 4-fluorophenyl (1b), 3-fluorophenyl (1c)] as potent antifungal agents.[1]

Being azole derivatives their mechanism of action was speculated to inhibit ergosterol synthesis and therefore the effect on ergosterol was assayed by HPLC-DAD. [2] Unlike fluconazole with *C. albicans*, the azoles studied did not reveal any inhibitory effect. Regarding *C. krusei,* the absence of effect was similar as observed with fluconazole. Therefore, despite the proposed mechanism, the compounds revealed no inhibition on the synthesis of ergosterol. The synergic activity between compounds 1a, 1b and 1c and fluconazole/amphotericin B against *Candida* spp. was evaluated, by checkerboard microdilution assay. The combination of the compounds with the commercial antifungals led to no significant synergism.

On a previous work the effect of the compounds on virulence factors of *C. albicans* was assessed and the inhibition of dimorphic transition was studied. The goal of the present work was to evaluate if the compounds affect other mechanisms of virulence involved in infections by yeasts, namely phospholipase and protease production. The effect in enzyme production was evaluated by inoculation on egg-yolk and bovine serum agar plates. Unlike observed with imidazole 1b, which greatly inhibited the dimorphic transition, no important effect was observed on phospholipase and protease production for all tested compounds.

Progress with the investigation related to the mechanism of action involved in the activity of these compounds is our goal.

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D-Penicillamine- and L-Cysteine-Derived Thiazolidine Catalysts: An Efficient Approach to Both Enantiomers of Secondary Alcohols

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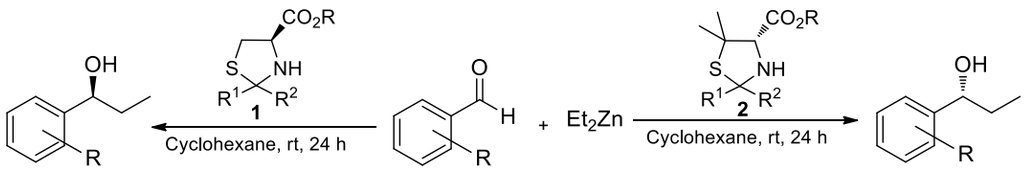
Synthetic procedures for efficiently obtaining chiral secondary alcohols are of major importance due to the vast application of these compounds in fine chemistry, pharmaceuticals, perfumes, herbicides, pesticides and because these alcohols incorporate many structures with biological activity. The enantioselective alkylation of aromatic aldehydes with diethylzinc in the presence of chiral ligands is a versatile and extremely valuable synthetic procedure for obtaining optically active secondary alcohols. Many types of chiral ligands have been used in this process, namely, diamines and their derivatives, diols and amino alcohols, among others giving products with high enantiomeric excesses.[1,2]

Chiral 1,3-thiazolidine-4-carboxylates derived from L-cysteine have been sparingly used as catalysts in the enantioselective alkylation of aldehydes with $ZnEt_2$, giving secondary alcohols with good to excellent enantiomeric excesses.[3,4] To the best of our knowledge, structurally identical D-penicillamine-derived thiazolidines **2** have not been used in these reactions. These two types of thiazolidines, easily obtained by a simple synthetic process could bring singular advantages to many catalytic processes. Because L-cysteine has a chiral center with (*S*) absolute configuration, while D-penicillamine's chiral center is (*R*), product alcohols with both (*S*) and (*R*) absolute configurations can be obtained when **1** or **2** is used, thus leading the way to a myriad of useful optically active products with opposite absolute configurations (ee up to >99%).

In this communication the synthesis of these thiazolidines will be described, as well as the results of enantioselective alkylations of aromatic aldehydes using these compounds as catalysts.



Scheme 1: Enantioselective alkylations using chiral thiazolidines **1** and **2**.

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In silico investigation of flavonoid bioactive scaffolds from *Senna* species as inhibitors at Q_i and Q_o sites in cytochrome bc_1 complex

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The flavonoids consist of a class of polyphenolic compounds with a benzo-γ-pyrone structure of natural origin, and they can be found in *different* structural forms and variety in the degree of unsaturation and oxidation of the heterocycle. Flavonoids are found in most plants and spite their antioxidant activity, most common in this class of metabolites, they present other important biological activities such as anti-inflammatory, antitumoral, antiparasitic and low toxic and side effects.[1] *Senna* species are natural sources of flavonoids what exhibit antimalarial activity against chloroquine-resistant *Plasmodium* falciparum parasites such as procyanidin B2 and quercetin, which showed antiplasmodial activity with IC_{50} values of 5.3 and 6.6 μmol.L^{-1} respectively.[2] In this communication, an i*n silico* investigation of 121 flavonoids isolated from *Senna* species identified several compounds as potential inhibitors of Q_o and Q_i sites of bc_1 complex which is an important component of the mitochondrial electron transport chain of various parasites. A molecular docking investigation was performed using the X-ray structure of bovine bc_1 complex (PDBID: 4D6T) and a homology model of cytochrome bc_1 Qo binding site developed based X-ray structure of the *Saccharomyces cerevisiae* bc_1 complex (PDBID: 3CX5). Drug-likeness of the compounds were considered using the Lipinsky "rule of five" to predict molecular properties related to adsorption, distribution, metabolism and excretion (ADME). The virtual screening procedure in Q_i and Q_o binding pockets of cytochrome bc_1 for flavonoids database showed good affinities and important interaction inside the binding pockets, showing a correlation to their antiplasmodial activity (**Figure 1**). This study identifies new potential antimalarial scaffolds from natural sources.

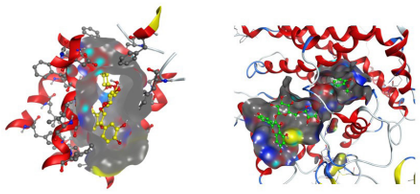

Figure 1: (a) Predicted binding poses in Q_i site of cytochrome bc_1 for rhamnetin-3-O-β-gentiobioside from of *Senna fistula* L; (b) Predicted binding poses in Q_o site of the homology model of *Plasmodium falciparum* for kaempferol-3-O-[6'''-O-trans-sinnapoyl- β -D- glucopyranosyl(1-->6)]-β-D-glucopyranoside) from of *Senna angustifolia* L.

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Structure-Based Pharmacophore Modeling and Virtual Screening of Metallo-Beta-Lactamase Inhibitors

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The hydrolytic activity of β-lactamases underlies one of the major mechanisms that drive bacterial resistance to β-lactam antibiotics, which target the DD-transpeptidase involved in the synthesis of the bacterial cell wall. The β-lactamases inactivate β-lactam antibiotics through hydrolysis of the β-lactam ring. The major strategy to overcome the activity of β-lactamases is the use of inhibitors that have little to none antibiotic activity, but that bind with greater affinity to β-lactamases, allowing an effective antibiotic therapy.
Class B β-lactamases, the MBL, are a structurally distinct class of β-lactamases that require one or two zinc ions to function. MBLs have a wide-spectrum of activity, hydrolysing almost all classes of β-lactams (except monobactams) and are not inhibited by any currently available inhibitor. They are increasingly produced by clinical bacteria and their prevalence will continue to grow as some types of MBL, particularly IMP-type, are encoded in mobile genetic elements like plasmids and integrons[1,2]. It is thus, evident, the need for solutions that allow β-lactam antibiotics to keep their effectiveness[2,3].
The study of inhibitors for MBLs has highlighted some classes of compounds potentially useful for the design of a successful inhibitor in the future. However, none have yet entered further development stages[3,4].
In this study, we have developed a structure-based pharmacophore model to screen large compound databases (NCI, ZINC and DrugBank) for candidate ligands to IMP-1 MBL, followed by docking of the successful results.

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Synthesis and Structure−Activity Relationships of Novel Pyrroloquinolone-based analogues as Potent Antimalarials

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The most virulent human malaria parasite, *Plasmodium falciparum*, is responsible for 198 million cases of malaria worldwide and its burden is heaviest in the Sub-Sharan region.[1] The emergence of drug-resistant pathogens is a major threat to human health, and *P. falciparum* exhibits high propensity to develop resistance to drug that has been deployed against it on a large scale.[2] Therefore, there is an urgent need to identify new antimalarial agents to combat emerging resistant strains with a new mode of action. Pyrroloquinolones are an extremely important class of heterocycles with wide applications in the areas of medicinal chemistry.[3-6] Pyrroloquinolones are potent and selective inhibitor of 3′,5′-cyclic nucleotide phosphodiesterases (PDEs). PDEs have been well studied as potential targets in various eukaryotic organisms. Recently, Deprez *et al*., reported the Plasmodium phosphodiesterase activity of tadalafil.[7]

On the otherside, The Liver stage of Plasmodium obligatorily precedes erythrocytic stages and therefore offers a potential drug target.[8] On the basis of these, we have designed and synthesized a series of highly substituted pyrroloquinolones (**Figure 1**), which has shown a potent activity against both the erythrocytic and exoerythrocytic forms of Plasmodium parasites. Our activity results represent a new structural lead for further optimization as dual-stage antimalarials.

