11th iMed.ULisboa Postgraduate | 4th i3DU

Students Meeting

Book of abstracts

July 15th 2019

INDEX

WELCOME MESSAGE

Dear participants,

On behalf of the Postgraduate Students Commission (ipSC) from the Research Institute for Medicines (iMed.ULisboa), we are pleased to welcome you to the 11th iMed.ULisboa Postgraduate Students Meeting and 4th i3DU Meeting.

The main goal of this Meeting is to complement the training of iMed.ULisboa postgraduate students', stimulating the cross-fertilization between the areas of the Institute and the network between students and fellow researchers.

This year, the programme will combine two keynote lectures, focusing on the interdisciplinarity and the cross-link between different scientific areas. Remaining lectures will be given by PhD Students from the different groups, presenting their work. All other students will also expose their work in the poster sessions organised through the day, allowing an environment of discussion and scientific sharing.

The programme will also include a round table - "The importance of interdisciplinarity: working in between scientific fields" - in which known top researchers from our institution will share their experiences.

This session will certainly provide some insight on the growing importance of multidisciplinary researchers and collaboration between the several groups from iMed.ULisboa. All of us are lucky to study and work in an interdisciplinary institute and, in a scientific world of increasing competition, it's time to take advantage on what can differentiate our work and science from the others.

We look forward to welcoming you at this event!

Scientific Committee

Cecília Rodrigues - Cellular Function and Therapeutic Targeting
Elsa Anes - Host-Pathogen Interactions
Ana Paula Leandro - Metabolism and Genetics
João Gonçalves - Molecular Microbiology and Biotechnology
Dora Brites - Neuron-Glia Biology in Health and Disease
Pedro Góis - Bioorganic Chemistry
Rui Moreira - Medicinal Chemistry
Maria José Umbelino - Natural Products Chemistry
Maria Henriques - Chemical Biology and Toxicology
Helena Florindo - BioNanoSciences - Drug Delivery and Immunotherapy
António Almeida - Nanostructured Systems for Overcoming Biological Barriers
Maria Beatriz Lima - Pharmacological and Regulatory Sciences
Nuno Taveira - HIV Evolution, Epidemiology and Prevention
Fernando Fernandez-Llimós - Pharmacoepidemiology and Social Pharmacy

Organizing Committee

Sara Oliveira - Cellular Function and Therapeutic Targeting
Gonçalo Garcia - Neuron Glia Biology in Health and Disease
João Ravasco - Bioorganic Chemistry
Ricardo Lopes - Bioorganic Chemistry
André Campaniço - Medicinal Chemistry
Cláudia Braga - Medicinal Chemistry
Shirley Sancha - Natural Products Chemistry
Ana Bárbara Carreira - BioNanoSciences - Drug Delivery and Immunotherapy
Nuno Costa - BioNanoSciences - Drug Delivery and Immunotherapy
Ana Henriques Mota - Nanostructured Systems for Overcoming Biological Barriers
Jacinta Pinho - Nanostructured Systems for Overcoming Biological Barriers
Inês Moranguinho - HIV Evolution, Epidemiology and Prevention

SPONSORS AND ACKNOWLEDGEMENTS

ipSC would like to thank its sponsors for their generous contributions and support to the 11th iMed.ULisboa Postgraduate Students Meeting and 4th i3DU Students Meeting.

GENERAL INFORMATION

Venue

Auditorium Maria Odette Santos-Ferreira of the Faculty of Pharmacy, Universidade de Lisboa
Av. Prof. Gama Pinto
1649-003 Lisbon
Portugal
Tel: +351 21 794 6490
Fax: +351 21 794 6491

Language

The official language of the Meeting is English.

Registration Desk

The registration desk will be at the main entrance of Faculty of Pharmacy.

Certificates of attendance

Certificates of attendance will be send by e-mail to all registered participants after the meeting.

Liability and Insurance

The organizer is not able to take any responsibility whatsoever for injury or damage involving persons and property during the Meeting.

SOCIAL PROGRAM

After the Round Table Session, the iMed.ULisboa Postgraduate Students Commission is pleased to invite all participants for a toast in the Professors' Living Room. The Sunset Party will take place at 6pm, promoting a social environment of networking between students, post-doctoral researchers, principal investigators, professors and speakers.

SCIENTIFIC INFORMATION

Oral Communications

A selected student from each group will present his work through an oral presentation. Each presentation will take 10 minutes with 5 extra minutes for questions. All presentations will be evaluated by the Evaluation Committee, composed by four top Principal Investigators, one for each area of iMed.ULisboa. The best presentation award, kindly sponsored by iMed.ULisboa, comprises a conference registration grant (up to 250€), which will be delivered at the Closing Session.

Poster Communications

All remaining students from iMed.ULisboa will have the opportunity to present their work in the two Poster Sessions scheduled through the day. The posters were placed in advance at the Professors' Hall and evaluated by the Evaluation Committee, composed by four top Postdoctoral Researchers, one for each area of iMed.ULisboa. Selected posters will be presented and discussed with the committee in the Poster Sessions. The best poster award will be delivered at the Closing Session.

All students are requested to stand next to their poster during viewing sessions for informal discussions regarding their work. The posters are numbered according to the Book of Abstracts. All posters will be displayed until the end of the Meeting. The Organizing Committee is not responsible for posters that have not been collected.

MEETING SCHEDULE

July 15th, 2019

08h30 **Registration**

09h00 **Opening Ceremony**

ipSC | Prof. Cecília Rodrigues

Session 1

Chairs: Dr Elsa Rodrigues and Dr Carlos Afonso, iMed.ULisboa

09h15 **Plenary Lecture**

Dr Pedro M. Baptista, Aragon Health Sciences Institute, Zaragoza, Spain

'*Whole-Organ Bioengineering: The Future of Transplantation Medicine*'

10h00 **Host-Pathogen Interactions**

Marta Calado

'*How HIV enters CNS - Microglia and astrocytes are infected in trans from CD4+ T-lymphocytes*'

10h15 **Bioorganic Chemistry**

João Ravasco

'*Creating diversity from biomass a tandem bio-/metal-catalysis for the chemoselective synthesis of heterosubstituted furans*'

10h30 **Coffee Break and Poster Sessions**

Session 2

Chairs: Dr Francisca Lopes and Dr Afonso Cavaco, iMed.ULisboa

11h15 **Chemical Biology and Toxicology**

Isabella Bramatti

'*Repurposing Thimerosal To Target Glioblastoma Cells: Comparison To Temozolomide*'

11h30 **HIV Evolution, Epidemiology and Prevention**

Francisco Martin

'*Broad And Potent Neutralizing Antibody Responses In HIV-1 Infected Angolan Patients: Implications For Vaccine Design And Efficacy*'

11h45 **Cellular Function and Therapeutic Targeting**

Maria Ribeiro

'*Diet-Associated Gut Microbiota Metabolites Dictate Mitochondrial Stress As A Key Regulator Of Adult Neurogenesis*'

12h00 **Medicinal Chemistry**

Jorge Grilo

'*New opportunities for an Old Disease: Click-Chemistry Tools to Unravel New Targets in Malaria*'

12h15 **BioNanoSciences - Drug Delivery and Immunotherapy**

Diana Fernandes

'*How To Successfully Spray Dry Biopharmaceuticals Targeting The Lungs: A Novel Integrated Methodology*'

12h30 **Pharmacoepidemiology and Social Pharmacy**

João Rafael Gonçalves

'*How are Pharmacists performing in Long-Term Integrated Care? A systematic review*'

12h45 **LUNCH**

Session 3

Chairs: Dr Maria João Catalão and Dr Liana Silva, iMed.ULisboa

14h00 **Metabolism and Genetics**

Inês Vieira da Silva

'*Aquaporin-3 is involved in interleukin-6 and interleukin-1ß release in macrophages*'

14h15 **Natural Products Chemistry**

David Cardoso

'*Generation of a library of indole alkaloid derivatives as ABC transporter modulators*'

14h30 **Nanostructured Systems for Overcoming Biological Barriers**

Beatriz Silva

'*Nanostructured Systems for Overcoming Biological Barriers: A Novel Approach to Ocular Delivery of Erythropoietin*'

14h45 **Molecular Microbiology and Biotechnology**

Pedro Gomes

'*Mining High-Throughput Sequence Data Towards a Global Perspective of the Mycobacterium Tuberculosis Resistome Diversity*'

15h00 **Plenary Lecture**

Dr Maria João Matos, University of Santiago de Compostela, Galicia, Spain & FCUP

'*Emerging drug discovery approaches: From small molecules to targeted therapies*'

15h45 **Coffee Break and Poster Session**

16h15 **Pharmacological and Regulatory Sciences**

Inês Janeiro da Silva

'*TNBS-Induced Colitis In Rodents: Preliminary Results of a Chronic Model*'

16h30 **Neuron-Glia Biology in Health and Disease**

Marta Barbosa

'*Benefits of VS in Counteract ALS-astrocyte Aberrancy Involves miR-146a Upregulation*'

Session 4

Chair: Dr Cecilia Rodrigues

16h45 **Round Table**

'*The importance of interdisciplinarity: working in between scientific fields.*'

Dr Helena Florindo | Dr Nuno Taveira | Dr Pedro Góis | Dr Rui Castro

17h30 **Closing Session and Awards**

18h00 **Sunset Party**

PLENARY LECTURES

Whole-Organ Bioengineering: The Future of Transplantation Medicine

Dr. Pedro Baptista

Biography

Dr Pedro M. Baptista (male): PharmD, PhD, is a senior researcher at Aragonese Foundation for Research & Development (ARAID) and a group leader at Health Research Institute of Aragon (IISA) and an expert in research related to biomaterials, whole-organ and tissue bioengineering and regenerative medicine. He is a Visiting Assistant Professor at the Biomedical and Aerospace Engineering Department of Carlos III University in Madrid, Spain and a member of the Internal Scientific Advisory Board of IISA. He is the founder and head of the Organ Bioengineering and Regenerative Medicine Laboratory at IISA. He is currently an elected member of the Governors Board of the European Society for Artificial Organs (ESAO) and Deputy Chairman of the European Society for the Study of the Liver (EASL) Consortium for Regenerative Hepatology. He is also a member of the Scientific Advisory Board of the company Cytes Biotechnology SL, and of the French National Consortium iLITE - Innovation for Liver Tissue Engineering. He is the co-author of 32 peer-reviewed publications, cited >1600 times [H index: 17 (Google scholar)], 1 patent licensed to Biorg, Inc. (USA), 2 books in Regenerative Medicine and more than 14 book chapters, has more than 50 participations in international conferences (16 as an invited speaker). Co-investigator in 12 research projects and principal investigator in 6 of them. Member of the editorial board of the journal Organogenesis and Frontiers in Medicine. “Pestle and Mortar Prize” winner (Pharmacist of the Year in Portugal) in 2011. His research resulted in the generation of the first human liver ever made in a laboratory, impacting the scientific community at large. His current research focuses on investigating liver stem cell biology and the development of novel methods to expand multiple foetal and adult human stem/progenitor cells to the required large numbers necessary for organ bioengineering, and in making the long-term transplantation of these lab-grown organs a reality. He is also developing novel advanced bioreactors and decellularization machines with automatic and non-destructive control of decellularization. Dr. Baptista is also interested in applying bioengineered hepatic tissues and organs to study developmental biology, physiology and in drug discovery.

Abstract

The Organ transplantation is presently the only proven therapy able to extend survival for end-stage organ disease. It is also the only treatment available for severe acute organ failures and to some forms of inborn errors of metabolism. Nevertheless, the waiting list for organ transplantation is long and many patients will not survive long enough to receive an organ due to the dramatic shortage of donors or lack of eligibility. This distressing donor shortage is common to most solid organs like the liver, lung, heart and particularly kidneys.

In light of the grim situation of organ transplantation, our laboratory has developed novel methods to generate an entire liver scaffold from whole animal livers, using tissue decellularization that preserves the organ's vascular network. This same method is also able to decellularize other solid organs generating specific acellular kidney, lung, intestine, pancreas or heart scaffolds. Our subsequent studies showed the possibility to efficiently recellularize the liver bioscaffolds by perfusing them with human liver progenitor and endothelial cells in a perfusion bioreactor. The outcome of this was a bioengineered human liver.

Although, most of the current generation of bioengineered organs lack *in vivo*-like functional tissue and physiological vascular networks, making their transplantation still a mirage. These challenges and potential solutions will be described in detail drawing a path to transplantation, to truly shape organ bioengineering into the Future of Transplantation Medicine.

Emerging drug discovery approaches: From small molecules to targeted therapies

Dra. Maria João Matos

Biography

Maria João Matos received her degree in Pharmaceutical Sciences in 2006 from the University of Porto, her MSc in Organic Chemistry in 2008 from the University of Santiago de Compostela, her first PhD in Pharmaceutical Science and Technology in 2010 from the University of Cagliari, and her second PhD in Medicinal Chemistry in 2013 from the University of Santiago de Compostela. She has worked in Chemical Biology at the University of Cambridge for three years as a Postdoctoral Fellow. Currently, Maria is Researcher at the University of Porto and University of Santiago de Compostela, where she leads the group of "I+D de Fármacos". Her research interests include new tools for drug design, discovery and delivery, in particular in the field of neurodegenerative diseases.

Abstract

The 21th century witnessed a significant demographic change in the human population of the industrialized world that is currently followed by a similar shift of life expectancy to upper age ranges in developing countries. Some types of cancer and neurodegenerative diseases are becoming increasingly prevalent with the lifestyle and aging of the general population. Understanding some of the molecular changes associated to these ubiquitous and widespread diseases has stimulated efforts to develop new approaches to achieve different targets in different tissues [1,2]. The effectiveness of a drug depends on accumulation at the site of action at therapeutic levels. However, challenges such as rapid renal clearance, degradation or non-specific accumulation require drug delivery enabling technologies. Targeted drug delivery is a very promising concept, which still needs improvement for better clinical outcomes [1,2]. Different approaches and illustrative case studies of their successful implementation in the search for the treatment of cancer and Parkinson's disease, will be presented and discussed [1,2]. Computer-assisted design of potent small molecules, together with the development of new carriers and delivery strategies, allow a wide range of possibilities for targeted therapies. Knowledge on biochemical processes brings the opportunity to provide treatments that are potentially less toxic and more effective than traditional therapeutic approaches.

Acknowledgements

This work was supported by the University of Porto, University of Santiago de Compostela (2018-PU067) and Xunta da Galicia Plan of Research, Innovation and Growth 2011–2015 (Plan I2C, ED481B 2014/086–0 and ED481B 2018/007).

References

[1] Nature Commun. 2016, 7, 13128; J. Am. Chem. Soc. 2017, 139(50), 18365; J. Am. Chem. Soc. 2018, 140(11), 4004; Chem. Sci. 2018, 9, 4185; Chem. Eur. J. 2018, 24, 12250; Nat. Protoc. 2019, 4, 86; Angew. Chem. Int. Ed. Engl. 2019, 131, 6712;

[2] J. Med. Chem. 2017, 60(16), 7206; Fut. Med. Chem. 2018, 10(9), 983; ACS Appl. Mater. Interfaces 2018, 10(46), 39557.

ROUND TABLE

‘The importance of interdisciplinarity: working in between scientific fields’

Interdisciplinarity can be seen as the ability to work in between fields. Research work on the edge of two or more areas with researchers not specialized in each, but qualified in both. A new and challenging way of scientific practice that increases the value, complexity and impact of the science. These are the pillars for the Round Table the ipSC is proposing. What are the real and practical advantages of interdisciplinarity? Is it something to promote in Masters’ and PhD students? iMed.ULisboa is a multidisciplinary institute with diverse scientific fields that can be explored in the making of a research project, but are we really taking advantage of it? Are there enough collaborations? Enough students with supervisors from different fields? And specially, speaking of students, is this something necessary for our future, something to look forward in our education? When you become an interdisciplinary researcher, you find new ways of looking at the same old problems, you find new crowds and new research outcomes and possibilities. But are we doing it enough?

Rui Castro

Rui Castro completed his PhD degree in Pharmacy (Biochemistry) by the Faculty of Pharmacy, Univ. of Lisbon (FF/UL) in 2006, having spent a year at the Dept. of Medicine, Univ. of Minnesota Medical School, USA. He was later appointed with an Assistant Investigator position at the Research Institute for Medicines (iMed.ULisboa) and recently became Assistant Professor at FF/UL. Castro’s most recent publications have contributed for understanding the role of miRNAs during non-alcoholic fatty liver disease (NAFLD) pathogenesis, as well as its significance as biomarkers and molecular targets in liver disease. He has been using well-established NASH in vitro and in vivo models, human samples and miRNA knockout strategies, combining multi-layered pharmacological approaches, aiming to ultimately bring miRNA therapies to the liver clinical setting.

Helena Florindo

Helena Florindo completed her PhD in Pharmaceutical Technology in 2008, by the Faculty of Pharmacy, University of Lisbon. Since then, she has been a researcher at iMed.ULisboa and an Assistant Professor in FFULisboa. In 2015, Florindo was appointed as Group Leader for the BioNanoSciences – Drug Delivery & Immunotherapy Research Group. Her research is focused on the development of multivalent nanotechnology-based systems to modulate the recognition, capture, processing and presentation of cancer antigens by dendritic cells. It includes the *in vitro* and *in vivo* studies of the anti-tumor effects induced by the combination of these nanovaccines with nanotherapeutics designed to modulate the function and activation of other key cells within tumor site, such as T cells, myeloid-derived cells and tumor cells.

Pedro Góis

Pedro M. P. Gois studied chemistry at the NOVA University of Lisbon from where he also received in 2005 his PhD in organic chemistry under the supervision of Prof. Carlos Afonso. From May 2005 to May 2008 he worked as a postdoctoral research fellow at the University of Sussex with Prof. F. Geoffrey N. Cloke FRS, at the University College of London with Prof. Stephen Caddick and at the Instituto Superior Técnico (Technical University of Lisbon) with Prof. Carlos Afonso. In May 2008, he joined the Pharmacy Faculty of the Lisbon University as an assistant research fellow of the medicinal chemistry group (iMed.UL – Research Institute for Medicines) and in July 2013, Gois was appointed Principal Investigator at the same institution and head of the Bioorganic group. In 2017 he received his habilitation in Pharmacy and was appointed assistant professor of the Pharmacy Faculty.

Nuno Taveira

Nuno Taveira is a full professor of microbiology and molecular biology at the Instituto Universitário Egas Moniz, a private university located in Monte de Caparica, Almada, Portugal. He is also a research associate and group leader at iMed.UL, Faculty of Pharmacy of Lisbon's University, leading a group of more or less 8 researchers (2 post-docs and 4 PhD students). Taveira's research is mainly on HIV, AIDS and, more recently, yellow fever and tuberculosis in Angola. His research group is interested in designing and producing new antivirals, new HIV vaccines and microbicides and new molecular diagnostic

assays that can be used in developing countries. He also has a long standing interest in investigating the molecular epidemiology of HIV, yellow fever and TB in Africa. Taveira dedicates a great deal of our time and effort to study HIV-2 infection, which is relatively frequent in Portugal. Many of his studies are done in Africa (Angola, Cape Verde and Mozambique) and the data produced is used by the local governments, to develop better public health interventions, and by the local clinicians, to better manage and treat their patients. Since 2016, he is a collaborator of the Global Burden of Disease Injuries and Risk Factor Study (GBD), led by the Institute of Health Metrics and Evaluation, University of Washington, USA.

ORAL COMMUNICATIONS

How HIV Enters CNS – Microglia And Astrocytes Are Infected In *trans* From $CD4^+$ T-lymphocytes

Calado M. (1), Vaz A.R. (2), Matos A.M. (3), Anes E. (1), Brites D. (2), Azevedo-Pereira J.M. (1)

1- Host-Pathogen Interaction Unit, Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; 2- Neuron-Glia Biology in Health and Disease, Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; 3- Chemical Engineering Processes and Forest Products Research Center, Faculty of Pharmacy, University of Coimbra, Portugal.

Background: During chronic HIV infection, 20-50% of the patients exhibit neurologic dysfunctions known as HIV-associated neurocognitive disorders (HAND). Entrance of HIV into central nervous system (CNS) occurs soon after HIV systemic infection, and is mainly mediated via infected $CD4^+$ T-lymphocytes and monocytes from the blood stream. In the CNS compartment, first cells to be infected are perivascular macrophages and microglia that together with astrocytes act as HIV reservoirs and may disseminate HIV to neighboring cells.

Our research goal is to define the mechanisms by which HIV establishes and maintains a local infection in the CNS. In particular, the results described here address the effectiveness of HIV transmission from $CD4^+$ T-lymphocytes (TCD4) to microglia and astrocytes. With this aim, we studied *trans*-infection of microglia and astrocytes using well characterized HIV strains.

Materials/methods: Donor (TCD4) and target cells (microglia and astrocytes) were maintained in two distinct conditions: (i) co-culture during seven days, and (ii) separated 42h after infection. The infected lymphocytes were co-cultured in mixed culture (cell-cell contact) and transwell co-cultures. Virus production was monitored by reverse transcriptase (RT) activity in culture supernatants, using an immunoenzymatic assay (Cavidy).

Results: When donor and target cells were maintained in condition (i) our results show a higher RT activity in mixed culture compared to transwell co-culture, both in microglia and astrocytes infected with HIV-2_{ROD} and HIV-$1_{92HT599}$. Considering HIV-2_{ROD} infection, when donor and target cells were maintained in condition (ii) we verified that viral replication was more efficient that in condition (i). It was also interesting to note that viral replication in TCD4, microglia and astrocytes directly infected was less efficient than in those resulting from mixed culture. Similar to what happens in HIV-2_{ROD} infection, the HIV-$1_{92HT599}$ replicates more efficiently in TCD4, microglia and astrocytes separated from mixed culture. Interestingly, when microglia and astrocytes were directly infected (*cis*-infection) no viral replication could be detected.

Conclusions: Although microglia displays low levels of CD4 expression and astrocytes are CD4-negative (the main HIV receptor), both cells were infected *in vitro*. We hypothesize that the infection of microglia, and particularly astrocytes, is mainly driven by extracellular vesicles, which are known to contain viral particles, genetic material and viral proteins. The potential role of these EVs in viral dissemination and chronic inflammation of CNS may help explain the pathogenic mechanisms underlying HAND.

Acknowledgements

Financial support for this research was provided by Programa Gilead Genese, Edição 2016 and Fundação para a Ciência e Tecnologia (Grant: PTDC/SAU-INF/28182/2017).

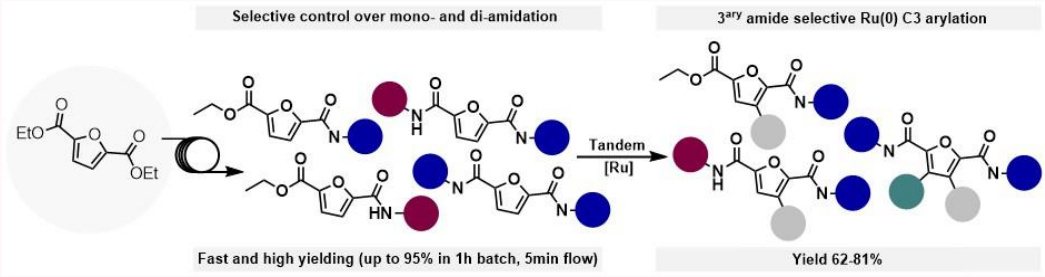
Marta Calado is supported by a PhD fellowship from Fundação para a Ciência e Tecnologia (Grant: SFRH/BD/131948/2017).

Creating Diversity From Biomass: A Tandem Bio-/Metal-Catalysis For The Chemoselective Synthesis Of Heterosubstituted Furans

Ravasco J.M.J.M. (1), Monteiro C.M. (1), Siopa F. (1), Oble J. (2), Poli G. (2), Trindade A.F. (1,3), Simeonov S. (1,4*), Afonso C.A.M. (1*)

1- Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy,Universidade de Lisboa, Lisbon, Portugal; 2- Sorbonne Université, Faculté des Sciences et Ingénierie, CNRS,Institut Parisien de Chimie Moléculaire, IPCM,4 place Jussieu, 75005 Paris, France; 3- School of Chemistry, University of Leeds, Leeds LS2 9JT, UK; 4- Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Acad. G. Bonchev str., bl. 9, 1113 Sofia, Bulgaria.

Biogenic furans obtained through biorefinery are considered one of the most promising core structures for chemical valorization of renewable resources. Given its rich chemistry and biomass availability, HMF-derivates have been employed to access an extensive variety of chemical scaffolds. Following the lead of furan-containing FDA-approved molecules in the market, this core undoubtedly poses as a promising platform for the synthesis of small molecules for drug discovery in a diversity-oriented manner. Yet, the synthetic access to densely decorated furans (hetero tri- and tetra- substituted), among others, still offer significant limitations as they often rely on synthetically demanding substrates, and/or possess limited tolerability to diversification. Such endeavor automatically calls for need towards the development of novel and environmentally benign processes which allow direct diverse chemoselective functionalization of HMF derived furanic platform. Driven by the obvious advantages of bio- and modern transition metal catalysis (TMC), the combination of both represents a privileged tool for substructure diversity-oriented synthetic strategies towards the construction of both nature-inspired and pharmacologically active compounds [5]. The usage Ru0 directing groups in furfural and HMF derivates have been reported by our group , amongst others, to, for instance, direct C3 arylation and addition of vinylsilanes to furfural imines, as well as C3 vinylation of N-methylfuran-2-carboxamide using vinylsilanes [3]. Herein we report a tandem and two-step diversity oriented synthesis of furan based scaffold, based on a fast and high yielding biocatalysed amidation (5 minutes 95% yield) of FDCA and chemo and regioselective C3-arylation via a tertiary amide hotspot [4].



Scheme 1. Synthesis of diverse hetero-substituted furans from a FDCA ester

Acknowledgements

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References

[1] Rosatella, A. A.; Simeonov, S. P.; Frade, R. F. M.; Afonso, C. A. M., Green Chem. 2011, 13, 754–793; [2] Rudroff, F.; Mihovilovic, M. D.; Gröger, H.; Snajdrova, R.; Iding, H.; Bornscheuer, U. T., Nat. Catal. 2018; [3] a) Pezzetta, C.; Veiros, L. F.; Oble, J.; Poli, G., Chem. - A Eur. J. 2017; b) Siopa, F.; Ramis Cladera, V. A.; Afonso, C. A. M.; Oble, J.; Poli, G., European J. Org. Chem. 2018; [4] Ravasco, J. M. J. M., et. al., Manuscript in preparation.

Repurposing Thimerosal To Target Glioblastoma Cells: Comparison To Temozolomide

Bramatti I.C. (1), Branco V. (1), Holmgren A. (2), Carvalho C. (1)

1- Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; 2- Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, Sweden.

Glioblastoma multiforme is the most aggressive and common form of malignant gliomas presenting a relatively high morbidity and mortality and often demonstrating resistance to chemotherapy. Additionally, the existence of the blood-brain barrier constrains drug-delivery [1]. Cancer cells rely heavily on antioxidant systems to keep redox homeostasis, thus glioma cells may present high levels of redox proteins, such as thioredoxin (Trx), thioredoxin reductase (TrxR) and glutathione peroxidase (GPx). The thioredoxin and glutaredoxin/glutathione systems regulate oxidative stress being determinant for cell survival and proliferation, and an inhibition of these systems leads to cell death by apoptosis [2,3]. There are a large number of inhibitors of these systems, including heavy metals, natural compounds and synthetic drugs. Mercury compounds in particular present a high affinity for binding thiols and selenols being the thioredoxin system a target for these compounds [4]. Therefore, the aim of this study is to test the efficacy of thimerosal (TM), in human glioblastoma cells in comparison to temozolomide (TMZ), frequently used in chemotherapy.Human glioma U87 cell line was used for the experiments. Following exposure of cells to TM and TMZ, cell viability (MTT assay), TrxR and Trx activity (insulin endpoint assay), expression of these enzymes and oxidation of peroxiredoxin 2 (Western Blot) were evaluated. Furthermore, the effect of treatment on vascular endothelial growth factor (VEGF) expression, which is related with tumor proliferation, was assessed. After 24 hours of exposure to different concentrations of TM the GI50 was approximately 13 µM. Cells demonstrated a high tolerance to the tested concentrations of TMZ (GI50 > 800 µM). Upon exposure to thimerosal TrxR and Trx activities decreased more than 50% but were not affected by TMZ alone. Despite the decrease in TrxR and Trx activity there were no alterations in their expression as verified by Western blot. TM and TMZ decreased the expression of VEGF, with co-exposure presenting a synergic effect leading to a further decrease in expression. Overall, results indicate that thimerosal alone or combined with temozolomide constitutes a promising therapeutic tool to tackle antioxidant defenses and overcame tumors therapy resistance, although new concentrations of temozolomide should be tested.

Acknowledgements

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References

[1] Koncarevic, S.; Urig, S.; Rahlfs, S.; Herold-Mende, C.; Sueltmann, H.; Becker, K., Free Radic. Biol. Med. 2009; 46: 1096–1108; [2] Branco, V.; Godinho-Santos, A.; Gonçalves, J.; Holmgren, A.; Carvalho, C., Free Radic. Biol. Med. 2014; 73: 95–105; [3] Branco, V.; Coppo, L.; Solá, S.; Lu, J.; Rodrigues, C.M.P.; Holmgren, A.; Carvalho, C., Redox Biol. 2017; 13:278–287; [4] Carvalho, C.M.L.; Chew, E.H.; Hashemy, S.I.; Lu, J.; Holmgren, A, J. Biol. Chem. 2008; 283: 11913–11923.

Broad And Potent Neutralizing Antibody Responses In HIV-1 Infected Angolan Patients: Implications For Vaccine Design And Efficacy

Martin F. (1), Palladino C. (1), Mateus R. (1), Diniz A.R. (1), Calado R. (1), Figueiredo I. (1), Bártolo I. (1), Borrego P. (1,2), Clemente S. (3), Taveira N. (1,4)

1- Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; 2- Centro de Administração e Políticas Públicas, Instituto Superior de Ciências Sociais e Políticas, Universidade de Lisboa, Lisbon, Portugal; 3- Hospital da Divina Providência, Luanda, Angola; 4- Centro de Investigação Interdisciplinar Egas Moniz CIIEM, Instituto Superior de Ciências da Saúde Egas Moniz, Caparica, Portugal.

Characterizing the neutralizing antibody response and virus evolution in Central and West African countries with old HIV epidemics driven by highly divergent viruses may provide useful insights for vaccine design. Herein, we make the first detailed characterization of the neutralizing antibody responses and identify its determinants in Angolan patients infected with HIV-1. A total of 204 plasma samples from Angolan patients were analysed against a reference cross-clade Env-pseudotyped tier 2 indicator virus panel (n=12). Binding antibody activity to polypeptides comprising the C2, V3 and C3 envelope regions from different HIV-1 clades was evaluated in ELISA assays. The envelope C2V3C3 region was sequenced in order to assess subtype, co-receptor use and virus evolution. Remarkably, ≈20% of the patients developed antibody responses with the capacity to potently neutralize at least half the viruses from the panel. Neutralizing antibody responses were positively associated with subtype C infection and patients' age, and negatively associated with CD4 counts. Patients infected with isolates more closely related to the viruses in the indicator panel had stronger neutralizing responses against the panel of reference viruses that was used. However, few patients developed broad neutralizing antibody (bnAb) responses despite the high genetic distance to the pseudoviruses in the indicator panel. There was a strong and positive correlation between neutralizing responses and titer of antibodies binding to C2V3C3-polypeptides of all clades. Viruses from patients with bnAb responses had far less variability in C2V3C3, as measured by entropy analysis and number of positively selected sites, relative to patients without bnAb responses. Important V3 bnAb recognition sites and sites associated with resistance to neutralization in the C2V3C3 region were under positive selection in patients with elite neutralizing capacity. In conclusion, binding antibody titer against the envelope C2V3C3 region was a good indicator of neutralization responses in HIV-1 infected individuals from Angola and it may also be a good indicator of vaccine efficacy. Taking into consideration the neutralizing responses of certain HIV-1 infected Angolan patients the design of a pan-HIV vaccine might not be an utopic concept.

Acknowledgements

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Diet-Associated Gut Microbiota Metabolites Dictate Mitochondrial Stress As A Key Regulator Of Adult Neurogenesis

Ribeiro M.F., Santos A.A., Castro R.E., Rodrigues C.M.P., Solá S.

Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal.

The influence of dietary factors on neuronal function has revealed important mechanisms that mediate the action of diet on brain health and mental function. Gut microbiota may affect central nervous system function and behavior through the microbiota-gut-brain axis. However, the molecular mechanisms responsible for the impact of diet and the associated microbiome in the adult neuroregeneration process is still largely unclear. Curiously, post-natal neurogenesis plays a role in neurological and psychiatric disorders. In the present study, we aimed to investigate whether and how changes in diet-associated microbiome and its metabolites impact on adult neurogenesis. Mice were fed a high-fat, choline deficient (HFCD) diet that usually associates with non-alcoholic steatohepaptitis (NASH) phenotype. Our results revealed that HFCD diet triggers premature neurogenesis, exhausting the NSC pool and neurogenesis in the long-term. HFCD diet resulted in neuroinflammation, oxidative stress, synaptic loss and cell death in different regions of the brain. Notably, dietary changes stimulated gut dysbiosis in small intestine and cecum, while upregulating metabolic pathways of short chain fatty acids (SCFAs), such as propionate and butyrate. By dissecting the effect of these specific SCFAs *in vitro*, we showed that propionate and butyrate enhanced mitochondrial biogenesis and promoted early neurogenic differentiation of NSCs through a ROS-pERK1/2-dependent mechanism. More importantly, neurogenic niches of HFCD-fed mice showed increased expression of mitochondrial biogenesis markers, decreased mitochondrial ROS scavengers and phosphorylation of ERK1/2 protein, corroborating the involvement of this mitochondrial stress-dependent pathway in adult neurogenesis alterations mediated by diet. Altogether, our results reveal a novel signaling mechanism of microbiota-gut-brain axis interaction upon dietary changes.

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New Opportunities For An Old Disease: Click Chemistry Tools To Unravel New Targets In Malaria

Grilo J. (1,2), Fidalgo L. (1), Fontinha D. (2), Anacleto B. (1), Moreira R. (1), Prudêncio M. (2), Ressurreição A.S. (1)

1- Research Institute for Medicines (iMed.ULisboa), Faculdade de Farmácia, Universidade de Lisboa, Lisbon, Portugal; 2- iMM – Instituto de Medicina Molecular, Faculdade de Medicina da Universidade de Lisboa, Av. Prof. Egas Moniz, 1649-028 Lisboa, Portugal.

With nearly half a million deaths a year reported for 2010-2017, and no reduction in the number of diagnosed cases in the last five years, Malaria continues to be a major public health concern [1]. To overcome parasite resistance to the therapeutic arsenal currently in clinical use, we have already shown that Torin2, a known ATP- competitive mTOR kinase inhibitor, [2] is a potent antimalarial with *in vivo* activity against both liver and blood stages, that is independent of the host mTOR pathway [3]. The next logical step was to develop biorthogonal chemical tools for *in vivo* imaging and drug target identification of Torin2-based compounds in malaria parasites. Herein, we report the development of a set of click probes through selective modifications of the Torin2 scaffold. These include: (i) "turn-on" fluorescent probes for bio-imaging purposes in live parasites and (ii) clickable photoprobes, for affinity-based protein profiling of the parasite proteome (Figure 1). These new chemical tools constitute a fundamental step to expand our understanding on the mechanism of action and protein target(s) of this class of inhibitors, providing important starting points for future pharmaceutical development of Torin2-based compounds as new antimalarials.

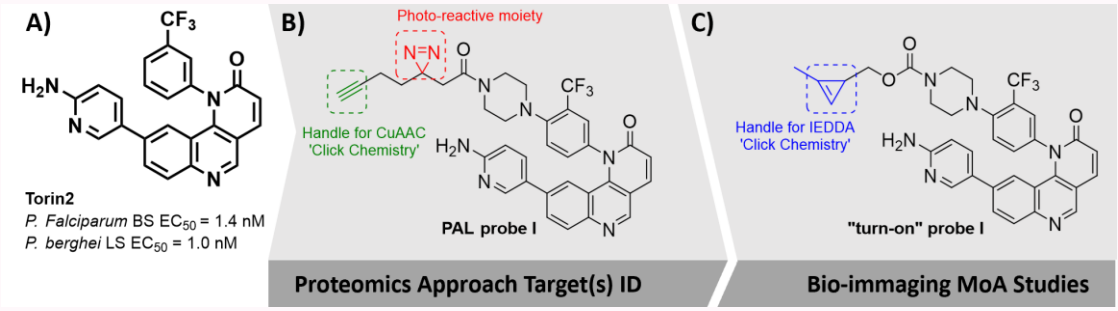

Figure 1 - A) Torin2 hit compound; **B)** Structure representative of the Photo-affinity labelling probes, derived from Torin2 by the introduction of a diazirine (for attachment to partner proteins) and a terminal alkyne (enabling protein visualization/enrichment by conjugation with a suitable tag); and **C)** Structure representative of the "turn-on" probes, derived from Torin2 by the introduction of a strained alkene enabling compound visualization in live cells through conjugation with a suitable quenched fluorophore.

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References

[1] World Health Organization, World Malaria Report 2018, WHO, Geneva, Switzerland, 2019.; [2] Liu, Q.; Wang, J.; Kang, S. A., et al, J. Med. Chem. 2011, 54 ,1473; [3] Hanson, K.K.; Ressurreição, A. S.; Buchholz, K.; Prudêncio, M., et al, Proc. Natl. Acad. Sci. U. S. A. 2013, 110, E2838.

How To Successfully Spray Dry Biopharmaceuticals Targeting The Lungs: A Novel Integrated Methodology

Fernandes D.A. (1,3), Costa E. (3), Leandro P. (2), Corvo L. (1)

1- BioNanoSciences, Drug Delivery and immunotherapy, Research Institute of Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; 2- Metabolism and Genetics, Research Institute of Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; 3- Hovione SA, Loures, 2074-506, Portugal.

One of the main challenges when developing a spray dried biopharmaceutical formulation for Dry Powder Inhalers is the generation of a stable aerosol able to reach the lungs while preserving the integrity of the biopharmaceutical, rendering it safe and effective. Hence, an appropriate excipient selection based on spray drying (SD) process development and biopharmaceutical stabilization is required to meet this balance. Herein, an integrated methodology is presented to expedite the selection of the best composite excipient matrix to formulate three inhalable model enzymes of increasing size and 3D structure complexity: Cu,Zn Superoxide Dismutase from bovine erythrocytes (SOD ≈ 32.5 kDa, homodimer – 2 subunits), Glucose Oxidase from Aspergillus niger (GOx ≈ 160 kDa, homodimer – 2 subunits) and Catalase from bovine liver (Cat ≈ 184 kDa, homotetramer – 4 subunits).Based on inhalation, spray drying process development precedence and biopharmaceutical stabilization, 4 non-reducing sugars – Mannitol, Sucrose, Raffinose, Trehalose – and four amino acids – Arginine, Alanine, Leucine, Lysine – were screened using High Throughput Differential Scanning Fluorimetry (DSF). The sugar and amino acid showcasing an increased stabilizing effect by DSF for each enzyme, were spray dried together (Mini Spray Drier BUCHI model B-290) using fixed process conditions (Drying flowrate, Fdrying = 35kgh^{-1}, Feed flowrate, Ffeed = 1.7 gmin^{-1}, Atomization flowrate, Ratom = 60 mm, Solids concentration, Csolids = 2% (w/w), Solvent system = deionized water) at three different ratios (20:80, 50:50, 80:20), to assess which formulation would display the best in vitro performance. The selected formulations were positively validated given their in vitro performance (Fine Particle Fraction) ranging from 60- 90% while keeping each enzyme 3D structure after SD. Thus, the present integrated methodology proved to be successful, allowing the narrow down of 48 formulations to only 1 for each enzyme, within one day, while requiring µg range of sample amount.

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How Are Pharmacists Performing In Long-Term Integrated Care? A Systematic Review

Rafael Goncalves J. (1), Lopes M.J. (2), Ramalhinho I. (3), Sleath B. (4), Cavaco A. (1)

1- Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; 2- School of Nursing, University of Évora, Portugal; 3- Faculty of Sciences and Technology, University of Algarve, Portugal; Eshelman School of Pharmacy, University of North Carolina at Chapel Hill, USA.

Introduction: In recent years, both Integrated and Long-Term Care systems (LTIC) have developed rapidly, driven by changes in healthcare models and worldwide demography. Pharmacists are delivering activities beyond their traditional dispensatory role e.g. in primary care, with a clear impact on well-defined health outcomes. Pharmacists´ participation in LTIC has thriving, however comprehensive studies mapping the field, quality and impact of pharmaceutical activities in institutional LTIC settings are scarce.
Aims: i) identifying pharmacist and/or pharmacy-based interventions in institutional LTIC settings; ii) characterize which medical conditions and/or therapeutic groups interventions have been most prevalent.
Methods: The systematic review was designed based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA). A set of 16 keywords divided into 3 domains – Professional, Type of Care and Type of Setting - were combined into search equations used to screen 3 electronic databases – PubMed, Scopus and Web of Knowledge. The selected studies quality assessment was performed using the Quality Assessment Tool for Quantitative Studies.
Results: Twenty-six studies met the inclusion criteria, out of 794 initial hits. Twelve studies (46%) were rated as Weak for quality assessment. Only five studies (20%) were classified as Strong. The mapping of pharmacist/pharmacy-driven interventions, shows that most studies [1,2] assessed Medication Management Reviews Programs impact in different endpoints or outcomes (e.g. Mortality, DRPs – Drug- Related Problems, MAI – Medication Appropriateness Index, DBI – Drug Burden Index), followed by three studies specifically assessing pharmacists' interventions on antipsychotics, benzodiazepines or anticholinergic drugs use. Good Administration Practices, new models of pharmaceutical care and antibiotics stewardship programs comprised other 6 studies. The rest of studies (5) assessed pharmacist interventions in diverse fields e.g. warfarin and vitamin D monitoring programs; pharmacy-managed informatics tool; educational program.
Conclusions: Despite the poor quality of the body of evidence identified on this systematic review, pharmacists are performing their activities in a wide array of fields in LTIC. Although fragmented, the implementation of pharmaceutical activities in LTIC, opens pharmacists' interventions opportunities, with scientific and healthcare outcomes. It is also highlighted the need for improvement in scientific reporting of pharmacists performing in LTIC institutional settings.

References

[1] Moher, D.; Liberati, A.; Tetzlaff, J; Altman, D. G., The PRISMA Group (2009) Preferred Reporting Items for Systematic Reviews and Meta- Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. [2] Thomas, B. H.; Ciliska, D.; Dobbins, M.; Micucci, S. (2004). A process for systematically reviewing the literature: Providing the research evidence for public health nursing interventions. [3] Worldviews on Evidence-Based Nursing, 1(3),176-184.

Role Of AQP3 In Inflammasome Priming And Activation In THP-1 Macrophages Like Cells

da Silva I. V. (1), Cardoso C. (2), Martinez-Banaclocha H. (3), Casini A. (4), Pelegrin P. (3), Soveral G. (1)

1- Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; 2- Clinical Chemistry Laboratory, Miraflores, Portugal; 3- BioMedical Research Institute of Murcia (IMIB-Arrixaca), Murcia, Spain; 4- School of Chemistry, Cardiff University, Cardiff, United Kingdom.

Aquaporins (AQPs) are protein channels that facilitate the transport of water and/or small solutes through cell membranes, essential for cell volume regulation and water and glycerol homeostasis. Variations in cell osmolarity and consequent cell volume regulation precede NLRP3 inflammasome activation [1] and AQP blockage was shown to limit IL-1β release from NLRP3-activated macrophages [2]. However, the role of AQPs in inflammation is still unclear.Human peripheral blood monocytes (HPBMs) and THP-1 macrophages cultured under stimuli for cell priming and inflammasome activation are valuable models to study inflammation. Here, we used HPBMs to evaluate AQPs expression in a healthy or pro-inflammatory phenotype. Then, we used THP-1 macrophages to uncover the mechanism where AQPs are players and potential new targets. Our results show that AQP9 and AQP3 are the most representative and are upregulated by LPS- priming in primary monocytes and THP-1 cells, respectively. PMA-differentiated macrophages-like cells and LPS-primed macrophages-like cells incubated with and without Auphen, a selective AQP3 inhibitor, showed similar water and glycerol permeability values, and glycerol permeability was affected by Auphen. Therefore, we investigated the role of AQPs during cell priming and inflammasome activation using the AQP3 inhibitor Auphen. LPS-priming was partially blocked by Auphen, decreasing mRNA expression and protein release levels of IL-6 and IL-1β. This suggests an involvement of AQP3 in macrophage priming by Toll-like receptor 4 engagement. NLRP3 inflammasome priming and activation was also blocked by Auphen, decreasing mRNA expression and protein release of IL-1β after NLRP3 activation with nigericin and ATP. Moreover, challenging LPS-primed cells with hyperosmotic solutions of glycerol increased IL-1β release. Altogether these data evidence AQPs as candidate players in the setting of the inflammatory response.

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References

[1] Compan, V., et al., Immunity, 2012, 37(3), 487-500; [2] Schorn, C. et al., The Journal of Biological Chemistry, 2011, 286(1): 35–41.

Generation Of A Library Of Indole Alkaloid Derivatives As ABC Transporter Modulators

Cardoso D.S.P. (1), Mulhovo S. (2), Santos D.J.V.A. (1,3), Falson P. (4), Ferreira M.J.U. (1)

1- Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; 2- Centro de Estudos Moçambicanos e de Etnociências (CEMEC), Faculdade de Ciências Naturais e Matemática, Universidade Pedagógica Campus de Lhanguene, Av. de Moçambique, 21402161 Maputo, Moçambique; 3- LAQV@REQUIMTE/Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, Rua do Campo Alegre, 4169-007 Porto, Portugal; 4- Drug Resistance & Membrane Proteins Team, Molecular Microbiology & Structural Biochemistry Laboratory, Centre National de la Recherche Scientifique (CNRS) and Claude Bernard University Lyon 1 Joint Research Unit 5086, Institut of Biology and Chemistry of Proteins (IBCP), 7, passage du Vercors, 69367 Lyon, France.

Multidrug resistance (MDR), a phenomenon in which cancer cells acquire simultaneous resistance to several functional and structurally unrelated anticancer drugs, has become the main reason of chemotherapy treatment failure. One of the principal mechanisms of MDR is related with the overexpression of transmembrane proteins of the human ATP-binding cassette (ABC) superfamily. To date, the main efflux pumps responsible behind this phenomenon are Breast Cancer Resistance Protein (BCRP or ABCG2), P-glycoprotein (P-gp or ABCB1) and Multidrug Resistance Protein (MRP-1 or ABCC1). This study aims at generating a new library of bioactive compounds through derivatization of indole alkaloids in order to evaluate their ability as ABC transporter inhibitors for reversing MDR. Therefore, two major isomers, isolated from the African medicinal plant Tabernaemontana elegans (Apocynaceae), were derivatized through alkylation of the indole nitrogen to generate a first set of compounds. In parallel, one of the main isomers was condensed with hydrazine hydrate to afford an imine that further reacted with different aldehydes to yield new azine derivatives. The chemical structures were established by 1D and 2D NMR experiments. The anti-MDR reversal activity of the N-alkylated compounds was evaluated using as models transfected cancer cells NHI-3T3, overexpressing P-gp, and a transfected HEK293 cell line overexpressing either MRP1 or ABCG2. The efflux activities of the pumps were monitored by flow cytometry. Docking experiments were performed in the three ABC transporters to evaluate binding affinities and mode of action for all derivatives.

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Nanostructured Systems For Overcoming Biological Barriers: A Novel Approach To Ocular Delivery of Erythropoietin

Silva B. (1,3), Marto J. (1,4), São Braz B. (3), Delgado E. (3), Gonçalves L.M.D. (1,2)

1- Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; 2- Cardiovascular Autonomic Function Laboratory, Faculty of Medicine, Universidade de Lisboa; 3- CIISA - Centro de Investigação Interdisciplinar em Saúde Animal, Faculty of Veterinary Medicine, Universidade de Lisboa; 4- Laboratório Edol, Produtos Farmacêuticos S.A., 2795-225 Linda-a-Velha, Portugal.

