EFMC-YMCS
Young Medicinal Chemists' Symposium
Virtual Event
September 9-10, 2021

Book of Abstracts

www.efmc-ymcs.org

Content

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Welcome

On behalf of the European Federation for Medicinal Chemistry and Chemical Biology (EFMC) and the Organising Committee, we again warmly e-welcome you for the 8th edition of the EFMC Young Medicinal Chemists' Symposium (EFMC-YMCS).

We had big hopes to be able to hold the meeting in Basel, Switzerland, but the lasting COVID-19 pandemic continued to impact our lives and the way we can meet and travel.

Today more than ever, we must keep interacting: sharing scientific work with peers, promote excellence and deliver leaders in the field... exactly what the EFMC-YMCS aims at since it was created in 2014. Because of this, it was crucial for us to run the 8th edition of the EFMC-YMCS as a fully virtual event – offering as much as possible the conditions for a successful event.

We welcome you all for this two-day programme consisting of 3 keynote lectures, 19 oral communications given by prize winners from national competitions around Europe, 20 Flash Poster Presensations and 100+ posters.

During the closing ceremony, the following prizes will be awarded to the European Champions in Medicinal Chemistry and Chemical Biology:
- EFMC-YMCS Presentation Prize, sponsored by the EFMC & Idorsia
- EFMC-YMCS Flash Poster Prizes, offered by MDPI/Molecules
- EFMC Poster Prizes, sponsored by ChemMedChem/ChemBioChem
- EFMC-YMCS Public's Choice Prize, offered by F. Hoffmann-La-Roche

The scientific programme will be further completed by a series of activities provided to you by the EFMC Young Scientists Network (EFMC-YSN).
- Soft-skill Training Lecture: Preparing a Funding Proposal: Setting Yourself up for Success
- Round Table Discussion: "The Stories Behind the Medicinal Chemists in Industry"
- Networking Evening

We also would like to thank our numerous sponsors (American Elements, Astex Pharmaceuticals, AstraZeneca, Charles River, ChemMedChem/ChemBioChem, Evotec, Idorsia, Merck, MDPI, MSD, Novartis, NS-MC Consulting, Roche, Sanofi, Sosei Heptares & Sygnature Discovery) and all the participating National Adhering Organisations for their support, without which we could not run this event, and we look forward to your participation!

EFMC-YMCS Organising Committee

Chairs
Kristina GONCHARENKO (Young Scientists Network, CH), Dennis GILLINGHAM (University of Basel, CH)

Members
David ALKER (David Alker Associates, UK), Maja BEUS (University of Zagreb, HR), Christina LAMERS (University of Basel, CH), Laetitia MARTIN (Roche, CH), Brieuc MATAGNE (LD Organisation, BE), Jessica REYNOLDS (University of Oxford, UK), Michal SHOSHAN (University of Zurich, CH), Luc VAN HIJFTE (Symeres, NL)

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Participating EFMC Organisations

Belgium	Société Royale de Chimie (SRC), Medicinal Chemistry Division
Croatia	Croatian Chemical Society, Section of Medicinal/Pharmaceutical Chemistry
Denmark	The Danish Society for Medicinal Chemistry and Chemical Biology
France	Société de Chimie Thérapeutique (SCT)
Germany	Division of Medicinal Chemistry of the German Chemical Society (GDCh) German Pharmaceutical Society, Section of Pharmaceutical/Medicinal Chemistry (GPhG)
Greece	Hellenic Society of Medicinal Chemistry
Hungary	Organic and Medicinal Chemistry Division (OMCD) of the Hungarian Chemical Society (HCS)
Italy	Division of Medicinal Chemistry of the Italian Chemical Society (Divisione di Chimica Farmaceutica - Società Chimica Italiana, DCF-SCI)
Poland	Polish Society of Medicinal Chemistry
Portugal	Division of Medicinal Chemistry of the Portuguese Chemical Society
Serbia	Serbian Chemical Society, Division of Medicinal Chemistry
Slovenia	Section for Medicinal Chemistry of the Slovenian Pharmaceutical Society
Spain	Sociedad Española de Química Terapéutica
Switzerland	Division for Medicinal Chemistry & Chemical Biology (DMCCB)
The Netherlands	Division Medicinal Chemistry & Chemical Biology, Royal Netherlands Chemical Society (KNCV-MCCB)
Turkey	Turkish Association of Medicinal and Pharmaceutical Chemistry
United Kingdom	The Biological and Medicinal Chemistry Sector (BMCS) of the Royal Society of Chemistry (RSC)