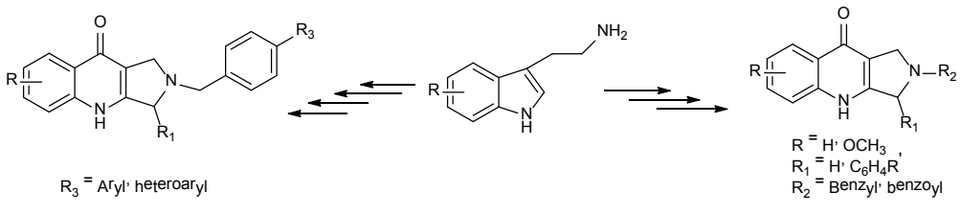

Figure 1: Synthesis and SAR of Pyrroloquinolone Analogues

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Highly Stable Platinum(II) fused-ring Chlorins as Near-Infrared Emitting Ratiometric Probes for Molecular Oxygen

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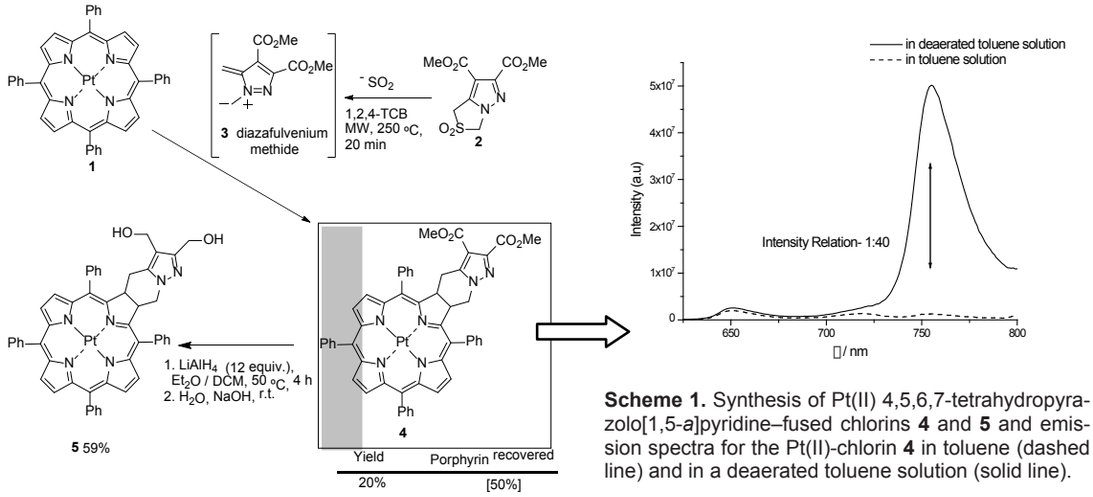
Molecular imaging is now of major importance in clinical diagnosis and biomedical research for studying biochemical processes at the cellular and subcellular levels.[1] Near infrared (NIR) emitters are particularly important for these applications since their light output is in a region where organisms are highly transparent. Until now the only long wavelength emission compound approved by the US Food and Drug agency (FDA) for direct usage in medical diagnostics is the cyanine dye indocyanine green.[2] There is, therefore, the urgent need to develop new materials with these properties. Novel, near-infrared luminescent compounds based on platinum(II) 4,5,6,7-tetrahydropyrazolo[1,5-a]pyridine–fused chlorins **4** and **5** will be presented (Scheme 1).[3] These compounds, with excellent thermal stability, show good light emission in the biologically relevant 700-750 nm near-infrared (NIR) spectral region, making them excellent materials for use in biological imaging. The intensity of the phosphorescence of these compounds is strongly quenched in the presence of oxygen whereas the fluorescence is relatively unaffected, which provides the possibilities of their application as ratiometric oxygen sensors in chemical and biological media.



Scheme 1. Synthesis of Pt(II) 4,5,6,7-tetrahydropyrazolo[1,5-*a*]pyridine–fused chlorins **4** and **5** and emission spectra for the Pt(II)-chlorin **4** in toluene (dashed line) and in a deaerated toluene solution (solid line).

Acknowledgements: This work was funded by *Fundação para a Ciência e a Tecnologia* (FCT), Portuguese Agency for Scientific Research (Coimbra Chemistry Centre, UID/QUI/00313/2013). We acknowledge the UC-NMR facility for obtaining the NMR data.

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Enrichment of Glycidamide Adducts of Human Serum Albumin

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Glycidamide (GA) is considered the toxic metabolite of the food carcinogen acrylamide, as it interacts covalently with DNA bases, predominantly forming N^7-adducts with guanine and N^3-adducts with adenine[1],and induces a tumor spectrum similar to acrylamide in rodent bioassays[2]. Besides DNA bases, glycidamide also binds with serum proteins to form protein adducts, which are useful biological markers of acrylamide exposure. Indeed, the GA-hemoglobin adduct, *N*2-(2-carbamoyl-2-hydroxyethyl)valine (GA Val) is a biomarker for the biochemical effect (reaction with nucleophiles) and is regarded as asurrogate for the determination of DNA adducts.[3]These adducts are usually detected by gas chromatography coupled with mass spectrometry (GC-MS) following a derivatization procedure, which raises some questions about the automation of the methodology due to the complexity of sample preparation.

Methodologies based on liquid chromatography – mass spectrometry analysis of protein digests provide better alternatives, involving conditions that result in low thermal input and mild ionization. Moreover, these methodologies offer a wider applicability, enabling the analysis of covalent adducts formed with polar electrophiles. Nonetheless, levels of protein adducts are small compared to the unmodified proteins, and therefore they are difficult to detect and identify without prior enrichment. To increase analytical sensitivity for detecting less-abundant protein adducts, we report herein a methodology to enrich GA adducts of Human Serum Albumin (HSA) prior to nano-electrospray ionization mass spectrometry analysis. Following trypsin digestion of HSA modified *in vitro* with glycidamide, the glycidamide-modified peptides were enriched through solid-phase borate-complex extraction and then analyzed by nano liquid chromatography coupled to quadrupole-time-of-flight mass spectrometry(Q-ToF) using a data-dependent auto-MS/MS method. This analytical method provides a straightforward approach for detecting glycidamide-protein adducts and, therefore, stimulating the commonuse of HSA adducts as biomarkers of acrylamide exposure.

Acknowledgements: This work was supported in part by Fundação para a Ciênciae a Tecnologia (FCT), Portugal (RECI/QEQ-MED/0330/2012, UID/QUI/00100/2013 and IF/01091/2013/CP1163/CT0001). ILM and CC thank FCT for doctoral fellowships (SFRH/BD/75426/2010andSFRH/BD/102846/2014, respectively). AMM also acknowledges ProgramaOperacionalPotencialHumano from FCT and the European Social Fund (IF/01091/2013), and the LRI Innovative Science Award. We thank the Portuguese MS network (IST node) for providing access to the facilities.

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Solid-Phase Synthesis of new Sulfo-Tyrosine Peptides

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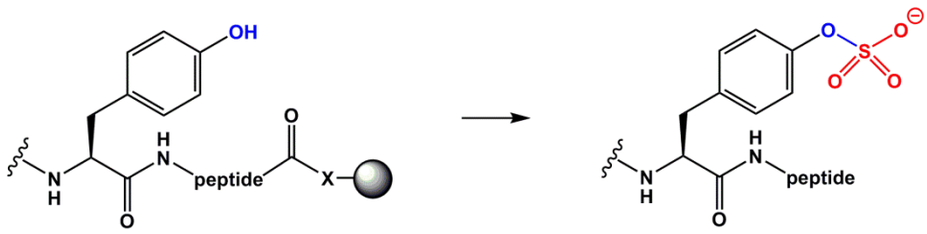
The post-translational modification (PTM) of proteins by sulfation of tyrosine residues has been the subject of several recent studies. Tyrosine sulfate has so far been found exclusively in secretory proteins, and it has been suggested that tyrosine sulfation may be one of those modifications that have a role in the secretion of proteins. Evidence suggests up to 1% of all tyrosine residues of the total protein content in an organism can be sulfated[1]. Tyrosine-sulfated proteins are involved in many biological processes, including hemostasis, leukocyte rolling on endothelial cells, optimal proteolytic processing (*e.g.* gastrin processing), visual functions, proteolytic activation of extracellular proteins (*e.g.* factor V and VIII activation), viral entry into cells (*e.g.* HIV-1 entry) and some ligand binding to receptors (*e.g.* chemokine/chemokine receptor binding)[2,3].
One of the factors hindering the study of the significance of sulfo-tyrosines in a protein is the absence of a general method that enables the synthesis of sulfo-tyrosine peptides in satisfactory yields and purity. Although several strategies for the synthesis of sulfated peptides have been reported, a general, efficient approach is still being sought. There are several ways to synthesize this type of peptides, which include chemical sulfation *after* they have been synthesized or incorporation of tyrosine sulfate monoesters *during* the course of peptide synthesis. This work presents some general approaches for the solid-phase synthesis (SPPS) of new sulfo-peptides of biological interest, including advantages and disadvantages of each method developed. The new sulfo-peptides produced were purified and characterized by HPLC and LC-MS, and are currently under study regarding their thrombin-inhibiting ability.



Acknowledgements: We thank the FCT for financial support through IF00092/2014 and UID/Multi/04378/2013 projects. Thanks are further due to ON.2 and FCUP for co-funding refurbishment of the Porto Peptide Synthesis Facility (POP-UP) through operation NORTE-07-0162-FEDER-000111.

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Exploring viral surface glycoproteins as potential targets against HIV infections

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HIV infection is a major threat to public health [1] and it has been marked by a long and elusive search for an effective HIV vaccine. There are two types of viruses, HIV-1 and HIV-2. While HIV-1 is responsible for a pandemic worldwide infection, HIV-2 causes localized epidemics mainly in West Africa, India, Brazil and in Europe (mainly in Portugal and France). There are several differences between them visible in the form how the virus proceed which can suggest a more effective immune response against HIV-1. Approved drugs target protease, reverse transcriptase, integrase, transmembrane envelope glycoprotein (gp) and the CCR5 co-receptor of HIV. Until now, no “cure” is available and drug efficacy is reduced with time and that has encouraged alternatives. HIV establishes a permanent link between the cell and the host and it integrates the genomic DNA like a latent provirus. New targets under investigation include the viral envelope gp of viral entry into cells. Computational tools allow us to rationalize the investigation and screen more targets to develop potential vaccine to the virus. [2,3]

Our goal relies on the study and modulation of the most important envelope surface glycoproteins. We are currently developing and optimizing an homology model of gp125 (HIV-2) due to the lack of the crystallography structure using MOE and Modeller softwares. Structure, interactions and dynamic behaviour are explored. Molecular dynamics simulations are being performed with Gromacs program package. [4]

Acknowledgements: We thank the Fundação para a Ciência e a Tecnolgia for financial support to Patrícia Serra for her PhD Grant SFRH/BD/100643/2014.