Studies using animal models of ophthalmic neurodegenerative diseases have shown that erythropoietin presents neuroprotective and neuroregenerative effects. Subconjunctival delivery of recombinant human erythropoietin beta (EPOβ) to the retina in a rat glaucoma model has been proven effective. Nevertheless, the topical ocular administration of erythropoietin has not been studied yet. The ocular defense mechanisms like tear film clearance, blinking, conjunctiva/ choriocapillaris blood flow, uveoscleral outflow and intraocular pressure, combined, can wash way up to 80 or 90% of the drug instilled in the eye. Therefore, effective topical ocular delivery systems must have mechanisms to increase ocular surface contact time, in order to improve drugs bioavailability. Drug delivery systems based on chitosan (CS) nanoparticles can improve drug topical ocular delivery by increasing contact time and mucoadhesion. CS is a hydrophilic biodegradable polymer with a polycationic nature that interacts with the polyanionic surface of the ocular mucosa. These properties can be further improved by using hyaluronic acid (HA), a natural polymer that has second generation mucoadhesion properties through CD44 receptor-mediated binding. Therefore, this study aimed to develop and evaluate a hyaluronic acid and chitosan nanoparticulate system for topical ocular delivery of EPOβ. Nanoparticles (NPs) were prepared by a modified ionotropic gelation technique using six different HAs (HA1-HA6), and characterized by size, zeta potential (ZP), polydispersity index (Pdi), cytotoxicity and mucoadhesion. Encapsulation efficiency and drug loading capacity were also determined. Ex vivo permeation was performed with fresh porcine corneas, scleras and conjunctivas, which were tested for EPOβ by immunohistochemistry. The nanoparticles presented a size under 300 nm, a ZP around +30 mV and low Pdi (0.167-0.539) at a 1:1 CS:HA mass ratio. HA6 (300 kDa - Eye), which presented the best mucoadhesive properties, was selected to the ex vivo permeation assay. CS/HA6- EPOβ formulation permeated more rapidly through porcine conjunctiva, followed by sclera and cornea, as confirmed by immunohistochemistry. All formulations did not present cytotoxicity effects on ARPE-19 and HaCaT cell lines, by the metabolic and membrane integrity tests. In conclusion, CS/HA6-EPOβ NPs could be a promising formulation to increase ocular bioavailability of EPOβ by enhancing its retention time and permeation into the ocular membranes.

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Mining High-Throughput Sequence Data Towards a Global Perspective of the *Mycobacterium Tuberculosis* Resistome Diversity

Gomes P. (1), Perdigão J. (1), Portugal, I. (1)

1- Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal.

Tuberculosis (TB), which is caused by the microbial pathogen *Mycobacterium tuberculosis (M.tb.*), is the leading infectious disease worldwide, having contributed to 1.6 million deaths in 2017. Moreover, it is estimated that 1.7 billion people carry latent tuberculosis infection, which may evolve to an active infection and ultimately develop TB. Although TB incidence has been steadily declining over the years, the increasing number of drug resistant (DR) TB cases and the lacking of worldwide TB diagnosis, especially in developing countries, continue to undermine the goal of total TB eradication (World Health Organization, 2018). The undergoing investigation at the Laboratory of Molecular Mycobacteriology (iMed.Ulisboa) is taking advantage of M.tb. genome sequence data to mine DR-associated mutations in a high-throughput fashion, using cutting-edge bioinformatic tools to better understand the global distribution of *M.tb.* resistome. An added phylogenomic clustering analysis is also being implemented to uncover the association between the *M.tb.* genetic background and its ability to acquire drug resistance, at a global scale. This investigation will hopefully shed a light on the complexity of *M.tb.* drug resistance, and further contribute to the elimination of TB disease.

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References

[1] World Health Organization. (2018). Global tuberculosis report 2018. World Health Organization. https://apps.who.int/iris/handle/10665/274453. License: CC BY-NC-SA 3.0 IGO

TNBS-Induced Colitis In Rodents: Preliminary Results of a Chronic Model

Silva I., Mateus V., Pinto R.

Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal.

Background: Inflammatory bowel disease (IBD) is a gastro-intestinal disorder characterized by a chronic inflammation of the intestinal epithelium [1,2]. The symptoms of IBD depend on the intestinal affected segment and usually include diarrhea often with blood, colic abdominal pain and fecal urgency. Beyond these, other unspecific symptoms may occur like fever, loss of appetite and weight, fatigue and primary amenorrhea [3]. Nowadays, used therapy in IBD consists in salicylates, corticosteroids, immunosuppressants, and biological therapy. These drugs aim to induce and/or maintain the patient in remission and ameliorate the disease's secondary effects, rather than modifying or reversing the underlying pathogenic mechanism [4,5].
Aim: Development of an animal model of trinitrobenzene sulfonic acid (TNBS)-induced chronic colitis in order to evaluate the influence of new drugs in the IBD.
Materials and Methods: Male CD-1 mice with 6-10 weeks age were used to the development of this model. The experimental colitis was induced by administering from one to three intracolonic injections of TNBS. Two different TNBS concentrations were also tested, namely 1% and 1.25%. The evaluation of the colitis induction was based on clinical symptoms/signs, fecal hemoglobin, tumor necrosis factor (TNF)-α, interleukin (IL)-10, urea and alanine aminotransferase (ALT) concentrations.
Results: Mice with TNBS-induced colitis presented a decreased body weight and an alteration of intestinal motility characterized by diarrhea, edema of the anus and morbidity, while the control group has no alterations in the clinical signs/symptoms. Fecal hemoglobin and TNF-α are also increased in the colonic mice. The mortality rate was 0% with TNBS 1% and 47.1% with TNBS 1.25%, at day 4. However, our results are still preliminary.
Conclusion: These findings allow us to propose 3 administrations at 1% of TNBS for the induction of colitis. However, it is important to increase the frequency of administrations and the number of animals per group to confirm our preliminary data.

References

[1] Wirtz, S.; Popp, V.; Kindermann, M.; Gerlach, K.; Weigmann, B.; Neurath, M. F., Nat Protoc., 2017, 12(7), 1295-1309; [2] Mowat, C., Gut, 2011, 60(5), 571-607; [3] Kaser, A.; Zeissig, S.; Blumberg, R. S., The Annual Review of Immunology, 2010, 28(1), 573-621; [4] Engel, M; Neurath, M., Journal of Gastroenterolgy, 2010, 45, 571-83; [5] Pithadia, A. B., Pharmacol Rep., 2011, 63(3), 629-642.

Benefits of VS in Counteract ALS-astrocyte Aberrancy Involves miR-146a Upregulation

Barbosa M. (1), Gomes C. (1), Carvalho L.A. (1), Moreira R. (1,3), Vaz A.R. (1,2), Brites D. (1,2)

1- Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; 2- Department of Biochemistry and Human Biology, Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; 3- Department of Pharmaceutical Chemistry and Therapeutics, Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal.

Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disease without effective treatment. Physiopathological changes in glial cells, miRNA dysregulation and neuroinflammation have been pointed to contribute for neurodegeneration in ALS. Our group identified an aberrant phenotype of cortical astrocytes isolated from the SOD1G93A transgenic (mSOD1) mice pups (GFAP/miR-146a^{low} and S100B/Cx43/vimentin^{high}) with neurotoxic properties [1]. More recently we demonstrated the anti- inflammatory potential of glycoursodeoxycholic acid (GUDCA) and dipeptidyl vinyl sulfone (VS) in mSOD1 N9-microglia [2]. Here, we aimed to i) assess the ability of GUDCA and VS in neutralizing mSOD1 astrocytic aberrant phenotype and ii) validate if miR-146 is involved in the recovery of the beneficial mSOD1 astrocyte phenotype and iii) evaluate the effects of mSOD1 astrocyte-derived secretome in naïve N9-microglia and whether the secretome from pre-miR-146a modulated astrocytes could prevent microglial alterations. Cortical astrocytes were isolated from mSOD1 and non-transgenic (wt) mice at 7-day-old and cultured for 13 days *in vitro* (DIV). Cells were incubated with GUDCA (50 µM) or VS (10 µM) at 12 days-in-vitro (DIV) for 24h. In parallel, miR-146a upregulation was performed by using pre-miRNA technology. WT-non-treated astrocytes were used as controls. Both compounds decreased Cx43 gene levels in mSOD1 astrocytes, while VS was also able to restore GFAP protein and S100B mRNA expression, together with a marked increase of miR-146, showing additional reparative benefits over those of GUDCA. Interestingly, direct upregulation of miR-146a in mSOD1 astrocytes mimicked the results observed with VS incubation, suggesting that the rescue of their aberrant phenotype involves miR-146a regulation. When we incubated the mSOD1 astrocyte-derived secretome in naïve N9-microglia for 24h, we observed increased levels of iNOS/TNF-α/apoptosis. Interestingly, the secretome derived from pre-miR-146a-treated mSOD1 astrocytes induced a recovery of these markers to the levels observed in the presence of WT astrocyte-derived secretome and a noticeable miR-146a upregulation, indicating a more anti-inflammatory microglia. Overall, we proposed that VS neutralizing effects towards the aberrant phenotype of cortical mSOD1 astrocytes involves the modulation of miR-146a. Importantly, miR-146a upregulation in mSOD1 astrocytes not only prevent their aberrancy but also the spread of inflammation to surrounding cells.

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References

[1] Gomes, C.; Cunha, C.; Nascimento, F.; Ribeiro, J. A.; Vaz, A. R.; Brites, D., Mol Neurobiol., 2019, 56(3), 2137-2158; [2] Vaz, A. R.; Pinto, S.; Ezequiel, C.; Cunha, C.; Carvalho, L. A.; Moreira, R.; Brites, D., Front. Cell. Neurosci, 2019, April 9.

POSTERS COMMUNICATIONS

Influence Of Culture Conditions On The Biophysical Properties Of Live Cells

Ventura A.E. (1,2,3), Santos T. (1,2), Pokorna S. (3), Prieto M. (2), Futerman T. (3), Silva L.C. (1)

1- Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; 2- iBB, Institute for Bioengineering and Biosciences, Instituto Superior Técnico – Department of Bioengineering, Universidade de Lisboa, Lisbon, Portugal; 3- Weizmann Institute of Science, Biomolecular Department, Rehovot, Israel.

Stress conditions have a pronounced effect on the behavior of cells promoting alterations in different levels of cell metabolism. Stress signals are very different and can range from a physiological condition to an alteration in a specific component. Most cells are very sensitive to changes and require specific culture conditions. While culturing cells, one of the factors that should be considered is their confluency. Many cell lines exhibit differences in growth rate or gene expression depending on the degree of confluence. In some cell types the contact between different cells will promote growth arrest, however, in immortalized cells this feature is not present, and cells can grow on top of the parent cells creating layers. The stress caused by the overconfluency might directly affect the physico-chemical properties of the membranes, including their fluidity. We used fluorescent microscopic techniques capable of reporting the fluidity state of the membrane to address this subject. Our results show that the overall Laurdan GP of HEK293 cells increased significantly with increased cell density (increased confluency), showing that membrane fluidity is affected by this parameter. In addition, cells grown to overconfluency presented and increased number of highly ordered intracellular structures and their formation was almost abolished if the medium of these cells was regularly replenished with nutrients during their growth. To investigate if the effects were common to different cell lines, we used a metastatic (SW620) and a resistant (HT29) colon cancer cell line. At normal confluency the Laurdan GP of SW620 cells was lower compared to resistant HT29 cells. Increasing SW620 cell density resulted in increased Laurdan GP, as observed for HEK293 cells. However, an opposite behavior was observed for HT29, which showed a decrease in Laurdan GP, suggesting that the fluidity of HT29 cell membranes increases in overconfluent cells. Our results show that membrane biophysical properties of different cells are differentially affected by stress factors originating by culture conditions and highlight the importance of controlling these factors when addressing cell properties and function.

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Interplay Between Breast Cancer Cells And Blood-Brain Barrier Endothelial Cells Along Extravasation

Garcia R. (1), Godinho-Pereira J. (1,2), Figueira I. (1,2), Kim K.S. (3), Botelho, H.M. (4), Malhó R. (4), Brito M.A. (1,5)

1- Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; 2- Faculty of Pharmacy Association for Research and Development (Farm-ID), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; 3- Division of Infectious Diseases, Johns Hopkins University School of Medicine, 600 North Wolfe Street Park 256, Baltimore, MD21287, USA; 4- BioSystems and Integrative Sciences Institute (BioISI), Faculty of Sciences, Universidade de Lisboa, Lisbon, Portugal; 5- Department of Biochemistry and Human Biology, Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal.

Brain metastases (BMs) are amongst the impact factors associated with decreased life expectancy of many breast cancer (BC) patients. The extravasation, a key step for the establishment of metastasis, is thought to involve the disruption of the microvascular endothelium that composes the blood-brain barrier (BBB) by cancer cells. However, little is known about the mechanism involved in BBB transposition by BC cells. So, we aimed to assess BC cells extravasation across the BBB that ultimately lead to brain metastases. To this end, human brain microvascular endothelial cells (HBMEC), which mimic the BBB, and a human BC cell line with brain tropism, MDA-MB-231 Br4 (tagged with GFP), were used in mixed cultures. HBMEC were cultured until confluence and incubated with CellTrackerTM Red CMTPX Dye, and MDA-MB-231 Br4 were added to HBMEC monolayers, being monitored over time. The interaction between endothelial and BC cells was evaluated by live-cell imaging using a Leica TCS SP8 confocal microscope at 37°C and 5% CO2, to perform a time-lapse image acquisition with 10min intervals until 1h and then every 3h up to 24h. Our results demonstrated that, after 4h, BC cells have already intercalated within endothelial cells and some have transmigrated through the endothelial monolayer, appearing underneath it. This was accompanied by morphological alterations in BC cells, revealed by an increase of their area and perimeter and a decrease in circularity and roundness, which are compatible with a migratory and invasive phenotype. Moreover, the release of small vesicles from BC cells was increasingly observed along time. Concomitantly, a progressive disruption of the endothelial monolayer integrity was evident. These studies, relying on a robust human in vitro model of BC brain metastasization, contribute to a better understanding of BC cells trafficking across brain microvascular endothelium, an essential step for the future development of novel strategies to avoid extravasation of malignant cells into the brain and thus to prevent brain metastases from occurring.

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Potential Use Of *Sambucus nigra* L. Extracts For Skin Applications

Mota A.H. (1), Andrade J.M. (1), Kuplennik N. (2), Sosnik A. (2), Viana A.S. (3), Rijo P. (1), Baby A. (4), Ascensão L. (5), Fattal E. (6), Almeida A. J. (1), Reis C.P. (1,7,8)

1- Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; 2- Laboratory of Pharmaceutical Nanomaterials Science, Department of Materials Science and Engineering, Technion-Israel Institute of Technology, De-Jur Building, Technion City, 3200003 Haifa, Israel; 3- Centro de Química e Bioquímica, Centro de Química Estrutural, Faculty of Sciences, ULisboa, Campo Grande, 1749-016 Lisboa, Portugal; 4- Departamento Farmácia, Escola de Ciências Farmacêuticas, Universidade de São Paulo, 580 Prof. Lineu Prestes Av., Bl. 15, Conjunto das Químicas, Cidade Universitária, 05508-900 São Paulo, SP, Brazil; 5- Centro de Estudos do Ambiente e do Mar (CESAM), Faculty of Sciences, ULisboa, Edifício C2, Campo Grande, 1749-016 Lisboa, Portugal; 6- UMR CNRS 8612 Institut Galien Paris-Sud, Faculté de Pharmacie, Université Paris Saclay, Châtenay-Malabry, France; 7- IBEB, Biophysics and Biomedical Engineering, Faculty of Sciences, ULisboa, Campo Grande, 1749-016, Lisboa, Portugal; 8- Dreams, Universidade Lusófona de Humanidades e Tecnologias, Campo Grande, Lisbon, Portugal.

Sambucus nigra L. (Adoxaceae) is a species widely used in traditional medicine to treat several diseases [1]. This study aims to scientifically evaluate the use of *S. nigra* for cosmetological use. In a previous study, twenty-seven (27) extracts from *S. nigra* were obtained from flowers and berries using different extraction processes (ultrasounds and microwave), solvents and then the *in vitro* bioactivities of resultant extracts were screened such as antioxidant activity (AA), collagenase (Coll) inhibition and photoprotection efficiency. This best method of extraction was the ultrasounds method using methanol as solvent extraction. The yield was 6.6 ± 0.5 % (n = 4). One of those 27 extracts revealed 93 ± 3 % (n = 3) of AA, 94 ± 1 % (n = 3) of Coll inhibition and 50+ of photoprotection efficiency, attracting high interest for skin application. The positive controls achieved to antioxidant activity was quercetin, to collagenase inhibition epigallocatechin gallate and to photoprotection efficiency was used titanium dioxide (TiO_2). To deliver this extract to the skin, nanoparticles were chosen as protective carriers. As nanotechnology plays, in cosmetic area, an important role in delivering active ingredients to the skin [2], different nanocarriers were developed and fully characterized.Ethosomes were developed showing small size (≈ 200 d.nm) and monodisperse distribution (PI ≈ 0.3) as shown in Figure 1. According TEM and AFM analyses, empty ethosomes were spherical in shape.

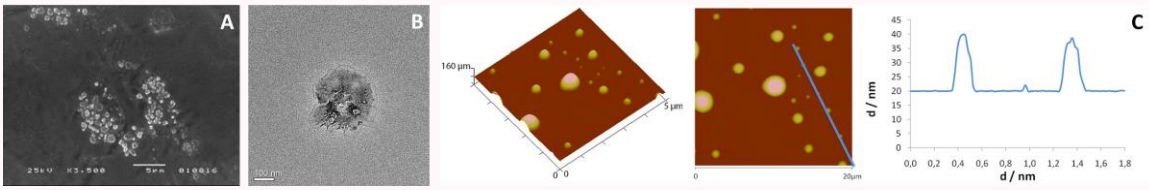

Figure 1 – Empty Ethosomes. **A, B,** SEM and TEM micrographs. **C,** AFM topographs.

Our next research step will involve the encapsulation of this extract into those ethosomes and its characterization using previous biological assays and in vitro permeation studies.

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References

[1] Committee on Herbal Medicinal Products. Assessment report on *Sambucus nigra* L., fructus. European Medicines Agency. 2013, 44(March), 1-26; [2] Mota, A. H.; Rijo, P.; Molpeceres, J.; Reis, C. P., International Journal of Pharmaceutics. Elsevier B.V., 2017, 532(2), 710-728.

Proimmunotoxin: A Novel Design Strategy Of Immunotoxins Applied To Breast Cancer

Manuel A.M., Gonçalves J.M., Vítor J., Madeira C., Leandro A.P.

Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal.

Breast cancer is the second leading cause of death among female cancer patients. One of the most used therapies against receptor tyrosine-protein kinase erbB 2 (HER2)+ metastatic breast cancer is the monoclonal antibody Trastuzumab. Immunotoxins have been pursued for a long time as a new and innovative type of biopharmaceutical against cancer, they combine the cytotoxicity of a variety of toxins with the specificity of antibodies. An immunotoxin combining Ricin A-chain [Ricin A] and an antibody targeting breast cancer has already been tried as a therapeutic. However, the observed non-specific toxicity prevented further studies. To address this problem, a new type of immunotoxin was envisioned, a proimmunotoxin which comprises Trastuzumab linked to a Ricin A neutralizing VHH, capable of delivering Ricin A toxin with high specificity to HER2+ breast cancer cells, while neutralizing its activity during blood circulation, overcoming the non-specific toxic side effects of a normal immunotoxin. The results here presented show that this new type of immunotoxin retains the specificity of the antibody Trastuzumab towards HER2, shows high affinity towards Ricin A, and most importantly, it can protect HER2- cells, while retaining the ability to deliver Ricin A toxin to HER2+ breast cancer cells, decreasing cell viability. The possibility of clustering HER2 at the cell membrane to enhance the internalization and trafficking of the proimmunotoxin was also studied by constructing one proimmunotoxin with two different binding sites to HER2. Preliminary results show that this proimmunotoxin has a quicker internalization by cells, shown by an enhanced decrease in cell viability.

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Herbicide-Loaded Chitosan Solid Lipid Microparticles: Formulation And Characterization

Neves A. (1), Marto J. (1), Aparício S. (2), Gaspar D. (2), Madeira P. (2), Ribeiro H.M. (1), Almeida A.J. (1)

1- Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; 2- Ascenza Agro S.A., Setúbal, Portugal.

Pesticides are widely used in agricultural production and are usually developed through very strict regulation processes to function with reasonable certainty and minimal impact on human health and the environment [1]. Therefore, exploring novel applications for approved excipients with a history of safe use in pharmacy and cosmetics is a smart strategy to obtain safer pesticides pharmaceutical products. The aim of this study was to formulate and characterize hybrid microparticles based on solid lipids and of chitosan (CS) to produce a less toxic herbicidal formulation for the effective and safe control of weeds in agriculture. For this purpose, solid-lipid microparticles (SLM) were formulated with different solid lipids (e.g. stearic acid; octadecylamine; gelucire 48/16; Compritol ATO; Lipocirel A SG; Precirol ato 5; Geleol; cetyl palmitate; stearyl acohol) and coated with different CS amounts (concentrations from 0.25 to 1.5% w/v were studied). The SLM were prepared using an herbicide-containing oil phase, while and aqueous phase was prepared by dissolving an appropriate amount of sodium lauryl sulfate (SLS) in purified water, followed by heating at the same temperature of the oil phase. Microparticles were then obtained by a hot emulsification method where the aqueous phase was added dropwise to the melted oil phase and then homogenized using a high-shear laboratory mixer (Ultra-Turrax®, IKA-Labortechnik) [2]. Afterwards, SLM were coated with the CS solutions, at a CS:SLM ratio of 1:4 for 1 h at room temperature, with a gentle stirring. Formulations were characterized for particle size distribution and zeta potential, as well as colloidal stability. The Compritol formulations demonstrated the best results. As expected, microparticle size increased with increasing CS concentrations. Zeta potential was highly influenced by the surfactant, but surface charge is drastically altered upon coating with CS. Herbicide-loaded solid-lipid microparticles showed potential as alternative for use in eco- friendly herbicide formulations.

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References

[1] Damalas, C. A.; Eleftherohorinos, I. G.; International Journal of Environmental Research and Public Health, 2011, 8, 1402-1419; [2] Gaspar, D. P.; Faria, V.; Gonçalves, L. M.; Taboada, P.; Remuñán-López, C.; Almeida A. J., Internacional Journal of Pharmaceutics, 2016, 497, 199-209.

Unveiling The Influence Of mSOD1 MNs-Derived Secretome On mSOD1 Mice Spinal Microglia Polarization

Colaço A.R. (1), Morgado P. (1), Vaz A.R. (1,2), Brites D. (1,2)

1- Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; 2- Department of Biochemistry and Human Biology, Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal.

Amyotrophic lateral sclerosis (ALS) belongs to the group of motor neuron (MN) diseases. MN dysfunction and microglia over-reactivity are important hallmarks in ALS pathology. It has been accepted that deregulated MN-Microglia signaling is associated to neuroinflammation, neurodegeneration and disease progression. However, changes in cell-to-cell communication and microglia response to neurotoxins and inflammatory mediators in ALS disease models are far from been clarified and need further investigation. Recent data from our lab showed a deregulation in N9 microglia when exposed to both secretome and exosomes derived from MNs mutated in SOD1G93A (mSOD1) [1,2]. Here, we aimed to define the phenotypic changes of microglia isolated from the spinal cord of wild-type (WT) and mSOD1 mice pups (8 day-old) upon exposure to secretome of mSOD1 MNs (G93A NCM). For that we cultured primary microglia after 2 days *in vitro* with G93A NCM for 4 hours. WT microglia exposed to G93A NCM presented a reactive profile, with increased levels of both pro- and anti-inflammatory associated markers, namely HMGB1, IL-18, IL-10 gene expression as well as iNOS protein levels. The phagocytosis-associated marker MFG-E8 mRNA was additionally upregulated in these cells upon treatment with G93A NCM. Interestingly, all of these markers were downregulated in mSOD1 microglia treated with G93A NCM, except HMGB1. TNF-α and Arg1 mRNAs were also decreased in these cells after exposure to G93A NCM, indicating that mSOD1 microglia are, overall, less responsive than their matched WT ones. In conclusion, our results suggest that G93A NCM produce distinct effects on WT and mSOD1 microglia that may partially justify the global cellular failure after transplantation of neural precursors, while also contribute to ALS disease progression.

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References

[1] Vizinha D., Master Thesis, 2018; [2] Pinto, S.; Cunha, C.; Barbosa, M.; Vaz, A. R.; Brites, D., Front Neurosci., 2017, 11, 273.

Affinity Maturation And Phage Display Selection Of Anti-NCL Antibodies Against Triple Negative Breast Cancer

Ribeiro R. (1, 2, 3), Moreira J.N. (2, 3), Gonçalves J. (1)

1- Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; 2- Center for Neuroscience and Cell Biology (CNC), University of Coimbra, Coimbra, Portugal; 3- Faculty of Pharmacy, University of Coimbra, Coimbra, Portugal.

Triple-negative breast cancer (TNBC) is an aggressive subtype of breast cancer that represents about 15% of all breast cancers. Because TNBC cells lack the molecular targets of therapeutic agents, patients do not respond well to hormonal or anti-HER2 therapies and are often given combination of chemotherapy. Nonetheless, the 5-year survival rate is only 22% [1]. Developing targeted therapeutic strategies is therefore an imperative need. In this context, nucleolin (NCL) arises as a relevant target. It is a protein located in several cell compartments but is overexpressed at the surface of both cancer and angiogenic endothelial cells of solid tumors, such as TNBC [2]. Small antibody formats, such as camelid heavy chain variable domains (VHH) can be engineered to present the desired high affinity to a chosen target and good tumour penetration, emerging as a promising strategy against this target. An anti-NCL VHH was previously developed by our group and its ability to target different cell lines of NCL-overexpressing cells was evaluated and confirmed [3]. This project aims at increasing the affinity of the aforementioned anti-NCL VHH. An *in vivo* random mutagenesis technique using a bacteria strand with a low fidelity polymerase was performed to create a library of mutated anti-NCL antibodies. This pool of mutated antibodies was then used in a phage display procedure, to select the mutation variants with affinity to recombinant human NCL. Clones presenting higher affinity to the target were selected in expression and binding assays. The next step of this project will be to assess the binding and possible internalization of the selected clones using TNBC NCL-overexpressing cells.

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References

[1] Oakman C.; Viale G.; Di Leo A., The Breast, 2010, 19(5), 312-321; [2] Gregório, A. C.; Lacerda, M.; Figueiredo, P.; Simões, S.; Dias, S.; Moreira, J. N., Critical Reviews in Oncology / Hematology 125, 2018, 89-101; [3] Romano, S.; Moura, V.; Simões, S.; Moreira, J. N.; Gonçalves, J., Scientific Reports, 2018, 8, 7450.

Frailty Index In Multiple Sclerosis Course: A Novel Approach To Better Understand Disease Outcome

Ribeiro A.R. (1), Howlett S. (2), Fernandes A. (1,3)

1- Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; 2- Department of Pharmacology and Medicine (Geriatric Medicine), Dalhousie University, Canada, 3- Department of Biochemistry and Human Biology, Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal.

Aging itself is associated with natural structural, functional and physiological changes, but how could it be measured in order to predict people vulnerability? For that, the concept of Frailty emerged, being defined as a biological and clinical syndrome resulting from deficits' accumulation and increased vulnerability to adverse outcomes. Many Frailty approaches have been developed to translate the results into the best reliable way. Here, we focused on the Frailty Index (FI), develop by Rookwood K. [1], which is defined as the proportion of accumulated deficits (e.g. symptoms, signs, functional impairments and laboratorial abnormalities). It is obtained by counting the number of deficits present and dividing this count by the number of all deficits considered. Since, this approach allows to understand the variability across adult life course, Frailty measure has been applied mostly to the aging phenomena. But could Frailty be used to predict disease courses/outcomes? Research concerning this topic is scarce and therefore its huge potential is still unknown. For instance in Multiple Sclerosis (MS), it is known that patients with other comorbidities or with increased age respond distinctly to the therapeutic interventions and show a faster progression of disability, but there is no current biomarker/marker for this clinical prognosis. So, as a first attempt to study the FI in MS, we adapted the deficits considered on the FI developed for mice models by Howlett S. [2], to the MS animal model: the Autoimmune Encephalomyelitis (EAE). As so, we will evaluate: alopecia, dermatitis, loss of whiskers and coat condition; tremor, forelimb grip strength; vestibular disturbance; nasal discharge; diarrhea; breathing rate/depth; piloerection and body weight. This evaluation will allow the detection of subtle differences in distinct animals of a cohort along disease course that may correlate with the magnitude of disease progression/recovery and associated CNS damage. More interestingly, we will also focus on: i) animals' age; and ii) animals ability to mount an inflammatory response, in order to best translate these findings to the human MS course. Overall, FI may have a tremendous potential in the correlation of physical changes, neuroinflammation and psychopathological comorbidities in MS progression.

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References

[1] Searle, S. D.; Mitnitski, A.; Gahbauer, E. A.; Gill, T. M.; Rockwood, K., BMC Geriatrics, 2008, 8, 1-10; [2] Whitehead, J. C., et al., Journals of Gerontology: Biological Sciences, 2014, 69, 621-632.

Polymeric 3D-Scaffolds As Platforms For Local-Delivery To The Bone: Materials Characterization And *In Vitro* Biological Studies

Saraiva A.S. (1,2), Ribeiro I. (2), Cláudio R.A. (3), Alves M.M. (4), Carmezim M.J. (3,4), Santos C. (3,4), Gonçalves L.M. (2), Bettencourt A.F. (2)

1- Departamento de Ciências da Vida, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Portugal; 2- Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; 3- EST Setúbal, CDP2T, Instituto Politécnico de Setúbal, Campus IPS, 2910 Setúbal, Portugal; 4- CQE Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais 1049-001, Lisboa, Portugal.

Secondary bone cancer is a frequently pathology with a golden standard treatment (surgical ablation, auto- or allografts replacements followed by chemo- and/or radiotherapy) [1], which has a variety of limitations and restrictions that need to be replaced by more effective strategies. 3D-printed polylactic acid (PLA) scaffolds are biocompatible, biodegradable and accomplish several biological and physical requirements for bone regeneration [1] with the additional possibility of design the macro- and microarchitecture of scaffolds according to the patient's needs. The combination of hydroxyapatite nanoparticles (nHA) enhance the osteogenic potential of PLA [2], promoting new bone formation, and the combination of metallic nanoparticles (mNPs) allows the elimination of the remain cancer cells, in alternatively to chemo- and/or radiotherapy, through their magnetic properties when applied an alternating magnetic field, causing a hyperthermia effect with a local increase of temperature to 40-45^{o}C [3]. Also, the addition of the antibiotic minocycline (MH) to PLA decreases the likelihood of an infection and have a positive effect on bone regeneration [2]. The aim of this work was to characterize and study the *in vitro* antimicrobial, biocompatibility and osteogenic properties of PLA scaffolds, produced by a 3D-printing technique previously optimized in our groups [2], functionalized with different combinations of nHA, mNPs and MH.The characterization of the scaffolds was performed through SEM and EDS analysis, were it was possible to conclude that the adsorption of nHA and mNPs was achieved and stable. Microbiological studies against *Staphylococcus aureus* (ATCC 25923) showed that the group with MH has antibiofilm properties. The biocompatibility of the scaffolds was evaluated by the AlamarBlue assay through which it was possible to confirm that the functionalized PLA scaffolds are not cytotoxic for the cells (MG-63 osteoblasts). The osteogenic capacity was assessed by the phosphatase alkaline (ALP) activity and Alizarin assays, both tests showed that the PLA scaffolds loaded with nHA have a slightly higher osteogenic potential. In conclusion, functionalized PLA scaffolds showed a promising application for directly targeting the bone. Further studies are needed to evaluate their potential application in bone tumours management.

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References

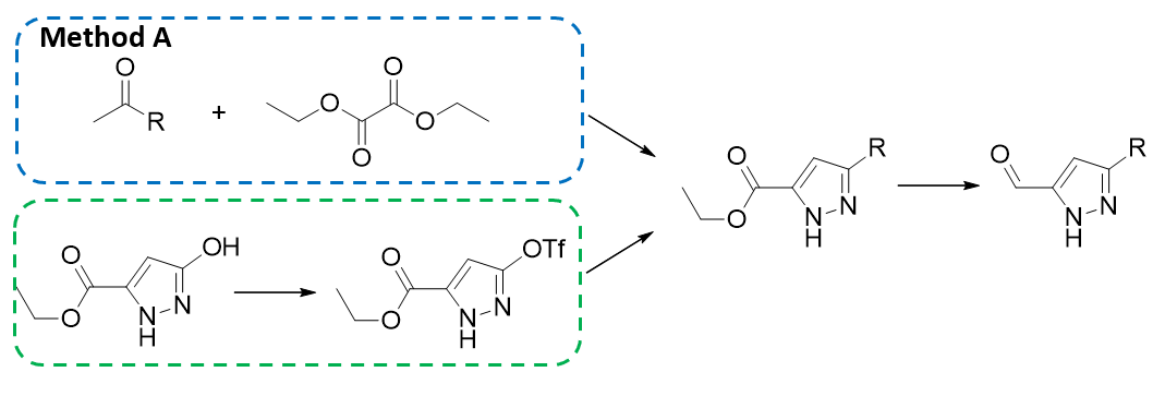
[1] Zhang J.; Zhao, S.; Zhu, M.; Zhu, Y.; Zhang, Y.; Liu, Z.; Zhang, C., J. Materials Chemistry B., 2014, 2, 7583-7595; [2] Martin V.; Ribeiro, I. A.; Alves, M. M.; Gonçalves, L.; Cláudio, R. A.; Grenho, L; Fernandes, M. H.; Gomes, P; Santos, C. F.; Bettencourt, A. F., Mat Sci and Eng C., 2019, 101, 15-26; [3] Herea, D.; Danceanu, C.; Radu, E.; Labusca, L.; Lupu, N.; Chiriac, H., Int J of Nanomedicine, 2018, 13, 5743-5751.

Synthesis Of 3-substituted 1H-pyrazole-5-carbaldehydes As Precursors Of Necroptotic Cell Death Inhibitors

Luz A.F.S. (1), Vieira A.C. (1), Guedes R.C. (1), Gomes-da-Silva L.C. (2), Moreira R. (1)

1- Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; 2- Chemistry Department, University of Coimbra, Coimbra, Portugal.

Necrosis has been traditionally regarded as passive and unregulated, therefore very little efforts had been made to investigate its mechanism, despite of its prevalence in human pathology. However, the identification of necroptosis' (a regulated form of necrosis) pathway mediators, such as receptor interacting protein kinase-1 (RIPK1) and 3 (RIPK3), and its impact in critical human illnesses, led to an intensive, but unaccomplished, search for high quality necroptosis inhibitors [1,2]. In order to find new necroptosis inhibitors, a high throughput cell-based phenotypic screening of AstraZeneca's compounds was performed in the iMed.ULisboa. New RIPK1 inhibitors hits, with IC50 values in the sub micromolar range and different scaffolds from the known necroptosis inhibitors [3], were identified. In this project, several 3-substituted 1H-pyrazole-5- carbaldehydes, precursors of the new antinecroptotic inhibitors' scaffold, were synthesised through two different methodologies (Scheme 1). The diversified groups introduced in the pyrazole C-3 position are crucial to synthesise dissimilar final structures, with the same new RIPK1 inhibitors' scaffold. These new molecules will be useful to develop relevant structure activity relationships, that will guide future derivations to create compounds with higher activity.



Scheme 1

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References

[1] Cho, Y. S.; Challa, S.; Moquin, D.; Genga, R.; Ray, T. D.; Guildford, M.; Chan, F. K., Cell, 2009, 137, 1112-1123; [2] Moreno-Gonzalez, G.; Vandenabeele, P.; Krysko, D. V., American Journal of Respiratory and Critical Care Medicine, 2016, 415-428; [3] Kopalli, S. R.; Kang, T. B.; Koppula, S., Expert Opinion on Therapeutic Patents, 2016, 1239-1256.

Activation Of miR-34a Correlates With NAFLD Progression And Disease Hallmarks

Simão A.L. (1), Rodrigues P.M. (2), Afonso M.B. (1), Santos-Laso A. (2), Jimenez-Agüero R. (2), Eizaguirre E. (2), Bujanda L. (2), Pareja M.J. (3), Bañales J.M. (2), Rodrigues C.M.P. (1), Castro R.E. (1)

1- Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; 2- Department of Liver and Gastrointestinal Diseases, Biodonostia Research Institute, Donostia University Hospital, University of the Basque Country (UPV/EHU), San Sebastian, Spain; 3- Hospital Juan Ramón Jiménez, Huelva, Spain.

Non-alcoholic fatty liver disease (NAFLD) pathogenesis remains incompletely understood. In this regard, we and others have shown that microRNAs (miRNA/miRs), particularly miR-34a, are key modulators of disease progression, from steatosis to non-alcoholic steatohepatitis (NASH), up to development of hepatocellular carcinoma. We now aimed to elucidate whether activation of miR-34a represents a universal event in etiological diverse diet-induced NAFLD models and which disease hallmarks correlate with miR-34a expression, pinpointing its main roles in disease pathogenesis. C57BL6 mice were fed five different NAFLD-inducing diets, namely a methionine and choline-deficient diet for 2 and 8 weeks; a high-fat choline-deficient diet for 14 weeks; a high fat, 2% cholesterol diet for 25 weeks; a high-fat/calorie diet with high fructose/glucose in drinking water for 16 weeks; and a choline-deficient amino acid-defined diet for 32 weeks. Liver biopsies were obtained from a cohort of 165 NAFLD patients, thoroughly characterized at the histological and metabolic levels (NAS≤2: n=26; NAS 3 to 4: n=62; NAS≥4: n=77). miRNAs were quantified by Taqman Advanced Real-Time RT-PCR. Mice fed any of the five diets developed different degrees of steatosis, NASH and fibrosis, with or without significant weight gains or development of insulin resistance. Nonetheless, liver miR-34a expression levels were significantly increased in all diseased mice ($p<0.05$), comparing with control diet-fed animals. NAFLD patients exhibited different degrees of steatosis and NASH, with or without the presence of fibrosis and concomitant diseases. Liver miR-34a expression was found to progressively increase with steatosis, lobular inflammation and NAS score ($p<0.05$ for all). Furthermore, miR-34a expression levels were significantly increased in patients with advanced fibrosis ($p<0.05$), as well as in those with concomitant diabetes, arterial hypertension and cholelithiasis ($p<0.05$). miR-34a expression also progressively increased with age, although women displayed lower expression levels comparing to men ($p<0.01$). Finally, bivariate analysis indicated that liver miR-34a expression positively correlated with histological findings (steatosis, lobular inflammation, fibrosis and NAS score), serum hepatic enzymes (AST and ALT), hepatic triglyceride content and age ($p<0.05$). In conclusion, activation of miR-34a appears to be a key event governing NAFLD pathogenesis and progression, correlating with specific, well-characterized disease hallmarks.

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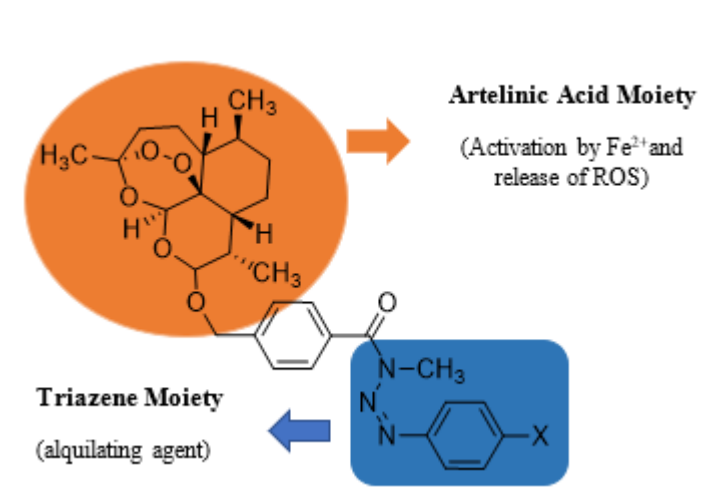
Lysosome As A Target For Antitumoral Tryazenes

Figueiredo A., Lopes F., Perry M.J.

Research Institute for Medicines (iMed.Ulisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal

Lysosome is an essential intracellular organelle since it is involved in vital cellular functions including the normal turnover of most proteins. It receives a large number of macromolecules delivered by endocytosis, phagocytosis and autophagy processes. In these, the reception of macromolecules containing iron, promote an iron-enriched environment, which can make it sensitive to oxidative stress [1]. Cancer cells have lysosomal alterations that cause an increased production of enzymes which lead to tissue invasion and tumour growth. Also, cellular iron metabolism pathways are disturbed in this type of cells – to maintain rapid growth and proliferation, cancer cells get larger amounts of iron by changing the iron metabolism proteins expression. Targeting enriched iron lysosome with substances that have accumulation in this cell component may lead to a selective toxic effect in tumoral cells and can be explored as a specific therapeutic target [2]. The development of small hybrid molecules (Figure 1) that combines Triazenes (DNA alkylating agent) with Artelinic Acid (an endoperoxide that is activated by Fe^{2+} and release Reactive Oxygen Species) constitutes a promising therapeutic approach [3].



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References

[1] Piao, S.; Amaravadi, R. K., Ann N Y Acad Sci., 2016, 1371(1), 45-54; [2] Zhou, L.; Zhao, B.; et al., Int J Mol Sci., 2018, 19(5); [3] Fröhlich, T.;, Kiss, A.; et al., ACS Med Chem Lett., 2018, 9(11), 1128-1133.

Genomic Characterization Of The Prophage Repertoire Of *Helicobacter pylori* Strains From Colombia

Muñoz A. (1,2), Trespalacios A.A. (1), Vale F.F. (2)

1- Infectious Diseases Group. Sciences Faculty. Pontificia Universidad Javeriana. Bogotá, Colombia; 2- Host-Pathogen Interactions Unit, Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal.

Helicobacter pylori is a bacterium associated with the most frequent chronic bacterial infection worldwide. In developing countries, its prevalence reaches up to 90%. Accordingly, in Colombia, the rate of infection is between 70% and 80% [1]. *H. pylori* is a genetically diverse bacteria, presenting a variability between strains that has been associated to the geographic origin of the human host. Such diversity may be influenced, among other things, by the presence of mobile genomic elements, such as prophages. There are currently four *H. pylori* prophage populations. This phylogeographic pattern discriminated one African, one Asian and two European populations [2]. This study seeks to investigate the characteristics of prophages present in Colombian *H. pylori* isolates. We have screened prophage presence in 213 *H. pylori* strains collected from Colombian patients suffering from chronic gastritis, which have been previously characterized for the presence of virulence factors. Integrase and holin genes were sought as markers of prophages presence, using the PCR technique with primers previously described by Vale et al. (2015) [3]. Among this group, 24 strains (11%) were positive for one of these genes, and only 8 strains (3.7%) were positive for both genes. As for the virulence factors, it was found that the most common phenotype was CagA positive/VacAs1am1/BabA positive/iceA1, which was present in 17 strains (56%). The frequency of the holin and integrase genes obtained in this study was lower than previous estimation of a frequency of prophages of around 20% in *H. pylori* strains. Whether there is a lower frequency of prophages in Colombian strains, or the primers used (designed over Asian and European prophage sequences) are not specific for South American prophage screening remains to be determined. So far there are no reports on the presence of prophages in the *H. pylori* isolated from Americas and if they exist, their sequences may be considerably different. To address this subject, the whole genome sequencing of positive strains for the presence of the holin or integrase genes along with some negative ones are currently being analyzed. These results are the first approach to understand and characterize the *H. pylori* prophages from Colombian.

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References

[1] Porras, C.; Nodora, J.; Sexton, R.; Ferreccio, C.; Jimenez, S.; Dominguez, R. L.; Cook, P.; Anderson, G.; Morgan, D. R.; Baker, L. H.; Greenberg, E. R.; Herrero, R., Cancer Causes Control., 2013, 24(2), 209-215; [2] Vale, F. F.; Nunes, A.; Oleastro, M.; Gomes, J. P.; Sampaio, D. A.; Rocha, R.; et al., Sci Rep., 2017, 7, 42471; [3] Vale, F. F.; Vadivelu, J.; Oleastro, M.; Breurec, S.; Engstrand, L.; Perets, T. T.; et al., Sci Rep., 2015, 5, 14333.

Targeting Mitochondria-Nucleus Crosstalk To Ameliorate Ageing-Impaired Neurogenesis

Estremores B., Sá Santos S., Costa M., Ribeiro M.F., Rodrigues C.M.P., Solá S.

Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal.

Although neurogenesis occurs in discrete areas of the adult mammalian brain, age-related decline of adult neurogenesis has been strongly correlated with ageing-associated cognitive dysfunction and neurodegeneration. Recent evidence also suggests that mitochondria affect the proliferative and differentiation potential of neural stem cells (NSCs), although the underlying molecular mechanisms are still not fully understood. The mitochondrial deacetylase Sirtuin 3 (SIRT3) has been described as a central player in mitochondrial metabolism and oxidative protection. In addition, tert-butyl hydroperoxide (tBHP) was shown to induce oxidative stress and ageing in NSCs. The aim of this study was to further dissect how SIRT3 regulates NSC ageing. Our results demonstrated that incubation of NSCs with tBHP resulted in decreased NSC viability, increased senescence, and decreased differentiation and proliferation potentials. More importantly, SIRT3 overexpression was able to revert changes in differentiation and stemness potential of NSCs treated with tBHP. SIRT3-induced changes in aged NSCs occurred concomitantly with increased activation of superoxide dismutase 2 (SOD2)-deacetylase, a major superoxide-scavenger in mitochondria, and telomerase reverse transcriptase (TERT), a key catalytic subunit of the anti-ageing enzyme telomerase. Interestingly, the inhibition of the long chain acyl-coenzyme A dehydrogenase (LCAD) protein, a putative downstream target of SIRT3 involved in mitochondrial lipid metabolism, was shown to decrease NSC differentiation potential. Ongoing co-modulation and immunoprecipitation experiments are now focused in clarifying whether LCAD is a direct target of SIRT3 and are required for rescuing NSCs from ageing. Overall, our results reinforce the role of mitochondrial activity in regulating age-related NSC fate alterations, while also suggest that targeting mitochondrial oxidative state and metabolism could be a promise strategy to arrest neurogenesis decline and cognitive deficits throughout ageing.

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Atypical And Canonical Sphingoid Bases Affect The Membrane Integrity And Membrane Biophysical Properties Of Membrane Models

Mestre B. (1), Sousa C. (1), Santos T. (1,2), Almeida R.F.M. (3), Silva L.C. (1)

1- Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; 2- Centro de Química Física Molecular and Institute of Nanoscience and Nanotechnology, Instituto Superior Técnico, Universidade de Lisboa, Lisboa, Portugal; 3- Centro de Química e Bioquímica, DQB, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal.

The sphingoid bases are produced at the initial step of the *de novo* biosynthesis of sphingolipids (SL), by the condensation of palmitoyl-CoA with the amino acid serine, a reaction catalyzed by serine palmitoyl transferase (SPT)[1]. Besides the canonical substrates, SPT can also use L-alanine as substrate forming atypical 1-deoxy-sphingolipids (1-deoxySL). 1-DeoxySL differ structurally from canonical sphingolipids by the lack of the C1-hydroxyl group and the position of the double bond (DB)[2]. The absence of this OH-group not only precludes the synthesis of complex sphingolipids, but also blocks their degradation by canonical degradation pathway. Consequently, 1-deoxySL might accumulate in cellular compartments disrupting its normal functioning. In healthy individuals, 1-deoxySL exist at low levels in plasma, but are found elevated in neurological and metabolic disorders [3]. To gain further insight into the mechanisms that might underlie deoxySLs pathological actions, we used fluorescence-based methodologies to investigate how the atypical 1-deoxysphinganine (1-deoxySA) and 1-deoxysphingosine (1-deoxySO) long chain bases (LCB) affected membrane permeability. For comparison, we also studied the effects of the canonical sphinganine (SA) and sphingosine (SO) counterparts on membrane permeability. Preliminary results showed that the interaction of the LCBs with the bilayer caused an increase in membrane permeability. This effect was more pronounced in the presence of DxSA and DxSO and for membranes containing higher liquid ordered phase fraction. These results show that deoxy-LCB bases have higher ability to disrupt the permeability of the membranes compared to their canonical counterparts, and suggest that elevated levels of deoxy-LCB may influence membrane integrity and subsequently impair cellular function. Further studies are being performed to understand the impact of these atypical SL in the biophysical properties of the membranes.

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References

[1] Gault, C. R.; Obeid, L. M.; Hannun, Y. A., Adv. Exp. Med. Biol., 2010, 688, 1-23; [2] Steiner, R.; et al., J. Lipid Res., 2016, 57, 1194-1203; [3] Othman, A.; et al., Diabetologia, 2012, 55, 421-431.

A Novel Strategy To Examine Dry Powder Biorelevant Dissolution In Lung Simulated Mucus

Noriega B. (1,2), Malmlöf M. (3), Gerde P. (3), Costa E. (1), Corvo M.L. (2)

1- Hovione FarmaCiencia SA, Portugal; 2- BioNanoSciences, Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; 3 - Inhalation Sciences, Sweden.

The therapeutic efficacy of inhaled drugs is limited by their rapid clearance in the lungs and a short residence time, which can imply frequent dosing (and thus lower patient compliance). The development of controlled release formulations (CRF) can be a strategy to maintain drug levels and improve therapeutic outcomes [1]. Hence, a biorelevant dissolution method is necessary capable of assessing the dissolution and permeation of the API which deposits in the lung, and recording particle dissolution to understand what controls it. DissolvIt® is a dissolution system which simulates the physiological conditions in the lung [2]. It is combined with an automated system simulating human breath to collect aerosolized powder on glass coverslips [3]. The main goal of the present work is to assess how two commercial dry powder inhalers (DPI) behave in the presented dissolution system, and evaluate its suitability to assess CRF performance. Flixotide Diskus (Fluticasone Propionate) and Pulmicort Flexhaler (Budesonide) were used. PreciseInhale® was employed to aerosolize and collect the DPI on coverslips, and three coverslips evaluated in the DissolvIt® system. The mucus simulant contains polyethylene oxide (MW 5 000 000) and 0.4% L-alpha phosphatidyl choline (both SigmaAldrich). The perfusate is phosphate buffer (7.4 pH) with albumin (ChemCruz). Dissolution takes place in the dissolution chamber (50 µm-thick layer of mucus and polycarbonate membrane) followed by diffusion to the perfusate, at 37°C. The particle dissolution was followed with a microscope.Particles in mucus (Figure 1) and dissolution/diffusion profiles (Figure 2) show the dissolution behavior of the two DPIs, containing lactose and drug particles. Most of the powder on the coverslip disappears after contacting the mucus, most likely the lactose particles. This is confirmed by the dissolution profiles, which do not show a drug concentration peak in the first minute. This shows that the system can differentiate the dissolution of the different components of a formulation, by crossing information. Moreover, the particles of the budesonide DPI completely disappear after 12 min, while the fluticasone's are still present after 4 hours. This is in accordance with the dissolution profiles. Hence, the system can differentiate the dissolution formulations with different solubilities.