Programme

(CEST time zone)

Thursday September 9, 2021

MORNING SESSION

Session Chair: Dr Kristina GONCHARENKO (YOUNG SCIENTISTS NETWORK, Basel, Switzerland)

09:30 **Opening Ceremony**
Prof. Rui MOREIRA (UNIVERSITY OF LISBON, Lisbon, Portugal)
Dr Kristina GONCHARENKO (YOUNG SCIENTISTS NETWORK, Basel, Switzerland)

09:40 **KL01 - Powerful New Tools for Augmented Medicinal Chemistry**
Dr Werngard CZECHTIZKY (ASTRAZENECA, Mölndal, Sweden)

YMCS Competition Presentations - Session 1

10:10 **OC01 - Design and Synthesis of Novel Benzothiazole-Piperazine Propanamide Derivatives for Multi-Targeted Approach in Alzheimer's Disease Treatment**
Winner of the Young Medicinal Chemist Meeting in Turkey
Ms Bengisu TURGUTALP (YEDITEPE UNIVERSITY, Istanbul, Turkey)

10:30 **OC02 - Design and Synthesis of Dual Inhibitors Of DYRK1A/CLK1 Kinases Involved in Neurodegenerative Diseases**
Winner of the Young Medicinal Chemist Meeting in France
Mrs Clementine PESCHETEAU (ICOA, ORLEANS, France)

10:50 **OC03 - Towards the Development of ACSL4 Selective Inhibitors to Prevent Ferroptosis in Neurodegenerative Diseases**
Winner of the Young Medicinal Chemist Meeting in Belgium (SRC)
Dr Romain MARTEAU (LOUVAIN DRUG RESEARCH INSTITUTE, Woluwe-Saint-Lambert, Belgium)

11:10 Networking Break

11:20 **OC04 - Small Organic Molecules to Trigger the Activity of NK Cells**
Winner of the Young Medicinal Chemist Meeting in Portugal
Dr Pedro PINHEIRO (INSTITUTO SUPERIOR TÉCNICO, Lisboa, Portugal)

11:40 **OC05 - Novel Hdac6-Selective Inhibitor for Glioblastoma Treatment**
Winner of the Young Medicinal Chemist Meeting in Croatia
Dr Maja BEUS (INSTITUTE FOR MEDICAL RESEARCH, Zagreb, Croatia)

12:00 **OC06 - Design and Synthesis of Brain Penetrant P38-Alpha Mitogen-Activated Protein Kinase Inhibitors**
Winner of the Young Medicinal Chemist Meeting in Germany (DPhG)
Dr Pierre KOCH (UNIVERSITÄT REGENSBURG, Germany)

Programme

(CEST time zone)

12:20 Flash Poster Presentations (Odd numbers)

FP01 - Electrophilic Warheads for The Assessment of Tractable Cysteines
Dr Peter ÁBRÁNYI-BALOGH(RESEARCH CENTRE FOR NATURAL SCIENCES, Budapest, Hungary)
FP03 - Synthesis and In Vitro Evaluation of Novel G6Pd Inhibitors
Mr Andrea BACCI (UNIVERSITY OF PISA, Pisa, Italy)
FP05 - Synthesis, Development and Evaluation of a Therapeutic Peptide Conjugate to Protect Biomaterials from Undesired Immune Attack
Mr Clément BECHTLER (UNIVERSITY OF BASEL, Basel, Switzerland)
FP07 - Inhibition of the Proteinâ€“Rna Interaction of Lin28 and Let-7 with Trisubstituted Pyrrolinones
Ms Lydia BORGELT (MAX PLANCK INSTITUTE OF MOLECULAR PHYSIOLOGY, Dortmund, Germany)
FP09 - Azaaurones as Novel Chemotypes against Mycobacterium Tuberculosis: Sar, Adme Profiling and Photo-Switching Properties
Mr André CAMPANICO (I.MED.ULISBOA - FFULISBOA, Lisboa, Portugal)
FP11 - Protac Technology to Investigate Dysregulation of the Ubiquitin-Proteasome System
Ms Carlotta CECCHINI (UNIVERSITY OF GENEVA, Geneva, Switzerland)
FP13 - High-Affinity Glycomimetic Ligands for Human Siglec-8
Mr Gabriele CONTI (UNIVERSITY OF BASEL, Basel, Switzerland)
FP15 - Synthesis and Biological Evaluation of Novel Variously Substituted 3-Benzyl-Quinolin-2(1H)-Ones as Potent Agonists of the Gpr55 Receptor
Mrs Ceni COSTANZA (UNIVERSITY OF PISA, Empoli, Italy)
FP17 - Design and Synthesis of Novel Androgen Receptor Splice Variant-7 Protacs for the Treatment of Castration-Resistant Prostate Cancer
Ms Jenny DESANTIS (UNIVERSITY OF PADUA, Padova, Italy)
FP19 - A Novel Covalent Approach for Stabilising 14-3-3σ Protein-Protein Interactions
Ms Marta FALCICCHIO (UNIVERSITY OF LEICESTER, Leicester, United Kingdom)