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Application of Molecular Dynamics and Alchemical Free Energy Calculations to study the interaction of β-2 microglobulin with thioflavin-T

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The development of new and better molecular probes that can be used for the early diagnosis of amyloid diseases such as Alzheimer's, Parkinson's and type II diabetes are of critical essence.[1] We employed molecular dynamics (MD) simulations and alchemical free energy calculations (AFEC) to better characterize the interaction of the protein β-2 microglobulin (β2m) with the well-known amyloid probe thioflavin-T (ThT). The crystallographic structure of β2m (PDB 3MZT) may be considered an amyloid-like oligomer consisting of 3 homodimers arranged in a ring where two alternative ThT conformations intercalate between the β-sheets of each dimer of the protein.[2] MD simulations were employed to evaluate the structural stability of both the β2m dimer and its complex with ThT. The results obtained in these simulations were in agreement with several reports found in the literature, where the Q8 and Y10 residues of β2m were shown to be crucial for the interaction with ThT. To better characterize the topological constraints imposed by these residues, we have mutated Q8A, Y10A and Y10F, and performed several MD simulations. Additionally, in order to quantify the energetic contribution of these residues to the stability of the β2m-ThT complex, we have also performed several AFEC. The MD results suggest that the benzene ring of the Tyr residue is crucial to the interaction of ThT and β2m, as with the mutation Y10A this interaction is strongly affected. Regarding the mutation Y10F, no significant differences within the complex dynamics could be observed. The AFEC results clearly demonstrate that energetically the most important residue is Y10, as the transformation of Y10A is highly unfavorable. Moreover, the transformation of Y10F is slightly adverse, hence indicating that the hydroxyl group may have a role in stabilizing the interaction of ThT with β2m. Finally, the mutation Q8A is energetically favorable supporting the previous MD results. In sum, the results suggest that the Y10 residues are crucial to the interaction of ThT with β2m while the role of Q8 residues in the interaction with ThT are not as relevant.

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Activation of cytotoxic responses of NK cells

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Natural killer (NK) cells are a type of cytotoxic lymphocyte critical to the innate immune system. NK cells provide rapid responses to viral-infected cells, and play a role in tumor immunosurveillance by directly inducing the death of tumor cells. Instead of acting via antigen-specific receptors, lysis of tumor cells by NK cells is mediated by alternative receptors, including NKG2D, NKp44, NKp46 and NKp30 [1].

In recent developments, B7-H6, a surface protein present on a broad panel of tumor cells including lymphoma, melanoma, and carcinoma, was identified as a ligand for the NKp30 receptor. The structure of the NKp30-B7H6 complex has also been resolved [2]. The comparison between the 3D structures of unbound and B7-H6-bound NKp30 demonstrated marked conformational changes that may be a key-factor for the NK-response activation role of B7-H6.

This work aims at designing a family of small organic molecules (SOMs) capable of mimicking the effect of B7-H6 on the NKp30 receptor. A combination of computational docking and molecular dynamics tools was extensively used to scan several ligand libraries, yielding core-structures as possible ligands for the receptor. These were further optimized to generate lead structures that are now being synthesized for experimental screening as NKp30 ligands through mass spectrometry tools.

Our main goal is to obtain an SOM capable of inducing the activation of an NK response, through binding of the NKp30 receptor, and structurally amenable to derivatization with specific tumor-targeting molecular units to produce a specific immune response against cancer cells.

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N-acylation of antimalarial classics towards dual-stage antimalarial leads

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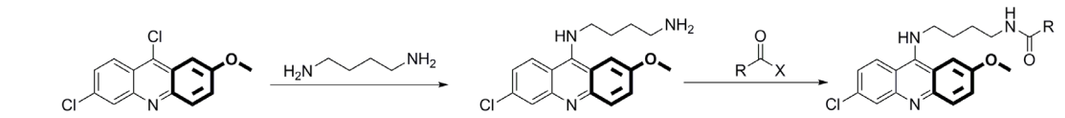
A child dies nearly every minute from malaria. Despite the fact that malaria-associated deaths have decreased by about 54% in Africa since 2000, eradication is far from being achieved in the near future. One strategy to accelerate the development of novel antimalarials is to start from the chemical frameworks of known ones to produce new drugs, i.e., to recycle classical drug scaffolds.[1] In this regard, our group has been extensively working on the chemical recycling of classical antimalarials with rather promising results.[2] Ensuing our reports on *N*-cinnamoylated chloroquine and quinacrine analogues as promising dual-stage antimalarials leads, displaying high *in vitro* potency against both blood-stage *Plasmodium falciparum* and liver-stage *P. berghei*,[2] we have now investigated the effect of replacing the cinnamoyl moiety by other acyl groups. Thus, a series of *N*-acylated chloroquine and quinacrine analogues (respectively, 1 and 2 in Scheme 1) were synthesized, and their activities against blood- and liver-stage *Plasmodium spp.*, as well as *in vitro* cytotoxicity, were assessed. Results obtained, to be presented, showed that the new *N*-acylated analogues were somewhat less active and more cytotoxic than their *N*-cinnamoylated counterparts. Still, they preserved nanomolar activities *in vitro* against blood-stage drug-sensitive and -resistant *P. falciparum*, and significant *in vitro* liver-stage activity against *P. berghei*.[3] Therefore, it is demonstrated that simple *N*-acyl surrogates of antimalarial classics are promising low-cost dual-stage antimalarial leads.



4,7-dichloroquinoline (thin only)
or
6,9-dichloro-2-methoxyacridine (thin&**bold**)

1, chloroquine analogues (thin only)
or
2, quinacrine analogues (thin&**bold**)

Scheme 1. General synthesis route towards *N*-acylated analogues of chloroquine (**1**) and quinacrine (**2**).

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In vitro effects of lycopene-loaded lipid-core nanocapsules on isolated human erythrocytes

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Lycopene (Lyc, Fig. 1) is one of the bioactive compounds that belong to the carotenoid group. Frequent ingestion of Lyc has been associated with the decreased risk of developing oxidative stress related chronic degenerative diseases, such as inflammation and cancer. In a previous study of our research group, Lyc-loaded lipid-core nanocapsules (Lyc-LNC) were developed to overcome the instability of Lyc in the presence of oxygen, heat, light and also its high hydrophobicity, improving its potential to be used for food and medical applications. Thus, aiming a preliminary *in vitro* toxicological evaluation of Lyc-LNC, synthesized with high purity Lyc (> 98%), we evaluated the effect of these nanocapsules on erythrocytes viability and also on the behavior of the most relevant biomarkers of oxidative stress in human erythrocytes. According to the release profile of Lyc from Lyc-LNC, it is possible to observe that 2.8 μM of Lyc was released after the incubation for 6 h at 37 °C in PBS at pH 7.4, corresponding to 2% of total content, suggesting that this compound tends to remain in the lipid core. The incubation of isolated human erythrocytes with Lyc-LNC (up to 6 μM equivalents of Lyc) did not decrease cell viability even after 4 h of incubation at 37 ºC. However, a decrease (> 80%) in cell viability was observed for concentrations higher than 6 μM after 4 hours of incubation. In relation to the biomarkers behavior, incubation of Lyc-LNC (0.004-0.125 μM equivalents of Lyc) with isolated erythrocytes at 37 ºC was not able to induce lipid peroxidation, hemoglobin oxidation or the depletion of glutathione, excepting for the hemolysis that exhibited low percentage (3.5% at 0.125 μM) after 3h-incubation at 37 ºC. These same effects were also observed for nanocapsules synthesized without Lyc inside, at the same experimental conditions. The results suggest that nanoencapsulation of Lyc might be a promising strategy to improve the delivery system of hydrophobic bioactive compounds to be used by food and pharmaceutical industries. Notwithstanding, further research is clearly required to understand the molecular mechanisms of these kinds of nanoencapsulated bioactive compounds in different cellular and *in vivo* systems.

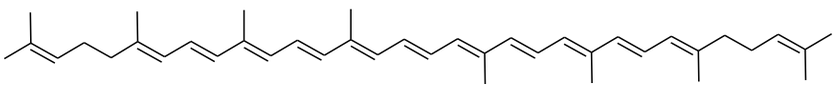

Figure 1. Chemical structure of lycopene.

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Clearance and biocompatibility of polyacrylic acid-coated iron oxide nanoparticles in mice

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This study aimed to clarify how polyacrylic acid-coated iron oxide nanoparticles (PAA-coated IONs) are recognized, internalized, and distributed within liver and spleen macrophages *in vivo*. PAA-coated IONs (8, 20, 50 mg/kg) were administrated, through the tail vein, to male mice (aged 8 weeks) of CD-1 strain. Mice were sacrificed 24 hours after administration, blood was immediately collected from the inferior vena cava, and liver and spleen were removed for histopathological analysis. Blood smears were stained using the Diff-Quik procedure, and a manual differential count of 100 leucocytes was performed under 100X lens. Iron detection was performed on Perls-stained sections. The results showed that liver and spleen are clearance pathways for the PAA-coated IONs in the blood. Kupffer cells (KC) and splenic macrophages form part of the important immune system known as mononuclear phagocytic system, which also include monocytes. No differences, however, were observed for monocytes in the differential leukocyte counts. On contrary, an increase in the neutrophils' frequency after the two highest doses of PAA-coated IONs was observed. These results show that body's immune system responds quickly to the presence of PAA-coated IONs, trying to eliminate them through phagocytic, metabolic and degradative processes in immune cells, i.e., by liver and spleen macrophages and by recruiting additional ones from circulation. KC were the most active phagocytes in uptaking the PAA-coated IONs. Due to iron presence, the cytoplasm of KC was granular and golden brown on Haematoxylin and Eosin staining. In electron microcopy it was observed that PAA-coated IONs were encapsulated in KC phagocytic vesicles. A positive correlation between iron and PAA-coated IONs concentrations was observed in splenic red pulp of mice. Further studies must be planned to: (1) to facilitate both PAA-coated IONs or monocyte–macrophages phagocytosis and targeting abilities, and/or (2) to prolong the circulation time of the PAA-coated IONs. Depending on the results of those studies it will be possible, in the future, extend the range of PAA-coated IONs biomedical applications.