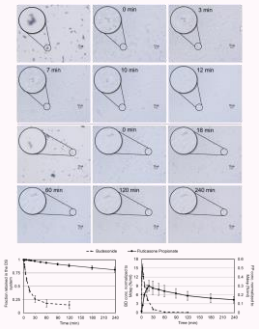

Figure 1 and 2

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References

[1] Loira-Pastoriza, C.; Todoroff, J.; Vanbever, R., Adv. Drug Deliv. Rev., 2014, 75, 81-91; [2] Gerde, P.; et al., Assay Drug Dev. Technol., 2017, 15(2), 77-88; [3] Noriega, B.; Malmlöf, M.; Corvo, M. L.; Gerde, P.; Costa, E., Respiratory Drug Delivery, 2018.

S100B Specific Inhibition Prevents CNS Damage And Inflammation In The *In Vivo* Model Of Multiple Sclerosis

Soromenho B. (1), Barros C. (1), Barateiro A. (1,2), Freitas R. (3), Brites D. (1,2), Graça L. (3), Fernandes A. (1,2)

1- Neuron-Glia Biology in Health and Disease, Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; 2- Department of Biochemistry and Human Biology, Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; 3- Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, Lisbon, Portugal.

Multiple Sclerosis (MS) is a chronic autoimmune disease of the central nervous system (CNS). Alongside with immune cell infiltration, MS is characterized by inflammation, demyelination and gliosis, leading to neurodegeneration. S100B, a small calcium-binding protein, has been emerging as an important inflammatory marker in neurodegenerative and neuroinflammatory disorders as MS. We showed that this protein was present in the CSF and serum of MS patients and, recently, increased levels of S100B were directly correlated with demyelination and inflammatory processes. Moreover, its inhibition using an *ex vivo* demyelinating model showed that S100B may be an emerging therapeutic target in MS. Here, we aimed to understand whether the targeting of S100B, using the specific small molecule pentamidine, in the *in vivo* model of MS, the experimental autoimmune encephalomyelitis (EAE), could modulate and even, ameliorate MS-like pathogenesis. EAE was induced in female C57BL/6J wild-type mice and two groups were formed: EAE vehicle group (saline) and EAE-treated group (pentamidine, 4 mg/kg, intraperitoneal, daily). Then, clinical score and body weight were evaluated during 30-days of experiment, along with locomotor performance (Rotarod and Pole Test) prior to the peak of disease. Brain and spinal cord (SC) were sectioned to evaluate demyelination extent, immune cell infiltration, oligodendrogenesis, astroglial reactivity and S100B expression. Animals treated with pentamidine showed a less severe EAE phenotype, reaching a lower disease clinical score and having a faster recovery. This was accompanied with less locomotor impairment when compared with the diseased ones. EAE-induced animals had increased demyelination and cell infiltration in brain and SC, which were prevented in pentamidine-treated animals. We also observed a marked astroglial reactivity with expression of S100B upon EAE induction. Most attractively, the treatment with pentamidine reduced not only oligodendrogenesis impairment, but also the astrocytic reactivity and S100B expression. Overall, our results indicate that S100B is involved in MS pathology, being expressed by reactive astrocytes present in the attacked white matter, and that its inhibition may be a new therapeutic strategy to reduce CNS damage and improve recovery.

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Restoring Drug Resistant Mycobacteria Susceptibility To Beta-lactam Antibiotics

Gama B., Olivença F., Carmo N., Pires D., Anes E., Catalão M.J.

Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal.

Tuberculosis is one of the top 10 causes of death worldwide, and the emergence of multi (MDR-TB) and extensively drug resistant (XDR-TB) *Mycobacterium tuberculosis* strains is a major public health concern, with an estimated number of 460 thousand cases in 2017. The potential use of antibiotics that are not usually included in tuberculosis treatment is currently being considered and recent studies have highlighted the potential use of carbapenems to treat MDR-TB. Carbapenems are a subclass of beta-lactam antibiotics, which target peptidoglycan biosynthesis, that are particularly resistant to inactivation by the BlaC beta-lactamase, produced by *Mycobacterium tuberculosis*. Mycobacteria have a characteristic cell envelope, consisting of a long chain mycolic acids layer, a highly branched arabinogalactan polysaccharide and a very cross linked and modified meshwork of peptidoglycan. This barrier contributes to the virulence, persistence and intrinsic resistance of mycobacteria to several drugs, and modulates host-pathogen immune response. In this project we intend to study if exposure to isoniazid and ethambutol – antibiotics that inhibit the synthesis of mycolic acids and arabinogalactan – lead to increased accessibility of peptidoglycan to antibiotics that target its biosynthesis. To address this, minimum inhibitory and bactericidal concentrations (MIC and MBC) to beta-lactams, isoniazid and ethambutol alone were determined. Carbapenems MIC values were lower than other beta-lactams, but all were bacteriostatic. Isoniazid and ethambutol were mostly bactericidal. We also tested if exposure of mycobacteria to isoniazid or ethambutol in subinhibitory concentrations (MIC/2 and MIC/4) and subsequent and/or simultaneous exposure to beta-lactams could improve beta-lactams efficacy. When mycobacteria were pre-treated with ethambutol, both MIC and MBC values for amoxicillin and meropenem (conjugated with the beta- lactamase inhibitor clavulanate) were significantly reduced, suggesting that the inhibition of the synthesis of cell envelope components with ethambutol, leads to peptidoglycan exposure to beta-lactams. In addition, mycobacteria immune recognition by human THP-1 macrophages will be addressed, allowing to correlate inhibition of mycolic acids synthesis with recognition of mycobacterial peptidoglycan. With this work we expect to establish a connection between unknown mechanisms of resistance and potential vulnerabilities in the cell envelope of mycobacteria, which could be exploited for therapeutic purposes.

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Immunogenicity In Fabry Disease: The Role Of Enzyme Replacement Therapy

Araújo C. (1), Gouveia P. (2), Olga Azevedo (3) Goncalves J. (1)

1- Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; 2- Hospital de Senhora da Oliveira, Guimarães, EPE, Portugal; 3- Serviço de Medicina Interna, Hospital de Santa Maria, Lisboa, CHU, Portugal.

Background: Fabry disease (OMIM #301500) is an X-linked disorder caused by alpha galactosidase A deficiency with two major phenotypes: classic and non-classic as males the most affected with the worst prognosis. Enzyme replacement therapy (ERT) are available since 2001 and is the most important therapeutic solution for those patients. Nevertheless, ERT may elicit anti-drug antibodies (ADA) as well as infusion associated reactions (IARs) leading to loss of clinical efficacy and safety issues in some patients.
Aim: We aimed to determine the epidemiology and functional characterization of anti-drug immune responses in a set of Fabry Portuguese patients, mainly the ADA with capability to neutralize the pharmacological activity of Agalsidase® alfa (Shire, Human Therapies, Inc).
Methods: Fabry patients from North of Portugal (n=52) and Lisbon area (n=125) were cross-sectional screened for anti-agalsidase IgG1, IgG4 and IgE antibodies (AAA) with an enzyme-linked immunosorbent assays (ELISA). Quantification of anti-agalsidase lambda and kappa light chain antibodies were further determined with specific ELISA assay. Furthermore Th1/Th2/Th17 cytokine expression profiles were measured from sera samples previous screened for ADA+ status with Luminex® xMAP® technology. Finally, screening of neutralizing antibodies is being carried out by microscale thermophoresis technology.
Results: Among patients exposed to agalsidase, 49% (n=86/177) had anti-agalsidase antibodies with 15.3% (n=27/177) for IgG1, 18.6% (n=33/177) for IgG4 and 31% (n=54/177) for IgE. Cytokine expression analysis have shown clearly an upregulation of pro-inflammatory response with a fold change of 6.1, 4.8, 4.6 and 7.8 for IL-1β, IL-2, IFN-γ and TNF-α respectively and relative to a healthy control ($p<0.05$) whereas for IL-4 and IL-10 a fold change of 0.01 and 0.15 suggested a downregulation of the anti-inflammatory immune response.
Conclusions: This study is underway with the development of assays to screen and characterize enzyme inhibition uptake by neutralizing antibodies. For that, we are employing sensitive thermophoresis to characterize binding affinity. Together these data will be correlated with clinical assessments in order to better understand the immunogenicity behavior of Fabry and therefore help clinicians to manage the disease with better outcomes.

Acknowledgements

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Neural Stem Cell Secretome As A Potential Therapeutic Tool For Neurodegeneration

Lopes C.R., Camões S., Estremores B., Sá Santos S., Ribeiro M.F., Rodrigues C.M.P., Miranda J., Solá S.

Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal.

Neural stem cells (NSCs) are present in well-defined neurogenic niches of the adult mammalian brain, where they maintain the ability to self-renew and differentiate into new functional neural cells throughout life. Curiously, emerging evidence suggests that NSCs may also induce neural regeneration and protection by cell-to-cell communication events, specifically through their paracrine activity. Additionally, three-dimensional (3D) culture systems, which better resemble the *in vivo* physiology, have already been proven to enhance the cell secretome profile. Thus, the aim of this project was to explore the therapeutic potential of NSC-derived secretome in different *in vitro* models of neural damage and test the best culture system for improving the therapeutic properties of NSC-released factors. Initially, different *in vitro* models of neurodegeneration were established using undifferentiated and differentiated mouse NSCs as well as a neuroblastoma cell line (N2a). Different exposure times and concentrations of 1-methyl-4-phenylpyridinium (MPP^+) and hydrogen peroxide (H_2O_2) were tested in order to mimic Parkinsonism and oxidative stress conditions, respectively, as assessed by decreased cell viability. Subsequently, for NSC secretome collection, a NSC spheroid culture model using Spinner flasks (3D system) was developed and characterized with respect to spheroid formation, cell phenotype and cell viability. NSC spheroid diameters were approximately 250 µm, and hematoxylin staining revealed compact spheroids with viable and evenly distributed cells and the absence of inner necrotic cores. However, the biochemical characterization of both systems, indicated that NSC spheroids present higher levels of neuronal differentiation markers when compared with the monolayer system, including βIII-tubulin, MAP2, as well as other metabolic markers associated with differentiation. Ongoing studies will clarify the effects of both NSC-derived secretomes in rescuing neural death induced by MPP^+ and H_2O_2, and establish comparisons between both culture systems. Ultimately, the molecular characterization of the most promising NSC-derived secretome will be performed to unveil the precise delivered molecules responsible for neuroprotection.

Acknowledgements

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Polymeric Micellar Formulation Enhances Anticancer Properties Of Salinomycin

Sousa C., Silva-Lima B., Videira M.

Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal.

Salinomycin (SAL) is a polyether antibiotic that exhibits a strong anticancer activity. It is known to interfere with the epithelial-to-mesenchymal transition (EMT), by upregulating epithelial markers, such as E-cadherin and keratin, and downregulating the mesenchymal markers, such as N-cadherin and vimentin (VIM). Highly linked with cell malignancy, the protein VIM is an intermediate filament whose function is dependent on phosphorylation/de-phosphorylation dynamics. The efflux of an anticancer drug to the cell microenvironment is accomplished by the intervention of three different members of the ABC family – MRP1, BCRP/ABCG2, and the most known, P-gp (P-glycoprotein) mediated membrane efflux. This ATP-dependent surface transporter is overexpressed in tumor cells, aiming to avoid intracellular drug accumulation. Class 2 (low solubility-high permeability) and class 4 (low solubility-low permeability) drugs - BCS– such as SAL, are considered P-gp's substrates. Therefore, nanoparticles are being developed to become (nano)carriers for such drugs. Nanocarriers are nanometer systems which provide both selectivity and safety once they are designed to target specific cell populations, avoiding drug adverse effects on healthy cells. In the present work, polymeric micelles were prepared using Pluronic® F127 (PM) to encapsulate SAL (PM_SAL) with the view of enhancing its anticancer activity. Effects of this formulation were assessed on cancer cell line A549, i.e. cell viability, prevention of P-gp efflux, VIM expression, and effects on migratory ability. PM_SAL demonstrated a 15-fold increase in P-gp's expression as well as a significant decrease of the cell's migration. PM_SAL can also interfere with the oncogenic protein VIM, involved in the crucial mechanism EMT, by downregulating its expression. Altogether the data obtained indicates that this antibiotic and the developed polymeric micelle system is a very promising inhibitor of tumor cell growth.

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References

Naujokat, C.; Steinhart, R., J Biomed Biotechnol., 2012, 2012, 44-46; Kidd, M. E.; Shumaker, D. K..; Ridge, K. M., Am J Respir Cell Mol Biol., 2014, 50(1), 1-6; Sulová, Z.; Šereš, M.; Barančík, M.; Gibalová, L.; Uhrík, B.; Poleková, L.; et al., Gen Physiol Biophys., 2009, 28, 89-95.

Multi-Targeting Approach In Glioblastoma Using Computational Tools To Overcome Blood-Brain Barrier And Target EGFR/PI3K Signaling

Franco C. (1,2), Kausar S. (1,2), Guedes R.C. (2,3), Falcão A. (1), Brito M.A. (2,4)

1- Department of Informatics, Faculty of Sciences, Universidade de Lisboa, Lisbon, Portugal; 2- Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; 3- Department of Pharmaceutical and Therapeutic Chemistry, Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; 4- Department of Biochemistry and Human Biology, Faculty of Pharmacy, Universidade de Lisboa, Portugal.

The discovery and development of new drugs for brain pathologies is challenging, mainly due to the blood-brain barrier (BBB) that limits access of almost all molecules into the brain. In this context, glioblastoma (GB) remains an incurable disease owing to the presence of the BBB and the molecular heterogeneity of the tumor. Amplification and mutation of the epidermal growth factor receptor (EGFR) gene represent signature genetic abnormalities encountered in GB, becoming an attractive target for therapeutic strategies. However, the activation of compensatory pathways that generates multiple inputs to downstream PI3K signaling leads to acquired anti-EGFR therapeutic resistance. Thus, the hallmark of this work is proposing a new cost-effective *in silico* strategy to predict the ability of candidate molecules to overcome the BBB and inhibit the EGFR/PI3K pathway in a multi-targeting approach. We used an automated and expandable framework and machine learning methods to build three quantitative structure-activity relationship (QSAR) models with reduced prediction error (calculated as the root mean square error, RMSE) and high percentage of variance explained (PVE): prediction of BBB permeability (RMSE=0.3, PVE=0.55), inhibition of EGFR (RMSE=0.17, PVE=0.70) and inhibition of PI3K (RMSE=0.16, PVE=0.71). These models were used in a virtual screening of ZINC15 commercial database, from which we selected the most promising candidate compounds that predictably cross the BBB (LogBB≥0.3, representing the logarithmic ratio between the concentration of a drug in brain and blood sides) and inhibit the considered targets according with their chemical structure ($-pIC_{50}\geq 6$, representing the scaled value of half-maximal inhibitory concentration). For each target was validated a 3D structural model (EGFR, $r^2=0.9036$, RMSD=1.251; PI3K, $r^2=0.8811$, RMSD=0.342) to perform a docking simulation and evaluate the interaction of selected molecules with the respective target. Finally, the ADMET properties were accessed to ascertain the drug-like phenotype of molecules, allowing the identification of 27 hit compounds that meets the requirements to enter in pre-clinical studies, namely 13 candidates for EGFR, 9 candidates for PI3K and 5 candidates for dual inhibition of both targets. These results suggest that this is a valuable approach to achieve a robust anti-tumor effect against GBM and speed up the drug discovery process.

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Polyoxometalates As Aquaporins Inhibitors With Potential Anticancer Properties

Pimpão C. (1), Coxixo A.S. (1), Aureliano M. (2), Rompel A. (3), Soveral G. (1)

1- Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; 2- Faculty of Sciences and Technology, University of Algarve, Faro, Algarve, Portugal; 3-Universität Wien, Fakultät für Chemie, Institut für Biophysikalische Chemie, Wien, Austria.

Aquaporins (AQPs) are transmembrane protein channels that facilitate the diffusion of water and glycerol across cell membranes, crucial for water and energy homeostasis. These proteins were found overexpressed in different cancer cells and tissues, being involved in cell proliferation and migration, tumor formation, and angiogenesis, suggesting their great potential as novel drug targets for cancer treatment. Identification of potent and selective aquaporin inhibitors to be used in cancer therapeutics is of utmost importance. Polyoxometalates (POMs) are transition metal complexes that exhibit a broad diversity of structures and properties. POMs are able to inhibit phosphatases, ectonucleotidases, and Ptype ATPases thus affecting several biochemical pathways, rendering them promising for biological purposes. In this work, we screened POMs as inhibitors of aquaporin-mediated membrane permeability in human red blood cells (RBCs) and further validated their potency and selectivity in yeast cells transformed with human AQP1 and AQP3. Among the various compounds tested, we identified one polyoxotungstate (POT) as a potent inhibitor of glycerol permeability via AQP3 (IC_{50}≈ 0.74 ± 0.14 µM) and lack of effect on water permeability *via* AQP1. Moreover, the effect of POT on tumor progression was investigated in pancreatic cancer cells (BxPC3). The obtained marked decrease in cell proliferation (IC_{50}≈ 9.15 ± 0.65 µM) and impairment of cell migration (20% reduction) revealed promising anticancer properties of this compound that correlate with its AQP3 inhibitory feature. Further studies are ongoing to fully characterize the selectivity, potency, and toxicity of this POT, establishing polyoxotungstates as novel AQP inhibitors with high potential for cancer therapeutics.

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Protection Of Human Phenylalanine Hydroxylase Activity By Small Molecules Targeting The Enzyme Catalytic Center

Lopes R.R. (1), Russo R. (1), Madeira C.A. (1), Tomé C. (2), Teixeira M. (2), Vicente J.B. (2), Guedes C.R.(1), Góis P.M.P. (1), Leandro P. (1)

1- Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; 2- Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Oeiras, Portugal.

Phenylketonuria (PKU) is an autosomal recessive inborn error of metabolism caused by a deficient activity of human phenylalanine hydroxylase (hPAH) a non-heme iron homotetrameric enzyme that hydroxylates L-Phe into L-Tyr in the presence of O_2 and tetrahydrobiopterin (BH4). In order to increase hPAH stability and/or activity, in this work, twelve small molecules were designed based on the properties and structural characteristics of the catalytic hPAH ligands (L-Phe, BH_4 and Fe). From this in-house library the most promising molecules were selected based on their effect over the activity and thermostability of the recombinant hPAH. The selected molecules were further tested by: (i) electron paramagnetic resonance (EPR), to confirm Fe interaction; (ii) limited proteolysis, to identify the mechanism of action; surface plasmon resonance, to determine the KD and; (iii) cytotoxicity to confirm the molecules biocompatibility. Selected compounds were also studied for their effect on the hPAH activity along incubation time at 42°C. Four molecules were considered the most promising candidates. They presented a slight inhibitory effect (17 to 40% enzyme activity decrease) but allowed further activation by L-Phe (1.3 to 1.7-fold). Two of the selected molecules stabilized the hPAH regulatory domain (ΔTm1: +8.3 and +4.0°C). An interaction with the catalytic iron was suggested by the observed changes in the EPR spectra and the molecular docking studies, which also indicate that the poses adopted by the quinoline ring and the side chain of the molecules significantly overlapped the position of BH4 and the substrate analog, respectively. At 100 µM the selected compounds did not present any toxic effect either on cell viability (62±4% to 101±5%) and membrane integrity (propidium iodide intake ≈1). Interestingly one compound was able to protect enzyme activity (1.5-fold increase compared to the control; 42°C for 60 min). From our series of molecules two compounds emerged as pharmacological chaperones and one as activity chaperone. Our data also provided proof-of-concept for the utilized strategy of compound design targeting the hPAH catalytic center.

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Improving Food Packaging Antimicrobial Properties To Enhance Food Safety: Can Wild-Fruit Extract Films Be A Viable Alternative?

Severo C., Bettencourt A., Ribeiro I.A.C.

Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal.

Numerous outbreaks of foodborne pathogens have been found to be associated with biofilms, which poses a concern to the food industry. There are current strategies to control this problem such as chemical treatments, however, there has been an arising problem of resistant strains that decreased the effectiveness of these conventional methods. Thus, there is a need for the development of novel strategies that can control biofilm formation, especially regarding food packaging material incorporated with antimicrobial agents such as phenolic compounds. Berries, for instance, have a high content in phenolic compounds and are suggested to have numerous health benefits for humans. The main aim of this research was to develop biodegradable films functionalized with berry extracts intended for food packaging and to study their antimicrobial impact against relevant biofilm forming bacteria. This study started by evaluating the antimicrobial properties and antioxidant capacity of three berry extracts sources, respectively fresh, dried and powder berry extracts. Then different formulations of (medium and high molecular weight) chitosan biodegradable films were prepared, their antimicrobial properties were assessed (i.e. the Kirby Bauer test) and their physical properties were evaluated (i.e. contact angle, swelling degree, total soluble matter, moisture content and UV transmission and transparency) to select the best formulations. The results revealed that all berry extracts presented antimicrobial and antioxidant properties especially powder and fresh berry extracts and, as expected, when incorporating berry extracts, films showed enhanced antimicrobial properties comparatively with the extract-free films. From physical properties evaluation, it was possible to select as best formulations the ones prepared with lower amount of medium weight chitosan. Results also showed that berry extracts incorporation increased films wettability and lowered films UV transmission and transparency. Overall, the investigation revealed the potential of berry extract films for the improvement of food packaging material antimicrobial properties.

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References

Zhao, X.; Zhao, F.; Wang, J.; Zhong, N., RSC Advances, 2017, 7(58), 36670-36683.

Small Molecule PGC-1α Activators: A Novel Approach To Anti-Neurodegenerative Therapeutics

Ranito C. (1), Rodrigues E. (1,2), Carvalho A.N. (1,2), Gama M.J. (1,2), Ruas J.L. (3)

1- Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; 2- Department of Biochemistry and Human Biology, Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; 3- Department of Physiology and Pharmacology, Molecular and Cellular Exercise Physiology Unit, Karolinska Institutet, Stockholm, Sweden.

Parkinson's Disease (PD) is a neurodegenerative movement disorder whose progression is highly influenced by mitochondrial dysfunction and reactive oxygen species (ROS) accumulation.Peroxisome proliferator-activated receptor γ (PPARγ) coactivator 1α (PGC-1α) is a transcriptional coactivator which, apart from its many distinct biological functions, is mainly responsible for the regulation of cellular bioenergetics through the regulation of genes involved in mitochondrial biogenesis and oxidative metabolism. PGC-1α is mainly expressed in tissues with a high energy demand, such as heart, muscle, liver and brain. In the brain, the isoform PGC-1α1 has been implicated in ROS abrogation and protection from neurodegeneration. Therefore, PGC-1α1 has been proposed as a potential therapeutic target for early intervention in PD. The main goal of this project is to explore proprietary small molecules, selected from a unique screening platform, that activate PGC-1α and its neuroprotective biological activities as new therapeutics for PD. Herein, we tested the potential of these small-molecule compounds to stabilize PGC-1α in a murine neuroblastoma cell line (N2a). For that, the expression levels of PGC-1α in the presence of the selected compounds were evaluated by Western Blot. Moreover, relative mRNA levels of PGC-1α target genes were evaluated by qRT-PCR. In order to exclude potentially cytotoxic compounds MTT and lactate dehydrogenase (LDH) assays were conducted for the tested compounds. Treating the N2a cells with 1-methyl-4-phenylpyridinium (MPP^+), as a cellular model of PD, we also tested the potential rescuing effect of the previously selected compounds, as assessed by different parameters of mitochondrial function. We were able to select some compounds that lead to increased expression of PGC-1α downstream targets in N2a cells. Moreover, preliminary results on the protection ability of those compounds against MPP^+ were also obtained. Overall these results contribute to elucidate the potential protective role of these compounds in a model of PD.

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Astrocytes Transdifferentiated From Als Patient-Fibroblasts Show Diverse Reactive Subpopulations And Downregulated Mir-146a As A Theragnostic Target

Gomes C. (1,2), Sequeira C. (1), Likhite S. (2), Dennys C.N. (2), Kaspar B.K. (2), Meyer K. (2-5), Brites D. (1,6)

1- Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; 2- The Research Institute, Nationwide Children's Hospital, Columbus, 43205; 3- Molecular, Cellular and Developmental Biology Graduate Program, The Ohio State University, Columbus, OH 43210; 4- Neuroscience Graduate Program, The Ohio State University, Columbus, OH 43210; 5- Biomedical Sciences Graduate Program, The Ohio State University, Columbus, OH 43210; 6- Department of Biochemistry and Human Biology, Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal.

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by progressive loss of motor neurons (MNs) and astrocyte aberrancy in the brain and spinal cord. Our previous work in the transgenic mouse SOD1G93A model identified a specific and neurotoxic astrocytic phenotype characterized by low GFAP and high Cx43, S100B and Ki-67 expression, together with decreased miR-146a (in the cortex) or increased miR-155 (in the spinal cord) [1,2]. Transposition of findings in these models to humans has been hampered by the limited access to CNS samples, now overcome by cell reprogramming. Here, we explored aberrant phenotypic diversity in astrocytes from sporadic (sALS) and SOD1 mutant ALS (fALS) patient fibroblasts, using direct transdifferentiation [3]. Fibroblasts from a total of 7 patients and 2 controls were directly converted into induced neural progenitor cells and subsequently transdifferentiated into induced astrocytes (iAstrocytes). All patient derived astrocytes displayed toxicity toward MNs by reducing neurite ramification. However, we observed that the aberrant astrocyte markers diverged among patient subpopulations. While almost all cell lines presented an increased proliferative capacity, reduced GFAP levels were found in most fALS cases and increased Cx43 expression mainly in sALS cell lines. Downregulated miR-146a was noticed in 1 fALS and 2 sALS, whereas 1 fALS and 1 sALS showed its upregulation, validating our previous findings in mice. Such up-/down-regulation discrepancies were replicated in astrocyte-derived small extracellular vesicles (EVs). Upregulation of miR-146a in deficient astrocytes counteracted some cell reactive and inflammatory aberrancies and had impact in improving axonal transport and synaptic signaling when co-cultured with NSC-34 motor neurons. In summary, our data identify miR-146a as a therapeutic target in a specific subpopulation of ALS patients and highlight transdifferentiated astrocytes as a promising personalized prediction model and a tool for assessing treatment benefits.

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References

[1] Gomes, C.; et al., Mol. Neurobiol., 2019, 56(3), 2137-2158; [2] Cunha, C.; et al., Mol. Neurobiol., 2018, 55(5), 4207-4224; [3] Meyer, K.; et al., Proc. Natl. Acad. Sci. USA, 2014, 111(2), 829-832.

Exploiting Exosomes From Human MSC 3D Cultures For Cutaneous Wound Healing

Dias C.T. (1)*, Camões S.P. (1)*, Rodrigues J.S. (1), Oliveira N.G. (1), Santos J.M. (2,3), Miranda J.P. (1)

*1- Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; 2- Centro de Estudos de Ciência Animal (CECA), Instituto de Ciências e Tecnologias Agrárias e Agro-Alimentares (ICETA), Universidade do Porto, Oporto, Portugal; 3- Instituto de Tecnologia Química e Biológica António Xavier (ITQB NOVA), Universidade Nova de Lisboa, Oeiras, Portugal * Both authors contributed equally.*

The success of wound healing is impaired in several medical conditions resulting in increased morbidity. The current available therapeutic options fail to promote full tissue regeneration and stem cell-based therapies have emerged as promising alternative strategies for wound healing. Within this context, mesenchymal stem/stromal cells (MSCs) gained particular relevance due to their role in tissue regeneration, not only due to cell differentiation, but mostly via paracrine mechanisms. Previous work has shown that the wound healing potency of umbilical cord tissue-derived MSCs (UC-MSCs) secretome can be primed by culturing cells as self-assembled spheroids under three-dimensional (3D) culture conditions. More recently, the secretion of exosomes has been suggested as a dominant mechanism by which MSCs exert their healing function. This study aimed at evaluating the role of exosomes derived from UC-MSCs primed by 3D culturing on cutaneous wound healing using *in vitro* methodologies. As such, the whole secretome was obtained by collecting and concentrating the conditioned media from MSCs cultured either under conventional two-dimensional monolayer conditions (CM2D) or in 3D spinner flask bioreactors (CM3D). Exosomes were isolated from CM2D (Exo2D) and CM3D (Exo3D) by size exclusion chromatography which allowed the purification without co-precipitation with other particles. The size distribution of the isolated exosomes (135 ± 54 nm and 265 ± 37 nm for Exo2D and Exo3D, respectively) pointed out the influence of the culture system in its morphology, although without compromising the presence of CD9 and CD81 exosomal surface markers. To assess the role of UC-MSC exosomes on the viability/proliferation and migration of skin cells, a human keratinocyte HaCaT cell line was used for MTS and scratch assays. Preliminary results revealed that both Exo3D and Exo2D enhanced the mitogenic and motogenic capacities of HaCaT cells. In particular, the data so far pointed out that Exo3D (5-100 µg/mL) induce the keratinocyte viability/proliferation whereas the same was only observed at lower concentrations of Exo2D. These results suggest that MSCs-derived exosomes promote motogenic and proliferative effects, both important in wound healing, granting their potential new role as active players in cell-free-based therapies.

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Design, Synthesis And Evaluation Of Hypoxia-Activated Triazene Prodrugs

Braga C. (1,2), Moreira R. (1), Bernardes G. (2), Perry M.J. (1)

1- Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; 2- Instituto de Medicina Molecular, Faculdade de Medicina da Universidade de Lisboa, Lisboa, Portugal.

Displaying a broad-spectrum chemistry, triazenes are best known for their cytotoxic properties, as exemplified by dacarbazine and temozolomide, well-known anticancer agents. Triazenes exert their chemotherapeutic activity through a unique mechanism of action that involves formation of a reactive alkyl diazonium intermediate capable of alkylating DNA and promoting cell death [1]. Herein we report a triazene-based platform that can be activated by nitroreductases (NTRs) [2] to undergo a self-immolative process that culminates with the release of the cytotoxic triazene. A series of nitrobenzyl and nitrofurfuryl carbamate prodrugs 1 (Figure 1) of cytotoxic triazenes was synthesized and NTR-triggered activation was investigated by HPLC and LC-MS. Corroboration of the reduction reaction was attained through chemical reduction using zinc/acetic acid and by means of the synthetic des-nitro analogue, underlining the importance of the nitro substitution. A549 cells (human epithelial lung carcinoma cells) were used as representative cell lines for bioreductive experiments. This approach unveils a novel triazene release system, particularly attractive to be broadly applicable to upregulated cancer-associated motifs.

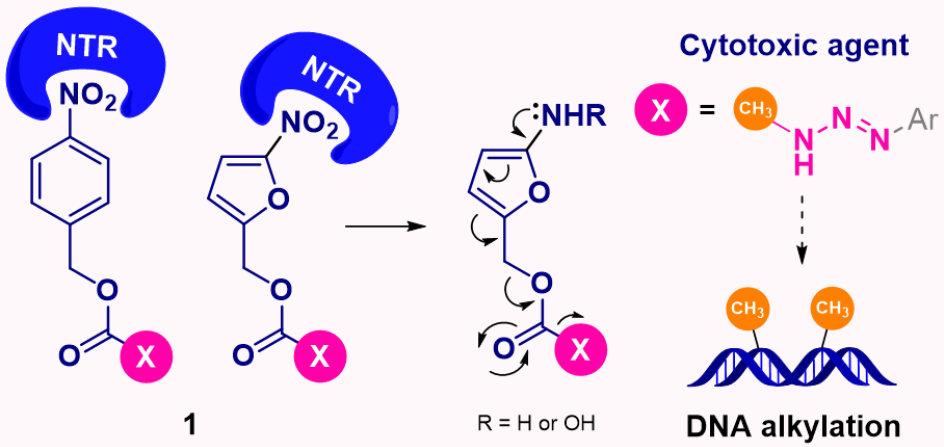

Figure 1

Acknowledgements

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References

[1] Marchesi, F.; Turriziani, M.; Tortorello, G.; Avvisati, G.; Torino, F.; De Vecchis, L., Pharmacol. Res., 2007, 56, 275-287;
[2] Chevalier, A.; Zhang, Y.; Khdour, O. M.; Kaye, J. B.; Hecht, S. M., J. Am. Chem. Soc., 2016, 138, 12009-12012.

Ectopic Expression Of CYP46A1 Ameliorates Niemann-Pick Type C Cellular Phenotype

Costa D.O. (1), Moutinho M. (1), Nunes M.J. (1), Silva M.F.B. (1), Gama M.J. (1), Castro-Caldas M. (1,2), Rodrigues C.M.P. (1) Cartier N. (3), Rodrigues E. (1)

1- Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Portugal; 2- Department of Life Sciences, Faculty of Science and Technology, Universidade NOVA de Lisboa, Portugal; 3- INSERM/CEA UMR1169, MIRCen Fontenay aux Roses, France.

Niemann Pick Type C (NPC) disease is a lysosomal storage disorder, characterized by cholesterol accumulation in the late endosomes/lysosomes (LE/L) compartment, which results from mutations in either NPC1 or NPC2 genes. The most dominant feature of the disease is the progressive neurodegeneration. Currently, there are no therapeutic options for NPC treatment. Since cholesterol homeostasis is deregulated in NPC, we hypothesize that the pathological phenotype might be ameliorated, by increasing the expression of a major regulator of brain cholesterol homeostasis, the cholesterol 24S-hydroxylase (CYP46A1). Human fibroblasts from patients bearing different NPC1 mutations (wild-type and heterozygous1920delG ontrols; homozygous NPC1I1601T, NPC1237S/I1061T and NPC11920delG/ IVS9-1009G>A) and NPC2 (homozygous NPC2G58T) were transduced with an adenovirus encoding GFP, or GFP and FLAG-tagged CYP46A1 and maintained for 96h. Ectopic expression of CYP46A1 led to a reduction in cholesterol accumulation and sequestration in the LE/L compartment. Moreover, CYP46A1 expression partially restored the mRNA levels of cholesterol homeostasis related genes. In human neuroblastoma cells with a stable NPC1-knockdown (NPC1-/-), CYP46A1 expression also could reduce cholesterol accumulation, and concomitantly rescue mitochondrial dysfunction, namely the decrease in polarized mitochondria content and increase in mitochondrial superoxide production. Moreover, the significant decrease in ATP levels, citrate synthase activity and mitochondrial copy number, can no longer be observed upon ectopic expression of CYP46A1. When analysing mitochondrial dynamics, the fission process seems to be favoured in NPC1-/- cells, since we observe a decrease in the expression levels of mitofusin 2 (MFN2) and a significant increase in mitochondrial fission 1 levels. Interestingly, CYP46A1 overexpression leads to a significant increase of MFN2 protein, a mitochondrial fusion and an endoplasmic reticulum (ER)-tethering protein. These data suggests that regulation of neuronal CYP46A1 might be a novel and promising strategy for NPC disease.

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Antitumor Activity And Interaction Studies Of New Derivatives Thiosemicarbazones

Duarte D.M.F.A. (1), Souza C. (1), Ribeiro A.G. (2), Alves J.E.F. (3), Silva-Lima B. (1), Moreira R. (1), Lima M.C.A. (2), Videira M. (1)

1- Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; 2- Laboratório de Química e Inovação Terapêutica, Departamento de Antibióticos, Universidade Federal de Pernambuco, Recife, Brazil; 3- Laboratório de Imunopatologia Keiso Asami (LIKA), Centro de Biociências, Universidade Federal de Pernambuco, Recife, Brazil.

Thiosemicarbazones are a class of compounds known for their chemical and biological properties. They are used as a strategy in the development of anticancer drugs, due to their ability to interact with DNA and interfere with the cell cycle. UV-Vis spectroscopy technique is used to study the interaction between chemical compounds and biomolecules that are potential tumorigenic targets, such as DNA, based on the identification of spectroscopic profiles of various substances. With this in mind, we evaluated the absorption profile and the cytotoxic effect of 9 thiosemicarbazones derivatives candidates for antitumor drugs (JF´s series), as well as its thiosemicarbazide intermediate (JF.01), used to obtain the other derivatives of the series. The cytotoxic effect was assessed in two distinct breast cancer cell lines – epithelial-like MCF-7 cell line and mesenchymal-like MDA-MB-231 cell line for 72 hours. The UV-vis scanning was performed using a GEHAKA-340 spectrophotometer, in the 200-900 nm range, with different DNA concentrations (0-100 µM) in Tris-HCl buffer pH 7.6, 25 µM of the compound. We observed that the compound JF.264 and the thiosemicarbazide intermediate compound (JF.01), presented an IC_{50} value of 5 µM and 3 µM, respectively, in the mesenchyme cell line (MDA-MB- 231). These two compounds were those with the lowest IC_{50} value, indicating an effective antitumor activity. One possible explanation is that the compound JF.264 has indole aromatic heterocycle present in its structure, which may contribute to the result of the activity. The UV-vis adsorption data showed a higher interaction with the DNA with the compound JF-264 with a Kb in the order of 106. As far as our results suggest, the derivative JF.01 and JF.264 are those that have the best antitumor activity those serie, and potentially interact with the DNA. Therefore can be exploited as compounds cancer therapy candidates.

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Novel Endoperoxide Platforms For Cancer Drug Delivery

Magalhães e Silva D., Rodrigues C., Lopes F., Moreira R.

Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal.

Cancer is a disease characterized by abnormal cell growth and is one of the main causes of death worldwide. In 2018, 18.1 million new cases were detected with 9.6 million deaths [1]. It has been demonstrated that tumor progression occurs by iron homeostasis disruption at a cellular or microenvironmental level leading to high amounts of Fe(II) in cancer cells [2-3]. Iron homeostasis deregulation in cancer allows the rational design of tumor-activated prodrugs (TAPs) with an endoperoxide core [3]. However, the development of selective anticancer drugs exploiting the iron metabolism remains an underexplored field. In this communication, we report the synthesis of key tetraoxane intermediate in route to the TAP, starting with the commercially available 1,3-cyclohexanediol. We will discuss the optimization of the acid-catalyzed cyclocondensation of gem- bishydroperoxides with ketones with different catalysts (Figure 1).

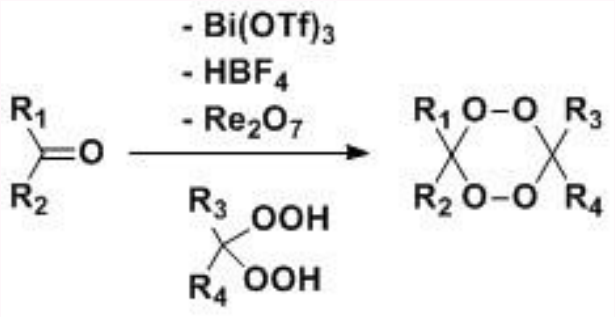

Figure 1

Acknowledgements

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References

[1] Dutta, S.; Mahalanobish, S.; Saha, S.; Ghosh, S.; Sil, P. C., Food Chem. Toxicol., 2019, 128, 240-255; [2] Crielaard, B. J.; Lammers, T.; Rivella, S., Nat Rev Drug Discov., 2017, 16, 400-423; [3] Spangler, B.; Morgan, C. W.; Fontaine, S. D.; Vander Wal, M. N.; Chang, C. J.; Wells, J. A.; Renslo, A. R., Nat. Chem. Biol., 2016, 12, 1-8.

New Azaaurones With Improved Solubility As Agents Against TB

Freitas E., Campaniço A., Harjivan S., Lopes F.

Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal.

Tuberculosis (TB) is an infectious disease caused by *Mycobacterium Tuberculosis* (Mtb) included in the top 10 causes of death worldwide. TB represents a major threat to public health due to the continuous emergence of new cases (10 million reported in 2017) and to the growing antimicrobial resistance [1]. Besides the resistance against current drugs, the absence of an efficacious vaccine, the duration of the treatment and the slow development of new drugs against TB are the main factors that prevent the eradication of TB [2]. It was found that azaaurone scaffold displays antimycobacterial activity. However, these compounds showed a poor aqueous solubility. This property represents a key role in drug discovery and development since a low solubility may affect the screening assays, lead to poor absorption and, consequently, low bioavailability [3]. In order to overcome these liabilities, more hydrophilic and non-planar moieties were introduced in the azaaurone scaffold. A solubility assay to study the kinetic solubility was set up and the solubility of these new compounds was measured.

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References

[1] WHO, Global Tuberculosis Report, 2018; [2] Sakamoto, K., Infectious Disease, 2012, 49(3), 423-439; [3] Savjani, K. T.; Gajjar, A. K.; Savjani, J. K., Drug Solubility: Importance and Enhancement, 2012, 10 pages.

Pure Dendrimers As Delivery Vehicle Of A Novel Small Molecule p53 Reactivator

Lopes E.A. (1), Pires R.F. (2), Saraiva L. (3), Bonifácio V.D.B. (2), Santos M.M.M. (1)

1- Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal. 2- CQFM/IN and IBB, Instituto Superior Técnico, Universidade de Lisboa, Lisboa, Portugal. 3- UCIBIO/REQUIMTE, Faculdade de Farmácia, Universidade do Porto, Porto, Portugal.

One of the most attractive targets for the treatment of tumors is the p53 protein which is inactivated in all human cancers, either by inhibition by negative regulators (e.g. MDM2 and/or MDMX), either by mutation or deletion of the TP53 gene. Thus, a promising therapeutic strategy against cancers with mutated p53 is the development of small molecules able to restore the wild type-like conformation to mutant p53 (mut p53) [1]. Recently, the tryptophanol-derived oxazoloisoindolinone SLMP53-1 was identified as a reactivator of human wt p53 and mut p53R280K-expressing tumor cells, enhancing p53 transcriptional activity and restoring wt-like DNA binding ability to mut p53R280K. In xenograft mice models, SLMP53-1 inhibited the growth of wt/mut p53-expressing tumors, without apparent toxicity [2]. Herein, we report our studies on the encapsulation of SLMP53-1 in a PUREG4 dendrimer, as well as the release studies. Polyurea (PURE) dendrimers are water-soluble, biocompatible, blue fluorescent and biodegradable polymers. Specifically, paclitaxel encapsulated formulations have 100-fold higher anti-proliferative activity in liver cancer cells compared with the paclitaxel alone [3]. So, the dendrimer nanoformulation of SLMP53-1 represents a valuable approach to improve the effect of this drug candidate in *in vitro* and *in vivo* models.

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References

[1] Lopes, E. A.; Gomes, S.; Saraiva, L.; Santos, M. M. M., Curr. Med. Chem., 2019 (in press, DOI: 10.2174/0929867325666181116124308); [2] Soares, J.; Raimundo, L.; Pereira, A.; Monteiro, Â.; Gomes, S.; Bessa, C.; Pereira, C.; Queiroz, G.; Bisio, A.; Fernandes, J.; Saraiva, L.; Santos, M. M. M., Oncotarget, 2016, 7, 4326-4343; [3] Restani, R. B.; Conde, J.; Pires, R. F.; Martins, P.; Fernandes, A. R.; Baptista, V.; Bonifácio, V. D. B.; Aguiar-Ricardo, A., Macromol. Biosci., 2015, 15, 1045-1051.

Pharmacophore-Based Drug Design: Novel EZH2 Inhibitors

Ramilo-Gomes F. (1,2), Bryant S.D. (3), Martini R. (4), Langer T. (4), Gul S. (5), Keminer O. (5), Sobral L. (6), Marques M.M. (1), Guedes R.C. (2)

1- Centro de Química Estrutural, Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais, 1049-001 Lisboa, Portugal; 2- iMed.ULisboa, Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; 3- Inte:Ligand Software Entwicklungs und Consulting, Mariahilferstrasse 74B, 1070 Vienna, Austria; 4- Department of Pharmaceutical Chemistry, Faculty of Life Sciences, University of Vienna, Althanstraße 14, A-1090 Vienna, Austria; 5- Fraunhofer IME-SP, Schnackenburgallee 114, 22525 Hamburg, Germany; 6- Hovione Farmaciência SA, Sete Casas, 2674-506 Loures, Portugal.

Epigenetic pathways are being recognized as determinants to cancer development and progression. Polycomb repressive complex 2 (PRC2) is an epigenetic regulator that catalyzes the trimethylation of lysine 27 in Histone 3 (H3K27me3), a process that facilitates chromatin compaction and gene silencing [1]. The overexpression of EZH2, the catalytic subunit of PRC2, is implicated in the development and progression of a variety of cancers with the worst prognosis. Thus, the therapeutic targeting of EZH2 emerged as a hot topic and the development of selective small-molecule EZH2 inhibitors is currently a promising research challenge for drug discovery [2]. A combination of state-of-the-art techniques, from computational drug design to synthetic methodologies and biological testing are being used to develop the new molecules. We performed a computer-aided drug design campaign to design new EZH2 inhibitors using LigandScout [3]. A panel of unique pharmacophore models were generated, validated and optimized. The prioritized models were used for two hit finding campaigns: Virtual Screening and De Novo Design. For Virtual Screening approach, several databases (e.g., DrugBank, NCI, MuTaLig Chemotheca, and our in-house libraries) were computed and screened. Interesting virtual hit molecules with high inhibition potential were found and tested in order to determine their EZH2 profiles. Notably, we found several hits with inhibition rates comparable to the reference compounds (in clinical trials). In parallel, we started a De Novo Design campaign based on selected pharmacophore models and we found new scaffold for EZH2 inhibitors. Those from de novo design are being synthesized. Finally, selectivity and binding mode of the most promising compounds are being elucidated and potential toxicity issues are being assessed through metabolism studies. The best drug candidates are expected to proceed to *in vivo* testing.

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References

[1] Brooun, A.; Gajiwala, K. S.; Deng, Y.-L.; et al., Nature Communications, 2016, 7, 11384; [2] Kim, K. H.; Roberts, C. W. M., Nat Med., 2016, 22, 128-134; [3] LigandScout Molecular Design Software from InteLigand GmbH (http://www.inteligand.com).

Shifting The Paradigm: Repurposing Beta-Lactam Antibiotics Against Drug-Resistant Clinical Isolates of *Mycobacterium tuberculosis*

Olivença F. (1), Carmo N. (1), Pires D. (1), Macedo R. (2), Nunes A. (2), Gomes J.P. (2), Anes E. (1), Catalão M.J. (1).

1- Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; 2- National Institute of Health Doutor Ricardo Jorge (INSA), Lisbon, Portugal.

Tuberculosis (TB), caused by *Mycobacterium tuberculosis*, remains as one of the top ten causes of death worldwide and is currently the most lethal infection. Ending the tuberculosis epidemic by 2030 is contemplated in the Sustainable Development Goals of the United Nations. However, the alarming rise of multidrug-resistant (MDR-) and extensively drug-resistant tuberculosis (XDR-TB) hinders this achievement. MDR-TB is caused by a strain resistant to, at least, isoniazid and rifampicin, while XDR-TB strains have additional resistance to any fluoroquinolone and at least one injectable agent.Since isoniazid and rifampicin are the most successful first-line anti-TB drugs, resistance to these antibiotics is a major concern and requires the use of less efficient and tolerable second-line drugs. Thus, there is an urgent need to reexamine the therapeutic options against MDR-TB/XDR-TB. One strategy consists in evaluating the potential use of antibiotics that are not usually considered for TB treatment, like beta-lactams. This class of antibiotics comprises potent inhibitors of the penicillin-binding proteins that synthesize peptidoglycan (PG) and includes penicillins and carbapenems, which target D,D- and L,D-transpeptidases, respectively. Historically, beta-lactams have been excluded from standard TB management because *M. tuberculosis* is considered innately resistant to these antibiotics, mainly due to the presence of non-classical transpeptidases and a potent beta-lactamase, BlaC.Here we present a screening performed on a collection of Portuguese clinical isolates of *M. tuberculosis* with diverse drug susceptibility patterns and one reference strain, H37Rv. The Minimal Inhibitory Concentration (MIC) to several beta-lactams, with or without a beta-lactamase inhibitor, clavulanate, was determined. Our preliminary results suggest that: (i) generally, addition of clavulanate reduces the MIC for beta-lactams by two to eight-fold; (ii) amoxicillin and ertapenem are the least efficient antibiotics, but when combined with clavulanate, amoxicillin is as potent as some carbapenems; (iii) within carbapenems, meropenem and biapenem have the lowest MIC values, combined or not with clavulanate. These findings are in accordance with previous studies, strengthening the notion that transpeptidases and beta-lactamase are therapeutic targets for *M. tuberculosis* eradication. Future assays with more clinical strains and mutants for PG biosynthesis genes will further elucidate on the clinical role of beta-lactams in TB.