12:40 End of Morning Session

13:30 Poster Session 1 (Odd numbers) & Networking

15:30 Round Table Discussion: "The Stories Behind the Medicinal Chemists in Industry"
Ms Helen AYLOTT (GSK, Stevenage, United Kingdom)
Dr Maude GIROUD (F. HOFFMANN-LA ROCHE, Basel, Switzerland)
Prof. Toni METSÄNEN (ORION PHARMA, Espoo, Finland)
Prof. Fabrizio MICHELI (EVOTEC, Verona, Italy)
Dr Nikolay SITNIKOV (NUVISAN, Berlin, Germany)
Dr Fionn O'HARA (F. HOFFMANN-LA ROCHE, Basel, Switzerland)

Programme

(CEST time zone)

AFTERNOON SESSION

Session Chair: Dr Michal SHOSHAN (UNIVERSITY OF ZURICH, Zürich, Switzerland)

YMCS Competition Presentation - Session 2

16:45 **OC07 - Metatacs: A Strategy for Metastasis Prevention Through Targeted Fascin Degradation**
Winner of the Young Medicinal Chemist Meeting in United Kingdom
Ms Sarah MEMARZADEH (UNIVERSITY OF GLASGOW, Glasgow, United Kingdom)

17:05 **OC08 - Structure Elucidation of Artificial, Self-Assembling Squalene-Conjugates and-Peptides**
Winner of the Young Medicinal Chemist Meeting in Hungary
Dr Dora BOGDAN (SEMMELWEIS UNIVERSITY, Budapest, Hungary)

17:25 **OC09 - Intracellular Receptor Modulation: Intracellular Ligands for Chemokine Receptors**
Winner of the Young Medicinal Chemist Meeting in The Netherlands
Dr Natalia ORTIZ ZACARIAS (LACDR, LEIDEN UNIVERSITY, Leiden, The Netherlands)

17:45 **OC10 - A Mating Mechanism to Generate Diversity for the Darwinian Selection Of DNA-Encoded Synthetic Molecules**
Winner of the Young Medicinal Chemist Meeting in Switzerland
Mr Luc FARRERA SOLER (UNIVERSITY OF GENEVA, Geneva, Switzerland)

18:15 End of Afternoon Session

19:00 **EFMC-YSN Virtual Networking Event**

Friday September 10, 2021

MORNING SESSION

Session Chair: Prof. Dennis GILLINGHAM (UNIVERSITY OF BASEL, Basel, Switzerland)

09:30 **Opening**

09:40 **KL02 - Discovery of Risdiplam (Evrysdi): A Medicine for the Treatment of Spinal Muscular Atrophy**
Dr Hasane RATNI (F. HOFFMANN-LA ROCHE LTD, Basel, Switzerland)

YMCS Competition Presentations - Session 3

10:10 **OC11 - Timing Multiple Contiguous Chiral Centers Through Dynamic Kinetic Resolution**
Winner of the Young Medicinal Chemist Meeting in Slovenia
Dr Andrej Emanuel COTMAN (FACULTY OF PHARMACY, UNIVERSITY OF LJUBLJANA, Ljubljana, Slovenia)

10:30 **OC12 - Modulators of Coactivator-Associated Arginine Methyltransferase 1 (CARM-1): There and Back Again**
Winner of the Young Medicinal Chemist Meeting in Italy
Dr Ciro MILITE (UNIVERSITY OF SALERNO, Fisciano, Italy)

10:50 **OC13 - Hydroxamic Acid-Functionalized Peptide Microarrays for the Study of Zn(II)-Dependent Histone Deacetylases**
Winner of the Young Medicinal Chemist Meeting in Denmark
Dr Carlos MORENO YRUELA (UNIVERSITY OF COPENHAGEN, Copenhagen, Denmark)

Programme

(CEST time zone)