Acknowledgements: We would like to thank FCT (Fundação para a Ciência e Tecnologia, Portugal) for the financial support to UCIBIO-REQUIMTE through the project UID/Multi/04378/2013. Couto, D, Freitas, M and Costa, VM acknowledge the FCT financial support for the PhD and Pos-doc grants (SFRH/BD/72856/2010, SFRH/BPD/76909/2011 and SFRH/BPD/63746/2009, respectively), within "POPH - QREN - Tipologia 4.1 - Formação Avançada" co-sponsored by FSE.

Metal ion complexes with 3-hydroxy-4-pyridinone ligands as inhibitors of neutrophil's oxidative burst

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Ligands of the 3-hydroxy-4-pyridinone (3,4-HPO) type have been reported to possess biomedical, chemical and analytical applications. In this first screening study aiming to uncover new promising agents to mitigate the oxidative damage highly present in several metabolic disorders, such as diabetes mellitus, we assessed the potential of twelve 3,4-HPO metal ion complexes to modulate oxidative burst in human neutrophils. Metal ion 3,4-HPO complexes of Ni, Fe, V, Co, Cu and Zn (Figure 1) were synthesized and tested up to 15 μM. Among all the compounds, $[Cu(mpp)_2]$ and $[Cu(dmpp)_2]$ exhibited the highest scavenging capacity against superoxide radical ($O_2^{\bullet-}$) (IC_{50}=0.36 ± 0.07 and 0.30 ± 0.06 μM, respectively) and against hypochlorous acid (HOCl) (IC_{50}=0.6 ± 0.3 and 0.4 ± 0.1 μM, respectively). In the particular case of $O_2^{\bullet-}$, $[Fe(mpp)_3]$ and $[Fe(dmpp)_3]$ (both at 15 μM) presented 35% and 22% of inhibition, respectively, while all the other compounds were neither able to scavenge $O_2^{\bullet-}$ nor stimulate its production. Regarding the scavenging capacity against hydrogen peroxide (H_2O_2), all the compounds showed low efficiency (from 6-39%). Finally, with exception of $[VO(mpp)_2]$ and $[VO(dmpp)_2]$, all compounds exhibited scavenging activity against HOCl (39-81%) and the most efficient compounds were Cu(II) and Zn(II) complexes. Thus, these preliminary results uncover promising new metal ion complexes, inhibitors of neutrophil's oxidative burst, with potential anti-inflammatory properties, which may constitute a new strategy for the treatment of the pathogenesis and/or complications of diabetes mellitus.

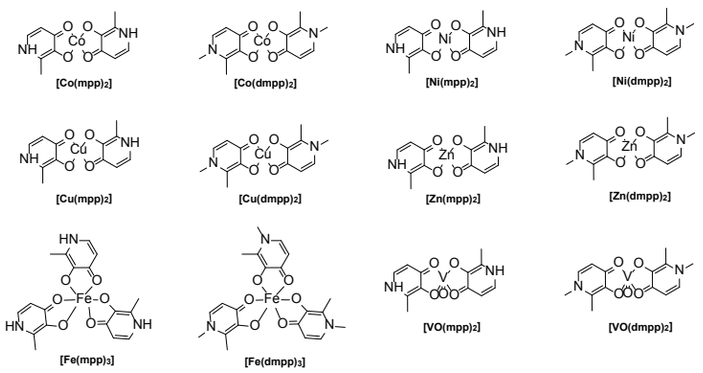

Figure 1: Chemical structures of the tested 3-hydroxy-4-pyridinone metal complexes.

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Production of surface carboxymethylated cellulose filters in aqueous medium.

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Cellulose, the most abundant natural biopolymer on earth, is considered as one of the most promising polymeric resource [1]. It has many advantages, such as low cost, biocompatibility and biodegradability [2]. Cellulose derivatives, in particular carboxymethyl cellulose (CMC) represents the most important ionic cellulose ether widely used where thickening, suspending, stabilizing, binding and film forming properties are important [3]. With the aim of producing cellulose filters able to adsorb positive proteins at low pH, we produced surface carboxymethylated cellulose using commercial filters has starting material.
Commercial filters were carboxymethylated using sodium hydroxide (NaOH) and monochloroacetic acid (MCA), in aqueous medium, under heterogeneous conditions. The carboxymethylation reaction was optimized to the NaOH concentration, MCA concentration, reaction temperature and reaction time. The degree of substitution (DS) was determined with respect to the reaction conditions using chemical methods. The produced CMC was identified by FTIR and protein adsorption capacity was performed by static method with lysozyme as control protein. The CMC surface modified filters had a DS between 0.139 and 0.179 and an adsorption capacity between 2.5 to 10.4 mg of lysozyme per gram of cellulose at pH 5.
This preliminary results show that it is possible to produce water insoluble, surface carboxymethylated cellulose filters for the adsorption of positively charged proteins at low pH in aqueous medium. Future work will focus on the optimization of the reaction process to increase the protein adsorption capacity of the filters.

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Synthesis and *in vitro* activity in HCT116 human colon cancer cells of new phenol-pyrimido[5,4-*d*]pyrimidine conjugates

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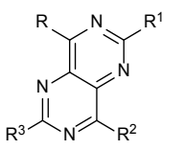
Colon cancer is the third cause of death worldwide and it is more frequent in industrialized countries. In the USA it is the second cause of death and in Europe approximately 25,000 cases are reported every year. The existent treatments are based in the combination of 5-Fluorouracil (5-FU) and LV or 5-FU alone[1,2]. However, these treatments are not effective in all patients due to multidrug resistance developed by tumour cells and the lack of selectivity.[1,2]

The pyrimido[5,4-d]pyrimidine ring system **1** has been attracted enormous attention of the scientific community mainly due to its activity as anti-tumour[3], antiviral[4], antioxidant[5,6],antifungal[7] and hepatoprotective[8] properties. In our research group, pyrimido[5,4-*d*]pyrimidines were identified as highly active against colon cancer cells (HCT116 p53-wt), with IC_{50} lower than that of 5-FU.

Recently, we synthesized new derivatives of pyrimido[5,4-*d*]pyrimidines, **2**, combining pyrimido[5,4-*d*]pyrimidine core with phenolic aldehydes. The *in vitro* activity of the new compounds was assessed in HCT116 human cancer cells. The new compounds showed high activity that depends on the R^2 group. The synthetic approach and the biological results will be presented.



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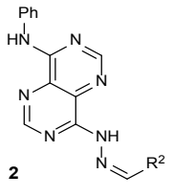

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Synthesis and Study of Ionic Liquids Based on Anti-malaria Drugs Primaquine and Chloroquine

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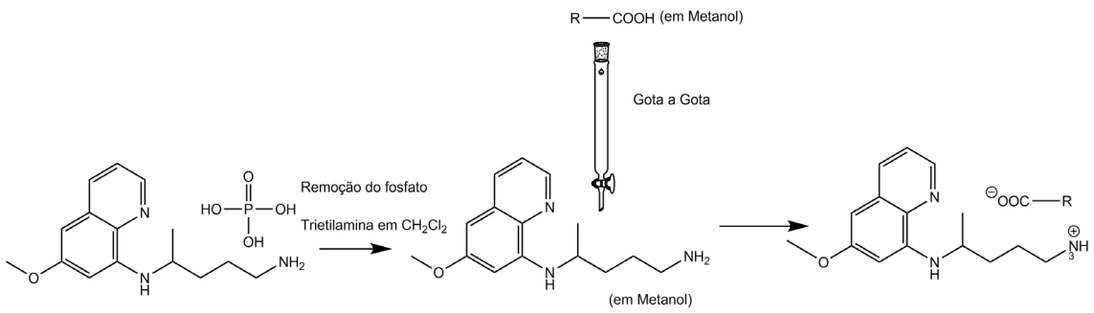
Ionic liquids (ILs) have been a hot topic in recent years. They are generally defined as organic salts, and many are liquids at room temperature. Ionic liquids (IL) come up as a resource to be used in the pharmaceutical industry, and have shown the ability to improve the characteristics of active pharmaceutical ingredients (APIs). In addition, antimicrobial properties also have been described for ILs. Hence, ILs combined with APIs or ILs as APIs may open new perspectives towards rescuing classical drugs whose clinical use is being or has been gradually abandoned.

In connection with the above, the purpose of this work was the synthesis and study of ILs derived from classical basic antimalarials, primaquine (PQ) and chloroquine (CQ), whose protonated forms (cationic) were combined with different acids, some of them also interesting from a therapeutic standpoint (Scheme 1). Fourteen ILs were produced and screened for their antimalarial properties. Interesting results were obtained, which will be presented in this communication.



Scheme 1. Synthetic scheme towards primaquine-derived ILs prepared in this work.

Discovery of 20S proteasome inhibitors: A strategy to anticancer therapy

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The ubiquitin proteasome system (UPS) is a nonlysosomal pathway by which cells regulate the controlled degradation of several proteins, not just in cell cycle regulation and apoptosis, but also in inflammatory and immune responses, antigen presentation, oxidative stress, hereditary disorders like cystic fibrosis, and carcinogenesis.[1,2] During normal protein homeostasis, the defective proteins are ubiquitinated and are specifically proteolysed into short peptides by the proteasome. The proteasome substrates include signalling molecules, tumour suppressors, cell cycle regulators, transcription factors, among others.[3]gene expression, the quality control of proteostasis and the response to geno- and proteotoxic stress. Prior to degradation, the proteasomal substrate is marked with a poly-ubiquitin chain. The key protease of the ubiquitin system is the proteasome. In dividing cells, proteasomes exist as holo-enzymes composed of regulatory and core particles. The regulatory complex confers ubiquitin-recognition and ATP dependence on proteasomal protein degradation. The catalytic sites are located in the proteasome core particle. Proteasome holo-enzymes are predominantly nuclear suggesting a major requirement for proteasomal proteolysis in the nucleus. In cell cycle arrested mammalian or quiescent yeast cells, proteasomes deplete from the nucleus and accumulate in granules at the nuclear envelope (NE Proteasome inhibition results in an interruption of the degradation of these substrates, leading to the activation of the apoptotic pathways and, eventually, cell death.[1,2] Rapidly growing cells, such as cancer cells which require a big amount of growth promotion proteins to make possible an accelerated and non-controlled mitosis, are particularly susceptible to proteasome inhibition.[4]

This work relies on a first computational based drug discovery campaign to discover alternative new, selective (and more effective) small molecule reversible proteasome inhibitors that can overcome the severe adverse drug reactions demonstrated by in use drugs. The efforts to discover new anticancer drugs described here combine different computer-aided drug design methodologies like pharmacophore modeling, molecular docking and structure-based virtual screening to find new insights that can improve chemical understanding of proteasome-small molecules interactions and identify potential hit compounds.