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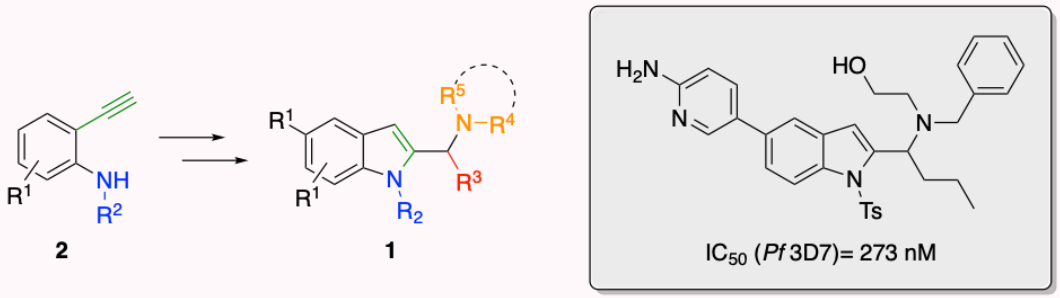
A New Lead Against Blood Stage Malaria Based On An Indole Scaffold

da Silva G. (1,2), Araújo D. (1,3), Silva V.L.M. (2), Nogueira F. (3), Silva A.M.S. (2), Moreira R. (1)

1- iMed.ULisboa, Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; 2- QOPNA, Departamento de Química da Universidade de Aveiro, Aveiro, Portugal; 3- UEI Malaria, Centro da Malária e Doenças Tropicais, IHMT, Universidade Nova de Lisboa, Lisboa, Portugal.

Indoles represent an important structural scaffold for the discovery of new drug candidates, being considered privileged structures for medicinal chemistry [1]. Several examples of this class have revealed antimalarial activity [2] and some have been included in the pathogen box [3]. In this communication, we report the results from a structure-activity relationship study, based on the optimization of an early indole hit with promising antimalarial activity against blood stage Plasmodium falciparum parasites. The facile access to a small library of polyfunctionalized C2-indoles 1 was explored using a domino multicomponent reaction with the corresponding ethynylaniline 2, eventual Suzuki coupling and tosyl-deprotection (Scheme 1). Our current lead compound revealed to be active against drug-sensitive and drug-resistant malaria parasites at a sub-micromolar range, with IC_{50} values lower than the most active C2-indole included in the Pathogen Box [3].



Scheme 1 - Facile synthesis of polyfunctionalized indoles 1 from the corresponding ethynylaniline 2.

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References

[1] de Sá Alves, F. R.; Barreiro, E. J.; Fraga, C. A., Mini-Reviews in Medicinal Chemistry, 2009, 9, 782-793; [2] Frederich, M.; Tits, M.; Angenot, L., Transactions of The Royal Society of Tropical Medicine and Hygiene, 2008, 102, 11-19; [3] van Voorhis, W. C.; et al., PLOS Pathogens, 2016; 12, e1005763.

A New Elastase Inhibitor Based Emulsion For Topical Inflammatory Diseases

Nunes A., Marto J., Félix R., Gonçalves L.M., Lopes F., Ribeiro H.M.

Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal.

Human neutrophil elastase (HNE) is a serine protease that belongs to the chymotrypsin superfamily. HNE is involved in the degradation of matrix proteins such as elastin, collagen, fibronectin, playing an important role in the modulation of inflammation. Its action is controlled by endogenous inhibitors and when imbalanced it can lead to several pathological conditions, such as psoriasis. HNE inhibitors have the potential to be used as therapeutic tools for this disease [1]. The first aim of this work is the synthesis of new HNE inhibitors with improved efficacy and reduced toxicity and the second is the evaluation of skin delivery of HNE inhibitor based emulsion emulsions. All synthesized compounds were characterized by NMR, melting point (solids) and IR. They were assayed against HNE for enzymatic inhibition and against HaCat cell line to study their cytotoxicity. Compound 4 was used as control. All compounds inhibited HNE in the nanomolar range and did not revealed any significant cytotoxicity, but AAN9 was chosen as a drug to be incorporated in topical innovative macro and microemulsions (ME). An *in vitro* drug release studies were performed using vertical Franz diffusion cells with Tuffryn®, 0.45 µm. Water:ethanol (50:50 w/w) with 2.5% of PEG-40 Hydrogenated Castor Oil were used as receptor phase. The amount of released drug was analyzed by a fluorescence method and data was expressed in drug cumulative amount of permeated as function of time. A solution with AAN-9 were used as a control. After 6 h the amount of AAN-9 released was 2.90±1.2 µg/cm^2, 7.94±2.1 µg/cm^2 and 17.93±1.6 µg/cm^2 for ME, emulsion and solution, respectively. ME have the advantage of being thermodynamically stable, but the emulsion released four times more the amount of AAN-9. In this study, it was demonstrated that the emulsions developed can be used for topical application to treat inflammatory diseases.

Acknowledgements

Pest-UID/DTP/04138/2013.

References

[1] Marto J et al, Eur J Pharm Biopharm. 2018;127:1-11.

Phenotypic-Driven High-Throughput Screening Strategy To Identify Novel Necroptosis Inhibitors

Brito H. (1), Marques V. (1), Afonso M.B. (1), Brown D.G. (2), Börjesson U. (3), Selmi N. (3), Smith D.M. (4), Roberts I.O. (4), Fitzek M. (5), Aniceto N. (1), Guedes R.C. (1), Moreira R. (1), Rodrigues C.M.P. (1)

1- Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; Hit Discovery, Discovery Sciences, R&D Biopharmaceuticals, AstraZeneca; 2- Boston, MA 02451 USA, 3- Gothenburg, 431 83 Sweden, 4- Cambridge, CB4 0WG UK, 5-Alderley Park, Macclesfield, SK10 4TG UK.

Regulated necrosis or necroptosis, mediated by RIPK1, RIPK3 and MLKL, contributes to the pathophysiology of inflammatory, infectious and degenerative human disorders. Despite recent efforts to identify necroptosis inhibitors, lack of specificity, off-target effects and toxicity have prevented these molecules from reaching clinical trials. Here, we developed a phenotypic high-throughput screening (HTS) cascade for the identification of small-molecule inhibitors of necroptosis. From the initial library of over 250,000 compounds, the primary screening cascade composed by three stages identified 356 compounds that strongly inhibited TNF-α-induced necroptosis, but not apoptosis, in human and murine cell systems, with EC_{50} < 6.7 µM. From these, 251 compounds were tested for RIPK1 and/or RIPK3 kinase inhibitory activity; only 21 were active. Based on specific chemical descriptors, 110 compounds proceeded into the secondary screening cascade, which then identified 7 compounds with maximum ability to reduce MLKL activation, IC_{50} > 100 µM, EC_{50} 2.5 - 11.5 µM under long-term necroptosis execution in murine fibroblast L929 cells, and full protection from ATP depletion and membrane leakage in human colon HT29 and murine microglial BV2 cells. Compound SN-6109, with binding mode to RIPK1 similar to that of necrostatin-1, confirmed RIPK1 inhibitory activity and appropriate pharmacokinetic properties. SN-6109 was further tested in mice, showing efficacy against TNF-α-induced systemic inflammatory response syndrome. In conclusion, a phenotypic-driven HTS cascade promptly identified robust necroptosis inhibitors with *in vivo* activity. Notably, this screening strategy yielded novel hits, thus revealing the opportunity for discovery of innovative hitherto unknown molecular targets.

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Evaluating *Burkholderia thailandensis* Ability To Synthesize Antimicrobial Rhamnolipids

Correia I., Farzadmanesh O., Frenzel J. L., Ribeiro I.A.C.

Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal.

Medical devices related-infections are responsible for significant raise of morbidity and mortality. Improvement of medical devices antimicrobial properties is a need and different approaches have been proposed such as incorporation of antimicrobials and development of antimicrobial device coating [1]. A possible approach can be the incorporation of biosurfactant antimicrobial compounds, such as rhamnolipids that besides killing bacteria can prevent their adhesion. This work aims the synthesis, identification and antimicrobial properties evaluation of rhamnolipids produced by *Burkholderia thailandensis*. Rhamnolipids were biosynthesised by the bacteria *Burkholderia thailandensis* using different culture media (i.e. Luria Bertani and Nutrient Broth) supplemented with hydrophobic carbon sources. Rhamnolipids production was evaluated through the contact angle measurement of culture media, Thin Layer Chromatography (TLC) and Ultra Performance Liquid Chromatography coupled to Mass Spectrometry (UPLC/ MS-MS). Minimum inhibitory concentration (MIC) of produced rhamnolipids was accessed through the microdilution method. Rhamnolipids were produced as a mixture and it was with Luria-Bertani culture media (LB) that the production was more evident. It was possible to identify mono and di-rhamnolipids with longer fatty acid chains, distinctive of this bacteria. Produced rhamnolipids crude presented a MIC of 0.125 mg/mL. In conclusion, the development of antimicrobial medical devices can provide patients with safer hospital environment.

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References

[1] Pontes, C.; Alves, M.; Santos, C.; Ribeiro, M. H.; Gonçalves, L.; Bettencourt, A. F.; Ribeiro, I.A.C., Int. J. Pharm., 2016, 513, 697-708.

Evaluation Of A Dual Function Minocycline Polymeric Bone Scaffold

Anjos I. (1,2), Zegre M. (2,3), Santos C. (4,5), Alves M.M (5), Ribeiro I. (2), Gonçalves L. (2), Bettencourt A.F. (2)

1- Departamento de Química, Faculdade de Ciências e Tecnologias, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal; 2- Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; 3- H&TRC- Centro de Investigação em Saúde e Tecnologia, ESTeSL- Escola Superior de Tecnologia da Saúde, Instituto Politécnico de Lisboa, Av D. João II, lote 4.69.01, 1990-096 Lisboa, Portugal; 4- EST Setúbal, CDP2T, Instituto Politécnico de Setúbal, Campus IPS, 2910 Setúbal, Portugal; 5- CQE Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais 1049-001, Lisboa, Portugal.

It is estimated that orthopedic procedures will rise due to population growth along with aging and increasing on chronic diseases. Consequently, orthopedic infections associated to these procedures can be a serious complication, leading to a state of morbidity [1]. Current strategies for treating bone infections and defects present several limitations, namely low local concentrations and systemic toxicity. To overcome these limitations, synthetic and biocompatible bone grafts substitutes (scaffolds) are being developed as platforms for local drug delivery, a strategy that allows high antibiotics' concentration in bone for orthopedic infections treatment [2,3]. Thus, this work aims to develop a drug delivery system with osteoconductive and osteoinductive properties for bone regeneration and capable of treating the infection. For this purpose, porous PDLLA scaffolds were produced by solvent casting technique, functionalized with bioglass (BG) and collagen (Col) and loaded with 0.5, 0.25, 0.1 or 0.05 mg/mL of minocycline hydrochloride (MH), a dual function drug that beyond its antibiotic role, also induce osteoblastic cells differentiation [2]. Scaffolds' surface morphology was characterized by scanning electron microscopy (SEM) and elemental chemical composition was performed by X-ray energy dispersive spectrometer (EDS). These drug delivery systems were also characterized in terms of drug release profiles and cytocompatibility through in vitro studies. SEM analysis demonstrated a porous surface and confirmed the functionalization. Regarding drug release profiles, the obtained results suggest a two-phase stage release, with an initial burst release of approximately 60%, 30% and 10% of MH in the first 15 min, for the two most MH concentrated groups, 0.1 mg/mL of MH group and 0.05 mg/mL of MH group, respectively, followed by a sustained release.In vitro cell studies were promising for scaffolds adsorbed with 0.1 and 0.05 mg/mL of MH, not revealing cytotoxicity, contrary to what was seen for scaffolds with higher concentrations of MH (0.5 and 0.25 mg/mL).In conclusion, due to release profiles of the drug and in vitro cell assays, scaffolds adsorbed with the two lowest MH concentration seem a promising strategy for an acute infection treatment, however antimicrobial assays must be conducted.

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References

[1] Tande, A. J.; Patel, R., Clinical microbiology reviews, 2014, 27(2), 302-345; [2] Martin, V.; Ribeiro, I. A.; Alves, M. M.; Gonçalves, L.; Claudio, R. A.; Grenho, L.; Fernandes, M. H.; Gomes, P.; Santos, C. F.; Bettencourt, A. F., Mat. Sci. and Eng. C., 101, 2019, 15-26; [3] Kluin, O. S.; Van der Mei, H. C.; Busscher, H. J.; Neut, D., Expert Opinion on Drug Delivery, 2013, 10(3), 341-351.

Deciphering The Role Of Cholesterol Homeostasis In Parkinson's Disease

Caria I. (1), Nunes M.J. (1,2), Carvalho A.N. (1,2), Costa D. (1), Castro-Caldas M. (1,3), Gama M.J. (1,2), Rodrigues E. (1,2), and Ruas J.L. (2,4)

1- Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; 2- Faculty of Pharmacy, Universidade de Lisboa, Portugal; 3- Department of Life Sciences, Faculty of Science and Technology, Universidade NOVA de Lisboa, Caparica, Portugal; 4- Department of Physiology and Pharmacology, Molecular and Cellular Exercise Physiology Unit, Karolinska Institutet.

Cholesterol has a key role in neuronal function and alterations in brain cholesterol homeostasis correlate with neurodegeneration. While disruptions in cholesterol homeostasis have been clearly associated with neurodegenerative disorders such as Alzheimer's and Huntington's disease, the role of cholesterol in Parkinson's disease (PD) remains controversial. To address this question, we started by characterizing changes in cholesterol intracellular localization and levels using N2a mouse neuroblastoma cells treated with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) toxic metabolite, 1-methyl-4-phenylpyridinium (MPP^+). Filipin III staining allowed to observe an increase in lysosomal accumulation of free cholesterol 16h after treatment with 1 mM MPP^+. Moreover, quantification of cholesterol levels also showed a significant increase in esterified cholesterol. The increase in total cholesterol levels, led to a significant decreased in the mRNA levels and transcriptional activity of sterol regulatory element-binding protein (SREBP) 1 and 2 proteins, which are the main regulators of fatty acids and cholesterol synthesis. Concomitantly, there is a down-regulation in the mRNA levels of SREBP-target genes, such as fatty acid synthase and hydroxymethylglutaryl-CoA synthase. To further investigate this issue, we proceeded to characterized changes in cholesterol homeostasis in the brain of MPTP-treated mice (40 mg/mL, i.p). Surprisingly, our preliminary results show an accumulation of the truncated forms of SREBP protein levels, 3 and 6 hours after MPTP administration, in both the midbrain and striatum, although we could not detect an increase in downstream target genes. Thus, our results show a deregulation of cholesterol homeostasis in the context of PD, which may contribute to the neurodegeneration associated with this disease.

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Increased Solubility And Stability Of Co-Amorphous Systems Containing Olanzapine And Sulfonic Acids

Santos I.A. (2), Bastos A.C. (1,2), Fernandes A.I. (2), Pinto J.F. (1)

1- Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; 2- CiiEM – Centro de Investigação Interdisciplinar Egas Moniz, Instituto Universitário Egas Moniz, Monte da Caparica, Portugal.

Introduction: A large number of active pharmaceutical compounds currently under development are poorly water soluble, which can limit their bioavailability and results in formulation challenges [1]. Co-amorphous systems (CAMs) enhanced value because of the increase of apparent solubility of a drug and dissolution rate [1,2]. Sulfonic acids were never investigated as possible co-formers for Olanzapine (OLZ). The aim of this work is to study the potential of the sulfonic acids, namely saccharin (SAC), cyclamic acid (CA), acesulfame (ACE) and their salts, on the formation of stable CAMs. Physical characterization, solubility, dissolution rate and stability tests of the produced CAMs were conducted.
Materials and Methods: Mixtures of OLZ and each co-former in molar ratios 1:1 were submitted to ball milling for 24h, solvent evaporation and quench cooling. Characterization of the samples by differential scanning calorimetry (DSC), X-ray powder diffraction (XRPD) and Fourier-transform infrared spectroscopy (FTIR) were performed. Solubility assessment, powder dissolution rate and stability studies were also conducted.
Results: Sulfonic acids had shown to be successful co-formers in the production and stabilization of OLZ-CAMs by the three different techniques. None of the sulfonic acid salts were capable of forming a CAM, leading to phase separation. In the FTIR results band shifts and broadening can be identified. The solubility and dissolution rate of OLZ were significantly increased. The produced CAMs were stable during time, as suggested by the DSC and XRPD results.
Discussion and Conclusions: Stable OLZ-CAMs were produced using SAC, CA and ACE as co-formers. FTIR results suggest an intermolecular interaction, namely a hydrogen-bond, between the N-H group in OLZ and the C=O group in SAC and ACE and the O-H group in CA. The inability of the sulfonic acid salts to form CAMs supports the FTIR results. The CAMs were stable during time supporting a strong intermolecular bonding. The increased solubility and dissolution rate suggest that the CAMs can lead to an improved bioavailability of OLZ.

Acknowledgements

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References

[1] Blaabjerg, L. I.; Lindenberg, E.; Rades, T.; Grohganz, H.; Löbmann, K., Int. J. Pharm., 2017, 521, 232-238; [2] Dengale, S. J.; Grohganz, H.; Rades, T.; Löbmann, K., Adv. Drug Del. Rev., 2016, 100, 116-125.

Infliximab Therapy Management – Development Of A Microfluidic Biosensor For Antibody Therapy Management

Iria I. (1,2), Danese S. (3,4), Fonseca J.E. (5,6), Conde J.P. (2,7), Gonçalves J. (1)

1- Molecular Microbiology and Biotechnology Unit|Drug Discovery Area, Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; 2- INESC-MN - Microsystems and Nanotechnologies, Instituto Superior Técnico, Universidade de Lisboa, Lisbon, Portugal; 3- Humanitas Clinical and Research Center, IBD Center, Rozzano, Italy; 4- Department of Gastroenterology, Istituto Clinico Humanitas, Rozzano, Italy; 5- Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, Lisbon, Portugal; 6- Rheumatology Department, Centro Hospitalar de Lisboa Norte, EPE, Lisbon, Portugal; 7- Department of Bioengineering, Instituto Superior Técnico, Universidade de Lisboa, Lisbon, Portugal.

Infliximab (IFX) is a biopharmaceutical product (BPs) that interacts with tumor necrosis factor-α (TNF-α), a cytokine whose levels are increased in inflammatory diseases, such as Crohn's Disease and Rheumatoid Arthritis. The mechanism of action of BPs is highly specificity making this type of pharmaceutical promising [1]. However, when administered to patients, BPs can lead to undesirable immune responses, namely the development of anti-drug antibodies (ADAs) [2-4]. These risks are increased due to the chronic nature of these diseases which require repetitive administration. To solve part of this problem, the therapy needs to be adapted to each patient, being necessary the quantification of IFX and ADAs. We are also interested in the biosimilars immunogenicity, by comparing it with the reference product (Remicade®). Biosimilars play an important role in the health system since they can reduce therapy costs up to 70%. However, there are major concerns about their immunogenicity, since it can affect the drug's pharmacokinetics and pharmacodynamics. To overcome these questions, an in a preliminary phase of the project, we studied the interactions between 21 immunoreactive peptides of IFX and the serum of 36 inflammatory bowel disease (IBD) patients treated with Remicade® or CT-P13 (biosimilar). To date significant differences have not been found between patients treated with Remicade® or CT-P13. Our results suggest that there is no difference in ADA produced by patients treated with biosimilar when compared to those treated with reference product, which may evidence that replacement of Remicade® by CT-P3 will not affect immunogenicity.

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PD/BD/128207/2016.

References

[1] Warnke, C.; Hermanrud, C.; Lundkvist, M.; Fogdell-Hahn, A., Drugs and Therapy Studies, 2012, 2, 56-63;. [2] Wadhwa, M.; Knezevic, I.; Kang, H.; Thorpe, R., Biologicals, 2015, 43, 298-306; [3] Schaeverbeke, T.; Truchetet, M. E.; Kostine, M.; Barnetche, T.; Bannwarth, B.; Richez, C., Rheumatology, 2015, 53, 1-11; [4] Harding, F. A.; Stickler, M. M.; Razo, J.; DuBridge, R. B., mAbs, 2010, 3, 256-265.

Inhibition Of Aquaporin-3 (AQP3) By Nanoformulated Metal-Based Compounds: A Novel Therapeutic Strategy Against Cancer

Lemos A.A. (1), Pinho J.O. (1), Rodrigues C. (1), Amaral J.D. (1), Rodrigues C.M.P. (1), Casini A. (2), Soveral G. (1), Gaspar M.M. (1)

1- Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; 2- School of Chemistry, Cardiff University, Cardiff, United Kingdom.

Aquaporins (AQPs) are transmembrane proteins that facilitate the bidirectional movement of water and small solutes (e.g.: glycerol) across cell membranes [1]. Taking into consideration their involvement in normal and diseased physiological states, the pharmacological modulation of AQPs emerges as a unique opportunity for novel and innovative therapeutic interventions in a variety of human disorders, namely cancer. The expression of AQP1, AQP3 and AQP5 in three human tumor cell lines was determined by qRT-PCR and the effect of two selected metal-based compounds, Cuphen and Auphen, on glycerol permeability of human and murine melanoma cells was evaluated. The expression of AQP3 was the highest in the analyzed human cells of colon cancer (HCT-116), melanoma (MNT-1) and breast cancer (MCF-7). In murine and human tumor cells, Cuphen and Auphen significantly inhibited the AQP3-mediated glycerol permeability (values between 32-50 and 39-22% of inhibition, respectively). The cytotoxicity of both metallodrugs against human and murine tumor cell lines, including melanoma patient-derived samples, was assessed. Both metallodrugs displayed high antiproliferative effect towards murine and human tumor cells, with IC50 values in the low micromolar range (<6 μM). To promote a preferential accumulation at tumor sites *in vivo*, the metallodrugs were nanoformulated in liposomes with different characteristics: non-pH sensitive, pH-sensitive and fusogenic. Highly homogeneous liposomal formulations were obtained, with mean size <130 nm, PdI < 0.1, and adequate metallodrug incorporation parameters. Thus, Cuphen therapeutic effect was evaluated in syngeneic murine models of melanoma and colon cancer [2,3]. The results from the syngeneic murine models of melanoma and colon cancer demonstrated a superior therapeutic benefit for mice treated with nanoformulated Cuphen, compared to control group and mice receiving the free compound. Finally, an *in vivo* model of metastatic melanoma was successfully established to most closely mimic the human pathology and the proof-of-concept in this model is on course. Altogether, the results demonstrate the promising potential of liposomal metallodrugs for AQP3 inhibition as a novel and effective therapeutic approach for cancer.

Acknowledgements

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References

[1] Verkman, A. S.; Anderson, M. O.; Papadopoulos, M. C., Rev. Drug Discov., 2014, 13, 259-277; [2] Nave, M.; Castro, R. E.; Rodrigues, C. M. P.; Casini, A.; Soveral, G.; Gaspar, M. M., Nanomedicine (Lond.), 2016, 11, 1817-1830; [3] Pinho, J. O.; Amaral, J. D.; Castro, R. E.; Rodrigues, C. M. P.; Casini, A.; Soveral, G.; Gaspar, M. M., Nanomedicine (Lond.), 2019, 14, 835-850.

Formulation Of Fast-Disintegrating 3D Printed Tablets Of Carbamazepine Containing Hydroxypropyl-β-cyclodextrin And Cellulose Ethers As Excipients

Conceição J. (1), Farto-Vaamonde X. (2), Goyanes A. (2,3), Adeoye O. (4), Concheiro A. (2), Cabral-Marques H. (4), Sousa Lobo J.M. (1), Alvarez-Lorenzo C. (2)

1- UCIBIO/REQUIMTE, MedTech-Laboratory of Pharmaceutical Technology, Department of Drug Sciences, Faculty of Pharmacy, University of Porto, Porto, Portugal; 2- Departamento de Farmacología, Farmacia y Tecnología Farmacéutica, R+D Pharma Group (GI 1645), Facultad de Farmacia and Health Research Institute of Santiago de Compostela (IDIS), Universidade de Santiago de Compostela, 15782 Santiago de Compostela, Spain; 3- FabRx Ltd., 3 Romney Road, Ashford, Kent TN24 0RW, United Kingdom; 4- Research Institute for Medicines (iMed.ULisboa), Department of Galenic Pharmacy and Pharmaceutical Technology, Faculty of Pharmacy, University of Lisbon, Lisbon, Portugal.

The purpose of this work was to study for the first time the use of cyclodextrins to prepare 3D printed tablets (printlets) of poorly soluble active pharmaceutical ingredients [1]. In this way, orodispersible printlets were developed by semisolid extrusion of wet masses of carbamazepine, hydroxypropyl-β-cyclodextrin and cellulose ethers, namely hydroxypropyl methylcellulose and croscarmellose sodium. Carbamazepine, an antiepileptic and anticonvulsant agent, was used as a model of a Biopharmaceutics Classification System class II drug (low solubility and high permeability). In addition, the feasibility of semisolid extrusion-based 3D printing on the formation of drug cyclodextrin complexes was evaluated. A 3D bioprinter (Regemat 3D S.L., Spain) with a fan attached (Fig. 1) was used. Physical properties of the 3D printed tablets such as weight variation, diameter, thickness, friability and hardness were assessed, as well as their drug content and structure features by scanning electron microscopy. Additionally, rheology, disintegration, dissolution, differential scanning calorimetry and X-ray diffraction studies were also performed. Three major conclusions were obtained from the results of the present work: i) hydroxypropyl-β-cyclodextrin showed to be a suitable excipient for the development of fast-disintegrating printlets of poorly soluble drugs; ii) orodispersible printlets of carbamazepine were successfully prepared and characterized according to a variety of techniques, including relevant pharmacopeial assays for solid oral dosage forms; and iii) semisolid extrusion, also known as 3D micro-extrusion, enabled *in situ* formation of drug-cyclodextrin complexes.

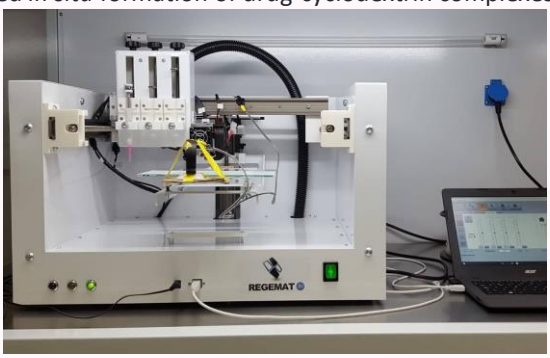

Figure 1 - The Regemat 3D bioprinter with fan, 5 mL extrusion syringe with a tapered extrusion tip (0.58 mm orifice), and computer connected.

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References

[1] Conceição, J.; et al., Carbohydr. Polym.; 2019, 221, 55-62.

Design Of New Traceless Linkers For Cysteine Bioconjugation

Carvalho J.I., António J.P.M., Góis P.M.P.

Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal.

Among the 20 canonic amino acids, cysteine (Cys) is a low abundant (1.6 %) residue that exhibits a highly reactive sulfhydryl side chain. For these reasons, native or engineered Cys on the protein surface emerged as a preferred "hot-spot" for the site-selective modification of proteins and may be modified by Michael reaction with Michael acceptors such vinyl sulfone [1]. A few years ago, our research group start to study diazaborines for bioorthogonal applications. The diazaborines developed resulted from reaction between FBBA and several hydrazides, these constructs demonstrated remarkable stability and fast reaction kinetics. Considering this, we develop a selective strategy for cysteine protein functionalization using a diazaborine heterocycle resulted from 2-FBBA and vinylsulfone minimal linker comprising a terminal hydrazine, which is a key moiety to give diazaborine construct. It was developed also a masking group for the vinylsulfone minimal linker to prevent hydrazide side-reactivity [2]. The minimal linker, diazaborine formation and reactivity towards laminin protein was evaluated by ESI-MS.

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References

[1] Faustino, H.; Silva, M. J. S. A.; Veiros, L. F.; Bernardes, G. J. L.; Gois, P. M. P., Chem. Sci., 2016, 7, 5052-5058; [2] Eldridge, G. M.; Weiss, G. A., Bioconjug. Chem., 2011, 22, 2143-2153.

Phototherapy And Nanotechnology: A New Therapeutic Strategy For The Treatment Of Superficial Tumors

Lopes J.M. (1), Albino M. (2), Mularczyk D. (3), Silva C.O. (2), Roberto A. (4), Gaspar M.M. (1), Viana A.S. (5), Vieira P. (6), Coelho J.M.P. (3, 7), Reis C.P. (1,3)

1- Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; 2- Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; 3- IBEB, Instituto de Biofísica e Engenharia Biomédica, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal; 4- Universidade Lusófona de Humanidades e Tecnologias, Lisboa, Portugal; 5- Centro de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal; 6- LIBPhys-UNL, Departamento de Física, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Lisboa, Portugal; 7- Laboratório de Óptica, Lasers e Sistemas, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal.

Cancer is considered to be a major public health issue, responsible for a high rate of morbidity and mortality. Currently, conventional treatment involves invasive methods like chemotherapy, radiation and surgery to remove the tumor (when it is possible), followed by more chemotherapy and radiation. However, these methods are far from being considered satisfactory in terms of efficacy and their use is also limited by serious side effects. Nowadays, a great evolution has occurred in the area of Nanotechnology; researcher's efforts have been focused on developing more selective therapies, able to target tumor cells without harming healthy tissues. In this subject, gold nanoparticles, are now being extensively studied for cancer therapeutic applications due to their physicochemical and optical properties. These nanoparticles absorb in the near infrared region (650-900 nm) enabling their activation with a laser as heat source, in contrast to commercial nanoparticles, that as absorb at lower wavelengths, do not allow light to penetrate into deep skin layers. In the present work, gold nanoparticles were prepared by using the Seed-growth method with some modifications [1]. These nanoparticles were characterized in terms of mean size, morphology and cytotoxicity in the presence of *Saccharomyces cerevisiae*, and two cell lines, the human keratinocytes (HaCat) and the murine melanoma (B16F10). Gold nanoparticles showed a spherical morphology with a mean size of 170 nm without cytotoxic effects towards yeast and tested cell lines. Nevertheless, after activation by a laser, a reduction of 40% in B16F10 cell line viability was observed. This work appears to be a highly promising strategy for the treatment of non-metastatic melanoma, taking into account that laser is able to penetrate only through the skin layers. The same approach may be applied to other types of superficial tumors, such as breast tumors.

Acknowledgements

Fundação para a Ciência e a Tecnologia (FCT) through projects: UID/DTP/04138/2019.

References

[1] Silva, C. O.; Rijo, P.; Molpeceres, J.; Ascensão, L.; Roberto, A.; Fernandes, A. S.; Gomes, R.; Pinto Coelho, J. M.; Gabriel, A.; Vieira, P.; Reis, C. P., Ther. Deliv., 2016, 7, 287-304.

Novel Water-In-Oil Emulsions As Vehicles For Khellin Topical Delivery

Pereira J. (1), Barreto M. (2), Dias C. (2), Carvalho F. (2), Almeida A.J. (1), Ribeiro H. (1), Marto J. (1)

1- Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; 2- LEF- Infosaúde, Rua das Ferrarias del Rei, 6, 2730-269 Barcarena, Portugal.

Water-in-oil emulsions development is a challenge due their thermodynamic instability. Therefore, emulsifier selection is crucial because it influences formulation properties and percutaneous delivery. In this work, the influence of two emulsifiers was evaluated on khellin (KHE)-containing W/O emulsions rheology, as well as on *in vitro* drug release and permeation. For this purpose a PEG-free emulsifier containing polyglyceryl-3 polyricinoleate, sorbitan sesquiolate, cetyl ricinoleate, glyceryl caprate, cera alba, magnesium stearate, aluminium tristearate (Symbio®muls WO), and a non-ionic emulsifier to create wax free emulsions, containing octyldodecanol, octyldodecyl xyloside, PEG-30 dipolyhydroxystearate (Easynov™), were used at a concentration of 10% and 3% (w/w), respectively. Rotational viscosity and dynamic oscillatory tests were performed with a controlled stress Kinexus Rheometer (Malvern) using cone and plate geometry (truncated cone angle 4° and radius 40mm). Droplet size distributions were determined using optical microscopy and the Image J software. Release and permeation studies were performed on artificial membranes and excised human skin using vertical Franz-type diffusion cells followed by drug quantification using HPLC. The Easynov™ formulation presented higher values of viscosity and smaller droplet size comparing with the Symbio®muls WO emulsion. Both formulations presented the storage module higher than the loss module. This is due to the presence of structures that, with the increase of the stress applied, disrupt the micellar structures present in formulations. Drug release from both emulsions was characterized using the Korsmeyer-Peppas model. Permeation results showed that KHE can penetrate the stratum corneum from both formulations: steady-state flux (Jss) 0.029±0.011 and 0.035±0.010 µg/cm^2/h, lag time 0.455±0.24 and 1.217±0.34h and permeability coefficient (Kp) 1.08E-05±0.00 and 1.33E-05±0.00 cm/h were the values obtained for Symbio®muls WO and Easynov™, respectively. In conclusion, both emulsifiers allowed the development of W/O emulsions resulting in suitable vehicles for the topical delivery of KHE.

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The Effect Of Insulin And Dexamethasone In Hepatocyte-Like Cells' Phenotype

Rodrigues J.S. (1), Cipriano M. (1), Correia J.C. (2), Camões S.P. (1), Ruas J.L. (2), Miranda J.P. (1)

1- Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; 2- Department of Physiology and Pharmacology, Molecular & Cellular Exercise, Physiology Unit, Karolinska Institutet, Stockholm, Sweden.

Liver diseases, such as non-alcoholic fatty liver disease, steatosis, cirrhosis and hepatitis, are responsible for approximately 2 million deaths per year worldwide. Therefore, hepatic in vitro models representative of human physiology are of great importance for non-clinical studies. Our lab has established a hepatic differentiation protocol for deriving hepatocyte-like cells (HLCs) from human neonatal mesenchymal stem cells (hnMSCs) [1]. However, the culture medium presents high concentrations of insulin and dexamethasone. Therefore, the aim of our work was to assess the effect of decreasing insulin and dexamethasone concentrations to more physiological levels in the obtained hepatocyte-like cells (HLCs). A three-step hepatic differentiation protocol lasting 21 days was applied to hnMSCs [1]. At day 21, HLCs were either maintained in differentiation medium (DM), containing 1.72 µM of insulin and 1 µM of dexamethasone or changed to maintenance medium (MM), which presents 1 nM of insulin and 100 nM of dexamethasone. HLC functionality was evaluated up to two weeks in culture regarding biotransformation and glycogen storage ability, presence of hepatic markers, urea and albumin production and gene expression profile. ECOD, EROD and UGT activities, urea and albumin quantification, PAS staining and IF were performed at days 27 and 34. Herein, HLCs were successfully adapted to a novel culture medium. HLCs in MM maintained a polygonal shape, glycogen storage ability and presented hepatic-specific markers, such as HNF-4a, Alb, CK-18 and the hepatic transporters OATP-C and MRP2. Overall, no significant differences between DM and MM regarding biotransformation capacity and urea and albumin production were observed. However, different concentrations of insulin and dexamethasone induced different gene expression profiles. Indeed, genes involved in energy metabolism such as Pepck, G6pase, Fxr and Ppargc1a were increasingly expressed by ≥ 2-fold in HLCs cultured in DM when compared to MM ($p < 0.001$). Concluding, we were able to successfully adapt HLCs to a more physiological culture medium with decreased concentrations of insulin and dexamethasone, maintaining their hepatic-specific functionality. Further modifications in the culture medium should be performed such as modelling glucose, fatty acid and bile acid contents to increase the relevance of our *in vitro* model for disease modelling and drug screening.

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References

[1] Cipriano, M.; Correia, J. C.; Camões, S. P.; et al; Arch. Toxicol., 2017, 91, 2469-2489.

Diazaborines: Boronic Acids Under Disguise For Selective Inhibition Of Human Neutrophil Elastase

Antonio J.P.M., Gonçalves L.M., Guedes R.C., Moreira R., Gois P.M.P.

Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal.

Boronic Acids are a preeminent functionality extensively used to design biologically active compounds such as the FDA-approved Bortezomib and Ixazomib. However, due to boron's open shell, this class of inhibitors also exhibits unspecific reactivity with endogenous nucleophiles that often results in poor pharmacokinetic profiles and off-target toxicity. Here diazaborines are presented as a new class of boron-based warheads for serine proteases inhibition, in which the boron functionality is stabilized in the form of an aromatic BN heterocycle. In this study, diazaborines were readily synthesized in a single step in yields up to 96%, without any chromatographic operation and were shown to selectively inhibit Human Neutrophil Elastase with IC_{50}'s values in the low µM range. Synthetic and theoretical studies performed on this system suggest that, like boronic acids, the reaction mechanism involves the formation of a reversible covalent bond between the diazaborine boron center and the catalytic serine oxygen. Finally and differently from boronic acids who have half-life of 2h in buffer, diazaborines were shown very stable in different biocompatible conditions like buffer and human plasma. This work demonstrates that diazaborines are an interesting starting point for the development of the next generation of serine proteases [1].

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References

[1] António, J. P. M.; Gonçalves, L. M.; Guedes, R. C.; Moreira, R., Gois, P. M. P., ACS Omega, 2018, 3, 7418-7423.

Targeted Effects Of Epigenetic Modulators On Central Players Of Mitochondrial Energy Metabolism

Caio J.A. (1), Moedas M.F. (1), Rivera I (1,2), Silva M.F.B (1,2)

1- Research Institute for Medicines (iMed.ULisboa), Metabolism and Genetics Group, Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; 2- Department of Biochemistry and Human Biology, Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal.

Homeostasis of metabolic networks and respective regulatory processes are affected by drug administration and also by enzymatic, genetic and epigenetic mechanisms. Acetylation of lysine residues in proteins, including histones, depends on the combined activity of acetyltransferases and deacetylases (KDAC) that involve NAD+ and acetyl-coenzyme A (acetyl-CoA). Microvesicular steatosis, induced by the lysine deacetylase inhibitor (KDACi) antiepileptic drug valproic acid (VPA), may be associated with specific mitochondrial targets, including: i) carnitine palmitoyltransferase 1A (CPT1A) [1]; ii) DLDH, the E3 subunit of 2 oxoacid dehydrogenase complex [2]; iii) pyruvate carrier [3]; iv) hepatic NAD+ biosynthesis [4]. The present study aimed to understand the drug-induced impact on the pyruvate dehydrogenase complex (PDH) which, like fatty acid oxidation (FAO), are crucial acetyl-CoA producing pathways, essential to the regulation of mitochondrial energy metabolism.Total activity of PDH complex, determined using a radiochemical assay in rat liver lysates, was significantly decreased in VPA-administered animals. Immunoblotting revealed a reduced expression of PDH complex subunits (E1-α, E2, E3-bp) in the acute regimen, which may relate with the decrease in PDH activity. A significant increase of acetylated proteins (hyperacetylation) was observed in rat liver which was associated with repeated VPA administration (subchronic regimen). Differences in metabolites levels such as succinate, lactate and 2-oxoglutarate, quantified by GC-MS analysis in rat urine samples, support the inhibited PDH activity. Impairment of central enzymes of mitochondrial energy metabolism, such as PDH complex, may underlie drug-associated hepatotoxicity. The mito-nuclear communication and the signaling role of acetyl-CoA via PDH complex-dependent pathways still warrant further investigation. Imbalance of mitochondrial or nuclear acetyl-CoA levels in liver cells suggests a critical role in drug-induced hepatotropic effects.

Acknowledgements

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References

[1] Aires, C. P.; et al., Biochem. Pharmacol., 2010, 79, 792-799; [2] Luís, P. B.; et al., Biochem. Biophys. Acta., 2007, 1767, 1126-1133; [3] Aires, C. P.; et al., FEBS Letters, 2008, 582, 3359-3366; [4] Moedas, M. F.; et al., Metabolomics; 2016, 12, 142-153.

Euphorbia pubescens As A Potential Source Of Bioactive Compounds

Carréu J., Duarte N., Ferreira M.J.U

Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal

Euphorbia species have been used in traditional medicine to treat cancer, tumours and warts. In the last decades, considerable attention has been focused on plants from this genus as a source of biologically active compounds (e.g. antitumour, cytotoxic, antibacterial and antiviral). These plants are characterized for biosynthesizing macrocyclic and polycyclic diterpenes, as well as triterpenes, steroids and phenols. In particular, the discovery of macrocyclic jatrophane- and lathyrane-type diterpenes as a new class of potent modulators of the transmembrane protein P-gp (P-glycoprotein), the main ABC transporter involved in multidrug resistance in cancer, has fostered an increasing interest in the research of this genus. In our continuing investigation to find out novel bioactive compounds, the phytochemical study of the methanol extracts of *Euphorbia pubescens* has been carried out. Two diterpenes with the jatrophane and abietane scaffolds, as well as several known triterpenes and steroids were isolated trough chromatographic techniques. Taraxerone, a pentacyclic triterpene, was isolated in major amounts. Therefore, taking advantage of the ketone function at C-3, some imine derivatives were prepared. The structures of the compounds, including stereochemical features, were deduced from their physical and spectroscopic data, which included: infrared spectroscopy, mass spectrometry (MS), and extensive one- and two-dimensional nuclear magnetic resonance studies (COSY, HMQC, HMBC and NOESY).

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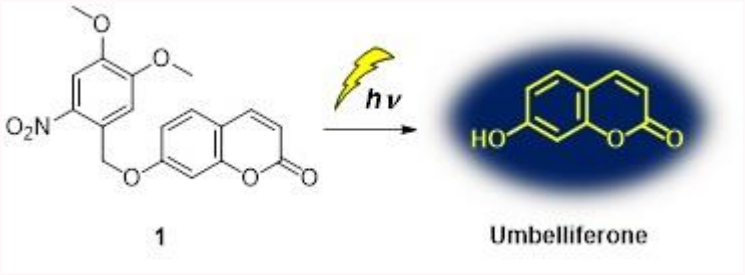
Improvement Of Tumor Identification And Targeting By Developing Photocaged Prodrugs

Vaz J. (1), Braga C. (2), Perry M. J. (2), Rosa N .J. (2), Moreira R. (2)

1- Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; 2- Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal.

Cancer is the second leading cause of death globally and is responsible for an estimated 9.6 million deaths in 2018. Globally, about 1 in 6 deaths is due to cancer [1]. Parallel to the development of new antitumor drugs, strategies to reduce the side effects often associated with these therapies have also been design. Photopharmacology tries to overcome lack of selectivity and emergence of resistance in anticancer treatments. In this approach, photoswitchable groups are incorporated into the molecular structure of bioactive compounds. The switching unit allows for the use of light as an external control element for pharmacological activity, which can be delivered with high spatiotemporal precision [2]. The photoswitchable groups are also called photoremovable/photocleavable protecting groups (PPGs) and the development of new PPGs in organic synthesis does not cease to increase, leading to the emergence of biology applications [3]. Nitrobenzyl derivatives are among the most commonly used. Following this background and the recent "boom" of probe research, the employment of a o-nitrobenzyl photocaging group as a possible photoswitchable moiety was attempted, using umbelliferone as fluorescent moiety, fully characterizing the obtained compound (1) and its release (Figure 1).



Acknowledgements

Funded, in part, by UID/DTP/04138/2019 from Fundação para a Ciência e a Tecnologia (FCT), Portugal.

References

[1] Velemea, W. A., J. Am. Chem. Soc., 2014, 136, 2178-2181; [2] Klán, P.; et al., Chem Rev, 2013, 119-191; [3] Horbert, R.; et al., ACS Chem. Biol., 2015, 10, 2099-2107.

Tetracyclic Bis-Quinolizidines: A Hidden Value In Food Processing Wastewater

Teixeira R., Gonçalves J.M., Afonso C.A.M.

Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal.

Lupin beans are the legume seeds of the Fabaceae family plants of the Lupinus genus. They are typically consumed as a pickled snack in the Mediterranean and Latin American regions. In order to be safe for human ingestion, lupin seeds require pre-treatment with aqueous extraction, generating high volumes of wastewater rich in highly toxic quinolizidine alkaloids, mainly lupanine [1,2]. Novel separations processes using low energy and chemicals at low cost are being developed using membrane processes and adsorbers capable of purification for in situ recycling of these effluents. Along with this concept, a valorization approach of lupanine is being developed consisting in: (a) chiral resolution of lupanine with tartaric acid, (b) transformation of each enantiomer in enantiopure sparteine and (c) conversion of these alkaloids in potentially added value compounds for pharmaceutical and chemical industries. Within this global approach innovation potential have been identified [3,4].

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WaterWorks (WaterJPI/0001/2014).

References

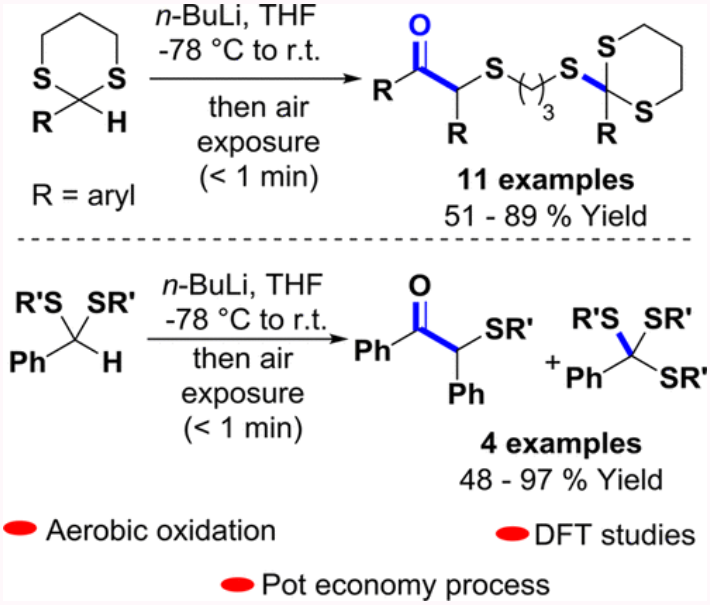
[1] Erbas, M. J., Food Qual., 2010, 33, 742-57; [2] Carmali, S.; Alves, V. D.; Coelhoso, I. M.; Ferreira, L. M.; Lourenc, A. M., Sep Purif Technol J., 2010, 74, 38-43; [3] Gawali, A. V. S.; Simeonov, S.; Drescher, M.; Knott, T.; Scheel, O.; Kudolo, J.; et al., ChemMedChem., 2017, 12, 1819-1822; [4] Parmaki, S.; et al., Chemosphere., 2017, 50-59.

Autoxidative Condensation Of 2-Aryl-2-lithio-1,3-dithianes

Vale J.R. (1,2), Rimpiläinen T. (1), Sievänen E. (3), Rissanen K. (3), Afonso C.A.M. (2), Candeias N.R. (1).

1- Laboratory of Chemistry and Bioengineering, Tampere University of Technology, Korkeakoulunkatu 8, 33101 Tampere, Finland ; 2- IResearch Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; 3- University of Jyvaskyla, Department of Chemistry, Nanoscience Center, P.O. Box 35, 40014 Jyväskylä, Finland.

Organolithium compounds can undergo autoxidation toward formation of highly unstable organolithium peroxides, which upon fast interaction with another organolithium leads to the ultimate formation of lithium alkoxides [1]. We recently reported the autoxidative condensation of 2-aryl-2-lithio-1,3-dithianes [2]. Treatment of 2-aryl-1,3-dithianes with n-buLi in the absence of an electrophile leads to condensation of three molecules of 1,3-dithianes and formation of highly functionalized α-thioether ketones orthothioesters in 51–89% yields upon air exposure (Scheme, top). The method was further expanded to benzaldehyde dithioacetals, affording the corresponding orthothioesters and α-thioether ketones in 48–97% yields (Scheme, bottom). The experimental results combined with density functional theory studies support a mechanism triggered by the autoxidation of 2-aryl-2-lithio-1,3-dithianes to yield a highly reactive thioester that undergoes condensation with two other molecules of 2-aryl-2-lithio-1,3-dithiane.



Scheme

Acknowledgements

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References

[1] a) Boche, G.; Lohrenz, J. C. W., Chem. Rev., 2001, 101, 697-756. (b) Wardell, J. L., In Comprehensive Organometallic Chemistry; Stone, F. G. A., Abel, E. W., Eds.; Pergamon: Oxford, 1982; pp 43-120. (c) Sosnovsky, G.; Brown, J. H., Chem. Rev., 1966, 66, 529-566; [2] Vale, J. R.; Rimpiläinen T.; Sievänen, E.; Rissanen, K.; Afonso, C. A. M.; Candeias, N. R. J., Org. Chem., 2018, 83, 1948-1958.

Structural Optimization Of n-Substituted Azaaurone Compounds As Anti-TB Agents

Ribeiro J., Harjivan S., Campaniço A., Freitas E., Moreira R., Lopes F.

Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal.

Tuberculosis (TB) is a deadly infection disease caused by a single infectious agent *Mycobacterium tuberculosis* (M.tb). The complexity and duration of the treatment leads to misuse and low compliance by patients, increasing the disease burden and the appearance of multidrug-resistant strains of M.tb. Thus, new antibiotics active against drug-resistant M.tb and useful for short period therapeutic regimens at lower required doses are urgently needed [1,2]. Recently, a family of azaaurones based derivatives, from a chemical library in iMedULisboa, revealed to be active against M.tb including multidrug- and extensively drug-resistant tuberculosis, from clinical isolates, at submicromolar level [3]. We now report the synthesis of novel azaaurones with improved solubility properties. These compounds were obtained by incorporating ionisable groups on the A-ring (Figure 1).