11:10 Networking Break

11:20 OC14 - Cleistanolate Analogues: Synthesis, Citotoxicity and SAR Study
Winner of the Young Medicinal Chemist Meeting in Serbia
Ms Jelena KESIC (FACULTY OF SCIENCES, NOVI SAD, SERBIA, Novi Sad, Serbia)

11:40 OC15 - deepSIBA: Chemical Structure-Based Inference of Biological Alterations Using Deep Learning
Winner of the Young Medicinal Chemist Meeting in Greece
Dr Christos FOTIS (NATIONAL TECHNICAL UNIVERSITY OF ATHENS, Athens, Greece)

12:00 OC16 - ITH15004, A Novel Blood-Brain Barrier-Permeable P2X7 Antagonist: From Drug Design to Pharmacological Applications
Winner of the Young Medicinal Chemist Meeting in Spain
Mr Francesco CALZAFERRI (CENTRE NATIONAL DE LA RECHERCHE SCIENTIFIQUE (CNRS), Montpellier, France)

12:20 Flash Poster Presentations (Even numbers)

FP02 - From Orthosteric and Allosteric Modulators to Dualsteric/Bitopic Ligands: a New Molecular Alliance at Cb2 Receptor
Dr Francesca GADO (UNIVERSITÀ DI PISA, Pisa, Italy)
FP04 - Rational Design of Light-Controlled Bioactive Compounds for Photopharmacology
Mr Piermichele KOBAURI (UNIVERSITY OF GRONINGEN, Groningen, The Netherlands)
FP06 - Neuroprotective, Neurotrophic and Anti-Inflammatory Activity of New Synthetic Dhea Neurotrophin Mimetic Derivatives
Ms Alessia LATORRATA (NATIONAL HELLENIC RESEARCH FOUNDATION, Athens, Greece)
FP08 - Novel Class of Antinecroptotic Inhibitors: Synthetic Approach and In Vitro Assays
Mr André LUZ (FACULTY OF PHARMACY, UNIVERSITY OF LISBON, Lagos, Portugal)
FP10 - Comparative Study of Ck1-Crbn and Ck2-Crbn Complexes using Computational Methods
Mrs Laura MÁRQUEZ (SAN PABLO CEU UNIVERSITY, Alcorcón, Madrid, Spain)
FP12 - Towards Protac-Mediated Degradation of Cbp/Ep300
Mr Leonardo PALAFERRI (UZH, Zurich, Switzerland)
FP14 - Optimization of Compound Metabolic Stability via Shap
Dr Sabina PODLEWSKA (MAJ INSTITUTE OF PHARMACOLOGY POLISH ACADEMY OF SCIENCES, Krakow, Poland)
FP16 - In Vivo Photocontrol of Adrenergic Neurotransmission
Dr Davia PRISCHICH (INSTITUTE FOR BIOENGINEERING OF CATALONIA (IBEC) , Barcelona, Spain)
FP18 - The E3 Platform: an Investigation of the 3D Pocket Space for Protac Design
Dr Tommaso PALOMBA (UNIVERSITÀ DEGLI STUDI DI PERUGIA, Perugia, Italy)
FP20 - Targeting Myeloid Leukemias using Human Dihydroorotate Dehydrogenase Inhibitors based on 2-Hydroxypyrazolo[1,5-A]Pyridine Scaffold: Overcoming of Metabolic Issues
Dr Chiara VIGATO (UNIVERSITY OF TURIN, Casale Monferrato, Italy)

12:40 End of Morning Session

13:30 Poster Session 2 (Even numbers) & Networking

Programme

(CEST time zone)

AFTERNOON SESSION

Session Chair: Dr Laetitia MARTIN (F. HOFFMANN-LA ROCHE , Basel, Switzerland)

16:00 **Preparing a Funding Proposal: Setting Yourself up for Success**
Dr Kaycie BUTLER (BUTLER SCIENTIFIC COMMUNICATIONS, Lausanne, Switzerland)

YMCS Competition Presentation Session 4

16:45 **OC17 - Far-Red Fluorescent DNA Binder Allows Host-Pathogen Interaction Studies of Multidrug-Resistant Bacteria**
Winner of the Young Medicinal Chemist Meeting in Germany (GDCh)
Dr Benedikt HEINRICH (LABORARZTPRAXIS, Bad Vilbel, Germany)

17:05 **OC18 - New 5-Arylideneimidazolones in the Ongoing Battle Against Bacterial and Cancer Multidrug Resistance**
Winner of the Young Medicinal Chemist Meeting in Poland
Ms Aneta KACZOR (JAGIELLONIAN UNIVERSITY, Kraków, Poland)