These *in silico* methodologies illustrate how important could be small-molecule key features in proteasome activity and, at the same time, can help in the discovery process of potential novel medicines with completely new chemotypes.

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Synthesis and antiproliferative evaluation of bile acid polyamine amides.

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Background: Cancer is a disease with high morbidity and mortality, as well as a severe socio-economic impact. Despite the enormous scientific advances, the majority of drugs currently used in the treatment of malignant tumours have low efficacy and severe side effects, which has stimulated research in the development of new drugs in this area over the past decades.[1]
Objectives: Bile acids have been widely applied in synthetic chemistry mainly due to their enantiomeric purity, high stability of the steroid nucleus, reactivity of the side chain groups, low cost and ready availability, being important building blocks in the construction of novel molecular assemblies. A relevant strategy consists in the development of molecules with potential anticancer action that combine its core base with polyamines.
Methods: The general synthesis procedure consisted firstly in the acylation of lithocholic or deoxycholic acid with succinic or phthalic anhydride, followed by the coupling of polyamines with different lengths and atoms donors using DCC as a reaction activator. Then, the compounds were evaluated for the antiproliferative action through 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay in LNCaP, MCF-7, T47D, NHDF and U87 cell lines and propidium iodide staining of dead cells (flow cytometry evaluation, MCF-7 cells).
Results and discussion: the strategy in this work consisted in the development of molecules combining lithocholic and deoxycholic acids with polyamines, since these can participate in electrostatic interactions, through their positively ionized amines, with the polyanionic sugar-phosphate backbone of DNA. The results show that the succinoyloxy-deoxycholic acid polyamines have an interesting potential as anticancer agents. In fact, product 7 revealed a selective cytotoxic effect on hormone-dependent tumours, as compared with non-transformed cell lines. Furthermore, this cytotoxic effect included cell death induction in MCF-7 cells. In contrast, compound 8, a phthaloyloxy-lithocholic acid polyamine showed a relevant anitiproliferative effect on LNCaP cells. Currently, more structure-activity studies are being performed to obtain new compounds with improved bioactivity.
Conclusion: it was possible to synthetize novel compounds some of which showing a promising biological activity and constitute interesting hit compounds to the development of novel analogues with increased anticancer activity.

Acknowledgements: This work was supported by FCOMP-01-0124-FEDER-041068 – EXPL/QEQ-MED/1068/2013.

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Anacardic Acid Derivatives from Cashew Nut Shell Liquid: NMR Characterisation and Evaluation of Its Biological Activities

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Cashew nut shell liquid is obtained in the processing industries of cashew nuts as a by-product of little commercial value but with high technological potential due to its phenolic constitution and its various biological properties such as antimicrobial, anti-inflammatory, anti-tumour, antioxidant, antiacetylcholinesterase activities,[1,2,3] showing a great therapeutic potential. Anacardic acid, the major constituent of CNSL, is a mixture of three types of salicylic acids with the side chain of fifteen carbon atoms with different degree of unsaturation (**Figure 1**). In this study, the major components of anacardic acid (15:1, 15:2 and 15:3) were isolated by means of column chromatography impregnated with silver nitrate and its structure was characterized by nuclear magnetic resonance, through a complete and unequivocal proton and carbon assignments. The effect of the side chain unsaturation in the antioxidant, and anticholinesterasic activities and also their toxicity against *Artemia salina* was also evaluated. Initially, the extraction of natural CNSL was made with the hexane solvent by maceration for further isolation of anacardic acid derivatives.[4] Antioxidant activity was assessed by inhibition of the free radical DPPH (1,1-diphenyl-2-picrylhydrazyl) to give an IC_{50} = 2.06 ± 0.28 mg/mL for monoene anacardic acid, 1.78 ± 0.01 mg/mL for the diene and of 0.13 ± 0.81 mg/mL for the triene, while the standard BHT gave 0.266 ± 0.005 mg/mL. The cytotoxicity against the *A. salina* was higher for the triene anacardic acid with and IC_{50} = 109.71 mg/mL, even lower, a cytotoxic effect also was observed for all other constituents studied for presenting LC_{50} <1000 g/mL.[5] Regarding antiacetilcolinesterase activity, the triene anacardic acid had the best inhibition (1.0 cm), followed by the diene (0.8 cm) and monoene (0.6), when compared to standard physostigmine (0.9 cm). Thus, among anacardic acids, the triene derivative showed better biological activities, followed sequentially by the diene and monoene. Therefore the greater the number of unsaturations higher is its action against free radicals, enzymes and organisms tested.

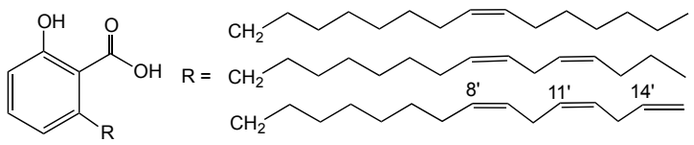

Figure 1. Anacardic acids from cashew nut shell liquid

Acknowledgements: We thank the Conselho Nacional do Desenvolvimento Científico e Tecnológico (CNPq), Brasil; University of Aveiro and QOPNA research Unit (FCT UID/QUI/00062/2013) for financial support.

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First evidence of efavirenz metabolism to a catechol – a plausible role in toxicity

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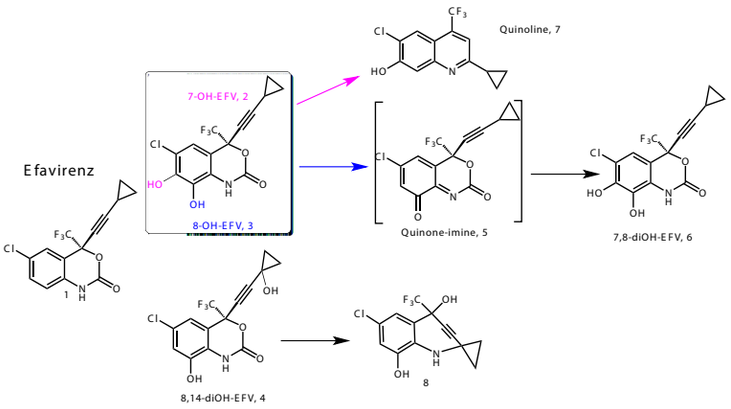
Efavirenz (EFV, **1**) is a non-nucleoside reverse transcriptase inhibitor administered as first-line treatment against HIV and prescribed as part of combination therapy. EFV is extensively metabolized by cytochrome P450, undergoing primary oxidation on the aromatic ring to the phenolic products 7-OH-EFV (minor, **2**) and 8-OH-EFV (major, **3**), and secondary oxidation on the cyclopropane ring (at C14) to 8,14-diOH-EFV (**4**) (**Scheme**). Despite the drug's efficacy, clinically restrictive neurotoxic and hepatotoxic events are a major limitation of EFV administration. Bioactivation to reactive electrophiles (*e.g.*, to catechol metabolites and their quinoid derivatives) capable of reacting with biomacromolecules is likely to be involved.



We obtained and fully characterized a new catechol (**6**) from direct oxidation of 8-OH-EFV with Frémy´s salt; its quinone-imine precursor (**5**) was also detected. Incubation of EFV and its mono-hydroxy metabolites **2** and **3** with rat and human liver microsomes, followed by LC-ESI-MS characterization, led to the first-time evidence for the formation of catechol **6** in metabolically competent systems. By contrast, direct oxidation of **2** afforded a stable quinoline derivative (**7**)[1] but no evidence of the catechol or its quinone-imine precursor was obtained. In addition, 8,14-diOH-EFV (**4**) afforded product (**8**) under bio-mimetic conditions. Given the propensity of quinone-imines to undergo nucleophilic addition, our results support a role for bioactivation of 8-OH-EFV at the onset of EFV-mediated toxicity.

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A new and efficient synthesis of N^3-cycloalkylamino-6,8-diaminopurines

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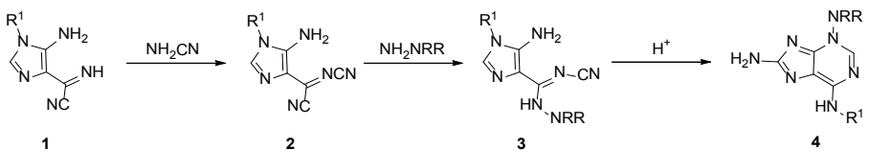
The purine core is a privileged scaffold in medicinal chemistry and the biological relevance of purine derivatives makes them attractive targets in the preparation of combinatorial libraries.[1,2] In particular, there is a great interest in the synthesis of 8-substituted purines due to their important potential as antiviral and anticancer agents.[3] Reports on 8-aminopurines are limited and general methods to obtain these purine derivatives are still needed.[4] Cyclic amines and hydrazines are key structural motifs in various bioactive agents.[5] Here we report a novel, efficient and inexpensive method for the synthesis of 6,8-diaminopurines **4** incorporating cycloalkylamino substituents at N^3-position of the purine ring.

In our research group, recent studies on the reactivity of 5-amino-4-cyanoformimidoylimidazoles showed that the 4-cyanoformimidoyl group reacts with amines under acidic conditions.[6] In a recent work, cyanoformimidoylimidazoles **1** were reacted with cyanamide to generate intermediates **2** that could be converted into novel N^3-substitued 6,8-diaminopurines by reaction with primary amines. In order to incorporate cycloalkylamino substituents at N^3-position of the purine ring, intermediates **2** were combined with cyclic hydrazines to obtain amidines **3** (**Scheme 1**). In the presence of acid, amidines **3** undergo an intramolecular cyclization to give the N^3-cycloalkylamino-6,8-diaminopurines **4** in good yields, which were fully characterized by IR and NMR spectroscopy.