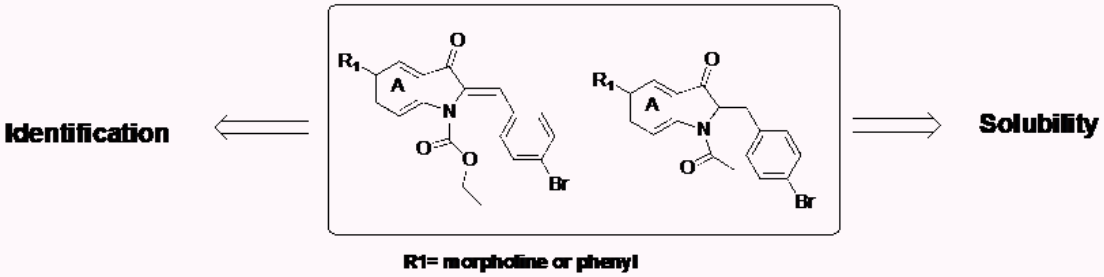

Figure 1

Acknowledgements

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References

[1] Campaniço, A.; Moreira, R.; Lopes, F., Eur J Med Chem., 2018, 150, 525-545; [2] Ehrt, S.; et al, Cell Microbiol., 2009, 11, 1170-1178; [3] Campaniço, A.; et al, ChemMedChem (accepted).

Synthesis, Potentiometric Studies As Antifungal *In Vitro* Evaluation Of thia-dioxo-polyaza Macrocyclic Compounds And Their Cu(II) And Ni(II) Complexes

Pereira J. (1), Torres N. (2), Costa J. (1,3), Castro M. (1,3), Lopes M.M. (1,3), Cabral M.F. (1,3)

1- Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; 2- Centro Hospitalar Lisboa Norte, Lisbon, Portugal; 3- Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal.

Over the past decades, fungi have emerged as a major cause of serious infections in immunocompromised patients. As known, the easily gained resistance is the main problem encountered in developing safe and efficient antifungal compounds. The standard antifungal therapies available for the treatment of Candida infections are scarce due to the high toxicity, low efficacy rates, and drug resistance [1]. As part of a continuing search for new antifungal agents, two dioxo-triaza macrocyclic compounds, the dioxo-[12]aneN3S (L1) and dioxo-[14]aneN3S (L2), a dioxo-tetraaza macrocycle dioxo-[15]aneN4S (L3) and its reduced derivative [15]aneN4S (L4) [2] presented in the scheme below, as well as, their Cu(II) and Ni(II) complexes were prepared. The antifungal activity of L1-L4 and their Cu(II) and Ni(II) complexes was evaluated *in vitro* against clinical isolates of multi-drug resistant strains of *Candida* spp from patients with candidemia or invasive candidiasis, using a microdilution reference method according to the EUCAST/ E.DEF 7.3.1. international guidelines [3].

Acknowledgements

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References

[1] Arendrup, M. C.; Patterson, T. F., J. Infect. Dis., 2017, 216, S445-S451; [2] Torres, N.;, Gonçalves, S.; et al., Molecules, 2014, 19, 550-567; [3] The European Committee on Antimicrobial Susceptibility Testing. Doc. E.DEF 7.3.1. (2017) 1-21.

Preparation Of Aminals In Water

Pereira J.G., Gomes R.F.A., Coelho J., Afonso C.A.M.

Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal.

Aminals are the condensation product of aldehydes and secondary amines, structurally similar to acetals. These compounds have been used as intermediates, chiral auxiliaries and protection groups in reactions and also used in biology due to its biological activity [1]. The most common methodology for the formation of aminals involves the condensation of aldehydes with amines in ethanol or toluene under high temperature using dehydrating agents to remove the water in the reaction, shifting the equilibrium to the product [2]. However, performing the reaction in aqueous media instead of organic solvents is a process environmentally competitive for the preparation of aminals. This work reports on the formation of aminals, from aromatic aldehydes and furfural derivatives with different secondary amines, in water under mild conditions, and on stability studies of different aminals.

Acknowledgements

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References

[1] Alexakis, A.; Lensen, N.; Mangeney, P., Tetrahedron Letters, 1991, 32, 1171; [2] Ramirez, M. A.; Ortiz, G.; Levin, G.; McCormack, W.; Blanco, M. M.; Perillo, I. A.; Salerno, A.; Tetrahedron Letters, 2014, 55, 4774.

Developing Tools For Metabolism Prediction Of New Psychoactive Substances – Preliminary Study With Buphedrone.

Carrola J. (2), Berkowicz K. (1,2,4), Florindo P. (1,2), Moreira R. (1,2), Perry M.J. (1,2), Lopes A. (2), Duarte N. (1,2), Bronze M.R. (2,3,4), de Mello-Sampayo C. (1,2)

1- Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; 2- Faculty of Pharmacy, ULisboa, Portugal; 3- iBET, Instituto de Biologia Experimental e Tecnológica; 4) ITQB, Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa, Portugal.

Novel Psychoactive Substances (NPS) is a term used to describe synthetic analogues or derivatives of controlled, more common illicit drugs. One of the most popular and sought for class of NPS are Synthetic Cathinones (SC), often marketed as “Bath Salts”/“Plant Food”. They started to appear on drug markets over two decades ago as a legal alternative to amphetamines, cocaine and MDMA, as the psychoactive effects they exert are often very similar to those substances. Chemically they derive from Cathinone, a stimulant alkaloid found in *Catha edulis* (Khat) plant leaves. Although structurally similar to amphetamines, their psychopharmacology, metabolism and half-life are often very different, posing a threat of toxicity and overdose among the users. Upon law banning NPS, emerging SC have been appearing as adulterants of MDMA and other stimulants or even as substitutes of older SC. A SC that emerged in 2010 right after banning of mephedrone was buphedrone. Nowadays, over 100 SC are monitored by the EMCDDA, with new ones being added to the list every year, but the negative public health implications of their use remain high. Develop measures to monitor the use/consumption of these newly synthesized substances, obtain sufficient knowledge on their metabolites and excretion profiles and evaluate their toxicity is of major relevance. AIMS: Access the main metabolites of selected SC firstly in animals and then in humans to either contribute to the metabolism knowledge of these drugs and subsequently evaluate the translation potential of using mice as specie with relevance for testing the toxicity and pharmacology of these drugs. For that, *in silico* metabolite prediction was carried out on buphedrone to map possible metabolism routes. Both buphedrone and selected metabolites were synthesized. *In vitro* preliminary studies were performed for detecting, by HPLC-MS/MS, the main metabolites present in the reaction mixture of buphedrone incubated in plasma or with liver microsomal preparations, at several time points. Results were compared with in-house recently obtained data showing that the most excreted metabolite, during 24 h following mice exposure to buphedrone, was that resulting from N-dealkylated reaction, followed by the N-dealkylated alcohol, and lastly by β-keto reduced N-alkylated metabolite.

Acknowledgements

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Cyclopentenones As New Anticancer Agents: Synthesis And Biological Evaluation

Andrade K.H.S., Gomes R.F.A., Estevão M.S., Guerra K.P., Frade R.F.M., Afonso C.A.M.

Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal.

Cyclopentenones (CPs) are intermediates for the synthesis of many biologically active compounds [1] and are usually present in several natural products. Its structure consists of an α, β-unsaturated carbonyl group attached to a membered ring. These compounds have been used as cytotoxic agents since the α-β- unsaturated carbonyl group can act as a Michael acceptor resulting in the alkylation of critical biomacromolecules for cancer cells. However, the reactivity of these Michael acceptors makes them also promiscuous to unwanted reactions with macromolecules critical to healthy cells [2]. Based on these, our group is involved in the preparation of novel CP with poor Michael acceptor character and in the evaluation of their cytotoxic activity in human cancer cells to elucidate their structure-activity relationship (SAR) [3].

Acknowledgements

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References

[1] a) Santoro, M.G., Trends in Microbiology, 1997, 5, 276-281; b) Lee, S. Y.; Ahn, J. H.; Ko, K. W.; Kim, J.; Jeong, S. W.; Kim, I. K.; Kim, J.; Kim, H. S., Prostag Oth Lipid M., 2010, 91, 30-37; c) Simeonov, S. P.; Nunes, J. P. M.; Guerra, K.; Kurteva, V. B.; Afonso, C. A. M., Chem. Rev., 2016, 116, 5744-5893; [2] a) C. Frederick M. et al, Org. Lett., 2000, 2, 20, 3143-3144; b) Diana S. Hamilton et al, J. AM. CHEM. SOC. 2003, 125, 15049-15058; [3] Manuscript under revision.

Trans-Cinnamic Acid Derivatives Of Triazenes As Anti-Cancer Hybrid Drugs

Ferreira L.M., Perry M.J.

Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal.

Gliobastoma Multiforme and Melanoma are two types of very aggressive cancer responsible for thousands of deaths around the world. The high incidence and mortality of these tumours has increased the research for new and more effective treatments [1]. The current Chemotherapy treatments are based on the prodrug Temozolomide (TMZ), a triazene that has capability to alquilate DNA of the tumor cells and therefore, stop cell growing. However, TMZ shows high toxicity and low efficiency, due to the resistance caused by DNA repair mechanisms and the increased drug efflux.New treatment approaches have been searched, such as, multiple therapy with Triazenes and Hystone Deacetylases inhibitors (iHDAC's) that have anti-tumor growth properties due to their ability to repress genes and repair DNA damages. An additional strategy was the development of hybrid molecules [2] containing differently substituted triazene moieties and HDAC inhibitor-related short chain fatty acids, including valproic and butyric acids. These hybrids revealed selective targeting of GL261 glioma cells and showed high induction of cell morphological alterations that led to cell migration impairment.Trans- cinnamic acid (tCA) has been found to display a broad spectrum of biological effects including anti-oxidant, anti-inflammatory and anti-cancer activities [3]. Based on the known HDAC inhibitory activity of tCA, four new hybrids were synthesised by binding of the tCA to different triazenes. Further studies on these compounds were also done to understand their pharmacokinetic and pharmacodynamics properties.

Acknowledgements

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References

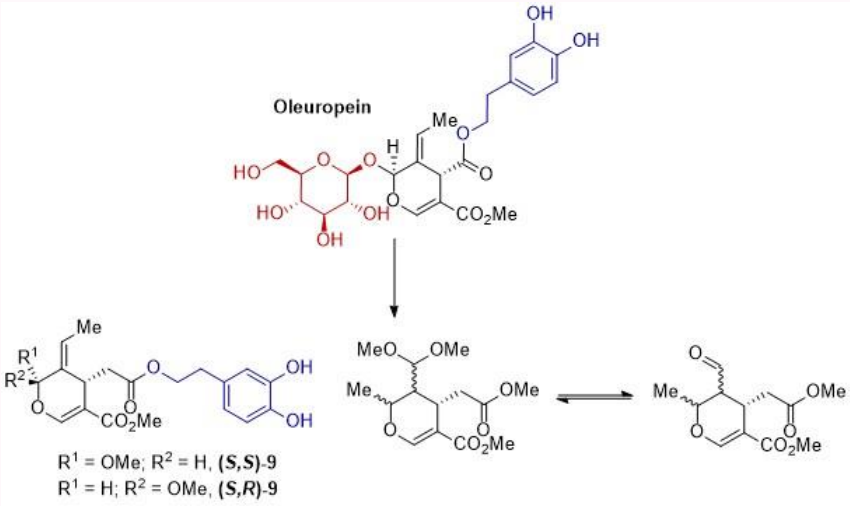
[1] GIOBIOCAN 2012: Estimate cancer incidence, mortality and prevalence worldwide. Available at: http://globocan.iarc.fr/Pages/fact_sheets_population.aspx. Accessed May 5, 2019; [2] Braga, C.; Vaz, A. R.; Oliveira, M. C.; Marques, M.; Moreira, R.; Brites, D.; Perry, M. J., Eur J Med Chem., 2019, 172, 16-25; [3] Anantharaju, P. G.; Reddy, D. B.; Padukudru, M. A.; Chitturi, C.H. M. K.; Vimalambike, M. G.; Madhunapantula, S. V., PLoS ONE 2017, 12(11), e0186208.

Valorization Of Oleuropein: Acid-Promoted Methanolysis

Cavaca L.A.S. (1), Rodrigues C.A.B. (1), Simeonov S.P. (1,2), Gomes R.F.A. (1), Coelho J.A.S. (1), Romanelli G.P. (3), Sathicq A.G. (3), Martínez J.J. (4), Afonso C.A.M. (1)

1- Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal, 2- Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences. Acad. G. Bonchev str., bl. 9, 1113, Sofia, Bulgaria, 3- Centro de Investigación y Desarrollo en Ciencias Aplicadas "Dr. J.J. Ronco" (CINDECA), Departamento de Química, Facultad de CienciasExactas Universidad Nacional de la Plata Argentina Calles 47 Nº 257, B1900 AJK, La Plata, Argentina, 4- Escuela de Ciencias Químicas, Facultad de Ciencias, Universidad Pedagógica y Tecnológica de Colombia UPTC; Avenida Central del Norte, Tunja, Boyacá, Colombia.

Oleuropein is one of the major secoiridoids found in the olive leaf (0.5-2% (w/w) on dry basis) [1]. Oleuropein structure can be divided in three subunits - glucoside, monoterpene and hydroxytyrosol (red, black and blue, respectively, Figure 1) [2]. The monoterpene unit is a highly functionalized moiety that includes two esters, one alkene, one enol ether, one acetal and a stable chiral center at C-4. This multifunctional structure makes it difficult to be obtained by other means than extraction from natural sources [3]. In this context, we became interested in the valorization of oleuropein towards the synthesis of diverse and synthetically rich building blocks. The acid-promoted methanolysis of oleuropein was studied using a variety of homogeneous and heterogeneous acid catalysts. Exclusive cleavage of the acetal bond between the glucoside and the monoterpene subunits or further hydrolysis of the hydroxytyrosol ester and subsequent intramolecular rearrangement were observed upon identification of the most efficient catalyst and experimental conditions. Furthermore, selected conditions were tested using Oleuropein under continuous flow and using a crude mixture extracted from olive leaves under batch.Formation of (-)-methyl elenolate was also observed in this study, which is a reported precursor for the synthesis of the antihypertensive drug (-)-ajmalicine [4].



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The authors acknowledge Fundação para a Ciência e a Tecnologia (FCT) (refs UID/DTP/04138/2013, SFRH/BPD/100433/2014, SFRH/BPD/109476/2015 PD/BD/128316/2017), COMPETE Programme (SAICTPAC/0019/2015) and European Research Area Network; ERANet LAC (ref ELAC2014/BEE-0341) for financial support.

References

[1] de Castro, M. L.; Japón-Luján, R., Trends Anal. Chem., 2006, 25, 501-510; [2] Erbay, Z.; Icier, F., Food Rev. Int., 2010, 26, 319- 334; [3] Cavaca L.A.S., Afonso C.A.M., Eur. J. Org. Chem., 2018, 581-589; [4] Cavaca, L. A. S., et al., ChemSusChem, 2018, 11(14), 2300-2305.

Activity-Based Protein Profiling Using 4-Oxo-β-Lactams Uncovers A New Chemotype For DPP8 And DPP9 Inhibition

Carvalho L.A.R. (1,2), Lum K. (2), Ross B. (3), Lucas S.D.L. (1), Huber R. (3), Cravatt B.F. (2), Moreira R. (1)

1- Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; 2- The Scripps Research Institute, La Jolla, California, USA; 3- Max-Planck-Institut fuer Biochemie Emeritusgruppe Strukturforschung, D-82152 Martinsried - Germany.

Activity-based protein profiling (ABPP) enables the profiling of enzymatic activity in complex biological systems [1]. We evaluated the 4-Oxo-β-Lactam (4OBL) [2] chemotype as a serine hydrolase reactive group in ABPP by synthesizing a library of 4OBL inhibitors and activity-based probes (ABPs). Our library was tested in a competitive ABPP approach against the well-established fluorophosphonate (FP) probes [3]. 4OBLs were shown to potently and selectively hit a small group of serine hydrolases in the presence of a complex proteome. Target deconvolution was performed using mass spectrometry-based ABPP to determine the full extent of target engagement by the compounds using LC-MS/MS. Our results confirmed previously known targets like human neutrophil elastase, but also selected members of the dipeptidyl peptidase (DPP) and the alpha beta hydrolase domain (ABHD) families of serine hydrolases, which were previously unreported as 4OBL targets. Target validation revealed high potency and preliminary SAR, which suggested that the substitution pattern at the N-aryl moiety affects target specificity. Compounds containing a meta-substituted N-aryl moiety were revealed to be selective and suited for inhibition of DPP8 and DPP9. The compounds were shown to have an unprecedented preference for DPP8 inhibition over DPP9 and crystal structures revealed a previously unknown mechanism of inhibition. Overall, we provided the first mass spectrometry-based analysis of target engagement by 4OBLs in selected proteomes, highlighting the promising features of the 4OBL warhead for ABPP applications and development of inhibitors selective for serine hydrolases.

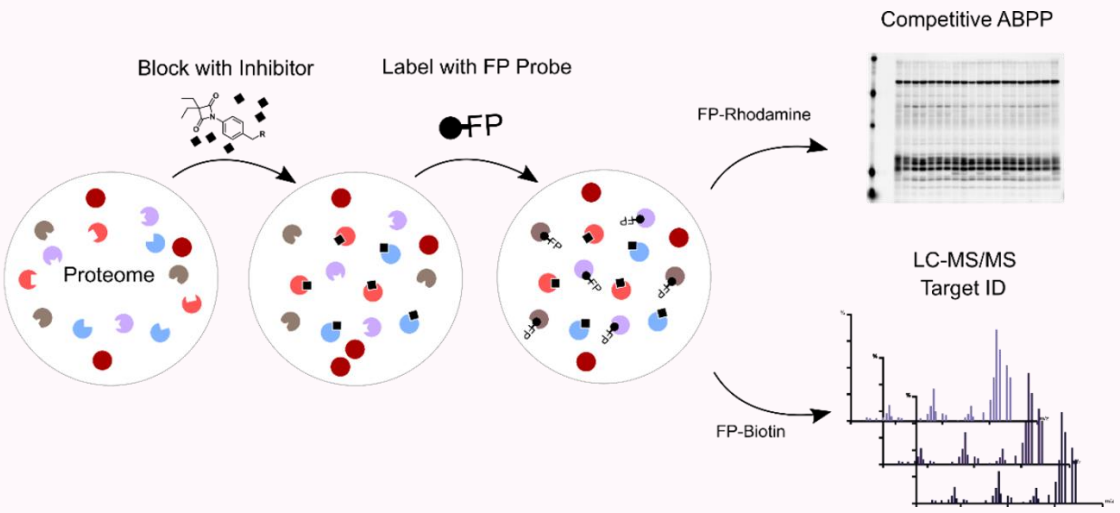

Figure 1

Acknowledgements

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References

[1] Niphakis, M.J.; Cravatt, B.F.; Annu. Rev. Biochem., 2014, 83, 341-377; [2] Mulchande, J.; Oliveira, R.; Carrasco, M.; Gouveia, L.; Guedes, R. C.; Iley, J.; Moreira, R., J. Med. Chem., 2010, 53, 241-253; [3] Liu, Y.; Patricelli, M. P.; Cravatt, B. F.; Proc. Natl. Acad. Sci., 1999, 96, 14694-14699.

Antibiotic Liposomal Nanoformulations As A Tool To Inhibit Mature *Staphylococcus aureus* Biofilms

Ferreira M.C. (1, 4), Pinto S. (2, 3), Ribeiro I.A. (1), Aires-da-Silva F. (4), Almeida A.J. (1), Bettencourt A. (1), Aguiar S.I. (4), Gaspar M.M. (1)

1- Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; 2- Centro de Química-Física Molecular e IN, Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais 1049-001 Lisbon, Portugal; 3- iBB-Institute for Bioengineering and Biosciences, Department of Bioengineering, Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais 1049-001 Lisbon, Portugal; 4- Centre for Interdisciplinary Research in Animal Health (CIISA), Faculty of Veterinary Medicine, Universidade de Lisboa, Lisbon, Portugal.

Staphylococcus aureus is responsible for most cases of bone infection, particularly when associated to orthopedic-implant devices. Infection eradication becomes a huge challenge, mainly due to S. aureus ability to form biofilms, which provides protection to bacteria tolerating higher concentrations of antibiotics than in planktonic form [1]. Lipid-based nanosystems, liposomes, can constitute an innovative and alternative therapeutic strategy against these infections, enabling a preferential interaction with biofilms and release of incorporated antibiotics [2]. In the present work, three antibiotics were selected: vancomycin (VCM), levofloxacin (LEV) and rifabutin (RFB). Susceptibility assays, of planktonic and biofilm form of *S. aureus* strain (ATCC®25923) to selected antibiotics, were performed by broth microdilution method followed by turbidity evaluation, MTS and MTT assay, and CFU counts. The minimal inhibitory concentrations (MIC_{95}) and minimal biofilm inhibitory concentrations ($MBIC_{50}$) were assessed. MIC_{95} and $MBIC_{50}$ were defined as the lowest concentration that reduced bacterial growth >95% and >50%, respectively. MIC_{95} obtained for RFB, LEV and VCM were 0.02, 0.18 and 1.54 µg/mL, and $MBIC_{50}$ were <0.01, 0.08 and >12.5 µg/mL, respectively. All antibiotics were incorporated in negative and positively charged nanoliposomes in the presence or absence of the fusogenic phospholipid, by dehydration-rehydration method followed by an extrusion step to reduce and homogenize liposomes mean [2]. Higher Incorporation Efficiencies (I.E.) were obtained for VCM and RFB, ranging from 32 to 88% and antibiotic loading between 23 and 40 µg/µmol of lipid, respectively. For the most promising antibiotic, RFB, anti-biofilm activity of liposomal forms was evaluated, against mature biofilm, by MTT assay. This effect proved to be lipid composition dependent: negative and positively charged liposomes presented the highest MBIC (0.04 µg/mL) while the lowest value was achieved for fusogenic liposomes (0.02 µg/mL). The internalization of nanoformulations, with different lipid composition, into *S. aureus* biofilm was evaluated by spectrofluorimetry and confocal microscopy imaging, with rhodamine-labeled liposomes [3]. This study showed that liposomes with fusogenic properties and positively charged were able to internalize with *S. aureus* biofilm in a very high extent. Therefore, these nanoformulations constitute a highly promising strategy to improve treatment of *S. aureus* infections.

Acknowledgements

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References

[1] Ferreira, M.; Rzhepishevska, O.; Grenho, L.; Malheiros, D.; Gonçalves, L.; Almeida, A. J.; Jordão, L.; Ribeiro, I. A.; Ramstedt, M.; Gomes, P.; Bettencourt, A., Int J Pharm., 2017, 532(1), 241–8; [2] Gaspar, M. M.; Cruz, A.; Penha, A. F.; Reymão, J.; Sousa, A. C.; Eleuterio, C. V.; Domingues, S. A.; Fraga, A. G.; Filho, A. L.; Cruz, M. E. M.; Pedrosa, J., Int J Antimicrob Agents., 2008, 31(1), 37–45.;[3] Jefferson K. K.; Goldmann, A. D.; Pier B. G., Antimicrob Agents Chemother, 2005, 49(6), 2467-73.

Development Of Quercetin Liposomal Formulations For Hepatic Ischemia/Reperfusion Injury Prevention

Ferreira-Silva M. (1,2), Faria-Silva C. (1), Carvalheiro M. (1), Baptista P.V. (2), Fernandes E. (3), Fernandes A.R. (2), Corvo M.L. (1)

1- Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; 2- Applied Molecular Biosciences Unit (UCIBIO), NOVA School of Science and Technology, Lisbon, Portugal; 3- Laboratório Associado para a Química Verde (LAQV), Faculty of Pharmacy, University of Porto, Porto, Portugal.

Ischemia/reperfusion injury (IRI) is a common complication in liver surgery and liver transplantation, due to oxidative stress and inflammation [1]. The current therapy in humans includes non-steroid anti-inflammatory drugs (NSAID) and corticosteroids do not show the desired outcome. Quercetin is known as a powerful antioxidant, able to scavenge reactive oxygen species, leading to a reduction of inflammation. The drawback of the treatments (low *in vivo* efficacy) is due to its poor bioavailability and fast liver metabolism [2]. These disadvantages can be surpassed by the incorporation of quercetin into nanoformulations. For that purpose, long-circulating liposomes that will preferentially target quercetin into inflamed liver areas of IRI were developed and optimized. The quercetin loaded long circulated liposomes present a size under 130 nm, displaying low polidispersity index values and zeta potential values around zero (mV), at pH 6.0. Quercetin loading saturation curves were also performed as well as release assays with PBS and 1% (w/v) BSA. The differences between using egg phosphatidylcholine (EPC) or soy phosphatidylcholine (SPC) are being analyzed. The different formulations were tested in an *in vitro* model of IRI, using human hepatocarcinoma cells in a hypoxic chamber, to evaluate the most suitable inflammatory biomarkers and the ideal temporal window for measurements. Using a range of doses equivalent to the one used in humans, the lipidic nanosystems did not decrease cellular viability and reduced the cytotoxicity of the free drugs. Further studies are ongoing to ultimately confirm the expected inflammation suppression and achieve a more effective therapeutic strategy.

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References

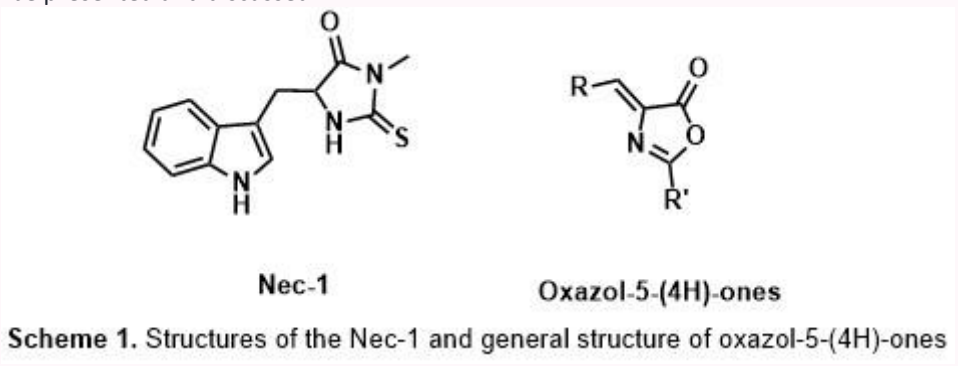
[1] Marcelino, P.; Marinho, H. S.; Campos, M. C.; Neves, A. R.; Real, C.; Fontes, F. S.; Carvalho, A.; Feio, G.; Martins, M. B. F.; Corvo, M. L., Eur. J. Pharm. Sci., 2017, 109, 464-471; [2] D'Andrea, G., Fitoterapia, 2015, 106, 256-271.

Oxazol-5-(4H)-ones Derivatives As Necroptosis Modulators

Ferreira M.B.T., Rodrigues C.A.B., Estevão M.S., Oliveira S.R., Amaral J.D., Ravasco J.M.J.M., Rodrigues C.M.P., Afonso C.A.M.

Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal.

Necroptosis is a regulated form of necrosis and occurs when apoptosis is inhibited. This is a form of cell death regulated caspase-independent realized by RIPK1, RIPK3 and MLKL. Necroptosis is associated with a variety of pathologies such as stroke, myocardial infarction, septic shock and acute pancreatitis, that currently lack effective therapies [1]. Necrostatin-1 (Nec-1) (Scheme 1) was the first reported necroptosis inhibitor and acts through inhibiting RIPK1 activity. However, this inhibitor is only modestly potent, and often used at concentrations in vitro, which can lead to off-target effects, also its metabolic stability is quite limited, resulting in a short *in vivo* half-life [1]. Due to the importance of treating various diseases associated with necroptosis, the development of more efficient and potent necroptosis inhibitors attracted a lot of attention. Thus, based on our experience on the synthesis of oxazolones [2] and after a preliminary screening of oxazol-5-(4H)-ones (Scheme 1) derivatives in cell lines (BV-2 microglia and L929 fibrosarcoma), it was found a lead compound which exhibits inhibition activity similar to the kwon inhibitor, Nec-1 [3]. These results motivated the synthesis and development of new oxazolones as potent necroptosis inhibitors. The synthetic details and results will be presented and discussed.



Scheme 1. Structures of the Nec-1 and general structure of oxazol-5-(4H)-ones

Acknowledgements

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References

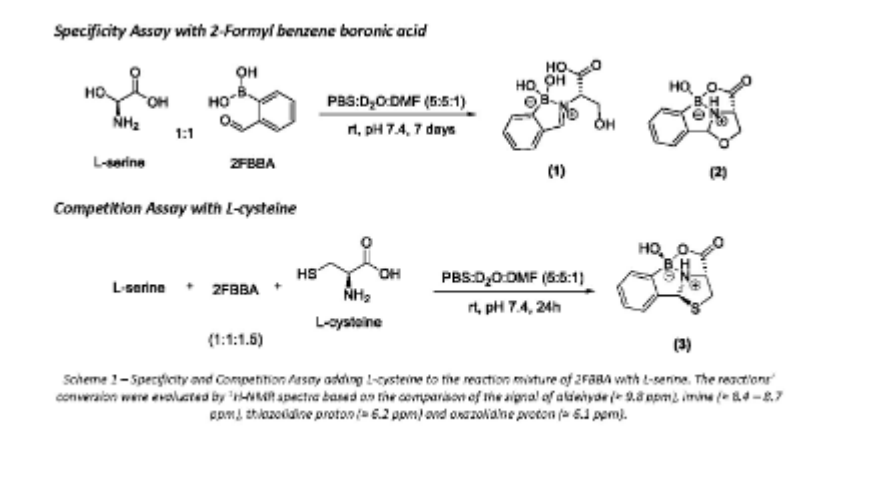
[1] Sun, L. M.; Wang, X. D. Trends Biochem. Sci., 2014, 39, 587; [2] a) Rodrigues, C. A. B.; Mariz, I.; Maçôas, E. M. S.; Afonso, C. A. M.; Martinho, J. M. G., Dyes and Pigments, 2012, 95, 713. b) J. Mater. Chem. C, 2015, 3, 10775. c) Rodrigues, C. A. B.; Martinho, J. M. G.; Afonso, C. A. M., J. Chem. Educ., 2015, 92, 1543; [3] Oliveira, S. R.; Dionísio, P. A.; Brito, H.; Franco, L.; Rodrigues, C. A. B.; Guedes R. G.; Afonso, C. A. M.; Amaral, J. D.; Rodrigues, C. M. P.; Cell Death Discovery, 2018, 4, 65.

Competitive Functionalization Of N-Terminal Amino Acids *Via* Iminoboronate Intermediates With B–N Bond Stabilization

Silva M.J.S.A, Faustino H., Gois P.M.P.

Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal.

By far the most explored property has been the nucleophilic reactivity of amino acid residues with respect to proteins bioconjugation. In fact, lysine and cysteine have been the election targets especially due their own reactivity since they bear the most reactive nucleophiles in their residue chain [1]. Despite this, there are also other nucleophilic substrates available to react, such as serine hydroxyl [2]. We have shown the high reactivity of formyl benzeno boronic acid (2FBBA) with N-terminal cysteines to form a boronated thiazolidine featuring a B–N bond under mild aqueous conditions (pH 7.4, 23°C) [3]. We reasoned that other type of N-terminal amino acids such as serine or threonine could participate in similar reactions. In fact, preliminary data shows that when 2FBBA reacts with serine, it generates a mixture of iminoboronate (1) and oxazolidine (2), although in low conversion (Scheme 1). Notwithstanding the addition of cysteine shifts the equilibrium to the cyclization of thiazolidine in the competition assay (3) (Scheme 1). Herein we will provide some results on the development of this methodology for orthogonal modification of N-terminal cysteine in peptides and proteins.



Scheme 1 – Specificity and Competition Assay adding L-cysteine to the reaction mixture of 2FBBA with L-serine. The reactions' conversion were evaluated by ^{1}H-NMR spectra based on the comparison of the signal of aldehyde (= 9.8 ppm), imine (= 8.4 – 8.7 ppm), thiazolidine proton (= 6.2 ppm) and oxazolidine proton (= 6.1 ppm).

Acknowledgements

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References

[1] (a) Bernardes, G. L.; Chalker, J. M.; Davis, B. G., Ideas in Chemistry and Molecular Sciences 2010 (b) Cal, P. M.; Vicente, J.o. B.; Pires, E.; Coelho, A. V.; Veiros, L. s. F.; Cordeiro, C.; Gois, P. M. J. Am. Chem. Soc., 2012, 134, 10299 (c) Cal, P. M.; Frade, R. F.; Cordeiro, C.; Gois, P. M. Chem. Eur. J. 2015, 21, 8182 (d) Cal, P. M.; Bernardes, G. J.; Gois, P. M. Angew. Chem. Int.l Ed. 2014, 53, 10585; [2] Koniev, O.; Wagner, A., Chem. Soc. Rev., 2015, 44, 5495; [3] Faustino, H.; Silva, M. J. S. A.; Veiros, L. F.; Bernardes, G. J. L.; Gois, P. M. P., Chem.l Sci., 2016, 7, 5052.

Novel Approach To Characterize Demyelination Using Newly Synthesized BASHY Molecules

Pinto M.V. (1), Santos F.M.F. (2), Barateiro A. (1,3), Brites D. (1,3), Gois P.M.P. (2,4), Fernandes A. (1,3)

1- Neuron-Glia Biology in Health and Disease, Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; 2- Bioorganic Chemistry, Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; 3- Department of Biochemistry and Human Biology, Faculty of Pharmacy, Universidade de Lisboa, Portugal; 4- Department of Pharmaceutical Chemistry and Therapeutics, Faculty of Pharmacy, Universidade de Lisboa, Portugal.

Demyelination, a pathological feature of many neurodegenerative disorders, is characterized by the progressive loss of myelin membrane normally enwrapping the axon, leading to axonal damage and severe neurodegeneration. As a multi-step process, lipid-rich myelin layers suffer initial decompaction, gradually detaching from the parent axon until being completely destroyed into vesiculated myelin debris that will be efficiently cleared away by microglia. Indeed, discrimination between different steps of myelin degradation, and identification of possible associations with cellular/molecular patterns is essential to understand myelin processing pathway, allowing remyelination-based therapies. BASHY dyes, recently synthesized boronic acid-based complexes, showed high specificity for lipid structures, particularly for the nonpolar lipid aggregates, therefore envisioned to be promising tools to assess later stages of myelin degradation. Here we aimed to test these BASHY dyes in the context of demyelination and address their location using an *ex vivo* cerebellar slice model, which attempts to reproduce the complexity and functionality of cell interactions found in vivo. *Ex vivo* cerebellar organotypic slice cultures (COSC) were obtained from 10 postnatal rats and treated with lysophosphatidylcholine (LPC, 0.5 mg/mL) to induce demyelination. Slices were immunostained for different markers to assess demyelination and detect glial cells and then incubated with BASHY dyes to further evaluate their co-localization. Firstly, we observed that BASHY fluorescence significantly increases in demyelinated slices, and specifically labels cholesterol-rich myelin debris, corroborated by its co-localization with filipin staining. Remarkably, these dyes were mostly confined to microglial cells with an increased presence along microglia morphological changes: from a more ramified microglia, to a bushy microglia and then an amoeboid microglia, which is in line with the previously described “foamy phagocytes” present in Multiple Sclerosis. Further, upon demyelination microglia containing BASHY/myelin positive vesicles were positive for iNOS, indicative of a more pro-inflammatory phenotype. Overall, we can conclude that BASHY dyes have high affinity for myelin debris present in activated microglia, the main inflammatory mediators in demyelinating conditions. Furthermore, knowing that BASHY complexes allow bioconjugation with small molecules, our study open new therapeutic strategies to prevent demyelination through microglial modulation, to reduce damage and enhance remyelination.

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Advancing Towards An Improved Melanoma Therapy Using A New Nanoformulated Hybrid Molecule

Pinho J.O., Matias M., Francisco A.P., Eleutério C.V., Perry M.J., Mendes E., Amaral J.D., Rodrigues C.M.P., Gaspar M.M.

Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal.

Melanoma is an aggressive form of skin cancer, with increasing incidence, high multidrug resistance and low survival rates. To overcome the therapeutic limitations, our team has recently synthesized a new hybrid molecule (HM), acting by two distinct mechanisms: 1) high specificity towards tyrosinase, an enzyme upregulated in melanoma cells; and 2) toxic alkylating properties [1]. *In vitro*, HM showed antiproliferative effect towards human (MNT-1) and murine (B16F10) melanoma cell lines, with IC_{50} values in the µM range (40-50 µM). Furthermore, in melanoma patient-derived samples, HM displayed a higher cytotoxic effect when compared to temozolomide, a clinically used chemotherapeutic drug. The inhibition of tyrosinase activity by HM was assessed in melanoma cell lines, showing a 2-fold decrease in relation to control. To promote a preferential targeting to tumor sites, HM was associated to long circulating liposomes with an incorporation efficiency of around 100%, a mean size of 100 nm and a polydispersity index below 0.1, demonstrating their high homogeneity. This lipid nanoformulation was highly stable in human plasma without eliciting hemolytic activity in human red blood cells and, after intravenous administration in healthy mice, no hepatotoxic side effects were observed. The therapeutic effect of HM formulations was evaluated in a syngeneic murine melanoma model [2,3]. Administering a dose of 12 mg/kg body weight, we achieved a remarkable antitumor activity for liposomal HM, with a 15-fold reduction in tumor volume compared to controls. On the other hand, for mice treated with free HM, only a 3-fold reduction was attained. Moreover, a strong correlation between tumor growth inhibition, increased caspase 3/7 activity and decreased tyrosinase activity in tumor protein extracts was observed for mice receiving HM nanoliposomes. The therapeutic effect of a lower dose (6 mg/kg) was also evaluated; an impaired tumor progression was observed for HM nanoformulations, although at a lower extent. An *in vivo* model of metastatic melanoma was successfully established that mostly mimics the human pathology. The therapeutic effect of HM formulations is on course. Overall, a new and highly effective therapy against melanoma has been established.

Acknowledgements

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References

[1] Granada, A. M. C., et al., 1st Symposium on Medicinal Chemistry, University of Minho, Braga, Portugal, 2013.[2] Sainz, V., et al., Acta Biomater., 2018, 76, 193-207.[3] Pinho, J. O.; Amaral, J. D.; Castro, R. E.; Rodrigues, C. M. P.; Casini, A.; Soveral, G.; Gaspar, M. M., Nanomedicine (Lond.), 2019, 14, 835-850.

A Phylogenomic Perspective On A Neglected Infectious Disease: Tuberculosis By *Mycobacterium bovis* In North Brazil

Conceição M.L. (1,2), Conceição E.C. (3), Furlaneto I.P. (1), Silva S.P. (2), Portugal I. (4), Lima K.V.B. (2) , Perdigão J. (4).

1- Universidade do Estado do Pará, Belém Brazil; 2- Evandro Chagas Institute, Belém, Brazil; 3- Oswald Cruz Foundation (FIOCRUZ), Rio de Janeiro, Brazil; 4- Research Institute for Medicines(iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal.

Besides a lack of routine surveillance data from countries where bovine Tuberculosis (bTB) is endemic, laboratories of limited capacity are unable to differentiate the Mycobacterium tuberculosis Complex (MTBC) species. It ensues that zoonotic TB is underdiagnosed and a possible cause for treatment failure due to *Mycobacterium bovis* natural resistance to pyrazinamide. Over recent years, the population dynamics of the Marajó Island in the State of Pará has alerted for this condition: the region is isolated from the mainland and, notification of zoonotic TB by *M. bovis* is inexistent along with a very close animal-human relationship (Figure 1). Herein, we used state-of-the-art genome-wide phylogenetic reconstruction to evaluate the transmission dynamics and genomic clusters of M. bovis among cattle and buffalo from abattoirs in the Marajó Island. Sixteen *M. bovis* clinical isolates from Marajó Island, representative from four municipalities, bearing two unusual spoligopatterns: SB822/SIT997 and SB885/SIT986 were included in the study. Whole Genome Sequencing (WGS) was carried out on an Illumina NextSeq (2x150bp) machine. Raw sequence reads were mapped to the *M. bovis* AF2122/97 (GenBank Accession NC_002945.4), BWA and SAMtools/GATK were used for variant calling. A maximum-likelihood phylogenetic tree was constructed using PhyML. Genomic transmission clusters were evaluated under a 5 and 12 SNP threshold. WGS showed a superior discriminatory power when compared to conventional genotyping. The phylogenetic scenario herein obtained demonstrates the presence of at least three recent transmission clusters using either a 5 or 12 SNP cut-off. The phylogenetic tree that was obtained was structured geographically with clusters composed of isolates from the same city and same animal species (Figure 2). This study demonstrates that recent transmission of *M. bovis* is ongoing at distinct sites in the Marajó Island, caused by strains evolved from a common ancestor likely introduced in the island at an uncertain point in time. Moreover, the phylogenomic approach taken in the present study provides a framework for understanding the host-pathogen adaptation in the region and, contributes with important novel data that can be integrated in the TB control strategy for the State of Pará.

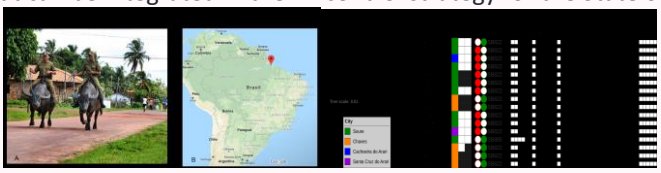

Fig. 1. A) Police officers patrolled on water buffaloes as they have done since the 1990s in Soure, the capital of Marajo Island, of about 23,000 people. (Picture: Alessandra Serrão/Agência Pará - http://g1.globo.com); B) Marajó Island - the world's largest fluvial island. Fig. 2. Whole genome phylogenetic tree of the 16 *M. bovis* strains (from Oct 2014 to Dec 2015) used in this study and reference AF2122/97. A maximum-likelihood phylogenetic tree was constructed using PhyML. The resulting tree was annotated using iTOL. The colored boxes indicate the city of Marajó Island; gray boxes delineate genomic clusters obtained using 5 and 12 SNP cut-offs; filled red and green circles indicate host species as buffaloor cattle, respectively. It is shown the SB profile number (https://www.mbovis.org/) and the isolate spoligotyping pattern.

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Lycopene-Enriched Extracts Formulated In Topical Microemulsions

Marques M. (1), Costa A. (2), Ascenso A. (2), Marto J. (2), Gonçalves L.M. (2), Carvalheiro M. (1), Paiva A. (1), Simões P. (1), Ribeiro H.M. (2), Simões S. (2)

1- LAQV-REQUIMTE, Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade NOVA de Lisboa, Caparica, Portugal; 2- Nano2B, Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal.

Lycopene is a natural carotenoid present in red-colored fruits and vegetables. Due to its well-known antioxidant properties, this natural compound is being used in cosmetic formulations for antiaging and skin care purposes [1]. In this work, lycopene-enriched extracts (LEE) from tomato residues (peel and seeds obtained from tomato processing industry) were obtained by CO_2 Supercritical Extraction. LEE were evaluated for cytotoxicity in HacaT cells. The capacity of LEE to reduce the ROS production was evaluated using HaCaT cells exposed to H_2O_2 or irradiated with a UV-B single dose. LEE showing no toxicity and antioxidant activity were formulated in microemulsions [2], with the goal of supplementing the skin levels of antioxidants. Microemulsion comprised a blend of monoacylglycerol and diacylglycerol as the oily phase. Polyoxylglyceride was selected as the surfactant agent at different concentrations (between 2.5-40% of total weight), and combined with a co-surfactant and purified water. After adding the oily phase to aqueous phase and stirred for 15 minutes at room temperature, the formation of a spontaneous transparent and isotropic microemulsion was observed at surfactant concentration higher than 30%. The formed microemulsion showed a small droplet size and a monodisperse population (mean size around 5-10 nm and a polydispersity index (PdI) of 0-15-0.30), and a pH approximately to 6.5. These microemulsions presented physical stability. Incorporation up to 2% (w/w) LEE was obtained by dissolving it in the oily phase. No influence on the droplet size and PdI were observed after incorporation of LEE. The lycopene skin penetration enhancement by means of microemulsions will be further studied.

Acknowledgements

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References

[1] Ascenso, A.; Pinho, S.; Eleuterio, C.; Praca, F. G.; et al., J. Agric. Food Chem., 2013, 61, 7284-7293; [2] Lopes, L. B.; VanDeWall, H.; Li, H. T.; Venugopal, V.; et al., J. Pharm. Sci., 2010, 99, 1346-1357.

Curcumin Monocarbonyl Analogs: Novel Potential Therapeutic Agents For Neurodegenerative Diseases

Clariano M. (1), Perry M.J. (1), Falcão A.S. (2)

1- Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; 2- CEDOC, Nova Medical School (UNL), Lisbon, Portugal.

Neurodegenerative diseases are related with defects in the autophagy-lysosome pathway (ALP). Autophagy is a complex self-degradative process mediated by lysosomes, which is responsible for the bulk degradation of intracellular components, preventing the occurrence of abnormal cellular functions [1,2]. Correcting the defects in the ALP may help treating neuropathies such as Parkinson disease (PD), Alzheimer disease (AD), Huntington disease (HD) and Creutzfeldt-Jakob disease (CJD) [1]. Curcumin is a natural polyphenolic compound, derived from the rhizome of turmeric (*Curcuma longa* Linn.), with an ample range of biological properties, such as antioxidant, antitumoral, neuroprotective, anti-inflammatory and wound healing activities [3]. Curcumin is also capable of improving retinal function in retinal degeneration and enhances autophagy [2,3]. Since the clinical use of curcumin is compromised by its inadequate pharmacokinetic properties, curcumin analogues have been developed in order to improve their potential therapeutic utility [1]. The attention has been focused on monocarbonyl analogs (Figure 1), where the stability, bioavailability and the pharmacokinetic profiles are improved and presents better activities *in vitro* and *in vivo* [1,3]. Several monocarbonyl analogs of curcumin were synthetized and their properties such as lipophilia, stability in physiological conditions and structure-activity relationship were evaluated.

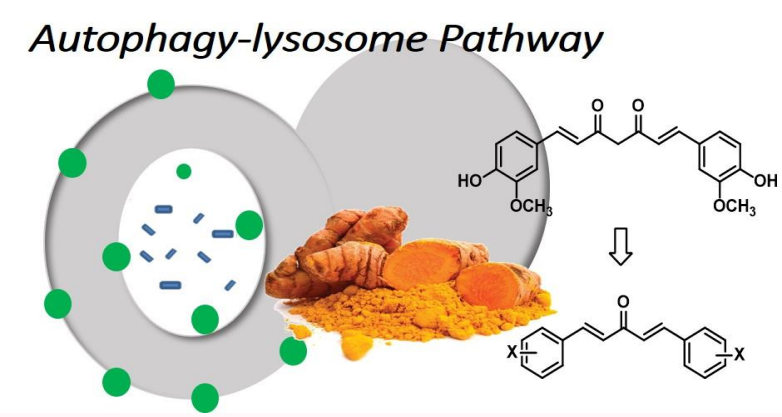

Figure 1

Acknowledgements

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References

[1] Song, J. X.; Sun, Y. R.; Peluso, I.; Zeng, Y.; Yu, X; Lu, J. H.; Xu, Z.; Wang, M. Z.; Liu, L. F.; Huang, Y. Y.; Chen, L. L.; Durairajan, S. S. K.; Zhang, H. J.; Zhou, B.; Zhang, H. Q.; Lu, A.; Ballabio, A.; Medina, D. L.; Guo, Z.; Li, M., Autophagy, 2016, 12, 1372-1389; [2] Shakeri, A.; Cicero, A. F. G.; Panahi, Y.; Mohajeri, M.; Sahebkar, A., Journal of Cellular Physiology, 2019, 234, 5643-5654; [3] Pan, Z.; Chen, C.; Zhou, Y.; Xu, F.; Xu, Y., Drug Development Research, 2016, 77, 43-49.

Nanoliposomal Formulations Of New Cytotoxic Metallodrugs Towards Murine Melanoma Cells

Albino M. (1), Lopes J.M. (1), Matos C. (2), Correia I. (2), Reis C.P. (1), Gaspar M.M. (1)

1- Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; 2- Centro de Química Estrutural, Departamento de Química, Instituto Superior Técnico, Lisbon, Portugal.

Cytotoxic treatments with metal-based drugs have shown great potential and it is also assumed that metallodrugs with endogenous metals are less toxic for normal cells. Therefore, this study is based on new metallodrugs, such as CMD143, in the following of research area of Correia and co-workers [1]. Nanolipidic systems are promising carriers for cytotoxic drugs following adsorption onto their surface or incorporation within their core. These nanolipid systems improve the stability of associated compounds and the inclusion of ligands at their surface promotes a specific targeting towards the site of action. The main objective of this study was to combine the features of the nanolipid system with a metallodrug to induce a pH-triggered compound release from long blood circulation time nanoliposomes. In order to do so, liposomes were prepared by a dehydration-rehydration method previously described [2]. The lipid compositions used were: Dioleoyl_phosphatidyl_choline (DOPC) : Dioleoyl_phosphatidyl_glycerol (DOPG) (80:20), DOPC : Cholesterylhemisuccinate (CHEMS) (60:40), DOPC : CHEMS : Distearoyl_phosphatidyl_ethanolamine, covalently linked to Poly(ethyleneglycol) (DSPE-PEG) (57:38:5). It is important to note that CHEMS becomes protonated when in contact with an acidic milieu leading to bilayer disruption and subsequent drug release at the microenvironment of solid tumors, slightly acidic, contrasting with healthy tissues thus meaning that nanoformulations will act with a target therapeutic effect. DSPE-PEG also has an important part promoting long blood circulation times. Nanoliposomes were characterized in terms of incorporation parameters, mean size and surface charge. The antiproliferative properties of the metallodrugs were evaluated in the free and liposomal forms by the MTT assay. Regarding CMD143, the results revealed the Incorporation Efficiency values ranged from 60 to 70%, demonstrating that the compound was easily incorporated. The nanoliposomes mean size ranged from 100 to 135 nm which is crucial because these carriers accumulate in regions of enhanced vascular permeability, such as tumor sites. Furthermore, the incorporation of CMD143 in DOPC:CHEMS and DOPC:CHEMS:DSPE-PEG formulations was able to preserve the cytotoxic properties of the compound with an IC_{50} value ranging from 5.8 to 9.0 µM. Concluding, the preliminary results of this study constitute a promising approach for melanoma therapy. CMD143 was efficiently incorporated in nanoliposomes and the cytotoxic properties of the compound were preserved, particularly for the pH sensitive nanoformulations.

Acknowledgements

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References

[1] Ribeiro, N.; Roy, S.; Butenko, N.; Cavaco, I.; et al., Journal of Inorganic Biochemistry, 2017, 174: 63-75; [2] Pinho, J.; Amaral, J. D.; Castro, R. E.; Rodrigues, C. M. P.; et al., Nanomedicine, 2019, 14(7), 835-850.

Construction Of Antibody-Drug Conjugates For The Development Of A Therapeutic Drug Against Breast Cancer

Madeira L.S. (1,2), Cardoso M. (1), Gonçalves J. (1)

1- Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; 2- Oswaldo Cruz Institute (IOC/Fiocruz), Oswaldo Cruz Foundation, Rio de Janeiro, Brazil.

In recent decades, antibodies have been widely studied as an alternative for the treatment of several tumors, including breast cancer [1]. Among several strategies, with the need to increase the cytotoxic response in the treatment of cancer, antibodies have been associated with different drugs for targeted delivery through specific receptors [2]. This study considered the development of antibody-drug conjugates (ADC), using a modified trastuzumab antibody in scFv-FC format and maytansine drug as payload, for selective delivery to the Human Epidermal growth factor Receptor 2 (HER2) in breast cancer cells. The conjugation linker is fluorescein isothiocyanate (FITC), since the antibody has a specific anti-FITC site. This project had stages of plasmid selection for the production of anti-IgG1 antibody in mammalian cells FreeStyleTM 293-F and its purification through Protein A affinity chromatography. The conjugates were constructed using the cytotoxic agent maytansine-FITC. *In vitro* assays were performed with HER2+ breast cancer to evaluate the efficiency of the cytotoxic agent delivery, through MTT colorimetric assay for ADC. The preliminary results showed an evident decrease in live cells for the construction of ADC. The next step will be to analyze the antibodies by resonance mass spectrometry of ion cyclotron transformed by Fourier (FT-ICR-MS) and thus evaluate the conjugation. In the future, we are planning experiments on animal models.

Acknowledgements

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References

[1] Reichert, J. M.; Rosensweig, C. J.; Faden, L. B.; Dewitz, M. C., Nature Biotechnology, 2005, 23(9), 1073-1078; [2] Bouchard, H.; et al., Bioorganic & Medicinal Chemistry Letters, 2014, 24, 5357-5363.

RIP3 Deficiency In NAFLD: A Lipidomic Approach

Mateus-Pinheiro M., Afonso M.B., Rodrigues P.M., Simão A.L., Gaspar M.M., Castro. R.E., Rodrigues C.M.P.

Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal.

Nonalcoholic fatty liver disease (NAFLD) comprises a spectrum of metabolic disarrays, ranging from accumulation of hepatic triglycerides to nonalcoholic steatohepatitis (NASH) and, ultimately, could progress to cirrhosis and/or of hepatocellular carcinoma. Recently, we have shown that receptor-interacting protein 3 (RIP3), a major participant in the necroptosis pathway, plays an important role in the pathogenesis of choline deficient dietary model of NASH. Intriguingly, RIP3 deficient mice displayed increased body weight gains, as well as increased liver fat accumulation in both NASH-inducing and control diets, suggesting a putative role for RIP3 as a lipid regulator. Here, we aimed to evaluate the impact of RIP3 signalling on the hepatic lipidomic profile during experimental NASH. Lipidomic analysis of liver samples from C57BL/6 wild-type (WT) or RIP3-deficient (RIP3-/-) mice fed a choline-deficient L-amino acid-defined diet (CDAA; n=14) or a control choline-sufficient L-amino acid-defined diet (CSAA; n=14) for 32 weeks was performed through ultra-high performance liquid chromatography - mass spectrometry (UHPLC-MS). A total of 392 metabolic features were detected in these samples. A multivariate analysis of samples depicted a clear segregation of samples depending on the diet and genotype. RIP3 deficiency increased levels of diglycerides (DG) and triglycerides (TG) with shorter acyl chains and low unsaturation in mice fed the CSAA diet, while species with longer acyl chains and high number of double bonds were decreased in RIP3-/- comparing to WT. These changes were also observed in CDAA-fed mice, although to a lesser extent, since the diet itself significantly increased TG and DG containing long and polyunsaturated fatty acids. These alterations in acyl chain length and saturation level might suggest changes in enzymes such as stearoyl-CoA desaturase-1 (SCD1) and those belonging to the elongation of very long chain fatty acids protein family (ELOVL) in RIP3-/- mice. The ratios between phosphatidylcholines and phosphatidylethanolamines (PC/PE) were also found reduced, mainly between diets, suggesting changes in methylation by phosphatidylethanolamine N-methyltransferase (PEMT). In summary, hepatic lipidomic profiles are influenced by choline deficient diet and RIP3, concomitantly or individually. A deeper understanding of these signatures will improve our understanding of NAFLD pathology towards effective treatment.

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Accessing Bicyclic Aziridines: A Flow Assisted Approach

Fortunato M.A.G., Siopa F., Afonso C.A.M.

Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal.

The discovery of potent biological properties of aminocyclopentitols and the promise that such effects might be exploited as an advantage in medicine have encouraged their synthesis [1]. An attractive approach for the synthesis of these compounds is the photochemical transformation of pyridinium salts to bicyclic-aziridines followed by aziridine ring-opening to afford aminocyclopentene derivatives [2]. However, photochemical reactions present a series of drawbacks, mainly due to the complexity of the processes and the difficult scale-up. Scalability is hampered due to the attenuation effect of photon transport which prevents the use of a simple dimension-enlarging strategy for scale-up. If larger reactors are used, over-irradiation of the reaction may become an essential issue as the reaction times are substantially increased, resulting in the formation of unwanted by-products. An increasingly popular solution to solve the aforementioned problem is the development of continuous-flow reactors [3]. We hereby present the development of a new home-made reactor with 12 parallel quartz tubes, with 95 cm of irradiation length, and its application on the photochemical transformation of 1-allyl pyridinium bromide salts to the correspondent α-hydroxycyclopenteno-aziridine.

Acknowledgements

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References

[1] Berecibar, A.; Grandjean, C.; Siriwardena, A., Chem. Rev., 1999, 99, 779; [2] Damiano, T.; Morton, D.; Nelson, A., Org. Biomol. Chem., 2007, 5(17), 2735. Zou, J.; Mariano, P. S., Photoch Photobio Sci 2008, 7 (4), 393; [3] a) Elliott, L.D., J.P.Knowles, P.J. Koovits, K.G. Maskill, M.J. Ralph, G. Lejeune, L.J. Edwards, R.I. Robinson, I.R. Clemens, B. Cox, D.D. Pascoe, G. Koch, M. Eberle, M.B. Berry, K.I. Booker-Milburn. Chem-Eur J, 2014, 20, 15226. b) Cambie, D., C. Bottecchia, N.J.W. Straathof, V. Hessel, T. Noel, Chem Rev, 2016, 116, 10276. c) Su, Y.H., N.J.W. Straathof, V. Hessel, T. Noel, Chem-Eur J, 2014, 20, 10562.

QbD Control Of Spray-Drying Encapsulated Process

Aguiam N, Sousa T., Lopes J.A.

Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal.

There are few microbial pathogens that have followed humankind throughout most of human history like tuberculosis. It was, and still is, one of the most prevalent diseases in humankind and its destructive nature was only curbed at the end of the XIX century with the discovery of the infectious nature of the disease. Exposure to *M. tuberculosis* generally doesn't lead to an infection. The progression of the disease when the subject is indeed infected leads to an immune system activation in roughly 90% of the cases, where the individual retains the disease in a latent form. Of those cases, other diseases or failures of the immune system can lead to a immunosuppression in 10% of latent cases, leading to the active form. Later on the first vaccine was produced by Albert Calmette which lead to a decrease in prevalence in Europe and North America. Today there are still 8 million people affected worldwide per year and certain stripes resistant to the staple antibiotics prescribed are appearing. The treatment for tuberculosis involves the use of isoniazid, streptomycin pyrazinamide and/or rifampicin in combination in OSD form. In particular isoniazid is used in isolation for the treatment of latent tuberculosis due to better tolerance, avoiding much of the undesired side effects of streptomycin. In the last decades of the XX century a resurgence of the disease led to new strategies of formulation, new combinations and/or dosage. One of such strategies is to create improvements in bioavailability through targeted drug delivery methods, particularly lung delivery. Isoniazid acts as a bactericidal agent for mycobacterium in active growth process. It is activated by the bacteria's catalase, working as a prodrug forming isonicotinic acyl radical, trough catalyzation with KatG enzyme. After activation the complex naturally inhibits fatty acid synthase (trough binding with InhA) and therefore the production of mycolic acids which form the structure of mycobacterial cell walls. (Figure 1). Isonicotinylhydrazide (INH) is mostly commercialized in the form of a white crystalline powder, it presents high solubility in water and a recorded melting point of 171°C. FT-IR and NIR can be used to identify the compound since it presents two very characteristic peaks of absorbance. Several studies explored the usage of mannitol as a carrier for microencapsulation. Mannitol is a common excipient and due to its crystallization profile, it can encapsulate drugs in particles smaller than 5 µm, a requirement for lung deposition. To this end the control of attributes such as particle size, particle distribution, morphology and overall flowability are deemed as critical for the final product quality. To microencapsulated INH in mannitol of process atomization trough solvent evaporation in a spray-drying may be used. It represents a quality by design (QbD) approach that lays the groundwork for continuous improvement and eventual design-space process regulatory filings.

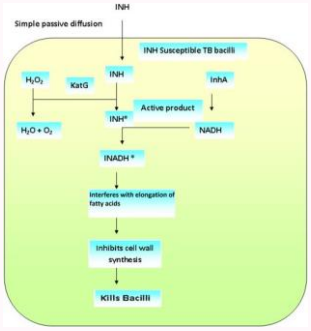

Figure 1

Acknowledgements

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Harnessing From Biomass: Copper(II)triflate As A Quick And Efficient Way To Prepare Trans- 4,5-diamino-cyclopent-2-enone From Furfural

Esteves N.R., Gomes R.F.A., Coelho J.A.S., Afonso C.A.M.

Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal.

It is known that trans-4,5-diamino-cyclopent-2-enones (CP) are usually prepared from furfural and its condensation with secondary amines using a Lewis acid as a catalyst [1]. Over the years new methodologies have been built upon this, creating more efficient ways to prepare CP. However, most of these methodologies are based on the use of organic solvents such as the use of ionic liquid 1- methylimidazolium tetrafluoroborate [2] and acetonitrile, and with catalysts like tosylamine [3], aluminum(III) chloride [4], DyIII /NiIII heteronuclear clusters [5,6], erbium(III) chloride in ethyl lactate [7] and erbium(III) chloride immobilized on silica as a reusable catalyst [8], with the reaction times of these procedures varying from 16h to 5 min. In this work we developed a methodology reducing the reaction time to 1-minute, with Copper(II) triflate as the catalyst in aqueous media under mild conditions, which performed well in terms of catalyst reusability and with an extraordinary functional group tolerance, having thus the requirements for use in chemical biology.

Acknowledgements

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References

[1] Li, S.-W.; Batey, R. A., Chem. Commun., 2007, 36, 3759; [2] Ramesh, D.; Reddy, T. S.; Narasimhulu, M.; Rajaram, S.; Suryakiran, N.; Mahesh, K. C.; Venkateswarlu, Y. Ã., Chem. Lett., 2009, 38(6), 5; [3] Liu, J.; Yu, J.; Zhu, M.; Li, J.; Zheng, X.; Wang, L., 2013, No. entry 7, 2; [4] Nunes, J. P. M.; Afonso, C. A. M., C. S. RSC Adv. 2013, 2; [5] Griffiths, K.; Gallop, C. W. D.; Abdul-Sada, A.; Vargas, A.; Navarro, O.; Kostakis, G. E. Chem. - A Eur. J., 2015, 21(17), 6358; [6] Griffiths, K.; Kumar, P.; Mattock, J. D.; Abdul-Sada, A.; Pitak, M. B.; Coles, S. J.; Navarro, O.; Vargas, A.; Kostakis, G. E., Inorg. Chem., 2016, 55(14), 6988; [7] Procopio, A.; Costanzo, P.; Curini, M.; Nardi, M.; Oliverio, M.; Sindona, G. ACS Sustain. Chem. Eng. 2013, 1 (5), 541; [8] Estevão, M. S.; Afonso, C. A. M., Tetrahedron Lett., 2016, No. Iii, 2.

Polymer Cyclodextrin Sub-Microcarrier For Enhanced Oral Delivery Of Antiretroviral Drug Lopinavir

Adeoye O. (1), Conceição J. (1,2), Bartolo I. (1), Francisco A. (1) Taviera N. (1), Cabral-Marques H. (1)

1- Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; 2- UCIBIO/REQUIMTE, MedTech - Laboratory of Pharmaceutical Technology, Department of Drug Sciences, Faculty of Pharmacy, University of Porto, Oporto, Portugal.

Lopinavir (LPV), a potent inhibitor of HIV-1 protease is currently used in the clinical management of Human Immunodeficiency Virus (HIV) infections. LPV's oral bioavailability is poor due to a combined effect of low aqueous solubility and P-glycoprotein (P-gp)/cytochrome P450 (CYP450 3A) mediated metabolism/low gastric permeability. Presently, LPV is clinically available as a co-formulation with sub-optimal doses of ritonavir (RTV) which enhances oral bioavailability by inhibiting LPV's pre-systemic and possibly systemic metabolism. The adverse effects of RTV such as; lipid elevation, perioral paraesthesia, glucose and gastrointestinal intolerance have necessitated the development of a RTV free LPV formulation. Thus, the principal aim of this study was to develop a novel cyclodextrin-polymer nanosystem (pCyD) of LPV for efficient oral bioavailability, and biodistribution without the need for RTV. Two types of pCyD were synthesised by a condensation polymerisation reaction using methyl Beta-; and (2-hydroxyl)propyl Beta cyclodextrin (MβCyD and HPβCyD) as monomers and pyromellitic dianhydride (PMDA) as the cross-linking agent in a molar ratio 1:4. The synthesised polymers were confirmed by 13-C CP/MAS NMR and Raman spectroscopy. The prepared pCyD were then loaded with LPV and fully characterised for using DSC, FTIR, PXRD, HPLC-MS/MS and DLS, cell-cytotoxicity and *in vitro* antiviral assays. Solid state characterisation of the synthesised polymers using with 13C CP/MAS NMR and Raman Spectroscopy revealed the addition of the carbonyl groups (C=O) of PMDA. The absence of a significant 13C chemical shift in the Carbons atoms of CyD suggests that its sugar rings retained their local conformation in the synthesised polymers. Thermal analysis of the synthesised pCyD revealed a different thermal transition compared to those of starting CyDs materials. Higher drug loading and lower particles sizes were observed in LPV-pHPβ compared to LPV-pMβ nanosystems. Cytotoxicity assays indicated that both pCyD and LPV-pCyD were safe. On-going studies are focused on evaluating the ability to the polymers to modulate enhanced bioavailability and biodistribution.

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Unravelling The Three-Dimensional Structure Of HIV-2 Viral Surface Glycoproteins By CADD

Serra P.A. (1), Taveira N. (1,2), Guedes R.C. (1)

1- Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; 2- Instituto Superior de Ciências da Saúde Egas Moniz (ISCSEM), Quinta da Granja, 2829-511 Monte da Caparica, Portugal.

The incidence of drug-resistant strains and toxicity associated with the current HIV drugs empowered the need for new and potent therapies. None of the current drugs effectively prevents entry into the cells and the efficacy of the available drugs is very limited against HIV-2. The viral entry mechanism being the initial and fundamental step for infection is looked as a potential and attractive target to inhibit the viral process [1]. In the absence of a crystallographic structure of HIV-2 envelope gp125 comprising variable domains, computer aided modulation is crucial to identify structural features in the variable regions that correlate with HIV-2 tropism and susceptibility to neutralization [2,3]. A 3D structure of HIV-2ROD gp125 was generated by homology modelling, using MOE2016 and MODELLER 9v19. Additionally, to disclose the importance of the main structural features and compare with experimental results, 3D-models of six mutants were also generated. These mutations revealed selectively impact in the behaviour of the protein. Additionally, molecular dynamics is being performed, using Gromacs 2006.3, in order to better characterize the full protein and disclose its the biological dynamic behaviour. It is primordial to understand the structural behaviour of these domains inserted in the main structure. The mutations revealed selectively impact in the behaviour of the protein. Structurally, the mutations studied leads to a loss of aromatic features, very important for the establishment of π-π interactions, which could induce a structural preference by a specific coreceptor.

Acknowledgements

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References

[1] Berinyuy, E.; Soliman, M. E. S., Interdiscip. Sci. Comput. Life Sci., 2017, 9, 406-418; [2] Uchtenhagen, lllH.; Friemann, R.; Raszewski, G.; Spetz, A.-L.; Nilsson, L.; Achour, A., PLoS One, 2011, 6, e18767; [3] Davenport, Y. W.; West, A. P.; Bjorkman, P. J., J. Virol., 2016, 90, 2112-2118.

Ablation Of RIP3 Protects From Dopaminergic Neurodegeneration And Replenishes Striatal GDNF Levels In A MPTP-Mouse Model Of Parkinson's Disease

Dionísio P.A., Oliveira S.R., Amaral J.D., Rodrigues C.M.P.

Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal.

Parkinson's disease (PD) is driven by dopaminergic neurodegeneration in the substantia nigra (SN) and striatum. Although apoptosis is considered the main mechanism for neurodegeneration, other cell death pathways may be involved. In this regard, necroptosis is a regulated form of cell death dependent on receptor interacting protein 3 (RIP3), which is implicated also in apoptosis and inflammation. Here we intended to explore the role of RIP3 in a MPTP-mouse model of PD. Wild-type (Wt) and RIP3 knockout (RIP3ko) mice were injected with 40 mg/kg MPTP intraperitoneally and sacrificed after 30 days. One hemisphere was processed for immunohistochemistry, while the striatum and midbrain, containing the SN, were isolated from the other hemisphere for Western blot (WB) analyses. Primary mixed glial cultures from Wt and RIP3ko mice were exposed to several pro-inflammatory stimuli, and pro-inflammatory gene expression and activation of signalling pathways were determined by qRT-PCR and WB, respectively. MPTP exposure decreased the levels of tyrosine hydroxylase, a marker of dopaminergic neurons, in the SN and striatum of Wt mice, which was reverted in RIP3ko mice. Intriguingly, MPTP-injected RIP3ko mice presented reduced caspase-3 activation in the SN, suggesting diminished apoptosis. Moreover, MPTP exposure increased astrogliosis in the striatum of Wt mice, which was exacerbated in RIP3ko mice. This effect was accompanied by absence of pro-inflammatory markers and reposition of glial cell line-derived neurotrophic factor (GDNF) levels in the striatum of MPTP-injected RIP3ko mice when compared to MPTP-injected Wt mice, which presented a massive decrease in GDNF levels. Of note, GDNF is a potent neurotrophin for dopaminergic neurons. Primary mixed glial cultures from RIP3ko pups also presented a general decrease in the expression of inflammation-related genes upon strong and mild pro-inflammatory stimulation, suggesting a pro-inflammatory role for RIP3 in this context. In conclusion, RIP3 knockout protected from apoptosis in the MPTP-mouse model, while potentiating a neurotrophic milieu in the striatum. Moreover, RIP3ko mixed glial cultures present decreased pro-inflammatory gene expression and activation of signalling pathways. Our results highlight RIP3 as a dual therapeutic target in PD, both in neurodegeneration and inflammation.

Acknowledgements

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Discovery-On-Chip: Integrated Microfluidic System For Antibody Discovery

Fontes P.M. (1,2), Condelipes P.G.M. (2), Brás E.J.S. (2,4), Serrão R.F. (2), Afonso M.B. (3), Marques V. (3), Rodrigues C.M.P. (3), Conde J.P. (2,5), Gonçalves J. (1)

1- Molecular Microbiology and Biotechnology Unit | Drug Discovery Area, Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; 2- INESC-MN - Microsystems and Nanotechnologies, Instituto Superior Técnico, Universidade de Lisboa, Lisbon, Portugal; 3- Cellular Function and Therapeutic Targeting Unit | Drug Discovery Area, Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; 4- IBB - Institute for Bioengineering and Biosciences, Instituto Superior Técnico, Universidade de Lisboa, Lisbon, Portugal; 5- Department of Bioengineering, Instituto Superior Técnico, Universidade de Lisboa, Lisbon, Portugal.

Phage Display is a versatile *in vitro* technology based on the genetic engineering of bacteriophages or phages (viruses that infect bacteria). It allows the selection of antibodies with unique features from a library of phages. Each phage carries the gene of interest fused to a phage coat protein which is displayed at the cell surface, creating a direct connection between phenotype and genotype. In order to find the antibody with the desired features, the phage library is subjected to several rounds of selection. However, conventional methodologies are time-consuming and laborious [1,2]. Microfluidic devices appear as a potential solution allowing a faster screening and the use of reduced working volumes [3]. The primary goal of this project is to develop a platform with controlled assay conditions for cell culture and consequently for the selection of target-specific and high-affinity antibodies [4,5]. In this project we will use this platform for the identification of specific colon cancer stem cell biomarkers. Cancer stem cells have been proposed as the driving force of tumorigenesis however their existence and role continue to be subject of intense debate [6]. Cancer stem cell research has been clearly hindered by the absence of reliable biomarkers since they share many characteristics with normal stem cells, including self-renewal and differentiation [7]. These biomarkers are thought to be important and a major unmet need, carrying enormous potential for improving colon cancer diagnostics and therapeutics [8].

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References

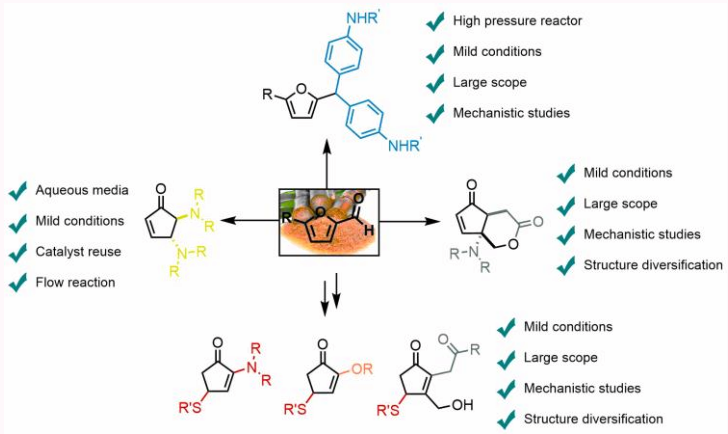
[1] Hoogenboom, H. R., Methods in Molecular Biology, 178, 1-37; [2] Russel, M.; Lowman, H. B., Phage Display: A Practical Approach, no. September, 2004, 1-26; [3] Cung, K.; et al., Lab Chip, 2012, 12, 562-565; [4] Halldorsson, S.; Lucumi, E.; Gómez-Sjöberg, R.; Fleming, R. M. T., Biosens. Bioelectron., 2015, 63, 218-231; [5] Kim, L.; Toh, Y. C.; Voldman, J.; Yu, H., Lab Chip, 2007, 7(6), 681-694; [6] Medema, J. P., Nat. Cell Biol., 2013, 15(4), 338-344; [7] Lobo, N. A.; Shimono, Y.; Qian, D.; Clarke, M. F., Annu. Rev. Cell Dev. Biol., 2007, 23, 675-701; [8] Abdul Khalek, F. J.; Gallicano, G. I.; Mishra, L., Gastrointest. Cancer Res., 2010, Suppl 1, S16-23.

Cyclopentenones As Novel Scaffolds For Drug Discovery

Gomes R.F.A., Andrade K., Frade R., Trindade A., Coelho J., Afonso C.

Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal

Given the increasing importance of sustainable development, the use of renewable resources has become an attractive way of synthesizing commodity chemicals [1]. Our group has been involved in the transformation of biomass-derived intermediates such as 5-hydroxymethylfurfural and furfural. On the other hand, there is an increasing drive in the pharmaceutical industry for strategies to diversify small molecules for drug discovery [2]. Thus, development of novel strategies to transform biomass-derived intermediates would constitute a powerful and sustainable strategy for the synthesis of biologically active small molecules. Herein we describe the preparation of diverse structures such as triarylmethanes, [3a] δ-Lactone-fused cyclopent-2-enones (LCP), [3b] trans-4,5-diamino-cyclopent-2-enones [3c] (DACP) and corresponding derivatives [4] (Scheme 1). These structures present both anticancer and antimicrobial activity [4]. We study the mechanism for the formation of the new products by 1H-NMR quantitative kinetic analysis, mass spectrometry (MS), Fourier-transform infrared spectroscopy (FTIR) and DFT-calculations.



Scheme 1

Acknowledgements

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References

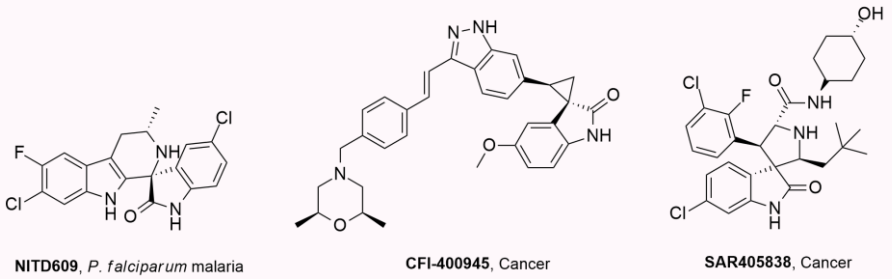
[1] Gomes, R.; Mitrev, Y.; Simeonov, S.; Afonso, C., ChemSusChem – VIP paper, 2018, 11(10), 1612-1616; [2a] Burke, M.; Schreiber, S., Angew. Chem. Int., Ed. 2004, 43, 46-58; [2b] Beckmann, H.; Nie, F.; Hagerman, C.; Johansson, H.; Wilcke, D.; Spring, D., Nat. Chem., 2013, 5, 861-867; [2c] Li, G.; Lou, H., Med. Res. Rev., 2018, 38, 1255-1294; [3a] Gomes R.; Coelho J.; Frade R.; Trindade A.; Afonso C., J. Org. Chem., 2015, 80 (20), 10404-10411; [3b] Gomes, R.; Coelho, J.; Afonso, C., ChemSusChem – VIP paper, 2019, 12, 420-425; [3c] Gomes, R.; Esteves, N.; Coelho, J.; Afonso, C., J. Org. Chem., 2018, 83(14), 7509-7751; [4] Manuscript under submission.

Spirooxadiazoline Oxindole Chemical Library

Mestre R., Lopes E.A., Santos M.M.M.

Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal

The spirooxindole core is a very attractive scaffold for organic and medicinal chemists. Indeed, several biologically active spirooxindoles have been reported as having biological activities such as anti-tumor, anti-microbial, anti-HIV, antimalarial, among others [1]. Therefore there is a huge interest in industry and academia to develop novel spirooxindoles with good potency against therapeutic targets. Several spirooxindoles (e.g.NITD609, CFI-40095 and SAR405838) have entered clinical trials for the treatment of diseases such as, malaria and cancers (Figure 1). In our research group, we have shown that spiro-[1,2,4]-oxadiazoline oxindoles have good antiproliferative activity in breast and colon cancer cell lines, by inducing apoptosis and cell cycle arrest, upregulating p53 steady-state levels while decreasing its main inhibitor MDM2 [2]. In this communication, our most recent results on the development of the regioisomer spiro-[1,3,4]-oxadiazoline oxindole skeleton to obtain novel reactivators of the p53 pathway will be reported. These spirooxindoles are normally obtained by a 1,3-dipolar cycloaddition of isatin derivatives with nitrile imines formed *in situ* from hydrazonoyl chloride derivatives using dichloromethane and triethylamine, as solvent and base, respectively [3]. Herein, we will disclose our results on the development of a greener method of synthesis, using different reaction conditions such as solvents, bases and catalysers that led to higher reaction yields.



NITD609, *P. falciparum* malaria **CFI-400945**, Cancer **SAR405838**, Cancer

Figure 1

Acknowledgements

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References

[1] Santos, M. M. M., Tetrahedron, 2014, 70, 9735-9757; [2] Ribeiro, C. J. A.; Amaral, J. D.; Rodrigues, C. M. P.; Moreira R.; Santos, M. M. M., MedChemComm., 2016, 7, 420-425; [3] Alizadeh, A.; Moafi, L., Helvetica Chimica Acta, 2016, 99, 457-461.

Protection Of Human Phenylalanine Hydroxylase Activity By Small Molecules Targeting The Enzyme Catalytic Center

Lopes R.R. (1), Russo R. (1), Madeira C.A. (1), Tomé C. (2), Teixeira M. (2), Vicente J.B. (2), Guedes C.R.(1), Góis P.M.P. (1), Leandro P. (1)

1- Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; 2- Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Oeiras, Portugal.

Phenylketonuria (PKU) is an autosomal recessive inborn error of metabolism caused by a deficient activity of human phenylalanine hydroxylase (hPAH) a non-heme iron homotetrameric enzyme that hydroxylates L-Phe into L-Tyr in the presence of O_2 and tetrahydrobiopterin (BH_4). In order to increase hPAH stability and/or activity, in this work, twelve small molecules were designed based on the properties and structural characteristics of the catalytic hPAH ligands (L-Phe, BH_4 and Fe). From this in-house library the most promising molecules were selected based on their effect over the activity and thermostability of the recombinant hPAH. The selected molecules were further tested by: (i) electron paramagnetic resonance (EPR), to confirm Fe interaction; (ii) limited proteolysis, to identify the mechanism of action; surface plasmon resonance, to determine the KD and; (iii) cytotoxicity to confirm the molecules biocompatibility. Selected compounds were also studied for their effect on the hPAH activity along incubation time at 42°C. Four molecules were considered the most promising candidates. They presented a slight inhibitory effect (17 to 40% enzyme activity decrease) but allowed further activation by L-Phe (1.3 to 1.7-fold). Two of the selected molecules stabilized the hPAH regulatory domain (Tm1: +8.3 and +4.0°C). An interaction with the catalytic iron was suggested by the observed changes in the EPR spectra and the molecular docking studies, which also indicate that the poses adopted by the quinoline ring and the side chain of the molecules significantly overlapped the position of BH4 and the substrate analog, respectively. At 100 M the selected compounds did not present any toxic effect either on cell viability (62 4% to 101 5%) and membrane integrity (propidium iodide intake ~1). Interestingly one compound was able to protect enzyme activity (1.5-fold increase compared to the control; 42°C for 60 min). From our series of molecules two compounds emerged as pharmacological chaperones and one as activity chaperone. Our data also provided proof-of-concept for the utilized strategy of compound design targeting the hPAH catalytic center.

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Synthesis And Evaluation Of Benzoic Acid Derivatives For The Treatment Of Tuberculosis

Freire R. (1,2), Pires D. (1), Carmo N. (1), Pais J.P. (1), Magalhães M. (1), Patrão P. (1), Pamplona T. (2), Anes E. (1), Francisco A.P. (1), Constantino L. (1)

1- Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; 2- Faculty of Science, Universidade de Lisboa, Lisbon, Portugal.

Tuberculosis (TB) is an infectious disease caused by *Mycobacterium tuberculosis*, which exists for thousands of years [1]. Despite the introduction 40 years ago of the inexpensive and effective four-drug (isoniazid, rifampicin, pyrazinamide [2] and ethambutol) treatment regimen, TB continues to cause considerable morbidity and mortality worldwide. There have been urgent calls for the inclusion of antimicrobials with some *in vitro* anti-tuberculosis (anti-TB) activity as part of the recommended drugs for the treatment to help solve the problems of high cost of therapy, inaccessibility of anti-TB drugs as well as bacterial resistance to existing drugs [1]. This motivated our interest to develop derivatives of compounds with interesting anti-TB activity. One interesting approach to its treatment can be the use of prodrugs that often have showed improved biological activities overactive drugs with poor absorption or difficulty to pass over membranes [3]. Previous studies have shown that weak acids have a significant antimycobacterial activity, which increases at acidic pH. Furthermore, esters of benzoic acid (BA) revealed to be a viable alternative since they diffuse more easily through the cell membranes, and inside the mycobacterial cell they will be converted into the weak acid [2,3]. Moreover, to be effective, they must be resistant to hydrolysis in human plasma and be activated once inside the mycobacteria [3]. In this work, we synthesised BA ester prodrugs containing electron withdrawing groups on the aromatic ring, in order to decrease the pKa of the liberated acid. In addition, alkyl chains with variable lengths, ranging from propyl to tetradecyl, were used for alkoxy group of the esters to modulate the absorption and resistance to mammalian esterases. The goal was to evaluate the effect of alkyl chains length on the plasma stability and activity of the esters. The chemical and enzymatic stability of these derivatives was studied in phosphate buffer and in human plasma by HPLC. The activity of the compounds was tested against *M. tuberculosis*.

Acknowledgements

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References

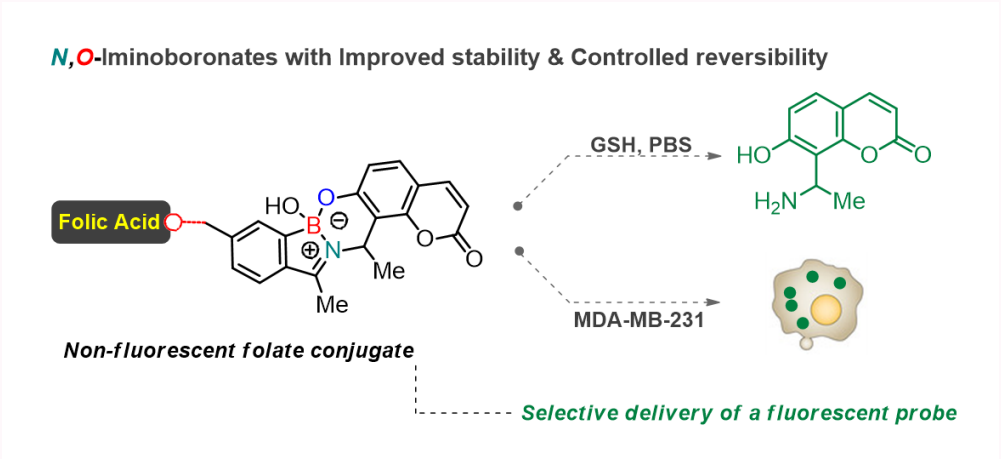
[1] Global tuberculosis report 2018. Geneva: World Health Organization; 2018; [2] Pires, D.; Valente, E.; Simões, M. F.; Carmo, N.; Testa, B.; Constantino, L.; Anes, E., Antimicrob. Agents Chemother., 2016, 59(12), 7693-7699; [3] Simões, M. F.; Valente, E.; Gómez, M. J. R.; Anes, E.; Constantino, L., Eur. J. Pharm. Sci., 2009, 37(3-4), 257-63.

Reversible N,O-Iminoboronates With Improved Stability For Cancer Cells Targeted Delivery

Lopes R.M.R.M. (1), Ventura A.E. (1,2), Silva L.C. (1,2), Faustino H. (1), Gois P.M.P. (1)

1- Research Institute for Medicines (iMed.ULisboa) Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; E-mail: rmr.lopes@campus.fct.unl.pt 2- Centro de Química-Física Molecular and Institute of Nanoscience and Nanotechnology, Instituto Superior Técnico, Universidade de Lisboa, Lisboa, Portugal.

Herein we present a new class of iminoboronates [1] obtained from 2-acetylbenzene boronic acids and aminophenols. The N,O-ligand topology enabled the formation of an additional B-O bond that locks the boron centre in a tetrahedral geometry [2]. This molecular arrangement decisively contributes to improve the construct stability in biocompatible conditions, retaining the iminoboronate reversibility in more acidic environments. 2-Acetylbenzene boronic acid was reacted with a fluorescent amino-coumarin to yield a stable and non-fluorescent N,O-iminoboronate. This mechanism was further used to assemble a folate receptor targeting conjugate that selectively delivered the fluorescent amino-coumarin to MDA-MB-231 human breast cancer cells [3].



Scheme 1 - Reversible N,O-Iminoboronates With Improved Stability For Cancer Cells Targeted Delivery

Acknowledgements

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References

[1] Cal, P. M. S. D.; Vicente, J. B.; Pires, E.; Coelho, A. V.; Veiros, L. F.; Cordeiro, C.; Gois, P. M. P., J. Am. Chem. Soc., 2012, 134, 10299-10305; [2] Lopes, R.; Ventura, A.; Silva, L.; Faustino, H.; Gois, P., 2018, Chem. Eur. J.. Accepted Author Manuscript; [3] P. M. S. D. Cal, R. F. M. Frade, V. Chudasama, C. Cordeiro, S. Caddick, P. M. P. Gois, Chem. Commun. (Camb)., 2014, 50, 5261-5263.

Modulation Of PD-1/PD-L1 Interaction By Small Molecules

Acúrcio R.C. (1), Leandro P.(1) Florindo H.F Guedes R.C(1)

1- Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal.

Immunotherapy is currently a powerful strategy in cancer therapy with very exciting outcomes. Modulation of immune checkpoint receptors have gain special attention. These immune regulators limit proliferation and activity of T cells and other immune cells enrolled in these signaling pathways. Under normal conditions, they are essential in modulation of immune responses; however, they are also one of the major mechanisms used by tumors to evade immune system recognition and destruction by suppressing their activation and effector functions. The modulation of immune checkpoint receptors has been explored to achieve the activation of the T-cell function, leading to the amplification of antigen-specific T-cell responses. To date, several immune checkpoint receptors have been identified and used as therapeutics in oncology, as programmed cell death protein 1 (PD-1). When engaged by one of its ligands (PD ligand 1 (PD-L1) and PD ligand 2) PD-1 limits autoimmunity. PD-1 ligands are upregulated in many human cancers and their blockade lead to activation of T cells and therefore enforce tumor recognition. In fact, PD-1/PD-L1 pathway is one of the most successful pathways in the context of clinical cancer immunotherapy with several approved drugs. These successful therapies rely on the use of monoclonal antibodies (mAbs). However, despite their outstanding success, they still have numerous disadvantages as severe immune-related adverse events. Recently, small-molecule modulators have emerged as a more affordable and accessible alternatives to mAbs. They exploit their benefits over recombinant protein approaches, namely (i) possible oral bioavailability, (ii) greater diffusion rate within the tumor microenvironment or (iii) improved pharmacokinetics. These immune modulators may also offer the possibility for avoiding the macrophage-mediated resistance observed in anti-PD-1 therapy.

However, limited efforts have been directed toward small-molecule immune system modulators. Our study focus the discovery of small-molecule inhibitors targeting PD-L1 in order to block PD-1/PD-L1 interaction and therefore overcome mAbs therapy disadvantages. Taking advantage of computational-aided drug design, we developed a computational approach where potential PD-1/PD-L1 inhibitors were identified following a structure based virtual screening campaign. Hit validation was ensured using a fluorescence PD-1/PD-L1 binding assay together with differential scanning fluorimetry. From ninety-six hits identified, ten were able to inhibit the interaction on a nanomolar scale. Currently, the most promising PD-1/PD-L1 inhibitors have been tested in vitro on breast and melanoma cancer lines by flow cytometry in which PD-L1 levels have been accessed. So far, three compounds with different chemical features were able to decrease the levels of PD-L1. Therefore, immune checkpoint blockade using small molecules represent a step forward in cancer immunotherapy.

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EMT Relay Race: TWISTing AKT2, FOXM1 And Vimentin As Suitable Therapeutic Targets

Costa R.(1), Albino S., Sousa C.(1), Silva-Lima B.(1), Videira, M.(1)

1- Research Institute for Medicines (iMED.UL), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal.

Among cell's malignancies is the ability to change their phenotype in order to degrade the basement and extracellular matrix membranes (ECM) allowing their migration and invasion to other tissues as the result of a biological process designated epithelial-mesenchymal transition (EMT). These biochemical changes are linked to an excessive activation of specific signaling pathways, namely PI3K/AKT, and include the expression of cell- surface proteins, loss of E-cadherin expression and overexpression of Vimentin, production of enzymes to degrade the ECM, and activation of transcription factors, such as TWIST and FoxM1. AKT2, one of the three AKT isoforms, is found to be overexpressed in numerous human cancers and is related with cell survival mechanisms mainly in response to stress conditions, being crucial in PI3K dependent signaling pathways and involved in cancer cells motility, invasion and metastization. Related to the EMT program is TWIST's capability of inducing AKT2's expression through its positive transcriptional regulation enhancing cell migration and invasion. Furthermore, active AKT2 directly phosphorylates TWIST and protects cells from apoptosis induced by DNA damage. Also, AKT2 has been reported to indirectly promote FoxM1 transcription by inhibiting the expression of pro-apoptotic proteins. FoxM1 activation, in turn, leads to its binding to the promotor of EMT related genes, such as TWIST and Vimentin, and their subsequent overexpression. These findings suggest a network of proteins that interact in a positive feedback loop that perpetuates tumor malignancy.To evaluate basal protein levels of AKT2, FoxM1, TWIST, and Vimentin we performed assays in a malignant glioma cell line - U-87MG (Glioblastoma Multiforme (GBM) - grade IV), thus correlating protein expression with tumor grade aiming to find intracellular therapeutic targets.

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Exploring Quenching Activity-Based Probes (qABP) To Detect β-lactamases In Biological Matrices

Félix R. (1), Mallo-Abreu A. (2,3), Moreira R. (1)

1- iMed.UL, Faculdade de Farmácia da Universidade de Lisboa, Av. Prof. Gama Pinto, 1649-003 Lisboa, Portugal; 2- Centro Singular de Investigación en Química Biolóxica e Materiais Moleculares (CIQUS); 3- Departamento de Química Orgánica, Facultade de Farmacia. Universidade de Santiago de Compostela, 15782. Santiago de Compostela, Spain.

Beta-lactamases comprise several serine and metallo-hydrolases that are responsible for the bacterial resistance to beta-lactam antibiotics (BLAs), thus posing a serious threat to the treatment of bacterial infections [1]. The serine-based enzymes are one of the most clinically relevant and challenging medicinal chemistry targets because pathogens have evolved to express previously rare or unknown beta lactamases, highlighting the need for new broad spectrum enzyme inhibitors. There has been an increasing interest in developing methods to profile enzyme activity in order to identify new therapeutic targets, biomarkers and understanding their molecular mechanisms. Quenched Activity-based probes (qABP) are molecules that contain in their structure a fluorophore (F) part and a quencher (Q) part, covalently tagging active enzymes but not their inactive form. In this particular case, qABP only shows fluorescence when it is linked to the enzyme, working as a mechanism-based (suicide) inhibitor (Figure 1). This work consists in the development and optimization of the synthetic methodology to obtain this type of compounds. Different linkers, quenchers and fluorophores will be the base for the new molecules, being evaluated by their ability to be used as beta-lactamase targeting qABP's.

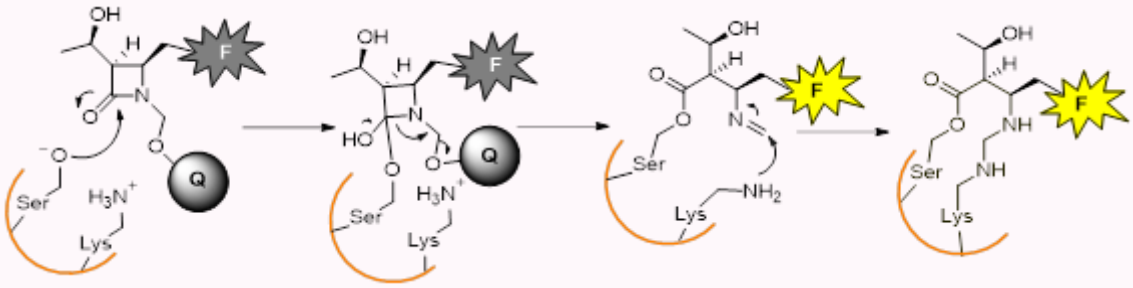

Figure 1

Acknowledgements

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References

[1] Bush, K., A resurgence of β-lactamase inhibitor combinations effective against multidrug-resistant Gram-negative pathogens. International Journal of Antimicrobial Agents, 2015. 46(5): p. 483-493; [2] Biondi, S., et al., Current Trends in b- Lactam Based b-Lactamases Inhibitors. Current Medicinal Chemistry, 2011. 18(27): p. 4223-4236; [3] Hu, M., Multicolor, One- and Two-Photon Imaging of Enzymatic Activities in Live Cells with Fluorescently Quenched Activity-Based Probes (qABPs). Journal of American Chemical Society, 2011. 113: p. 12009-12020.

Potential Necroptosis Inhibitors Based On Oxazolone Derivatives

Gonçalves-Pereira R. (1,2), Ferreira M.B.T. (1), Rodrigues C.A.B. (1), Estevão M. (1), Afonso M. (1), Oliveira S.R. (1), Rodrigues C.M.P. (1), Afonso C.A.M. (1)

1- Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, University of Lisboa, Av. Prof. Gama Pinto, 1649- 003, Lisboa, Portugal; 2- Center of Chemistry and Biochemistry, Faculty of Sciences, University of Lisboa, Campo Grande, 1749- 016 Lisboa, Portugal.

The mechanisms and regulation of cell death have a large interest in biology and medicine research. Necroptosis is a recently discovered form of programmed necrosis. Two protein kinases, known as RIPK1 and RIPK3 promote signalling that leads to cell death by necroptosis [1]. Based on these targets, studies have been initiated to search for necroptosis inhibitors as a promising target for the treatment of various conditions where necrosis plays a prominent role, such as neurodegenerative diseases like cancer, Alzheimer's, Parkinson's or multiple sclerosis [2, 3]. So far, no drug has reached the market, still there are few molecules in clinical trials. Therefore, the discovery of new specific and potent inhibitors of necroptosis is relevant and of utmost importance. We present herein the synthesis of oxazolones derivatives and performing a preliminary screening for their ability to block TNF-α induced necroptosis in cell lines (BV-2 microglia and L929 fibrosarcoma). Some of the newly synthesized oxazolones showed an inhibition activity similar to the know inhibitor Nec-1, rendering them promising lead molecules as necroptosis inhibitors.The synthetic work and the results of the bioactivity assessment will be presented and discussed herein.

Acknowledgements

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References

[1] W. Zhou and J. Yuan, Semin Cell Dev Biol. 35 (2014) 14; [2] Y. S. Cho, BMB Rep. 51 (2018) 219; [3] S. Oliveira et al., Cell Death Discov. 4 (2018) 65.

A Boron Hot-Spot Mass Study: 3-Hydroxy-Quinolinones Conjugates

Padanha R. (1), Russo R. (1), Fernandes F. (2,3), Veiros F. L. (4), Gois P. M. P. (1)

1- Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; 2- Centro de Química-Física Molecular and Institute of Nanoscience and Nanotechnology, Instituto Superior Técnico, Universidade de Lisboa, Lisbon, Portugal; 3- IBB-Institute for Bioengineering and Biosciences, Instituto Superior Técnico, Universidade de Lisboa, Lisbon, Portugal; 4- Centro de Química Estrutural, Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais 1, 1049-001 Lisbon, Portugal.

Bioconjugation has recently been the basis of antitumor targeting therapy, allowing the development of selective conjugates with improved stability. Reversible bioconjugation methods are of particular of therapeutical interest due to their ability for engaged biological processes by structural modification in different chemical environments [1-2]. Boronic acids (BA) are known to establish reversible covalent bonds with vicinal oxygen/nitrogen nucleophiles and are present in FDA-approved drugs' structures [3]. Here, we studied the boron hot-spot (BHS) technology, a 3-hydroxy-quinolinone heterocicle (3HQ) used in the coordination of iminoboronates onto the structure of peptides. This technology allows the installation of a boron ligand at a specific site of the perptide chain that could direct the carbonyl group towards a specific amino function, enabling the selective formation of an iminoboronate. The incubation of the BHS-Cys with 2-FBBA in ammonium acetate solution (20 mM, pH 7.0) afforded the desired imine within 2h at 37°C. ESI-MS studies were performed, showing the compatibility of this BHS with different amino acid side chain and competing functionalities. In more complex peptides, the BHS favors the N-terminal iminoboronate over the formation of in-chain iminoboronates in c-Ovalbumin and the RGD peptides. Exhibiting an N-terminal and an in-chain Cys residues, RGD was used to install 2 BHS, but only the N-terminal modification promoted the assembly with 2FBBA. The resulting iminoboronates are shown to be stable in ammonium acetate solution pH 7 and 4.5 or in the presence of BSA, although reversible in the presence of glutathione.