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

Scheme 1: Synthesis of N^3-substituted-6,8-diaminopurines **4** from imidazole precursors **1**.

Acknowledgements: The authors gratefully acknowledge the University of Minho and FCT for financial support to NMR portuguese network (PTNMR) FCT and FEDER-COMPETE-QREN-EU for financial support to the Research Centre [Pest-C/QUI/UI0686/2011 (FCOMP-01-0124-FEDER-022716)].

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STD-NMR and fluorescence quenching towards understanding astringency: a molecular study on procyanidins/salivary protein (mucin) interaction

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Procyanidins (PC) are a class of polyphenols that enter the human diet mainly through fruits and vegetable-based beverages like red wine. They are polymeric flavan-3-ols, in which elementary units are linked by C-C and occasionally C-O-C bonds yielding large ramified structures[1]. These compounds have the ability to interact with biological proteins, namely salivary proteins. Regarding the sensory analysis, PC interaction with salivary proteins is directly related to astringency sensation which has been defined as dryness, tightening and puckering sensations perceived in the oral cavity during the ingestion of food/beverages[2]. In the case of red wine, astringency is a desirable sensation and a parameter of quality when perceived in balanced levels[3]. However, most of the works done in astringency research area do not characterize this interaction at molecular level.

In this work, it was intended to study the interaction between mucin (salivary) protein and PC by two spectroscopic techniques, such as fluorescence quenching and Saturation Transference Difference-Nuclear Magnetic Resonance (STD-NMR). For this purpose, we focused in: a) synthesize/isolate PC with different molecular structures commonly present in red wine (dimers B3 and B4, tetramer and fractions of oligomeric procyanidins) and b) characterize the molecular interaction between these PC and mucin (salivary) protein (determination of dissociation constants, binding stoichiometry, structural binding epitopes, type of bonding involved).

The results obtained showed that the values of binding constants obtained by fluorescence extinction increase with procyanidins polymerization degree. It was also observed that, depending on the PC, hydrophobic and hydrogen bonds are the major driving forces for the interaction. In fact, the addition of 10% of ethanol or dimethyl sulfoxide (solvents known for disrupting hydrophobic and hydrogen bonds, respectively) decreased significantly the constants obtained by both fluorescence quenching and STD-NMR.

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Synthesis and spectroscopic characterization of a new dithiosquarylium cyanine dye

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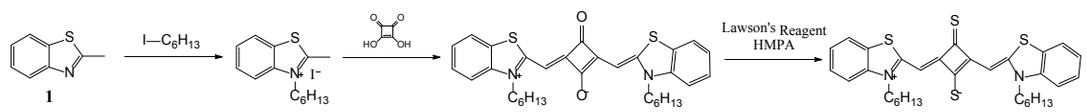
The discovery of new and better photosensitizers for alternative therapies, such as Photodynamic Therapy (PDT), has aroused the interest of the scientific community. The squarylium cyanines dyes are usually synthesized by condensation of an equivalent of squaric acid and two equivalents of electron rich aromatic or heterocyclic methylene bases. These organic compounds possess some of the essential properties for a good photosensitizer for PDT, including a strong absorption in the Visible (Vis) or NIR region and the significant production of reactive oxygen species[1]. However, some modification in their structure may improve these properties enhancing its biological application. Previous studies suggest that the replacement of the oxygen atoms bonded at central ring by sulfur atoms increase the singlet oxygen production[2].

In this study a new symmetric dithiosquarylium cyanine dye **4** was synthesized with a moderated yield (**Scheme 1**) and its structural characterization was elucidated by, m.p., IR, UV-Vis, ^{1}H-NMR, ^{13}C-NMR and HRMS-ESI-TOF. The citotoxicity in dark and under by LEDs irradiation, with an appropriate wavelength, was also evaluated in the cell lines HepG2 and Caco-2.



Scheme 1: Synthesis of a new dithiosquarylium cyanine dye

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Synthesis of a new squarylium cyanine dye and cytotoxicity evaluation in HepG2 and Caco-2 cells

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The continuous study and development of organic chemistry has enabled the discovery of compounds with different applications. Among them, the discovery of new synthetic dyes, such as squarylium cyanine dyes which have shown great interest in many areas and applications[1]. The squarylium cyanine dyes are a class of compounds, discovered in 1965 by Treibs[2], whose photochemical and photophysical properties showed ideal behaviour for many biotechnological applications, such as photodynamic therapy[3].

In this work a squarylium cyanine dye (Figure 1) was synthesized and fully characterized by melting point, IR, UV/Vis, ^{1}H-NMR, ^{13}C-NMR and HRMS-ESI-TOF spectra. This dye was obtained, according to methods adapted from literature[4], with moderate yield. Later, its cytotoxic effect was evaluated on Caco-2 and HepG2 cells, in the dark and after LEDs irradiation with an appropriate wavelength.

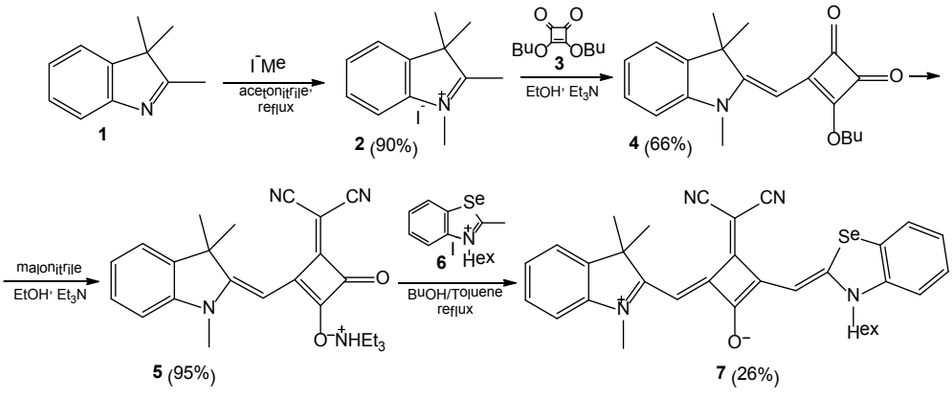

Figure 1: Synthesis of the new squarylium cyanine dye (**7**).

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Synthesis of Biflorin-Based Nitrogen Derivatives and Their Antibacterial Activity

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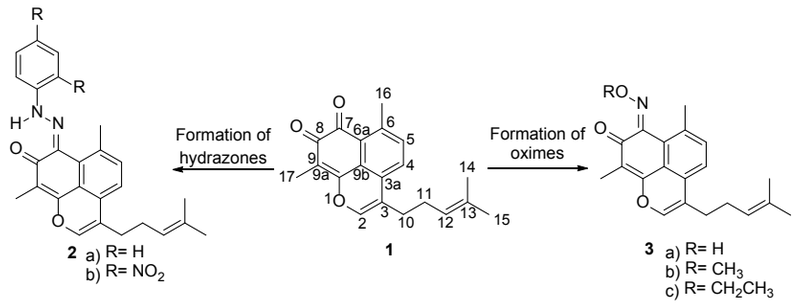
The quinones represent a wide and varied family of secondary metabolites of natural occurrence. The interest in these substances has intensified in recent years due to their pharmacological importance and great structural variety. Several natural and synthetic quinones possess potent and diverse pharmacological effects such as antitumor,[1] anti-inflammatory,[2] analgesic,[3] antifungal,[4] and trypanocidal.[5] Biflorin **1** is a quinone, biologically active, isolated from *Capraria biflora L.*. Five new biflorin-based nitrogen derivatives were synthesized (Scheme 1); two biflorin derived hydrazones **2a**,**b** and three biflorin derived oximes **3a-c**. In the case of the hydrazones **2a,b** only the *Z* isomers were obtained, while the oximes **3b** and **3c** exhibit mixtures of *E* and *Z* isomers (Figure 1).The compounds were characterized by 1D and 2D NMR spectroscopy and mass spectrometry. Their antibacterial activity was also investigated using the microdilution method for determining the MIC against six bacterial strains. Tests have shown that these derivatives have potential against all bacterial strains.



Scheme 1: Synthesis of biflorin-based nitrogen derivatives: modification in carbonyl C-7.

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Synthesis and evaluation of cytotoxic activity of spirooxadiazole oxindoles

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Cancer is one of the modern world's most common non-infectious diseases, figuring among the leading causes of morbidity and mortality worldwide. According to WHO Cancer Report (2014) it caused approximately 8.2 million deaths in 2012.[1] The non-selectivity and acute toxicity of many antitumor agents has prompted the search for new antitumor agents with improved tumor selectivity, efficiency and safety. In this area of research, our group has developed several novel scaffolds with *in vitro* anti-tumor activity (**Figure 1**).[2] Herein, we will present our latest results on the synthesis of a novel chemical family of spirooxindoles containing an oxadiazole five-membered ring. These two scaffolds (spirooxindole and oxadiazole), which are present in compounds that have *in vitro* antitumor activity, were merged in the target molecules.[3,4] The spirooxadiazole oxindoles were synthesized by 1,3-dipolar cycloaddition between derivatives of indoline-2,3-diones and hydrazonyl chlorides. *In vitro* screening of the compounds for cell growth inhibitory activity in human colorectal adenocarcinoma cell lines revealed that spirooxadiazole oxindoles may represent a promising scaffold for the development of new antitumor agents. Ongoing studies are directed to ascertain their molecular mechanism of action and cellular toxicity, in order to confirm their potential as antitumor agents.