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References

[1] Koniev, O.; Wagner, A. Developments and Recent Advancements in the Field of Endogenous Amino Acid Selective Bond Forming Reactions for Bioconjugation. Chem. Soc. Rev. 2015, 44 (15), 5495–5551; [2] Boutureira, O.; Bernardes, G. J. L. Advances in Chemical Protein Modification. Chem. Rev. 2015, 115 (5), 2174–2195; [3] Cal, P. M. S. D.; Vicente, J. B.; Pires, E.; Coelho, A. V; Veiros, L. F.; Cordeiro, C.; Gois, P. M. P. Iminoboronates: A New Strategy for Reversible Protein Modification. J. Am. Chem. Soc. 2012, 134 (24), 10299–10305.

Impact Of E3330 In Non-Small Cell Lung Cancer Cells Viability And Migration Upon Cisplatin Treatment

Manguinhas R. (1), Fernandes A.S. (2), Costa J.G. (2), Saraiva N. (2), Camões S.P. (1), Gil N. (3), Rosell R. (4), Castro M. (1), Miranda J.P. (1) and Oliveira N.G. (1)

1- Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; 2- CBIOS, Universidade Lusófona Research Center for Biosciences & Health Technologies, Lisbon, Portugal; 3- Champalimaud Clinical Centre (CCC) – Lung Cancer Unit, Lisbon, Portugal; 4- Dr Rosell Oncology Institute (ROI)/Catalan Institute of Oncology, Barcelona, Spain.

Lung cancer (LC) is the leading cause of cancer-related deaths for both men and women. Non-small cell lung cancer (NSCLC) is the most frequent LC sub-type, presenting low survival rates due to metastasis progression, and is often resistant to platinum-based chemotherapy. Elevated expression levels of APE1 have been correlated with more aggressive phenotypes and poor prognosis of NSCLC. Besides being a key DNA repair enzyme, APE1 also works as a redox signaling protein, modulating the activation of several transcription factors related to cancer progression. For this reason, the quinone derivative E3330 has been tested as a direct inhibitor of APE1's redox function in different types of tumors. In this context, the aim of the present study was to assess in NSCLC cells the effects of E3330 per se or in combination with cisplatin in terms of cytotoxicity and cell migration. Firstly, the human H1975 cells were exposed to E3330 (5-50 µM) and/or cisplatin (1-50 µM) for 72 h and cell viability was assessed using the crystal violet (CV) and MTS assays. Cisplatin clearly decreased cell viability in a concentration-dependent manner, with IC_{50} values of 9.6 µM for CV and 15.9 µM for MTS. The co-incubation of E3330 (30 µM) and cisplatin (5, 10 and 20 µM) markedly decreased cell viability compared to cisplatin alone, for all the concentrations tested and for both CV and MTS assays. The effect of E3330 in the migration of cisplatin-treated cells was also observed using non-cytotoxic concentrations of both compounds. In fact, E3330 reduced both collective (wound-healing assay) and chemotactic (transwell assay) migration of H1975 cells exposed to cisplatin. Overall, these results pointed out E3330 as a promising compound to boost cisplatin therapy that warrants further investigation in NSCLC.

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Enhancing Medical Devices Antimicrobial Properties Through Functionalization With Glycolipid Biosurfactants

Mendes R. (1), Bettencourt A.F. (1), Costa J. (1), Ribeiro I. A.C. (1)

Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal.

The active use of medical devices such as silicone catheters can easily promote surface bacteria colonization and related infections. Prevention of biofilm formation and growth of several bacteria in these devices is mandatory and new strategies are needed [1]. A possible alternative is the functionalization of surfaces with antimicrobial and/or anti-adhesive compounds such biosurfactants.In this study, different glycolipid biosurfactants, sophorolipids, were isolated to evaluate their ability to inhibit biofilm formation on silicone rubber aimed for medical catheters. Sophorolipids (SLs) were biosynthesized by Starmerella bombicola in a glucose peptone yeast extract (GPY) seed culture media, supplemented with borage oil or oleic acid, at 25°C under orbital shaking [2]. The biosurfactants were extracted after 168h, purified through automated flash chromatography and identified by Ultra Performance Liquid Chromatography (UPLC) coupled to Mass Spectrometry (UPLC/MS). Optimization of the flash chromatography and UPLC methods was performed in order to isolate the most active compounds and allow a faster identification of the SLs, respectively.
Antimicrobial activity was also evaluated for planktonic bacteria (i.e. minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC)) and sessile bacteria (i.e. biofilm inhibition crystal violet staining, colony forming units counts (CFU) and Scanning Electron Microscopy (SEM)). Different lactonic compounds were tested with Starmerella aureus and it was possible to verify that most active were L-C18:1 SLs (MIC= 50 µg mL^{-1}) and L-C18:0 SLs (MIC=100 µg mL^{-1}). Acidic SLs mixture and individual acidic SLs, presented a higher MIC (MIC> 800 µg mL^{-1}). With the crystal violet staining method a biofilm inhibition of 98%-100% was achieved with L-C18:1 and L-C18:0 in concentrations ranging from 0.1 to 3 mg mL^{-1} and a decrease of 4 log units in CFU was observed with L-C18:1 (1.5 mg mL^{-1}) in coated silicone. The SEM analysis also showed a concentration-dependent biofilm inhibition. These compounds may be on the right path to prevent biofilm formation on medical devices surface. Further studies will be performed on surface functionalization to improve the anti-adhesive and antimicrobial properties of medical devices surface colonization.

Acknowledgements

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References

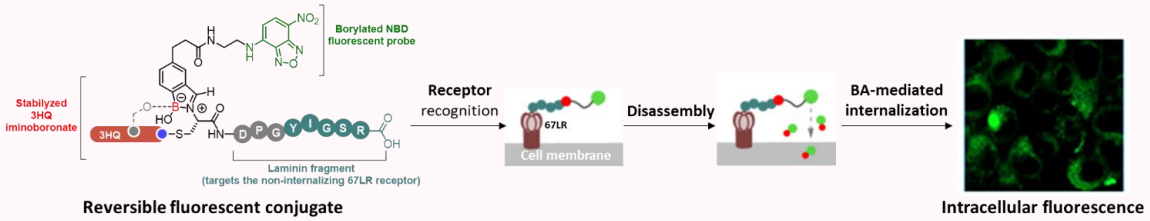
[1] Pontes, C.; Alves, M.; Santos, C.; Ribeiro, M.H.; Gonçalves, L.; Bettencourt, A.F.; Ribeiro, I.A.C., Int J Pharm 2016, 513(1–2), 697–708; [2] Ribeiro, I.A.C.; Faustino, C.M.C.; Guerreiro, P.S.; Frade, R.F.M.; Bronze, M.R.; Castro, M.F.; Ribeiro, M.H.L., J Mol Recognit 2015, 28(3), 155–65.

3HQ-Iminoboronate Formation For The Construction Of A Non-Internalizing Drug Conjugate

Russo R. (1), Padanha R. (1), Fernandes F. (2,3), Veiros L.F. (4), Gois P. M. P. (1,*)

1- Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; 2- Centro de Química-Física Molecular and Institute of Nanoscience and Nanotechnology, Instituto Superior Técnico, Universidade de Lisboa, Lisbon, Portugal; 3- IBB-Institute for Bioengineering and Biosciences, Instituto Superior Técnico, Universidade de Lisboa, Lisbon, Portugal; 4- Centro de Química Estrutural, Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais 1, 1049-001 Lisbon, Portugal.

In the field of modern bioconjugation, there is an increasing demand for reactions that are not only bioorthogonal, but also reversible under selected conditions. Boronic acids (BAs) are excellent candidates for this kind of applications as they are stable under physiological conditions, have good biocompatibility and reversibly bind to diols in aqueous environment.3-Hydroxy Quinolinones (3HQs) are isosters of Glycine that are also known to chelate various metals, and also proved to allow reversible boronic acid conjugation in aqueous medium. The 3HQs derivatives that we synthesized showed remarkable binding capabilities with boronic acids in buffer solution (Ka= 698.7±17 M-1) and were further developed to be inserted in peptidic fragments as pseudopeptidic platforms. The attachment of our platform to peptides allowed for the formation of stabilized iminoboronates on N-terminal Cysteine residues. This technology was used to prepare reversible fluorescent conjugates with the Laminin peptide, which allowed to validate the 67LR as a potential target for the delivery of payload to HT29 cancer cells.



Scheme 1 - 3HQ-Iminoboronate Formation For The Construction Of A Non-Internalizing Drug Conjugate.

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New Anticancer Proteasome Inhibitors: Computer-aided Drug Design, Biological Evaluation And Statistical Analysis Of Molecular Descriptors

Guedes R.A. (1,2), Aniceto N. (1), Gallerani E. (3), Gavioli R. (3), Carvalho N.A. (1), Gama M.J. (1), Andrade M.A.P. (4), Salvador J.A.R. (2,5), Guedes R.C. (1)

1- Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; 2- Center for Neurosciences and Cell Biology, University of Coimbra, Coimbra, Portugal; 3- Department of Life Sciences and Biotechnology, University of Ferrara, Ferrara, Italy; 4- ISCTE-IUL/ISTAR-IUL, Lisbon, Portugal; 5- Laboratory of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Coimbra, Coimbra, Portugal.

The ubiquitin proteasome system is a non lysosomal pathway by which cells regulate the controlled degradation of several proteins, not just in cell cycle and apoptosis but also in inflammatory and immune processes, carcinogenesis, among other examples. Usually, in protein homeostasis the defective proteins are ubiquitinated and are proteolysed into short peptides by the proteasome. Proteasome substrates include, for example, signalling molecules, tumour suppressors, cell cycle regulators and transcription factors. Proteasome inhibition results in an interruption of the degradation of these substrates, leading to the activation of apoptotic pathways and, eventually, cell death. Rapidly growing cells, such as cancer cells, are particularly susceptible to proteasome inhibition mechanisms [1,2]. This work relies on a computational-based drug discovery approach to find alternative new, selective small molecules as reversible proteasome inhibitors that can overcome the severe adverse drug reactions of the drugs on clinics. After the validation and refinement of the structure of the target protein, a virtual screening campaign was performed and were found new scaffolds of 20S proteasome inhibitors. The compounds were evaluated through biological assays, being performed proteasome inhibition assays in different human cancer cell lines and in isolated 20S proteasomes from human red blood cells. Cell viability assays were made in the same cancer cell lines and in fibroblasts. Was performed an intensive analysis of molecular descriptors of 680 chymotrypsin-like proteasome inhibitors (divided by four classes according to the half maximal inhibitory concentration value) described in the literature. The determination of the chemical space led to the conclusion that there is a difference of distribution of the four classes. A decision tree was trained and were defined two decision rules that allow to classify 63.60% of class A and efficiently separate this class from the others.

Acknowledgements

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References

[1] Ruggeri, B.; Miknyoczki, S.; Dorsey, B.; Hui, A.M., Adv Pharmacol. 2009, 57, 91-135; [2] Huber E.M.; Groll, M., Angew. Chem. Int. Ed. Engl 2012, 51, 8708–20.

Novel Tetraoxane-Pyrimidine Hybrids With Potential Antimalarial Activity In Liver Stage

Coutinho R.(1), Moreira R.(1), Capela R.(1)

1- Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal.

Malaria is a disease caused by parasites from *Plasmodium genus*. Development of antimalarial drugs has been predominantly focused on the blood stage of the malaria parasite, which is responsible for the symptoms associated to the disease. The liver stage of *Plasmodium* infection is an obligatory step in the maturation and replication of mosquito-delivered parasites toward generating the erythrocyte-infective forms [1]. It is highly desirable to target the live stage in order to eradicate malaria for efficacious and safe tools for malaria prophylaxis [2], furthermore, there is a limited number of available compounds actives against non-erythrocytic parasites [3]. Currently, only a few drug targets are fully validated for the hepatic stage of malaria. Building on our previous results on liver stage (active endoperoxides) [4], we now report the synthesis of tetraoxane-pyrimidine hybrid compounds containing a reactive warhead that may lead to reversible inhibition of parasitic cysteine hydrolases (Figure 1). These compounds can undergo a reaction with a catalytic cysteine residue to form a reversible abduct, and have the potential to be used as probes to gain a better understanding of the mechanism of action as well as identify the potential active cysteine containing target(s) for this class of liver stage inhibitors. In this communication we will present the synthetic strategy to prepare the hybrid inhibitors and their potential as antimalarial agents.

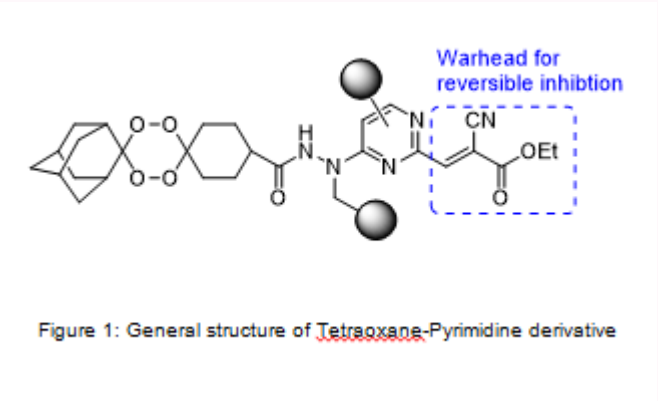

Figure 1: General structure of Tetraoxane-Pyrimidine derivative

References

[1] Prudencio, M.; Rodriguez, A.; Mota, M. M. The silent path to thousands of merozoites: the *Plasmodium* liver stage. Nat. Rev. Microbiol. 2006, 4, 849-856; [2] Douglass, A. N.; et al. Host-based prophylaxis successfully targets liver stage malaria parasites. Mol. Therapy. 2015, 23, 857-865; [3] Kori, L. D.; Valecha N.; Anvikar, A. R. Insights into the early liver stage biology of *Plasmodium*. J. Vector Borne Dis. 2018, 55, 9-13; [4] Oliveira, R.; et al. Tetraoxane-pyrimidine nitrile hybrids as dual stage antimalarials. J. of Medicinal Chemistry. 2014, 57.

Antioxidant-loaded Mucoadhesive Nanoparticles For Eye Drug Delivery: Evaluating The Reducing Of Generation Of Oxidative Stress

Cordeiro S. (1,2), Gonçalves L.M.D. (2), Marto J. (2,3)

1- Departamento Ciências da Vida, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Portugal; 2- Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; 3- Laboratório Edol, Produtos Farmacêuticos S.A., 2795-225 Linda-a-Velha, Portugal.

There are several techniques of drug delivery to treat ocular diseases, which can be invasive or non-invasive. In the area of the non-invasive techniques, new pharmaceutical strategies based on nanotechnology and mucoadhesive polymers are emerging approaches to reach an efficient treatment of ocular diseases. With that objective, our group are investigating innovative nanoparticulate systems for ocular drug delivery [1,2]. The aim of this work is to develop novel nanoparticle systems with mucoadhesive properties, intended to encapsulate antioxidant molecules and, aiming to reduce the generation of oxidative stress and consequently ocular diseases. To improve the retention time of antioxidant molecules on the surface of the eye, our strategy involves the use of nanoparticles composed by chitosan (CS) and hyaluronic acid (HA), with known mucoadhesive properties [1,2]. The optimization strategies involved the use of quality by design approach for the development of nanoparticles composition aiming a nanoparticle size lower than 400 nm and a positive zeta potential with a high yield. The nanoparticles were characterized by hydrodynamic mean particle size, dispersity index, zeta potential and yield (<350nm, <0.300, >+35mV, >90%, respectively) and the antioxidant encapsulation efficiency and the ophthalmic formulations were characterized in terms of pH, osmolality, viscosity and zeta potential. Mucoadhesion studies achieved by the interaction between the nanoparticles and mucin were conducted using different methods, namely: zeta potential measurements, viscosity measurements and tackiness testing, and they showed a high interaction between them, which means that the nanoparticles are able to increase the residence time in the eye surface. The *in vitro* release drug profiles trough synthetic membranes in Franz diffusion cells of the antioxidants were conducted to understand the kinetic mechanism involved on the drug release profile. The safety and efficacy of the novel formulation were tested in vitro using ARPE-19 cell line, where it was possible to see a high efficacy of the antioxidant activity and its low cytotoxicity on the cells. In conclusion, CS/HA nanoparticles is a promising platform for antioxidants delivery in the eye by increasing its residence time and controlled drug delivery.

Acknowledgements

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References

[1] Silva, M.M.; Calado, R.; Marto, J.; Bettencourt, A.; Almeida, A.J.; Gonçalves, L.M.D., Chitosan Nanoparticles as a Mucoadhesive Drug Delivery System for Ocular Administration, Marine Drugs. 2017; 15,370:1-16; [2] Graça, A.; Gonçalves, L.M.; Raposo, S.; Ribeiro, H.M.; Marto, J., Useful *In Vitro* Techniques to Evaluate the Mucoadhesive Properties of Hyaluronic Acid-Based Ocular Delivery Systems. Pharmaceutics 2018, 10, 110: 1-16.

Structure-based Virtual Screening Validation Toward Hexokinase 2 Inhibitors

Sara N. Garcia (1,2), M. Matilde Marques (1), Rita C. Guedes (2)

1- 1 Centro de Química Estrutural, Instituto Superior Técnico, University of Lisbon, Av. Rovisco Pais, 1049-001 Lisboa, Portugal; 2- Research Institute for Medicines (iMed.Ulisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal.

Glucose is regarded as the main fuel of cancer cells and the glycolytic pathway has been demonstrated as a potential target to be explored for cancer treatment. Several enzymes involved in glycolysis are overexpressed in different types of cancer cells, namely hexokinase 2 (HK2) [1]. This enzyme is not only involved in the first and most determinant step of glycolysis and subsequently in the different branched pathways [2,3], but also in the immortalization of cancer cells. When catalytically active, HK2 is able to bind to the voltage-dependent anion channel (VDAC) in the mitochondrial outer membrane, preventing the normal pro-apoptotic signalling. HK2- VDAC disruption would promote the binding of pro-apoptotic proteins to VDAC, promoting the enhancement of apoptosis in cancer cells4.In this way, the inhibition of the HK2 catalytic centre is proposed as a strategy to reduce the main source of energy to cancer cells, thus significantly decreasing cancer cell proliferation and avoiding HK2 binding to VDAC, enhancing the apoptosis process. As an effort to find hit compounds able to interfere with the HK2 catalytic activity, a structure-based drug design strategy was implemented, leading to the virtual screening of several general databases such as DrugBank (~2000 molecules), NCI (~265 000 molecules), Chemoteca (~800 molecules) and some specific natural product derivatives databases such as Ambinter (~10 000 000 molecules) and InterBioScreen Natural Products (~84 000 molecules). The virtual screening was carried out using molecular docking calculations through Gold 5.20 software. Molecules were prepared using Molecular Operating Environment (MOE2016 0802) and then docked into the HK2 catalytic site. Our results have suggested 2981 molecules with the potential to act as new HK2 inhibitors. Biochemical validation of the above-mentioned protocol was conducted with 64 molecules.

Acknowledgements

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References

[1] Hay, N. Nat. Rev. Cancer 2016, 16 (10), 635–649; [2] Martinez-Outschoorn, U. E.; Peiris-Pagès, M.; Pestell, R. G.; Sotgia, F.; Lisanti, M. P. Nat. Rev. Clin. Oncol. 2017, 14 (1), 11–31; [3] Hamanaka, R. B.; Chandel, N. S. J. Exp. Med. 2012, 209 (2), 211-215; [4] Krasnov, G. S., Dmitriev, A. A., Lakunina, V. A., Kirpiy, A. A. & Kudryavtseva, A. V. Expert Opin. Ther. Targets 17, 1221–1233 (2013).

Searching For Antibacterial Compounds From The African Medicinal Plant *Grewia hexamita*

Jogo S. (1), Duarte N. (1), Madureira A.M. (1), Mulhovo S. (2), Duarte A. (1), Ferreira M.J.U. (1)

1- Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; 2- Centro de Estudos Moçambicanos e de Etnociências (CEMEC), Faculdade de Ciências Naturais e Matemática, Universidade Pedagógica Campus de Lhanguene, Av. de Moçambique, 21402161 Maputo, Moçambique.

The genus Grewia (Malvaceae family), comprising approximately 150 species of small trees and shrubs, is well known for its ethnobotanical uses [1]. Various parts of plants of this genus have been used in African traditional medicine for the treatment of malaria, diarrhea, dysentery, typhoid fever, and small pox among other infection diseases [2]. Previous studies have demonstrated that plants belonging to this genus are rich sources of biologically active compounds, such as triterpenes, steroids and phenolic compounds [3]. *Grewia hexamita* is commonly used in Mozambique traditional medicine to treat infectious diseases. However, until date there have been relatively few phytochemical studies on this species. Aiming at evaluating and validating the antibacterial activity of *G. hexamita* and finding out antibacterial compounds, several extracts from the plant roots, with different polarities, were screened against Gram-positive species such as *Staphylococcus aureus* strains (sensitive, methicillin and vancomycin-resistant) and *Enterococcus faecalis* and Gram-negative such as *Pseudomonas aeruginosa* and *Salmonella typhymurium*. The best result was obtained for the methanol extract when tested against the Gram-positive strains with a MIC of 15 $\mu g.mL^{-1}$. Bioassay-guided fractionation of this extract allowed the isolation of several compounds, namely pentacyclic and tetracyclic triterpenes, including a new 3β-caffeoyl-cycloartane. Two steroids, a lactone and phenolic compounds were also isolated. Their structures were assigned based on spectroscopic methods namely 1D-NMR and 2D-NMR experiments. When evaluating the antibacterial activity of the pure compounds, the lowest MIC values were found for betulin (15 $\mu g.mL^{-1}$) against sensitive and methicillin-resistant *S. aureus* strains. A chemosensitization assay was also performed in order to evaluate the type of interaction of compounds with antibiotics. Some of the compounds showed a synergistic effect when combined with antibiotics.

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References

[1] Zia-Ul-Haq, M., Stanković, M.S., Rizwan, K., and De Feo, V. (2013). *Grewia asiatica* L., a food plant with multiple uses. Molecules 18, 2663–2682; [2] Chopra, R.N., Nayar, S.L. and Chopra, I.C. (1956). Glossary of Indian medicinal plants. New Delhi: Council of Scientific and Industrial Research; [3] Ullah, W., Uddin, G., and Siddiqui, B.S. (2012). Ethnic uses, pharmacological and phytochemical profile of genus Grewia. Journal of Asian Natural Products Research 14, 186–195.

Circulating miRNAs And Implications In Parkinson's Disease Patients

Oliveira S.R. (1), Dionísio P.A. (1), Correia Guedes L. (2,3), Gonçalves N. (2), Coelho M. (2,3), Rosa M.M. (2,3), Amaral J.D. (1), Ferreira J.J. (2,3), Rodrigues C.M.P. (1)

1- Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; 2- Instituto de Medicina Molecular, Universidade de Lisboa, Lisbon, Portugal; 3- Department of Neuroscience and Mental Health, and Department of Neurology, Hospital de Santa Maria, Centro Hospitalar Universitário Lisboa Norte, Lisbon, Portugal

Parkinson's disease (PD) is instigated by dopaminergic neurodegeneration in the substantia nigra. Although mostly sporadic, 5-10% of cases are related to heritable forms of PD, of which mutations in the leucine-rich repeat kinase 2 gene (*LRRK2*) are among the most common. MicroRNAs (miRNAs/miRs) are small non-coding RNAs, whose deregulation contributes to neurodegeneration. Here, we profiled candidate miRNAs in the serum of idiopathic (iPD) and LRRK2 mutated PD patients (LRRK2-PD), and healthy controls. Moreover, *in vitro*, we investigated if LRRK2 is a direct target of miR-335, as well as the downstream effect of miR-335 modulation. Total miRNAs were extracted from serum samples of discovery (20 iPD and 20 healthy controls) and validation (16 iPD, 41 LRRK2-PD and 15 healthy controls) cohorts. Expression levels of a selected panel of miRNAs (miR-21, miR-34a, miR-34c, miR-146a, miR-155, miR-335-3p/5p) were determined by Taqman qRT-PCR and analyzed using the ΔΔCt method. From miRNA target prediction bioinformatics (TargetScan, MiRTarBase), miR-335 was predicted to bind to LRRK2 and was further assessed *in vitro* using dual-reporter luciferase assays and Western blot. Production of proinflammatory mediators and activation of downstream signalling pathways were evaluated by RT-PCR and Western blot, respectively, in SH-SY5Y neuroblastoma cells with or without overexpression of alpha-synuclein (ASYN) in the absence or presence of miR-335. Our results showed that, in the discovery cohort, miR-146a and miR-335-3p/5p were significantly downregulated in the serum of iPD patients ($p < 0.05$) as compared with healthy controls. Importantly, the validation cohort confirmed discovery data. The same significant differences were observed in LRRK2-PD patients versus healthy controls, but not in iPD versus LRRK2-PD. *In silico* and *in vitro* experiments confirmed LRRK2 as a target of miR-335. Moreover, *in vitro*, miR-335 attenuated ASYN-induced inflammatory processes, decreasing TNF-α, IL-6 and COX2 mRNA levels and ERK1/2 activation. In conclusion, miRNAs are evolving as important biomarkers of PD as well as molecular tools for the development of innovative therapeutic strategies. miR-335 directly inhibits LRRK2, which may result into reduced inflammation. Thus, continuing to decipher the role of LRRK2 modulation by miR-335 may pave the way to a deeper understanding of PD pathogenesis.

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Lipid-based Nanoparticles Containing Centrally Acting Drugs: Development And Influence On Drug Encapsulation

Silva S. (1,2), Marto J. (2), Gonçalves L. (2), Almeida A.J. (2), Vale N. (1,3,4)

1- Laboratory of Pharmacology, Department of Drug Sciences, Faculty of Pharmacy, University of Porto, Rua de Jorge Viterbo Ferreira 228, 4050-313 Porto, Portugal; 2- Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; 3- Institute of Molecular Pathology and Immunology of the University of Porto (IPATIMUP), Rua Júlio Amaral de Carvalho, 45, 4200-135 Porto, Portugal; 4- Instituto de Investigação e Inovação em Saúde (i3S), University of Porto, Rua Alfredo Allen, 208, 4200-135 Porto, Portugal.

Lipid-based drug delivery systems have been widely used to overcome current drug limitations and biological barriers. Solid nanoparticles (SLN) and nanostructured lipid carriers (NLC) are colloidal carriers that provide controlled drug release for many different bioactive substances. The use of this nanotechnological platform can improve drug targeting to the brain, with an increase of efficacy and a decrease in toxicity. In this project, our studies aimed at developing and evaluating SLN and NLC able to incorporate three centrally acting drugs with potential application in the treatment of neurodegenerative diseases, i.e., rasagiline (RAS), tacrine (TAC), and tranylcypromine (TCP). Different formulations of SLN and NLC were produced using the hot high shear homogenization (HSH) method, as described elsewhere [1]. Further characterization was performed in terms of drug lipid solubility, particle size, potential zeta, encapsulation efficacy, physical stability and toxicity. The effects of various parameters, such as lipid concentration were evaluated in order to select the best candidate formulation to be used in subsequent studies. The NLC composed of glyceryl dibehenate and 25% of transcutol and 3% of Tween 80 revealed to be the more stable formulation, presenting suitable particle size (<150 nm), while cell viability studies demonstrated low toxicity in Caco-2 cell line. In addition, we are currently stipulating a protocol to also establish a primary culture of neurons in order to evaluate its toxicity and intracellular delivery.

Acknowledgements

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References

[1] Gaspar DP, Faria V, Gonçalves LM, Taboada P, Remuñán-López C, Almeida AJ (2016). Rifabutin-loaded solid lipid nanoparticles for inhaled antitubercular therapy: physicochemical and *in vitro* studies. Int J Pharm. 497: 199-209.

The Use Of 3D-Cultured MSCs' Secretome For Rheumatoid Arthritis Treatment

Camões S.P. (1), Gaspar M.M. (1), Rodrigues J.S. (1), Carvalheiro M. (1), Simões S. (1), Santos J.M. (2,3)*, Miranda J.P. (1)*

*1- Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; 2- Centro de Estudos de Ciência Animal (CECA), Instituto de Ciências e Tecnologias Agrárias e Agro-Alimentares (ICETA), Universidade do Porto, Oporto, Portugal; 3- Instituto de Tecnologia Química e Biológica António Xavier (ITQB NOVA), Universidade Nova de Lisboa, Oeiras, Portugal. *Both authors supervised this work.*

Rheumatoid arthritis (RA) is an autoimmune disorder whose treatment is mostly restricted to pain and symptom management and to the delay of joint destruction. Mesenchymal stem/stromal cells from the umbilical cord tissue (UC-MSCs) have previously been proven to be immunomodulatory and more efficient than bone marrow-derived MSCs in causing remission of local and systemic arthritic manifestations in vivo. Given these effects are essentially mediated through a paracrine mechanism, UC-MSC administration can be replaced by their secretome, thus avoiding allogeneic rejection and safety issues related to unwanted grafting. This work aimed at demonstrating the viability of applying a 3D-culture-based UC-MSC priming strategy to improve the efficacy of the resulting secretome for the treatment of RA. A proteomic analysis was performed to both, media conditioned by UC-MSC cultured either under conventional two-dimensional monolayer conditions (CM2D) or in 3D spinner flask bioreactors (CM3D). The analysis of relevant trophic factors confirmed secretome profiles with very significant differences in terms of therapeutic potential. Whereas CM3D was characterized by a prevailing expression of anti-inflammatory cytokines such as IL-10 and LIF, along with trophic factors involved in different mechanisms leading to tissue regeneration, such as PDGF-BB, FGF-2, I- 309, SCF and GM-CSF; CM2D presented relatively higher levels of IL-6, MCP-1 and IL-21, with recognized pro- inflammatory roles in joint disease and pleiotropic effects in the progression of RA. Accordingly, CM3D promoted a ~1.5-fold increase ($p < 0.001$) in chondrocyte migration capacity 24 h post-scratch when compared to CM2D while CM2D produced a ~2-fold increase ($p < 0.05$) in GAG induction when compared to CM3D. Finally, the evaluation of arthritic manifestations *in vivo*, using a rat adjuvant-induced model for arthritis (AIA), suggested a significantly higher therapeutic potential of CM3D over CM2D and even UC-MSCs. Histological analysis confirmed a faster remission of local and systemic arthritic manifestations of CM3D-treated animals. Overall, the results show that the use of CM3D is a viable and better strategy than direct UC-MSC administration for counteracting AIA-related signs. This strategy highlights the promising use of UC-MSC secretome as an ATMP representing a novel MSC-based but nonetheless cell-free treatment for arthritic conditions such as those characterizing RA.

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Bioactive Alkaloids From Species Of Amaryllidaceae Family

Sancha S., Priska S., Ferreira M.J.U.

Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal.

The main goal of this study is to develop a library of amaryllidaceae-type alkaloids, through isolation and molecular derivatization, for reversing multidrug resistance (MDR) in cancer cells, the major obstacle for cancer treatment. Plants from Amaryllidaceae family, traditionally used to treat cancer, can synthesize a high content and a wide variety of bioactive alkaloids [1]. The phytochemical study of the methanol extract of bulbs and flowers of *Narcissus bulbocodium* L. subsp *obesus* (Salisb) (Amaryllidaceae) led to the isolation of an alkaloid, an alkamide and four steroids. In addition, the study of the alkaloid fraction of the methanol extract of the bulbs of *Pancratium maritimum* L. (Amaryllidaceae) led to the isolation of several amaryllidaceae-type alkaloids. The structures of the compounds were established from their physical and spectroscopic data (IR, MS, 1D and 2D NMR -COSY, HMQC and HMBC and NOESY experiments).

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References

[1] Kornienko, A.; Evidente, A., Chem Rev., 2008, 108(6), 1982-2014.

Modular Construction Of Boronate Complexes For Drug-Conjugates Targeting

Silvia Baldo, Fabio M.F. Santos, Pedro M.P. Gois

Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal.

Neoplastic state represents the main focus at which medicinal chemistry strategies point nowadays to tackle cancer. Interrupting its evolution involves a variety of biological process be considered, therefore multifunctional constructs that combine the lethality of a cytotoxicity drug with the targeting ability of specific biomolecules, are required. In addition, stability and reversibility are necessary to internalize and release cargo into cells. On the basis of our experience [1] we envisioned that a promising strategy to create such compounds, known as Targeting Drug Conjugates (TDC) could take advantage of iminoboronate formation. We conceived that TDC could be readily created by assemblage of simple building blocks promoted by a boron tether and easily post functionalized. Here is presented the development of multifunctional second generation boronate complex (B-complex). Based on our previous experience we convinced that boron-core (B-core) stability would be affected by the imine carbon substituent [2]. Therefore 2-hydroxy-4-methoxy(R)phenone with different R substituent were synthesized and then assembled with phenyl boronic acid and aminophenol. The stability of this resulting B-cores has been evaluated in aqueous media at neutral pH through UV/Vis absorption measurements. Taking into consideration the stability and reversibility of the B-core, the building blocks corresponding of those exhibiting the best results will be selected as the components to build the multifunctional conjugates featuring a cytotoxic drug, a polar small chain and a target unit (Figure 1).

Subsequently the final boron-core complex assembly, biological assays will be performed to evaluate its selectivity and cytotoxicity against human cancer cells.

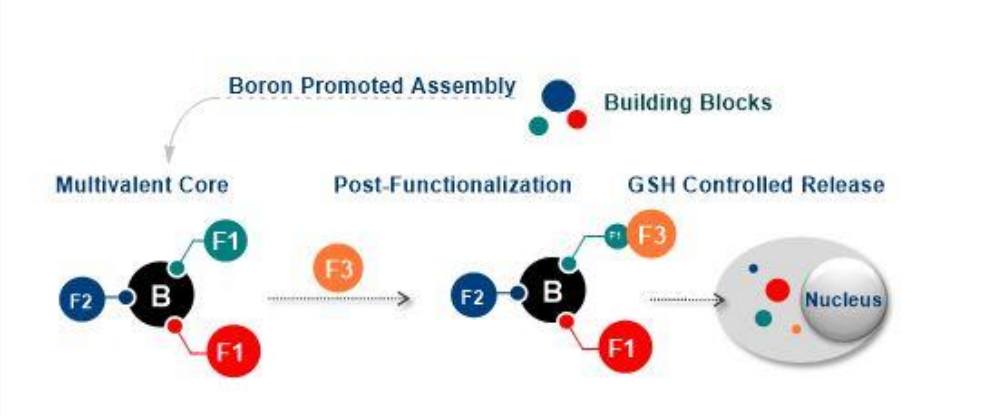

Figure 1

Acknowledgements

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References

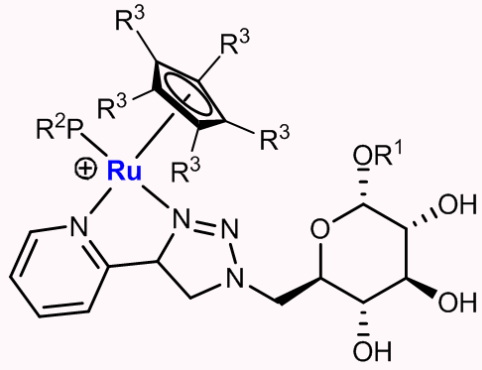
[1] Faustino, H.; Silva, M. J. S. A.; Veiros, L. F.; Bernardes, G. J. L.; Gois, P. M. P., Chem. Sci., 2016, 7, 5052-5058; [2] Santos, M. F., et al., Angew. Chemie - Int. Ed. 2017, 56, 9346-9350.

Ru(II) Complexes As Anticancer Agents

Milheiro S.A. (1), Gonçalves J. (1), Marques V. (1), Rodrigues C.M.P. (1), Costa P.J. (2,3), Florindo P.R. (1), Moreira R. (1)

1- Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; 2- Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal; 3- BioISI, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal.

Metal-based compounds as cisplatin and its analogues, carboplatin and oxaliplatin, are extensively used in the clinic as antitumor agents. However, its clinical utility has been limited due to side effects and drug resistance. Thus, these limitations have encouraged a search for more effective and less toxic antitumor agents based on organometallic compounds [1]. Known for high cytotoxicity and low general toxicity, ruthenium complexes may be the best candidates for anticancer agents as an alternative to platinum drugs in a near future [2]. Glucose transporters (GLUT) are transmembrane proteins responsible for cellular carbohydrate supply. These and other glycolytic proteins are overexpressed in most cancer phenotypes to comply with cancer cells increased glycolysis rate (Warburg effect). GLUT1 overexpression levels are correlated with cancer invasiveness, metastatic potential and poor survival prognosis. Glycoconjugation of a cytotoxic agent thus provides a route for its selective delivery at cancer tissues. This strategy led to the discovery of the first organo-ruthenium(II) anticancer glycoconjugates with cellular uptake mediated by glucose transporters in HCT116 colon cancer cells [3]. Here we report an extension of our library of cyclopentadienylruthenium(II) glycoconjugates in which we study the influence of phosphine, cyclopentadienyl and carbohydrate moiety (Figure 1) in colon anticancer properties. Compounds were evaluated in colon (HCT116) cancer cells. New complexes were docked at XylE (GLUT1 bacterial homologue) outward open conformation, and results compared with experimental data.



$R^1 = CH_3$ or H

$R^2 = Ph_3$, Cy_3, Ph_2CH_3, $(C_4H_8N)_3$ or PTA

R^3 = H or CH_3

Figure 1

Acknowledgements

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References

[1] V. Brabec, O. Nováková, Drug Resist. Updat. 2006, 9, 111–122; [2] A. Bergamo, C. Gaiddon, J. H. M. Schellens, J. H. Beijnen, G. Sava, J. Inorg. Biochem. 2012, 106, 90–99; [3] P. R. Florindo, D. M. Pereira, P. M. Borralho, P. J. Costa, M. F. M. Piedade, C. M. P. Rodrigues, A. C. Fernandes, Dalt. Trans. 2016, 45, 11926–11930.

PROTAC-mediated LRRK2 Degradation As A Novel Tool To Address Parkinson Disease

Domingos S. (1), Alessi D.R. (2), Ciulli A. (3), Moreira R. (1)

1- Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; 2- Medical Research Council Protein Phosphorylation and Ubiquitylation Unit, School of Life Sciences, University of Dundee, Dundee, UK; 3- Division of Biological Chemistry and Drug Discovery, School of Life Sciences, University of Dundee, Dundee, UK.

Missense mutations in the leucine-rich repeat kinase 2 (LRRK2), a multi-domain serine-threonine kinase, have been associated with autosomal dominant forms of Parkinson's Disease (PD) [1]. Collectively, while the structural biology of LRRK2 remains largely unknown, accumulated evidence has suggested that LRRK2 pathogenic variants generally increase kinase activity.2 Therefore, inhibition of LRRK2 kinase function with small molecules has been considered one of the most promising therapeutic strategies for the treatment of PD and several potent, selective and brain-penetrant LRRK2 inhibitors have been developed. However, the clinical efficacy of such compounds has not yet been validated [1]. In this context, PROteolysis TArgeting Chimeras (PROTACs) that can reduce pathogenic LRRK2, promoting its selective and controlled degradation, represent an interesting approach for the development of innovative PD therapies [2,3]. PROTACs are bifunctional molecules composed by one moiety that binds to the protein target of interest, an E3 ubiquitin ligase ligand, and a linker that connects both (Figure 1) [3]. In contrast to protein inhibition, PROTACs trigger a targeted catalytic protein degradation process inside cells via hijacking of the ubiquitin-proteasome machinery [3]. Following the analysis of the LRRK2 kinase domain, a library of LRRK2 PROTACs was designed and synthesized using a convergent synthetic strategy by combining an LRRK2 inhibitor with different linkers and E3 ligase ligands. In order to establish the target degradation profile, the developed PROTACs were screened in two different cell lines expressing LRRK2. According to the results, several compounds showed the ability to penetrate the cells and promote LRRK2 degradation, highlighting the potential of these compounds in the development of new therapies for PD.

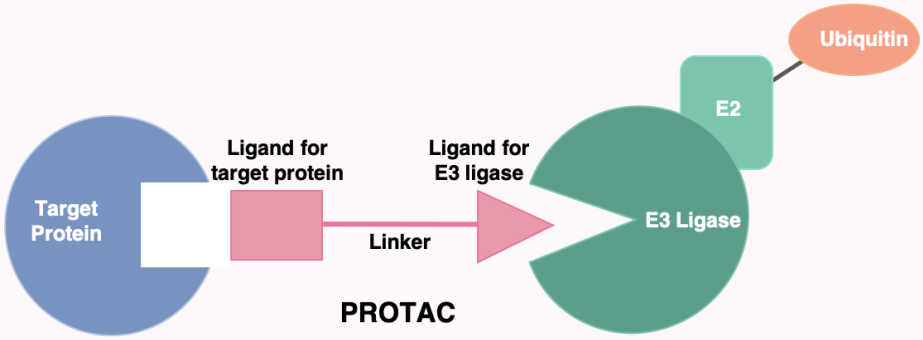

Figure 1. Schematic of the PROTAC technology.

Acknowledgements

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References

[1] Kang, U. B.; Marto, J. A., Proteomics 2017, 17, 9-20; [2] Jones, L. H., Cell Chem. Biol. 2018, 25, 30-35; [3] Lai, A. C.; Crews, C. M., Nat. Rev. Drug Disc. 2017, 16, 101-114.

Emerging Role Of MEF2C As Biomarker Of Human Brain Metastasization Of Breast Cancer

Galego S. (1), Sereno M. (1), Azevedo Kauppila L. (2), Malhó R. (3), Pimentel J. (4,5), Brito M.A (1,6)

1- Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; 2- Neurology, Department of Neurosciences and Mental Health, Hospital Santa Maria, Centro Hospitalar e Universitário Lisboa Norte, Lisbon, Portugal; 3- BioISI, BioSystems and Integrative Sciences Institute, Faculty of Sciences, Universidade de Lisboa, Lisbon, Portugal; 4- Faculty of Medicine, Universidade de Lisboa, Lisbon, Portugal; 5- Laboratory of Neuropathology, Department of Neurosciences and Mental Health, Hospital de Santa Maria, Centro Hospitalar e Universitário Lisboa Norte, Lisbon, Portugal; 6- Department of Biochemistry and Human Biology, Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal.

Myocyte enhancer factor 2C (MEF2C) is a transcription factor involved in development and differentiation processes. However, its role in metastasization is unclear. Our studies using a mouse model of triple-negative breast cancer (TNBC) showed that MEF2C is expressed in breast cancer (BC) brain metastases (BCBM) and translocates into the nucleus in later stages of metastasization. Therefore, we aimed to assess MEF2C expression in BCBM and to analyse if MEF2C is also expressed in human samples of BC primary tumours. We analysed 25 resected brain metastases derived from women with BCBM, with simple or multiple metastases, between 1 and 6 cm size, and 29-78 years old. The majority of cases corresponded to primary tumours human epidermal growth factor receptor 2 (HER2)-positive (HER2+) and hormone receptor-positive, whereas only 4 cases were TNBC. We further analysed resected tissue from 3 patients with primary BC. Double-labelling immunofluorescence analysis of MEF2C and pan-cytokeratin was performed in all samples. Analysis of brain tissue revealed MEF2C staining in pan-cytokeratin-positive cells, reflecting the expression of the transcription factor in metastasis. MEF2C presented distinct labelling patterns, presenting an extranuclear location, considered as phenotype (P)1, or showing ~50% cells with only extranuclear location and ~50% with both extranuclear and nuclear staining, denoted as P2, or with nearly all cells presenting MEF2C all over the cell, referred as P3. The majority of cases of brain metastases exhibited P2 and P3, whereas only 4 cases did not exhibit translocation into the nucleus (P1). Interestingly, as the tumour size and metastasis number increased, the percentage of cases with P1 decreased alongside with P2 and/or P3 increase, reflecting MEF2C translocation into the nucleus. We also observed MEF2C staining in primary tumours. MEF2C-positive cells were mostly located outside mammary ducts and corresponded to the lowest pan-cytokeratin expression, presumably corresponding to invading cells. This study reveals MEF2C as a new oncogene and a potential biomarker of BC prognosis and BCBM development.

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Synthesis And Evaluation Of New Triazene Hybrid Compounds As Anticancer Agents

Serra S., Mendes E., Gaspar M.M., Francisco A.P.

Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal

Hybrid molecules 1 were designed to be active in malignant melanoma. These new compounds 1, are based on two drug pharmacophores: a sulfur substituted phenol and a triazene. These pharmacophores covalently joined can act through two different mechanisms of action. On one hand sulfur substituted phenols can induce a specific apoptosis mechanism in melanoma cells [1] and on the other hand triazene moiety is a DNA alkylator agent [2]. Metastatic melanoma is the most aggressive and lethal form of skin cancer. In the advanced stages the prognosis is poor, and despite all current treatments, low response rates (less than 10% in 10 years) and several side effects are observed. Consequently new approaches are urgently needed to deal with this lethal disease and improve the cure rate [3]. In this communication, we present the design, synthesis and stability of new triazene hybrid compounds 1 as potential anticancer agents for the treatment of metastatic melanoma. These new hybrid compounds were designed to specifically target melanoma tumour sites, by incorporating in their structure a tyrosinase substrate. Tyrosinase is overexpressed in melanoma cells and can be used as a target [4]. The studied molecules were stable in plasma in order to allow the interaction with tyrosinase only in the melanotic tumour cells. The reaction of activation by the enzyme promotes the simultaneous release of both pharmacophores, increasing the targeting properties of the hybrids 1.

Acknowledgements

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References

[1] Y. Minamitsuji, K. Toyofuku, S. Sugiyama, K. Yamada, K. J. J Investig Dermatol Symp Proc. 1999, 4, 130; [2] A. P. Francisco, E. Mendes, A. R. Santos, M. J. Perry. Curr Pharm Des. 2019, 25: 1 (E-pub Abstract Ahead of Print). https://doi.org/10.2174/1381612825666190617155749; [3] B. Domingues, J. M. Lopes, P. Soares, H. Pópulo, H.. ImmunoTargets Ther. 2018, 7, 35); [4] A. Sousa , F. Santos , MM Gaspar, S Calado, JD Pereira, E. Mendes , AP Francisco, MJ Perry. Bioorg Med Chem, 2017, 25. 3900.

Manipulation Of Cystatin C To Control Mycobacteria Infection In Human Macrophages

Valente S., Pires D., Anes E.

Host-Pathogen Interactions Unit, Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal.

Tuberculosis (TB) is a disease caused by the facultative intracellular pathogen *Mycobacterium tuberculosis* (Mtb) which has the ability to subvert the immune system in order to survive in host cells. Macrophages are the first line of defense against the pathogen and trigger phagocytic mechanisms that engulf the bacteria in a structure called the phagosome that after fusing with lysosomes will be designed to destroy the bacteria.
Among the killing mechanisms delivered and activated within phagolysosomes are acidic hydrolytic enzymes cathepsins. These proteolytic enzymes participate in pathogen destruction and processing of antigens to be presented to lymphocytes. Recent studies revealed interactions between MTb, cathepsins, and their inhibitors, cystatins. It was shown that Mtb induces a decreased gene expression and activity of cathepsins, enabling their survival inside macrophages. Thus cathepsins are regulated by cystatins, which act by inhibiting cathepsins through binding to their proteolytic active site. Since Mtb was shown to down-regulate cathepsins activity we hypothesize that the cystatin pathway might be explored to improve the processes dependent on cathepsin activity such as pathogen destruction and antigen processing by type II HLA complexes.In this study we manipulated by siRNA, cystatins in order to prevent inhibition of cathepsins activity during Mtb infection. With this strategy we do expect to induce an increased pathogen killing within phagolysosomes and the processment of their antigens together with an improvement of HLA-DR receptors and antigens presentation in the surface of the infected macrophage. Our main target here was cystatin C (CSTC) since it is a major inhibitor of cathepsin S, which is required for HLA-DR processing, as well as for several other cathepsins.For this purpose we used small interfering RNA (siRNA) to silence CSTC, during *Mycobacterium bovis* bacillus Calmette-Guérin (BCG) and *Mycobacterium smegmatis* infection in human macrophages. We monitored the killing of the BCG and *M. smegmatis* through the counting of colony-forming unit (CFU) recovered from infected cells and the expression of the HLA-DR machinery and antigen presentation by flow cytometry. Our results indicate that the manipulation of CSTC induced down-regulation improved killing of mycobacteria in macrophages, and increase the expression of HLA-DR machinery in the surface of the cells. All together these results point for the cystatins activity manipulation in infected macrophages as a tool to improve the host cellular response against Mtb.