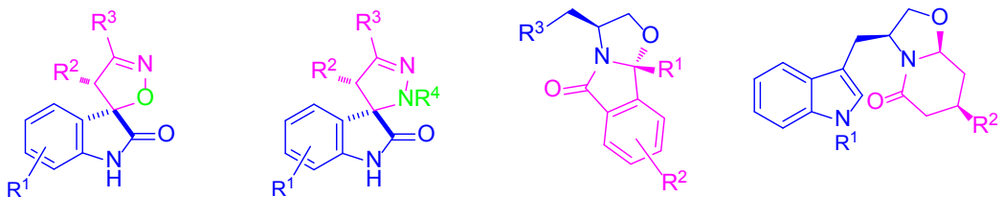

Figure 1: Scaffolds with potential anticancer activity previously developed in our group.

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Enantiopure tryptophanol-derived small molecules: synthesis and cytotoxicity evaluation

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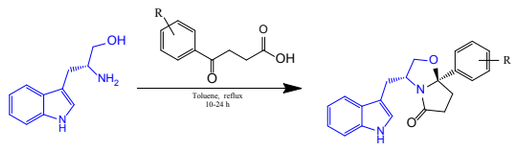
Efficient methodologies for the generation of several stereogenic centres with high diastereo- and enantioselectivity in a single synthetic step is one of the most challenging subjects in organic synthesis. Moreover, the synthesis of enantiopure drugs is a highly important issue in pharmaceutical industry, as different enantiomers are known to have different activities against a target. The chiral pool, is a cheap source to easily obtain enantiopure compounds that can be used as chiral auxiliaries/inductors to control the stereochemical course of a diastereoselective reaction. 1,2-Aminoalcohols containing a stereogenic centre are useful for the syntheses of natural products or biological active molecules that contain the aminoalcohol functionality.[1]

Starting from the enantiopure aminoalcohol tryptophanol, we have recently developed several biologically active small molecules.[2] Herein, we present our most recent results on the synthesis of (*S*) and (*R*)-tryptophanol-derived compounds, that were synthesized by cyclocondensation reaction of enantiopure forms of tryptophanol and several γ-ketoacids. In this reaction, the chiral inductor (tryptophanol) besides being responsible for the stereo-outcome of the final product, it is also part of the main skeleton of biologically active molecules (**Scheme 1**). For those reasons, this asymmetric reaction is highly efficient/atom economic. This specific one-step synthesis approach allows an asymmetric strategic construction of a new chiral center. The library of compounds was screened for cytotoxicity activity and a hit-to-lead optimization is ongoing.



Scheme 1: Synthesis of enantiopure tryptophanol-derived small molecules.

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Synthesis of Biocompatible Phthalocyanines Using Palladium Catalyzed Aminocarbonylation Reactions

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With the increasing number of applications of phthalocyanines and derivatives in the field of biomedicine, particularly in medical imaging techniques[1], research has been focused on the modeling of these macrocycles structures in order to increase their biocompatibility. Hence, the amide linkage (peptide bond) is one of the most natural conjugations available[2], present in many biological synthons, such as peptides, proteins or amino acids. Nevertheless, when compared with other functionalities, amide substituted phthalocyanines are quite rare[3].

In this study we present a new strategy for direct modulation of phthalonitriles through palladium catalysed amino-carbonylation reactions. Particularly, we have synthesised for the first time a phthalonitrile-DO_3A conjugate (DO_3A = amino functionalised 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic carboxylic acid ester), a versatile and easily accessible chelator, with great potential for the development of highly targeted contrast agents for optical imaging, namely magnetic resonance imaging (MRI) or positron emission tomography (PET). Additionally, we have also prepared the corresponding zinc phthalocyanine conjugates, as promising probes to be further exploited as suitable sensitizers in a variety of *in vivo* imaging techniques.

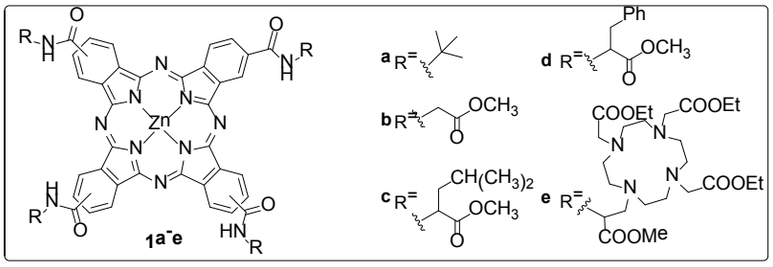

Figure 1: Amide substituted phthalocyanines

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The first biomimetic approach of ethionamide activation and its conversion into active and non-active metabolites

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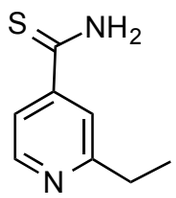
Ethionamide (ETH), a second-line antitubercular drug that is regaining lot of interest sight the increasing cases of drug-resistant tuberculosis, is structurally related to the frontline drug isoniazid (INH) and shares the same enzymatic target, the enoyl-acyl carrier protein reductase InhA. InhA is an essential enzyme of *Mycobacterium tuberculosis* (MTB) involved in the mycolic acids synthesis, which are specific components of the MTB cell wall. Both molecules are pro-drugs that require an enzymatic activation step to behave active and exert their therapeutic effect. Although both activation processes concern oxidative reactions, their mechanisms differ completely, as the involved enzymes. In the case of INH, the activation is ensured by the MTB catalase-peroxidase enzyme KatG, generating the isonicotinoyl radical. This radical then reacts covalently with the NAD cofactor (nicotinamide adenine dinucleotide) to form the active metabolite, the so-called INH-NADH adduct which is the ultimate inhibitor of InhA. As for ETH, the enzyme responsible for its bioactivation in MTB, the monooxygenase EthA, was identified independently by two research teams in 2000. The activation of ETH by EthA is known to proceed in two oxidative steps however, some questions remain open. In this work we present a biomimetic system of EthA and we propose a mechanism for the ETH metabolic activation.



ethionamide (ETH)

A new curcumin derivative with more potent antitumor and anti-P-glycoprotein activity than curcumin

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Cancer multidrug resistance (MDR) is a major limitation to the success of chemotherapy and may be highly associated with the overexpression of drug efflux pumps such as P-glycoprotein (P-gp) [1]. In order to achieve a more effective chemotherapy, it is important to develop P-gp inhibitors to block/decrease its activity [2]. Curcumin (**Figure 1**) is a secondary metabolite of turmeric, derived from *Curcuma longa*, which has antitumor activity and MDR modulatory activity, through the downregulation of *MDR-1* expression, and also decreasing the activity of P-gp [3]. However, curcumin under neutral and basic pH is very unstable and has poor bioavailability and extensive metabolism [3].

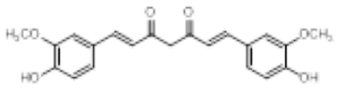

Figure 1. Curcumin structure

This work aimed to synthetize new curcumin derivatives and analogues that were more stable than curcumin itself and presented dual activity: antitumor and P-gp inhibitor. Several synthetic strategies were used to successfully synthesize the new curcumin derivatives and analogues: Claisen-Schmidt condensation reactions, S_N2 reactions and click chemistry. The structure elucidation of the synthesized derivatives was established on the basis of IR, UV-Vis, MS, HRMS, and NMR techniques. Stability and photostability studies were conducted, by comparing curcumin with two synthesized building blocks. Different assays were performed in order to evaluate the compounds stability subjected to several pH buffers, biological medium buffer, temperatures and storage times. Both building blocks have proven to be chemically more stable than curcumin. To analyse the biological effect of the compounds, two pairs of MDR and drug sensitive cell line counterparts were used (chronic myeloid leukemia, CML, and non-small cell lung cancer, NSCLC), in which the MDR phenotype was mainly due to overexpression of P-gp. Cell growth and viability analysis were carried out following cellular treatment with the compounds, using the Sulforhodamine B and the trypan blue exclusion assays and using doxorubicin and curcumin as controls. The drug-efflux activity of P-gp was analysed in the CML model, with the rhodamine 123 efflux assay. Results showed that from all the curcumin analogues and derivatives tested, one of the curcumin derivatives has more potent antitumor and anti-P-gp activity than curcumin itself. Moreover, the GI_{50} concentrations determined for all the compounds in the NSCLC model were similar in both the MDR cells and the drug sensitive counterpart cells. This may indicate that the new compounds are probably not P-gp substrates.
In conclusion, this work shows the potential of synthetizing new curcumin derivatives, to improve curcumin stability and possibly to improve its dual activity.

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Enantioselective synthesis of enantiopure bicyclic lactams and evaluation as NMDA receptor antagonists

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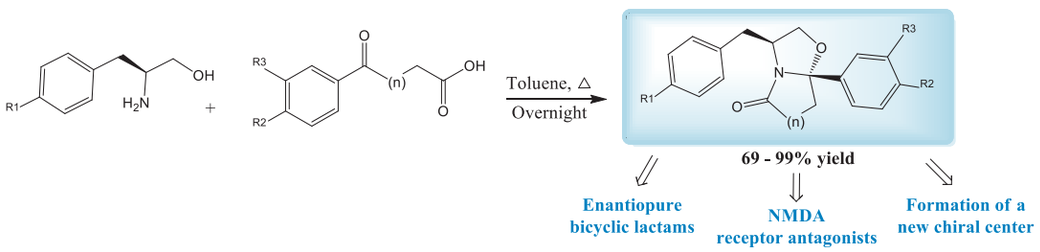
N-Methyl-*D*-Aspartate (NMDA) receptors are fundamental for the normal function of central nervous system and are responsible for memory and learning. These receptors are neuronal ionotropic channels, ligand-gated and voltage dependent, which are activated by their co-agonists, glycine and glutamate. An overactivation of these receptors leads to an increase of Ca^{2+} levels and consequently neuronal loss, which is associated with major degenerative disorders, including Parkinson's and Alzheimer's disease. The design of NMDAR antagonists that can modulate the excitotoxicity phenomena without disturbing the normal NMDAR physiologic functioning in the regulation of synaptic plasticity seems to be a promising therapeutic approach. Unfortunately, the majority of NMDAR antagonists synthesized so far exhibit poor pharmacokinetic profiles, low selectivity towards to other receptors and also side effects.[1]

In the last years, our research group has identified novel small molecules that act as NMDA receptor antagonists.[2] Herein, we will present our most recent results on the hit-to-lead optimization of a NMDA receptor antagonist previously identified by our group. The hit compound revealed to be 1.5 more active than amantadine (used in the clinic).[2a] Twenty six derivatives were synthesized by cyclocondensation reaction of the appropriate enantiopure amino alcohols and several keto-acids (scheme 1), in general with good to excellent yields. These cyclocondensation reactions allow the stereoselective formation of a new chiral center. The compounds were biologically evaluated as inhibitors of Ca^{2+} increases in embryonary rat cortical neurons. Six compounds showed an improvement of 3.6-fold in activity than the hit compound, and the most promising compound revealed to be 5.5 times more active than memantine.