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Nanosystem in Cancer Chemotherapy, a strategy to Multi-drug Resistance mediated by P-glycoprotein

Albino, C. Sousa, M. Videira,

1- Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal;

One of the main characteristics of cancer cells is the overexpression in their membrane surface of drug efflux pumps. One of the most studied is P-glycoprotein (P-gp), an ATP-binding cassete (ABC). Due to their presence in the cell's membrane, cancer cells can acquire resistance to a wide range of ant cancer drugs, resulting in a multidrug resistance (MDR) phenotype, and the main reason for chem therapy failure. Paclitaxel is an example of an anticancer drug, that belongs to a class of taxane broadly used as treatment for a variety of cancers, such as breast, lung, ovary and colon cancer. However, its poor water solubility and unsatisfactions/undesired bioavailability repre-sents a serious clinical limitation. Nanocarriers such as Solid Lipid Nanoparticles (SLNs), micelles, dendrimers, liposomes, have attracted attention in cancer therapy. Micelles are a promising system owing its capability to entrap class II drugs, that have been stud-ied in clinical trials as drug carriers. This system comprises a coreshell structure which enables the nanoparticles to incorporate drugs and shield healthy cells from adverse cytotoxic effects. Due to their small size, these systems exhibit many advantages, such as targeting ability, long circulation and easy production on effective delivery of drugs. The aim of this study was to develop micelles containing Paclitaxel, and to evaluate the activation of P-gp after the internalization of the system by cancer cells.

Acknowledgements
Projeto ENMed/0009/2015

References

[1] Cavaco MC, Pereira C, Kreutzer B, Gouveia LF, Silva-Lima B, Brito AM, Videira M. (2017), Evading P-glycoprotein mediated-efflux chemoresistance using Solid Lipid Nano-particles. Eur J Pharm Biopharm , 110:76-84. [2] Wang TH1, Wang HS, Soong YK. (2000), Paclitaxel-induced cell death: where the cell cycle and apoptosis come together. Cancer, 88(11):2619-28. [3] Videira M, Arranja AG, Gouveia LF. (2013), Experimental design towards an optimal lipid nanosystem: a new opportunity for paclitaxel-based therapeutics. Eur J Pharm Sci, 49(2):302-10. [4] Lee KS, Chung HC, Im SA, Park YH, et al. (2008), Multicenter phase II trial of Genexol-PM,a cremophor-free, polymeric micellesformulation of paclitaxel, in patients with meta-static breast cancer. Breast Cancer Res Treat, 108, 241-50. [5] Torchilin VP.. (2001), Structure and design of polymeric surfactant-based drug delivery sys-tems, J Control Rel, 73, 137-72.

1-deoxy(methyl) Sphingoid Bases Impact In Membrane Fluidity and Lateral Organization

Santos T.C.B. (1,2), Saied E.M. (3), Prieto M. (2), Arenz C. (3), Silva L.C. (1)

1- Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; 2- CQFM- IN and IBB-Institute for Bioengineering and Biosciences, Instituto Superior Técnico, Universidade de Lisboa, Lisboa, Portugal; 3- Humboldt Universität zu Berlin, Institute for Chemistry, Berlin, Germany.

The biological actions of Sphingolipids (SLs) have been associated to their effects in the biophysical properties of membranes. These effects depend on the structure of the SLs, which influences the interactions with the surrounding lipids changing their lateral organization and forming lipid domains. These membrane regions might function as platforms for the activation of specific processes and regulation of cell function. To better understand the relation between the structure and biophysical properties of SLs, we used complementary fluorescence-based methodologies and membrane model systems containing POPC and sphingoid backbones [long chain bases (LCB)] with different degrees of hydroxylation and unsaturation. We compared the effects of the canonical sphinganine (SA) and sphingosine (Sph) to their naturally occurring derivatives lacking the OH group in the C1 position – the 1-deoxy-LCBs - or both the OH and CH3 group in the C1 position – the 1-deoxymethyl-LCBs. We also studied the Sph derivatives with the canonical trans-double bond at position C4-5 (DB 4-5) or with the atypical but natural cis-double bond at C14-15 (DB 14-15). The interest in the study of these derivatives arose from their association with HSAN1 and diabetes type II development.Our results show that both the C1 position structure and the unsaturation of the LCB influence the formation of ordered domains. 1-deoxymethyl-LCBs showed the highest ability to form ordered domains, while the intermolecular interactions of 1-deoxy-LCBs were weaker leading to less packed domains. The presence of the DB 4-5 in the LCB decreased their ability to segregate into ordered domains, whereas the DB at 14-15 position completely abolished ordered domain formation.In conclusion, the different structure of 1-deoxy(methyl)-LCB lead to different changes in membrane fluidity and lateral organization, suggesting a possible mechanism to mediate the distinct biological action of these species.

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Identification Of Novel miRNAs Associated With Experimental NAFLD Pathogenesis

Islam T. (1), Simão A. (1), Afonso M. (1), Rodrigues P. (1), Carvalho M.G. (2), Rodrigues C.M.P. (1), Castro R.E. (1)

1- Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; 2- BioFIG - Center for Functional and Integrative Genomics, Faculty of Sciences, Universidade de Lisboa, Lisbon, Portugal.

microRNAs (miRNAs) are modulated in non-alcoholic fatty liver disease (NAFLD) and are being studied as putative diagnostic and prognostic biomarkers. Further, as post-transcriptional modulators of gene expression, miRNAs appear to contribute to NAFLD pathogenesis by regulating distinct cellular pathways, impacting on lipid and glucose metabolism, cell death, cell proliferation and differentiation. Here, we aimed to identify and validate novel metabolism-associated miRNAs modulated during experimental NAFLD.C57BL/6N male mice were fed distinct NAFLD-inducing diets, namely a methionine and choline-deficient diet (MCD; n=30) for 2 and 8 weeks; a high fat, 2% cholesterol diet with high fructose - fast-food diet (FF; n=12) - for 25 weeks; and a choline-deficient amino acid-defined diet (CDAA; n=28) for 32 and 66 weeks. Liver samples were collected and processed for histological and miRNA analyses. Liver RNA from 8 week MCD-fed mice was analyzed using TaqMan™ array microRNA cards, containing sequences for ~740 of the most relevant human or rodent miRNAs. qPCR array data were analyzed using the HTqPCR package in Bioconductor. We found significant differences in the expression of 52 miRNAs, with 25 miRNAs increasing and 27 miRNAs decreasing in the liver of MCD diet-fed mice, compared with chow-fed animals. Differentially expressed miRNAs were subjected to an in silico analysis to exclude previously reported associations with NAFLD or related metabolic liver diseases.

Based on this, a panel of different upregulated (miRNA-127, miRNA-136, miRNA-411) and downregulated (miRNA-107, miRNA-455) miRNAs were selected for downstream validation analyses.miR-136 was found to be up-regulated in MCD-fed animals alone. In turn, both miRNA-127 and miR-411 were found consistently upregulated in both MCD and CDAA diets, but unaffected in the FF diet, suggesting a possible association with liver damage rather than overall metabolism. Interestingly, miRNA-107 and, in particular, miRNA-455, were consistently downregulated in all diets, compared with control diet-fed animals, suggesting that they may constitute major regulators of disease pathogenesis. In conclusion, we have identified novel miRNAs modulated during experimental NAFLD, associating with distinct pathogenic signalling pathways. A better characterization of the role of these miRNAs in disease could translate into prospective therapeutic strategies as well as putative biomarkers in NAFLD.

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Metabolite Synthesis And Characterization Of A Novel Scaffold With Anticancer Properties In p53-targeted Therapies

Barcherini V. (1), Antunes A.M.M. (2), Santos M.M.M. (1)

1- Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; 2- CQE, Instituto Superior Tecnico, Universidade de Lisboa, Lisbon, Portugal.

Cancer survivorship is constantly increasing as result of more efficacious anticancer drugs or combinations of drugs used in cancer therapy [1]. For this reason, it is important to identify, study and overcome possible side effects in order to guarantee a high rate of success of these therapies. Furthermore, in the drug development process a possible drug candidate is evaluated based on the information about its metabolic pathway and pharmacokinetics. In this sense, it results crucial to investigate possible active metabolites that can correspond to chemically reactive intermediates [2]. In previous research, we have identified oxazoloisoindolinones SLMP53-1 and DIMP53-1 as promising small molecules with anticancer activity in vitro and *in vivo* in colon and breast cancer models [3]. In this communication, we want to show our most recent results in the characterization of the *in vitro* Phase I metabolism of these two compounds and our achievements in the metabolite synthesis of these two small molecules.

Acknowledgements

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References

[1] Shapiro, C. L., New England Journal of Medicinal Chemistry, 2018, 379, 2438-2450; [2] Hrynchak, I.; Sousa, E.; Pinto, M.; Costa, V. M.; Drug Metabolism Reviews, 2017, 49, 2, 158-196; [3] a) Soares, J.; Espadinha, M.; Raimundo, L.; Ramos, H.; Gomes, A. S.; Gomes, S.; Loureiro, J. B.; Inga, A.; Reis, F.; Gomes, C.; Santos, M. M. M.; Saraiva, L.; Molecular Oncology, 2017, 11, 6, 612-627; b) Soares, J.; Raimundo, L.; Pereira, N. A. L.; Monteiro, A.; Gomes, S.; Bessa, C.; Pereira, C.; Queiroz, G.; Bisio, A.; Fernandes, J.; Gomes, C.; Reis, F.; Gonçalves, J.; Inga, A.; Santos, M. M. M.; Saraiva; L.; Oncotarget, 2016, 7, 4, 4326-4343.

miR-ELISA Assay Proof-Of-Concept: A Novel Approach For The Detection And Quantification Of Circulating miRNAs

Marques V. (1), Afonso, M.B. (1), Flehmig B. (2), Normann A. (2), Rodrigues C.M.P. (1)

1- Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; 2- Mediagnost GmbH, Reutlingen, Germany.

Circulating microRNAs (miR/miRNA) have emerged as prime candidates for non-invasive biomarkers of disease. Currently, the gold-standard method for miRNA expression evaluation is the reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) requiring expensive laboratory equipment and time-consuming workflows. Here, we aimed to develop an enzyme-linked immunosorbent assay (ELISA)-based strategy for miRNA detection and quantification, as ELISA represents an easy and inexpensive laboratory procedure. Hence, we developed the miR-ELISA assay based on the sandwich-ELISA method, where a synthetic DNA oligonucleotide serves as a catcher probe for the complementary circulating miRNA. This DNA:RNA hybrid is detected by a primary antibody that specifically targets these hybrids; final signal detection is mediated by an HRP-conjugated antibody. A 2-day experimental protocol was established using hsa-miR-16-5p as target and cel-miR-39-3p as spike-in, which encompassed: 1) testing of dilution, hybridization and washing buffers; 2) titration of antibodies and catcher probes; 3) hybridization temperature optimization; and 4) time-course experiments for incubation steps. Both miRNAs bound specifically to their complementary DNA probe without evidence of any cross-binding reactions. In nuclease free water simulated samples, with controlled concentrations of synthetic miRNAs, limits of detection reached 10 pg/mL for hsa-miR-16-5p and 100 pg/mL for cel-miR-39-3p. Further, miRNAs were isolated from a pool of human serum, but only cel-miR-39- 3p was detectable by miR-ELISA at 10-ng/mL spike-in concentrations. These results were corroborated by RT- qPCR for both miRNAs. Dilution series of known concentrations of synthetic hsa-miR-16-5p and cel-miR-39-3p (ranging from 10 ng/mL to 100 ag/mL) were used to generate standard curves and interpolate absolute quantification of miRNA concentrations in human serum. Correlations between sample concentration, ct values and absorbance signal were established. In conclusion, the miR-ELISA assay can effectively detect miRNAs; however, it is still not sensitive enough to achieve the detection of endogenous hsa-miR-16-5p in human serum, described to be at 1 pg/mL concentration. Future work encompasses the redesign of the initial concept of the miR-ELISA sandwich to increase assay sensitivity.

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Mercury Compounds As Potential Antitumorals: Efficacy In Mouse Glioma Cells

Pires V., Branco V., Carvalho C.

Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal.

Glioblastoma multiforme (GMB) is the most aggressive and common form of glioma [1]. GMB, as many other tumors, present high levels of redox proteins, like thioredoxin (Trx) and thioredoxin reductase (TrxR), which is linked to therapy resistance [2]. Therefore, targeting antioxidant systems becomes increasingly more important in chemotherapy, since cancer cells present a higher vulnerability to oxidative stress. Indeed, inhibition of TrxR/Trx has been shown to promote cancer cell death.Mercury presents a high affinity to bind thiols and selenols, making the thioredoxin system a major target for this element. Moreover, most mercury compounds can effectively cross the blood brain barrier exerting neurotoxicity [3,4,5]. This study aims to verify if the inhibition of the thioredoxin system by thimerosal (TM) and ethylmercury (EtHg), promotes generates oxidative stress leading to cell death in mouse glioma cells, GL261. Cell viability was assessed by MTT assay for 24, 48 and 72 hours of exposure to TM and EtHg. The activities of Trx and TrxR as well as their expression were evaluated by the insulin endpoint assay and Western Blot, respectively. The increase in ROS by after exposure to TM and EtHg was verified by different endpoints including peroxiredoxin (Prx) 2 oxidation that was evaluated by Western Blot assay. The results show that GL261 cells are very sensitive to mercury compounds after 24 hours since GI50 values of 2.6±0.27 µM and 2.4±0.37 µM for EtHg and TM, respectively. There was a significant inhibition of TrxR activity (>50%) with EtHg and TM, with TM being more toxic, in spite the increased expression of TrxR. Thioredoxin was also inhibited but to a lower extent. The overall inhibition of the thioredoxin system was related with higher levels of Prx 2 dimerization, which reflects higher ROS levels and oxidative stress. Ongoing experiments are being conducted to evaluate the pathways of cell death including apoptosis.

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References

[1] Kyani, A.; Tamura, S.; Yang, S.; Shergalis, A.; Samanta, S.; Kuang, Y.; Ljungman, M.; Neamati, N., ChemMedChem 2018, 13(2), 164–77; [2] Carlsson, S.K.; Brothers, S.P.; Wahlestedt C., EMBO Molecular Medicine. 2014, 6(11), 1359–70; [3] Branco, V.; Godinho-Santos, A.; Gonçalves, J.; Lu, J.; Holmegren, A.; Carvalho, C., Free Radic. Biol. Med. 2014, 73, 95–105; [4] Branco, V.; Coppo, L.; Solá, S.; Rodrigues, C.; Holmegren, A.; Carvalho, C., Redox Biol. 2017, 13, 278–287; [5] Carvalho, C.; Chew, E.; Hashemy, S.; Lu, J.; Holmegren, A., J. Biol. Chem. 2008, 283, 11913–11923.

Molecular Docking As A Tool To Design Royleanone Derivatives From *Plectranthus spp.* As PKC-δ Modulators

Isca V.M.S. (1,2), Ntungwe E. (2), Tavares J. (2), Almeida J. (3), Siopa F. (2) Saraiva L. (3), Mori M. (4), Botta M. (4), Afonso C.A.M. (1), Rijo P. (1,2)

1- Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; 2- CBIOS, Universidade Lusófona, Campo Grande 376, 1749-024 Lisboa, Portugal; 3- Departamento de Ciências Biológicas, Faculdade de Farmácia da Universidade do Porto, Portugal; 4- Dipartimento di Biotecnologie, Chimica e Farmacia, Università di Siena, Italy.

Cancer is one of the most common causes of death worldwide. Protein Kinase C (PKC) family has wide ranging effects in crucial processes of tumorigenesis and metastatic dissemination. Moreover, the overexpression of PKC-δ induced growth inhibition in colon cancer cells [1]. Diterpenoids such as royleanones, commonly found on *Plectranthus genus*, exhibit promising anticancer activity [2]. One example is the compound 7α-acetoxy-6β- hydroxyroyleanone (Roy, Figure 1), obtained from *P. grandidentatus* [3]. A small library of Roy derivatives, previously prepared, was tested on a yeast-based screening assay [4] and showed promising ability to activate PKC isoforms. Furthermore, the patented diterpene 6β-benzoyloxy-12-O-benzoylroyleanone (RoyBz) shown selective modulation in PKC-δ [5]. In this work, Roy and RoyBz assist the structure-based drug design of new bioactive derivatives, through modification of the C-12 and C-6 hydroxyl groups. The molecular docking program FRED was used to dock libraries of new theoretical derivatives against 1PTR (Crystallographic structure of human PKC-δ regulatory domain). *In silico* screening allowed the identification of the most promising compounds for further synthesis. The most promising hits are currently been prepared by hemi-synthesis using Roy as starting material (Figure 1) for structure-activity relationships.

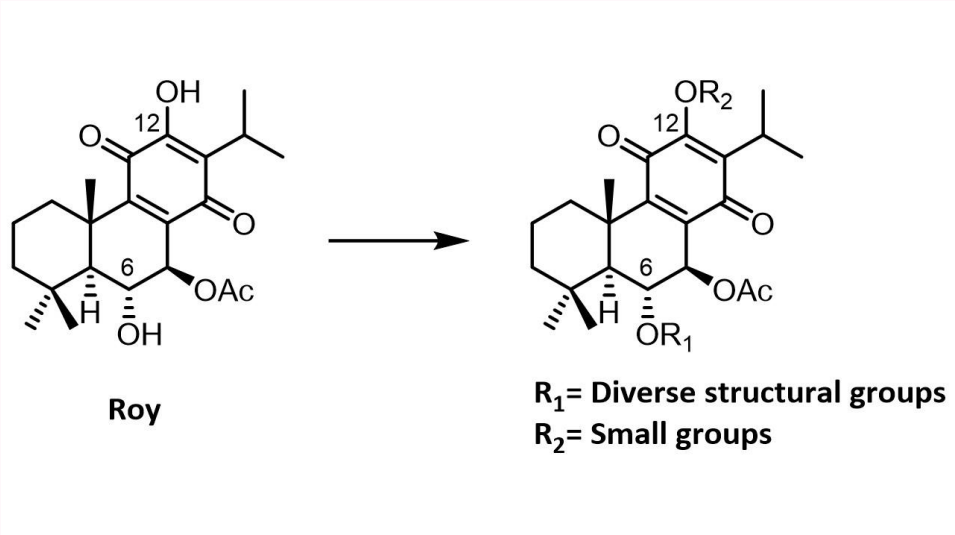

Figure 1

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References

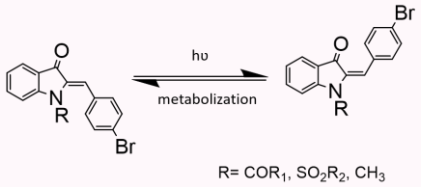
[1] C. Bessa et al., Cell Death Dis., 2018, 9; [2] D. Matias et al., ACS Omega, 2019, 4, 8094-8103; [3] C. E. S. Bernardes et al., Mol. Pharmacol., 2018, 15, 1412–1419.

Photoisomerization And Microsomal Metabolism Of Antimycobacterial *N*-substituted Azaaurones

Campaniço A., Perdigão J., Portugal I., Moreira R., Lopes F.

Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal.

Tuberculosis stands as one of the most lethal diseases worldwide, with 1.6 million deaths reported in 2017 [1]. Regarding current therapies, the emerging resistance alongside with low therapy compliance are key factors in increasing disease burden and mycobacteria proliferation [2]. It is urgent to find new potent drugs against *Mycobacterium tuberculosis*, that may overcome the resistance problem and simplify the treatment. Azaaurones are potent antimycobacterial agents, with MIC_{99} values as low as 0.37 µM against *M. tuberculosis H37rv* strain [3]. SAR analysis revealed an improved activity of *N*-acetyl azaaurones when compared to their NH counterparts. To this moment, azaaurones have been described as isomeric mixtures. We now report the isolation and differential study of E and Z isomers from diversely *N*-substituted azaaurones. Furthermore, the phenomena of metabolic- and photoisomerization were studied and the effect of different stereoelectronic moieties in the isomerization rates is disclosed (Scheme 1).



Scheme 1 - Photoisomerization of *E* and *Z* isomers of *N*-substituted azaaurones.

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References

[1] WHO. Global Tuberculosis Report. 2018; [2] Wellington S, Hung DT. ACS infectious diseases. 2018;4:696-714; [3] Campaniço A, et al, ChemMedChem, Accepted.

Precision Nanotechnology-based Therapeutics To Regulate Tumor-immune Cells Crosstalk Against Melanoma

Carreira B. (1,2), Scomparin A. (2,3), Krivitsky A. (2), Matos A.I. (1), Acúrcio R.A. (1), Conniot J.M. (1), Satchi-Fainaro R. (2), Florindo H.F. (1)

1- Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; 2- Department of Physiology and Pharmacology, Sackler Faculty of Medicine, Tel Aviv University, Israel; 3 - Department of Drug Science and Technology, University of Turin, Via P. Giuria 9, 10125 Turin, Italy.

Melanoma is the most lethal skin cancer and patients presenting the disseminated form of the disease have a very low survival rate, with highly restricted therapeutic alternatives. Thus, new therapeutic options are urgently need. To that end, we focused on the development of a rationally-designed multifunctional nanomedicine to successfully modulate melanoma-immune cell interactions by expanding the range of targeted cells in a site-specific manner. Biological dual precision nanoparticles (NP) were designed to target dendritic cells (DC) and the melanoma tumor microenvironment (TME), aiming for the delivery of combinations of melanoma-associated antigens with adjuvants and gene regulators, to effectively modulate the function of these antigen presenting cells (APC) and natural killer (NK) cells. Poly(lactic acid) (PLA) and Poly(lactic-co-glycol) (PLGA)-based NP were prepared by a modified double emulsion (w/o/w) solvent evaporation method, using a new grafted PLGA-polyethylene glycol (PEG)-mannose polymer to enhance the DC targeting efficiency of the NP. The mannosylated polymer was characterized by 1H-NMR and Fourier-transform infrared spectroscopy (FTIR) spectroscopy prior to NP formulation. The physico-chemical properties of these multifunctional NP were fully evaluated including hydrodynamic size, zeta potential and surface morphology, by Dynamic Light Scattering, Laser Doppler Electrophoresis, and Atomic Force Microscopy, respectively. The amount of melanoma antigens and gene regulators entrapped was determined by the fluorescamine assay and using the Picogreen® reagent, respectively. Immature DC (ATCC® CRL-11904™) were used to evaluate the impact of these NP on the viability of these phagocytic cells (propidium iodide assay), as well as to assess the NP cellular uptake kinetics by flow cytometry. The covalent conjugation between PLGA and PEG-Mannose was confirmed. Mannose-PEG-grafted NP presented an average diameter of 180 nm, narrow polydispersity index, surface charge close to neutrality, spherical morphology, and high loadings of antigens and gene regulators. No cytotoxic effect was observed on immature DC up to 48h of incubation. Cy5.5-labeled NP were extensively internalized by DC, with a significant increase in the uptake rate for NP synthesized with the modified PLGA-PEG-mannose polymer, when compared to that obtained for non-mannosylated NP. Having in consideration the physico-chemical properties of our NP, their internalization kinetics and ability to regulate the expression of immune-related players within the TME, this multifunctional nanomedicine is a promising immunotherapeutic approach to selectively modulate the function of DC and NK cells towards a strong and long-lasting immune-mediated anti-tumor cytotoxic response.

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Targeted nanovaccine as a powerfull platform to treat colorectal cancer

Ana I. Matos (1,2,3*), Bárbara Carreira (1), Liane Moura (1), Carina Peres (1,3), Ana S. Viana (4), João P. Conde (2), Luís Graça (3), Ronit Satchi-Fainaro (5) & Helena F. Florindo (1)

1 - Instituto de Investigação do Medicamento (iMed.ULisboa), Faculdade de Farmácia da Universidade de Lisboa, 1649-003 Lisboa, Portugal; 2 - INESC-MN – Microsystems and Nanotechnologies, 1000-029 Lisboa, Portugal; 3 - Instituto de Medicina Molecular, Faculdade de Medicina da Universidade de Lisboa, 1649-025 Lisboa, Portugal; 4 - Centro de Química e Bioquímica, Faculdade de Ciências da Universidade de Lisboa, 1749-016 Lisboa, Portugal; 5 - Department of Physiology and Pharmacology, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel

Colorectal cancer (CRC) is the third most commonly diagnosed cancer and the fourth cause of cancer death worldwide. This work focused on the development of a combinational nanoplatform for CRC immunotherapy and immunomodulation based on the design of nanoparticles (NP) to deliver a combination of CRC antigens, adjuvants and gene regulators according to targeted dendritic cell (DC) and CRC cells. Poly(lactic-co-glycolic) (PLGA) NP were prepared by the double emulsion solvent evaporation method. To potentiate a targeted delivery, NP surface was modified to target CD206 and αvβ3 receptors overexpressed on DC and tumor microenvironment (TME) cells, respectivelly. NP were characterized in terms of size, zeta potential and surface morphology. CRC neoantigens, adjuvants and immunomodulator loadings were quantified by fluorescence. Immature DC and CRC cells were used to evaluate the in vitro NP cytotoxicity and NP cellular uptake profile by flow cytometry. NP uptake in vivo by myeloid antigen presenting cells and the expression of maturation and co-stimulatory molecules at the DC cell surface within draining lymph nodes, were also evaluated by flow cytometry. The immunotherapeutic efficacy of our multivalent nanovaccines was assessed in MC38 murine model.NP presented a mean diameter close to 200 nm, low polydispersity index, neutral surface charge, spherical shape, and high loadings for neoantigens, adjuvants and immunomodulators. No cytotoxic effect was observed on immature DC and CRC cells up to 48 h of incubation. NP were extensively internalized by immature DC in vitro and by migratory DC in vivo, but also by CRC cells. A noteworthy tumor remission was observed in tumor-bearing mice treated with the nanovaccine. This approach can guide the design of effective treatments for CRC patients.

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Breast cancer brain metastasization: unraveling key features of extravasation and brain colonization

Galego S. (1), Godinho-Pereira J. (1,2), Vicente R. (1), Custódio-Santos T. (1), Molnar K. (3), Haskó J. (3), Malhó R. (4), Videira M. (1,5), Wilhelm I. (3), Krizbai I. (3), Brito M.A. (1,6)

1- Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; 2- Farm- ID, Faculty of Pharmacy Association for Research and Development, Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; 3- Institute of Biophysics, Biological Research Centre, Hungarian Academy of Sciences, Szeged, Hungary; 4- BioISI, BioSystems and Integrative Sciences Institute, Faculty of Sciences, Universidade de Lisboa, Lisbon, Portugal; 5- Department of Galenic Pharmacy and Pharmaceutical Technology, Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; 6- Department of Biochemistry and Human Biology, Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal.

Brain metastases have become a major limitation of the expectancy and quality of life in most the breast cancer (BC) patients. How the malignant cells transpose the blood-brain barrier (BBB) and form metastases in the brain parenchyma remains poorly understood. As such, we aimed to assess BBB properties and the signaling pathways involved in BC cells migration and colonization to the brain. We examined hippocampal sections of mice inoculated with 4T1 triple-negative BC cells along the brain metastasization process (5 hours, 3, 7, and 10 days) by haematoxylin-eosin staining and immunohistochemical/immunofluorescence analysis. Haematoxylin-eosin staining revealed well-established metastases after 7 days, which augmented thereafter. Along metastasization, BBB properties disruption occurred due to deposition of blood-borne molecule thrombin in the brain parenchyma. Paracellular and transcellular BBB hyperpermeability associated with the downregulation of tight and adherens junctions and upregulation of caveolin-1 and plasmalemma vesicle associated protein-1 was also observed. Along transmigration, the expression of myosin light chain kinase and smooth muscle a-actin was increased in mural cells, pointing to pericytes' activation. In addition, malignant cells exhibited an increased expression of platelet-derived growth factor-B, of proliferation marker Ki-67 and of Rac-1, indicating the involvement of autocrine regulation and mesenchymal-cell migration in the spread of metastatic cells, to which also account the expression of β4 integrin and focal adhesion kinase. It was also observed the expression of a gap junction' protein, connexin-43, at malignant-malignant and malignant-
endothelial cells' contacts, indicating its role in the cellular communication along migration and metastasization. Overall, alterations in both junctional and vesicular proteins occur along transendothelial migration of malignant cells that impact BBB integrity. Moreover, growth factors and signaling molecules seem to be involved in malignant cells dissemination and brain colonization, with intercellular communication appearing as a player in the brain metastatic process.

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Magnetic enzyme-based platforms in microbioreactors towards neuroprotective biocompounds

Lage P. (1), Lopes M.M. (1), Rosa M.E. (2), Ribeiro M.H. (1)
(1) Research Institute for Medicines (i-Med.ULisboa), Faculdade de Farmácia, Universidade de Lisboa, Lisboa, Portugal
(2) IDMEC, Instituto Superior Técnico, Universidade de Lisboa, Instituto Superior Técnico, Universidade de Lisboa, Lisboa, Portugal

Magnetic cross-linked enzyme aggregates (mCLEAs) have emerged as interesting biocatalyst design for purification and immobilization of enzymes. The CLEAs technology present many advantages, as it is simple and amenable to rapid optimization, leading to low costs and short time to-market processes.
The group of polyphenols has an important therapeutic value, with emphasis on anti-oxidant and anti-inflammatory activities, and recent studies point to considerable neuroprotective effects in different pathologies, including neurodegenerative diseases. One of the problems associated with these compounds is their reduced availability on the market, since they are obtained from the extraction of plants. This group includes flavonoids such as naringin, hesperidin or rutin, which naturally occur in some fruits. Some studies have shown that the biological activity is dependent on the (de)glycosylation of the compounds. The rhamnopyranosidase (Rhnase) catalyzes the hydrolysis of a broad spectrum of natural bioactive compounds, including polyphenolic compounds. The major sources for this enzyme production are filamentous fungi and yeasts. Rhnase is an enzyme commercially attractive, due to is potentially useful in food and pharmaceutical industries.
The main goal of this work was the development of a viable and economic process for the production and purification of rhamnopyranosidase (Rhnase) from the filamentous fungi - Aspergillus niger and Beauveria among others, using a microscale approach in batch mode. Several parameters were evaluated in the production of Rhnase with optimum activity: type of fungus, growth time, presence of inducers (rhamnose, naringin, sucrose), use of specific substrates for α-L-rhamnosidase and β-D-glucosidase activity (p-NPR, p-NPG) and nonspecific substrates (rutin, naringin, hesperidin, among others). The enzyme was concentrated and purified using different conditions and was immobilized onto magnetic particles in cross-link enzyme aggregates (CLEAS). Afterwards the biocatalyst was used in a microreactor in batch and packed bed mode, at different flow rates with evaluation of activity, volumetric productivity and operational stability. The biosynthetic compounds target the microglial cells playing their neuroprotective role.

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Glucosidase and pectinase co-encapsulated in hydrogels nanofibers with application in the wine industry

Machado M.L. (1), Rosa M.E. (2), Ribeiro M.H. (1)

(1) Research Institute for Medicines (i-Med.ULisboa), Faculdade de Farmácia, Universidade de Lisboa, Lisboa, Portugal
(2) IDMEC, Instituto Superior Técnico, Universidade de Lisboa, Instituto Superior Técnico, Universidade de Lisboa, Lisboa, Portugal

The use of glucosidases and pectinases in the wine industry has an interesting potential because these enzymes can promote the release and intensification of the aroma compounds existing in the grapes and reduce the turbidity of the must and wine. However, their use has been limited mainly due to the influence of the physicochemical characteristics of musts and wines (pH, temperature, ethanol, sugars) on enzyme activity. A major fraction of monoterpenes and norisoprenoids in wines is conjugated to sugars representing a significant reservoir of aromatic precursors. These compounds may undergo acidic or enzymatic hydrolysis. However, due to acid hydrolysis aglycones cause rearrangements, therefore enzymatic hydrolysis has attracted much interest in improving the flavor of wine, since it can efficiently release the glucosidic compounds without modifying the aglycone.

Therefore the aim of this work was the co-encapsulation of β-glucosidase and pectinase in hydrogels, in order to obtain the biocatalysts with high activity and stability, that will be easily removed from the final product, reduce the microbial content, maintain the antioxidant properties and improve the quality of the wines.

β-glucosidase and pectinase from a commercial Aspergillus niger preparation, were co-encapsulated onto chitosan and calcium alginate. Several parameters were optimized, like enzymes concentration (glucosidase and pectinase) in chitosan, calcium alginate and chitosan plus alginate. Optimization of activity and operational stability was carried out, in model solutions of acetate buffer pH 4.5 and in wine conditions. An increase on stability was observed in wine conditions.

The optimized biocatalysts co-encapsulated in chitosan plus alginate was studied in different Portuguese wines and yielded similar outcomes and in the end wines with a better quality and low microbial content.

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Chitosan and starch nanofibers towards active packaging

Carvalho M. (1), Rosa M.E. (2), Ribeiro M.H. (1)

(1) Research Institute for Medicines (i-Med.ULisboa), Faculdade de Farmácia, Universidade de Lisboa, Lisboa, Portugal
(2) IDMEC, Instituto Superior Técnico, Universidade de Lisboa, Instituto Superior Técnico, Universidade de Lisboa, Lisboa, Portugal

In the European Union, there has been an exponential growth in food waste. This food waste represents not only an environmental and ethical problem, but also an economic and social one. One of the main reasons for this waste is the poor packaging conditions of the products. In order to minimize this situation, alternatives to conventional packaging have been developed, as is the case with active packaging. In this case the packaging components interact with the product itself and the environment where it is inserted in order to increase its shelf life, improve its sensorial and safety characteristics while maintaining product quality. In this case it is possible for the consumer to make an informed and conscious choice regarding the quality and safety of the product to be purchased.

This project aims the development of nanofibers of hydrogels for application in active packaging. The eletrospinning method was used with the polymers chitosan / PVA and spirulina as bioactive compound. Spiruline was the bioactive compound used, as it shows antioxidant activity, among others.

In this work spiruline was encapsulated in chitosan (2%) beads and in chitosan with plasticizer beads, as well as in nanofibers. PVA was also tested in nanofibers. Different concentrations of spirulina were tested in release assays. Starch and chitosan allowed the formation of consistent fibers. The development of nanofibers with incorporated spiruline showed anti-oxidant activity.

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LIST OF PARTICIPANTS

Name	iMed.ULisboa Group
Adelaide Fernandes	Neuron-Glia Biology in Health and Disease
Afonso M Cavaco	Pharmacoepidemiology and Social Pharmacy
Ana Paula Francisco	Medicinal Chemistry
Ana Bettencourt	Chemical Biology and Toxicology
Ana Catarina Faria da Silva	BioNanoSciences, Drug Delivery and Immunotherapy
Ana Costa	Nanostructured Systems for Overcoming Biological Barriers
Ana Ester Ventura	BioNanoSciences, Drug Delivery and Immunotherapy
Ana F. M. Gonçalves	BioNanoSciences, Drug Delivery and Immunotherapy
Ana Garcia	Neuron-Glia Biology in Health and Disease
Ana Henriques Mota	Nanostructured Systems for Overcoming Biological Barriers
Ana Isabel Matos	BioNanoSciences, Drug Delivery and Immunotherapy
Ana Margarida Manuel	Molecular Microbiology and Biotechnology
Ana Martins	Nanostructured Systems for Overcoming Biological Barriers
Ana Neves	Nanostructured Systems for Overcoming Biological Barriers
Ana Rita Colaço	Neuron-Glia Biology in Health and Disease
Ana Rita Macário Ribeiro	Molecular Microbiology and Biotechnology
Ana Rita Valente Ribeiro	Neuron-Glia Biology in Health and Disease
Ana Rita Vaz	Neuron-Glia Biology in Health and Disease
Ana Sofia Coxixo	Metabolism and Genetics
Ana Sofia Ressurreição	Medicinal Chemistry
Ana Sofia Saraiva	Chemical Biology and Toxicology
André Campaniço	Medicinal Chemistry
André F. R. M. Dias	Bioorganic Chemistry
André F. S. Luz	Medicinal Chemistry
André Santos	Cellular Function and Therapeutic Targeting
André Simão	Cellular Function and Therapeutic Targeting
Andreia Bento da Silva	Structural Analysis Lab
Andreia Figueiredo	Medicinal Chemistry
Andreia Fortuna	Bioorganic Chemistry
Andreia Neves Carvalho	Cellular Function and Therapeutic Targeting
Angela B. M. Delgado	Host-Pathogen Interactions
António J. Almeida	Nanostructured Systems for Overcoming Biological Barriers
António Lemos	Metabolism and Genetics
Armanda Rodrigues	Chemical Biology and Toxicology
Bárbara Carreira	BioNanoSciences, Drug Delivery and Immunotherapy
Beatriz Anacleto	Medicinal Chemistry
Beatriz Estremores	Cellular Function and Therapeutic Targeting
Beatriz Mestre	BioNanoSciences, Drug Delivery and Immunotherapy
Beatriz Noriega Fernandes	BioNanoSciences, Drug Delivery and Immunotherapy
Beatriz Silva	Nanostructured Systems for Overcoming Biological Barriers
Beatriz Soromenho	Neuron-Glia Biology in Health and Disease
Bianca C. R. Gama	Host-Pathogen Interactions
Bruna Azevedo de Araujo	BioNanoSciences, Drug Delivery and Immunotherapy
Carina Peres	BioNanoSciences, Drug Delivery and Immunotherapy
Carlos Araújo	Molecular Microbiology and Biotechnology
Carlos R. Lopes	Cellular Function and Therapeutic Targeting

Carlos São-José	Molecular Microbiology and Biotechnology
Carlota M. L. Sousa	Medicinal Chemistry
Carolina Sousa	Pharmacological and Regulatory Sciences
Catarina Barros	Neuron-Glia Biology in Health and Disease
Catarina Franco	Neuron-Glia Biology in Health and Disease
Catarina G. Pimpão	Metabolism and Genetics
Catarina Madeira	Metabolism and Genetics
Catarina Monteiro Severo	Chemical Biology and Toxicology
Catarina Ranito	Cellular Function and Therapeutic Targeting
Catarina Reis	Nanostructured Systems for Overcoming Biological Barriers
Catarina Sequeira	Neuron-Glia Biology in Health and Disease
Catarina T. Dias	Chemical Biology and Toxicology
Cecilia Rodrigues	Cellular Function and Therapeutic Targeting
Cláudia Braga	Medicinal Chemistry
Cláudia Mourato	Cellular Function and Therapeutic Targeting
Cristina M. L. Carvalho	Chemical Biology and Toxicology
Cristina Sampayo	Pharmacological and Regulatory Sciences
Daniela F. O. Costa	Cellular Function and Therapeutic Targeting
David Cardoso	Natural Products Chemistry
Denise M. F. A. Duarte	Medicinal Chemistry
Diana A. Fernandes	BioNanoSciences, Drug Delivery and Immunotherapy
Diogo M. T. F. M. Silva	Medicinal Chemistry
Elisabete Freitas	Medicinal Chemistry
Elizabeth A. Lopes	Medicinal Chemistry
Elsa Anes	Host-Pathogen Interactions
Fábio M. F. Santos	Bioorganic Chemistry
Filipa Ramilo-Gomes	Medicinal Chemistry
Filipa Siopa	Bioorganic Chemistry
Filipa Vale	Host-Pathogen Interactions
Francisca Lopes	Medicinal Chemistry
Francisco Martin	HIV Evolution, Epidemiology and Prevention
Francisco Olivença	Host-Pathogen Interactions
Gabriela pereira	BioNanoSciences, Drug Delivery and Immunotherapy
Gecioni Loch Neckel	Neuron-Glia Biology in Health and Disease
Graça Soveral	Metabolism and Genetics
Gustavo A. L. F. Silva	Medicinal Chemistry
Helena Florindo	BioNanoSciences, Drug Delivery and Immunotherapy
Helena M Ribeiro	Nanostructured Systems for Overcoming Biological Barriers
Hélio Faustino	Bioorganic Chemistry
Hugo Brito	Cellular Function and Therapeutic Targeting
Igor Correia	Molecular Microbiology and Biotechnology
Inês Anjos	Nanostructured Systems for Overcoming Biological Barriers
Inês Bartolo	HIV Evolution, Epidemiology and Prevention
Inês Caria	Cellular Function and Therapeutic Targeting
Ines dos Santos	BioNanoSciences, Drug Delivery and Immunotherapy
Inês Figueira	Neuron-Glia Biology in Health and Disease
Inês Janeiro da Silva	Pharmacological and Regulatory Sciences
Inês Moranguinho	HIV Evolution, Epidemiology and Prevention
Inês Raquel da Silva Iria	Molecular Microbiology and Biotechnology
Inês Vieira da Silva	Metabolism and Genetics
Isabel B. Moreira da Silva	Pharmacological and Regulatory Sciences

Isabel Ribeiro	Chemical Biology and Toxicology
Isabella Bramatti	Chemical Biology and Toxicology
Jacinta Pinho	Nanostructured Systems for Overcoming Biological Barriers
Jaime A. S. Coelho	Bioorganic Chemistry
Jaime Conceição	FFUP
Joana Carrola	Structural Analysis Lab
Joana Godinho Pereira	Neuron-Glia Biology in Health and Disease
Joana Inês Carvalho	Bioorganic Chemistry
Joana Lopes	Nanostructured Systems for Overcoming Biological Barriers
Joana Marto	Nanostructured Systems for Overcoming Biological Barriers
Joana P Miranda	Chemical Biology and Toxicology
Joana Pereira	Nanostructured Systems for Overcoming Biological Barriers
Joana Pereira	Neuron-Glia Biology in Health and Disease
Joana Saraiva Rodrigues	Chemical Biology and Toxicology
João António	Bioorganic Chemistry
João Caio	Metabolism and Genetics
João Carréu	Natural Products Chemistry
Joao Miguel Ferreira Vaz	Medicinal Chemistry
João Miguel Gonçalves	Bioorganic Chemistry
João R. C. Vale	Bioorganic Chemistry
João Rafael Gonçalves	Pharmacoepidemiology and Social Pharmacy
João Ravasco	Bioorganic Chemistry
João Ribeiro	Medicinal Chemistry
Jorge Grilo	Medicinal Chemistry
Jorge Pereira	Chemical Biology and Toxicology
José Marcelino	HIV Evolution, Epidemiology and Prevention
Judite Nunes Costa	Chemical Biology and Toxicology
Juliana Pereira	Bioorganic Chemistry
Katarzyna Berkowicz	Pharmacological and Regulatory Sciences
Katelene Lima	Pharmacological and Regulatory Sciences
Késsia Andrade	Medicinal Chemistry
Laura Maria Ferreira	Medicinal Chemistry
Liane Moura	BioNanoSciences, Drug Delivery and Immunotherapy
Lídia A. S. Cavaca	Bioorganic Chemistry
Lidia Gonçalves	Nanostructured Systems for Overcoming Biological Barriers
Lilia Pedrosa	Chemical Biology and Toxicology
Luciana da Silva Madeira	Molecular Microbiology and Biotechnology
Luciana M. A. Pinto	BioNanoSciences, Drug Delivery and Immunotherapy
Luís Carvalho	Medicinal Chemistry
M. Luísa Corvo	BioNanoSciences, Drug Delivery and Immunotherapy
Madalena	BioNanoSciences, Drug Delivery and Immunotherapy
Magda Ferreira	Nanostructured Systems for Overcoming Biological Barriers
Margarida Castro-Caldas	Cellular Function and Therapeutic Targeting
Margarida Ferreira-Silva	BioNanoSciences, Drug Delivery and Immunotherapy
Maria Alexandra Brito	Neuron-Glia Biology in Health and Disease
Maria B. T. Ferreira	Bioorganic Chemistry
Maria de Fátima Cabral	Chemical Biology and Toxicology
Maria de Jesus Perry	Medicinal Chemistry
Maria João Catalão	Host-Pathogen Interactions
Maria Joao Gama	Cellular Function and Therapeutic Targeting

Maria João Matos	University of Santiago de Compostela & FCUP
Maria João Nunes	Cellular Function and Therapeutic Targeting
Maria João Silva	Metabolism and Genetics
Maria José Silva	Bioorganic Chemistry
Maria Luisa Machado	Chemical Biology and Toxicology
Maria M. M. Santos	Medicinal Chemistry
Maria Manuela Gaspar	Nanostructured Systems for Overcoming Biological Barriers
Maria Ribeiro	Cellular Function and Therapeutic Targeting
Maria Vaz Pinto	Neuron-Glia Biology in Health and Disease
Mariana Carvalho	Chemical Biology and Toxicology
Mariana Mateus	BioNanoSciences, Drug Delivery and Immunotherapy
Mariana Ruivo Matias	Nanostructured Systems for Overcoming Biological Barriers
Marília Lima da Conceição	Molecular Microbiology and Biotechnology
Marta Barbosa	Neuron-Glia Biology in Health and Disease
Marta Bento Afonso	Cellular Function and Therapeutic Targeting
Marta Calado	Host-Pathogen Interactions
Marta Carrasco	Medicinal Chemistry
Marta Marques	Biocatalysis and Bioenergy (FCT-UNL)
Marta S. S. S. V. Clariano	Medicinal Chemistry
Martin Vojtek	FFUP
Maryam Heydarpour Klidsar	Medicinal Chemistry
Maryam Malmir	Pharmacological and Regulatory Sciences
MD Kamrul Hasan	Pharmacological and Regulatory Sciences
Melissa Albino	Nanostructured Systems for Overcoming Biological Barriers
Miguel Cardoso	Molecular Microbiology and Biotechnology
Miguel Mateus Pinheiro	Cellular Function and Therapeutic Targeting
Milene Fortunato	Bioorganic Chemistry
Nádia Romeu Aguiam	BioNanoSciences, Drug Delivery and Immunotherapy
Noélia Duarte	Natural Products Chemistry
Nuno Esteves	Bioorganic Chemistry
Nuno Taveira	HIV Evolution, Epidemiology and Prevention, iMed Ulisboa
Oluwatomide Adeoye	BioNanoSciences, Drug Delivery and Immunotherapy
Paloma Gonçalves	HIV Evolution, Epidemiology and Prevention
Patricia Lage	Chemical Biology and Toxicology
Patrícia Rijo	Natural Products Chemistry
Patrícia Serra	Medicinal Chemistry
Paula Leandro	Metabolism and Genetics
Paula Matos de Brito	Molecular Microbiology and Biotechnology
Paulo Faria	Neuron-Glia Biology in Health and Disease
Paulo Salústio	BioNanoSciences, Drug Delivery and Immunotherapy
Pedro A Dionísio	Cellular Function and Therapeutic Targeting
Pedro Florindo	Medicinal Chemistry
Pedro Gomes	Molecular Microbiology and Biotechnology
Pedro M. Baptista	Aragon Health Sciences Institute
Pedro M. P. Gois	Bioorganic Chemistry, iMed Ulisboa
Pedro Mendes Fontes	Molecular Microbiology and Biotechnology
Rafael Gomes	Bioorganic Chemistry
Raquel A. T. Mestre	Medicinal Chemistry
Raquel Lopes	Metabolism and Genetics
Raquel Maria S. Freire	Medicinal Chemistry

Ricardo Lopes	Bioorganic Chemistry
Ricardo Paiva	Neuron-Glia Biology in Health and Disease
Rita Acúrcio	Medicinal Chemistry
Rita Costa	Pharmacological and Regulatory Sciences
Rita Félix	Medicinal Chemistry
Rita Gonçalves-Pereira	Bioorganic Chemistry
Rita I. C. Padanha	Bioorganic Chemistry
Rita Manguinhas	Chemical Biology and Toxicology
Rita Mendes	Chemical Biology and Toxicology
Rita Ramos	Neuron-Glia Biology in Health and Disease
Rita Serrano	Pharmacological and Regulatory Sciences
Roberto Russo	Bioorganic Chemistry
Romina Guedes	Medicinal Chemistry
Rúben A. D. Coutinho	Medicinal Chemistry
Rui Castro	Cellular Function and Therapeutic Targeting
Rui Moreira	Medicinal Chemistry
Rui Silva	Neuron-Glia Biology in Health and Disease
Rute Cordeiro Dias	BioNanoSciences, Drug Delivery and Immunotherapy
Rute Marcelino	HIV Evolution, Epidemiology and Prevention
Sandra Cordeiro	Nanostructured Systems for Overcoming Biological Barriers
Sandra Pena	BioNanoSciences, Drug Delivery and Immunotherapy
Sandra Simões	Nanostructured Systems for Overcoming Biological Barriers
Sara C. R. M. Bom	Nanostructured Systems for Overcoming Biological Barriers
Sara Garcia	Medicinal Chemistry
Sara Jogo	Natural Products Chemistry
Sara Oliveira	Cellular Function and Therapeutic Targeting
Sara Silva	Nanostructured Systems for Overcoming Biological Barriers
Sérgio P Camões	Chemical Biology and Toxicology
Shabnan Sabiha	Pharmacological and Regulatory Sciences
Shirley Sancha	Natural Products Chemistry
Shrika harjivan	Medicinal Chemistry
Silvia Baldo	Bioorganic Chemistry
Sofia Alexandra Milheiro	Medicinal Chemistry
Sofia Domingos	Medicinal Chemistry
Sofia R. Néné Galego	Neuron-Glia Biology in Health and Disease
Sofia Serra	Medicinal Chemistry
Sofia Valente	Host-Pathogen Interactions
Sónia Sá Santos	Cellular Function and Therapeutic Targeting
Stephanie Esteves Albino	Pharmacological and Regulatory Sciences
Susana Tracana	HIV Evolution, Epidemiology and Prevention
Tania C. B. Santos	BioNanoSciences, Drug Delivery and Immunotherapy
Tawhidul Islam	Cellular Function and Therapeutic Targeting
Tetyana Rybchynska	Metabolism and Genetics
Tiago Caetano	Chemical Biology and Toxicology
Valentina Barcherini	Medicinal Chemistry
Vanda Marques	Cellular Function and Therapeutic Targeting
Vanessa S. A. Pires	Chemical Biology and Toxicology
Vasco Branco	Chemical Biology and Toxicology
Vera M. S. Isca	Bioorganic Chemistry
Zohorul Islam	Pharmacological and Regulatory Sciences