Scheme 1: Enantioselective synthesis of target compounds.

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Therapeutic bioconjugates: Evaluation of iminoboronates as payload delivery system for cancer

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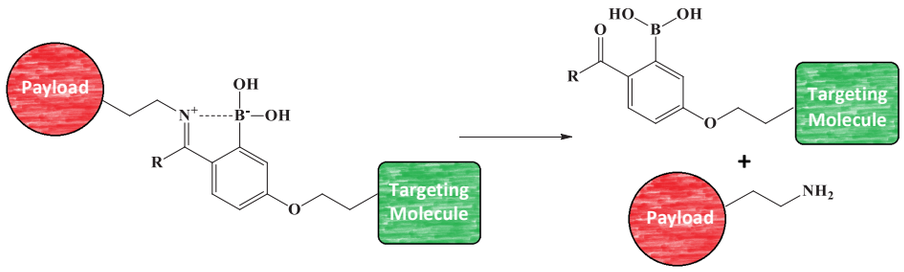
Chemotherapy uses small potent molecules with high activity towards specific tumour targets. However, with such activity comes high off target toxicity and severe side effects. Fortunately, chemotherapy can be now targeted thanks to powerful linkers that connect a ligand molecule with affinity to interesting biological receptors and a cytotoxic drug. This linkers must have very specific properties, such as high stability in plasma, no toxicity, no interference with ligand affinity nor drug potency, and at the same time, be able to self-lyse once inside the target cell. Bipolar environments as seen between tumoural extracellular and intracellular medias are usually exploited by this linkers in order to release the therapeutic warhead. This work explores a new model for the same task, specific cancer drug delivery. [1] Iminoboronates were studied due to its remarkable selective stability towards a wide pH range and endogenous molecules. [2] Bioconjugates were design to prove this iminoboronate linker's effectiveness. The ability to be uptaken by a cancer cell through endocytosis process and delivery of specific payload are two features expected for this construct.



Scheme 1: Iminoboronate as payload delivery system model.

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PORTUGUESE AWARD FOR BEST YOUNG ORGANIC CHEMIST 2015

The ORGANIC flavour in a materials CHEMISTRY journey

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After graduating in Applied Chemistry (Organic Chemistry major), with many hours of lab practices preparing and characterizing molecules, it was clear to me that Organic Chemistry would not be an end in itself but a key to push forward other Chemistry fields. For that reason, I started a mixed Ph.D. in hybrid materials for asymmetric catalysis. My Ph.D. project was focused on the preparation of new chiral catalysts based on salen ligands, which after careful functionalization, were anchored onto different supports. The synthetic strategy for the preparation of the new ligands was tuned to the type of support (carbon materials, mesoporous silicas, polymers). These new catalysts were used in different reactions: enantioselective epoxides ring opening; asymmetric synthesis of cyanohydrins; Suzuki couplings in water; and CO_2 insertion into epoxides.[1] The expertise acquired in the preparation and modification of materials provided me a competitive advantage during my postdoc, in the development of polymeric fluorescent sensors for continuous recording of chemical species or physical parameters. The combination of organic fluorescent molecules with polymeric materials improved the performance of the sensors and increases their range of applicability.[2]
The quest for new challenges lead my journey to move forward, and after the postdoc period I established an independent research program on the development of new platforms for imaging, theranostic and sensing, based in multifunctional fluorescent molecules.[3] In the last years we have worked with different families of molecules that interact with light, such as diphenylanthracenes for oxygen controlled release, fullerene-naphthalimide diads for light-harvesting systems, new chemiluminescent luminol derivatives, and new perylenediimides for vis-NIR imaging or photoactive materials.[4] This communication will show how organic chemistry as shaped my scientific work, and how new molecules can potentiate other Chemistry fields.

Acknowledgements: I'm grateful to: my Ph.D. supervisors, Dr. Bárbara Gigante (INETI, Portugal), Prof. Hermenegildo Garcia (UPV, Spain) and Prof. Avelino Corma (UPV-CSIC, Spain); my PostDoc supervisors Prof. Mário Santos (IST, Portugal) and Prof. Otto S. Wolfbeis (U. Regensburg, Germany); and my present research line director Prof. José Gaspar Martinho (IST, Portugal). During the last 15 years, in different ways, they have helped me to grow as a researcher.

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Organic chemistry: an important tool in drug discovery

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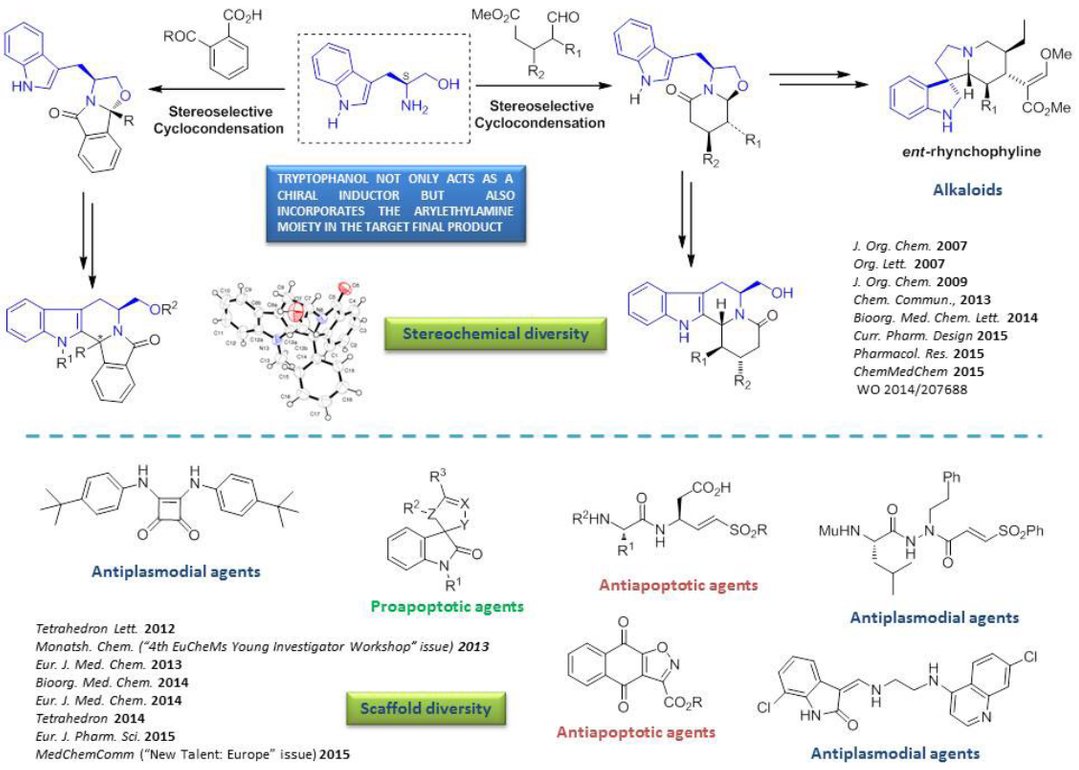
Research in Santos's lab combines organic synthesis and medicinal chemistry with applications for the synthesis of bioactive molecules.Our ultimate goal is the development of general synthetic methods for the synthesis of small molecules to target proteins, as well as protein-protein interactions, relevant to clinical indications.



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Efficient Synthetic Routes for New *N*-Glycosyl Compounds Containing Glucuronic Acid and Glucuronamide-based Moieties

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Uronic acids and their derivatives are components of many biologically active compounds, reported to display antitumor, antibacterial, or antiviral properties.[1] In particular, glucuronic acid is a common building block of glycosaminoglycans, polysaccharides that contribute to both intracellular and extracellular functions such as regulation of cell growth, metabolism, cell adhesion and control of assembly and function of extracellular matrices.[2] Moreover, many drugs have been conjugated to glucuronic acid in order to improve absorption, bioavailability or to reduce toxicity, leading sometimes to compounds with higher biological efficacies.[3] Such conjugation is normally achieved by glucuronidation, a process that is also involved in drug metabolism.[4]

The chemical synthesis of glucuronides is particularly difficult since the presence of the electron-withdrawing carboxylic group at C-5 decreases the reactivity of the anomeric center. Hence, methodologies that allow the efficient access of glycosides of glucuronic acid as well as of related analogs are required. On the other hand, the carboxylic acid function can have an effect on the stereochemical outcome of a glycosylation reaction as well as it allows the selective introduction of additional moieties at C-6 via typical reactions that this functionality, or a derived lactone system, may undergo, enabling structural variations. These aspects motivate the exploitation of glucuronic acid as a synthons for the stereoselective access to a variety of glycosyl derivatives.

In this communication, the stereoselective synthesis of glucuronic acid and glucuronamide-derived *N*-glycosyl compounds, such as *N*-glycosylsulfonohydrazides and nucleosides, as novel potentially bioactive molecules, will be presented. Glucuronic acid or reducing glucuronamides, arising from opening of glucurono-6,3-lactone with amines, were the glycosyl donors used for N-glycosylation of a sulfonohydrazide. Azide-alkyne “click” cycloadditions were used for the access to triazole-containing *N*-glycosyl derivatives. Glucuronic acid-derived nucleosides were synthesized from glucurono-6,3- or 6,1-lactones. In particular, a (triazolyl)methyl amide-linked disaccharide nucleoside, whose structure is analogous to that of nucleoside diphosphate sugars, was accessed.

The synthetic strategies and methodologies will be disclosed.

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