17:25 **OC19 - Phosphorylation-Inducing Chimeric Small Molecules**
Winner of the Trainee Pre-Conference Forum at the virtual 9th Annual Conference of the International Chemical Biology Society
Dr Dhanushka MUNKANATTA GODAGE (BROAD INSTITUTE OF MIT AND HARVARD, Cambridge, United States)

17:45 **KL03 - Covalent Inhibitors - From Discovery to Functionalization**
Dr Nir LONDON (THE WEIZMANN INSTITUTE OF SCIENCE, Rehovot, Israel)

18:15 **Prize Ceremony & Closing**
Prof. Rui MOREIRA (UNIVERSITY OF LISBON, Lisbon, Portugal)

18:30 **End of the event**

YSN Activities

Soft-skill Training | Preparing a Funding Proposal: Setting Yourself up for Success

In this lecture included in the conference programme, **Dr Kaycie Butler** will give hints and recommendations on how to prepare a successful funding proposal.

About Kaycie Butler

Dr Kaycie Butler is founder of Butler Scientific Communications, helping scientists communicate their work through specialized editing services, workshops, and online courses. Through this work, she has helped over 50 labs across both the US and Europe compose funded research grants and publish hundreds of papers.
As a graduate student who struggled with academic writing, a large focus of her work is on ensuring that young scientists build a solid foundation in this area that will support them throughout their career. Her workshops and online courses are designed with these students in mind, breaking down paper and grant writing into clearly identified steps and formulas to reduce stress and time spent writing. Before switching to science communication, Kaycie earned her PhD in chemistry from Caltech (2014) and completed a postdoc in chemical biology at EPFL; she remains based in Switzerland.

Abstract

Securing funding is crucial for academic success, but preparing a proposal can be one of the most stressful tasks an early career researcher has to learn to navigate. However, universities seldom offer training in preparing proposals, and much of the freely available advice is not presented in an easily applicable manner for scientists without significant experience. This introduction to preparing a proposal is designed to bridge that gap and provides the backbone for early career researchers (rising PIs and postdocs, specifically) starting their journey to securing research funding. We will start with a brief introduction to finding funding opportunities, what to keep in mind when planning your proposed research, and where to go for more information. The talk will focus on the general structure of a research proposal, highlight what information to include and what to keep in mind for each part, and "formulas" to help you in the writing and revision process. Throughout, I provide tips for rapidly elevating any proposal, writing with your reviewer in mind, and the difference between writing for a research paper and for a grant proposal, as these mindset shifts can quickly correct many of the issues seen in early career proposals. Overall, this talk will provide immediately applicable tools for getting started and writing and formatting your proposal, so you can instead spend time on what matters most – your science.

Round Table Discussion: "The Stories Behind the Medicinal Chemists in Industry"

Planning for a career in industry or simply curious to hear some successful career experiences? Do not miss the round table discussion.

The concept of the panel discussion, organised by the EFMC Young Scientists Network, will be for attendees to listen to the career paths, experiences, and overall stories of early career scientists working in industry; get inspired and ask questions.

Confirmed Panelists

- *Ms Helen AYLOTT (GSK, Stevenage, United Kingdom)*
- *Dr Maude GIROUD (F. HOFFMANN-LA ROCHE, Basel, Switzerland)*
- *Prof. Toni METSÄNEN (ORION PHARMA, Espoo, Finland)*
- *Prof. Fabrizio MICHELI (EVOTEC, Verona, Italy)*
- *Dr Nikolay SITNIKOV (NUVISAN, Berlin, Germany)*
- *Dr Fionn O'HARA (F. HOFFMANN-LA ROCHE, Basel, Switzerland)*

When?

On September 9 from 15:30 to 16:30 CEST.

EFMC-YSN Activities

How does it work?

After a general who's who, registered participants will be split in smaller groups to interact with each panelist and directly ask questions. For practical reasons, **registration to the round table is mandatory**, at no additional costs.

Networking Evening

Join the EFMC Young Scientists Network (EFMC-YSN) on September 9 at 19:00 CEST for an evening of networking and games!

We all have been missing the networking moments where we could have a chat with peers around a few drinks and bites. While this year again we have to offer a virtual event, we would like to try to bring back those happy times with our virtual networking evening.

So, grab a glass of a characteristic drink of your home region, a few snacks and join us for some informal chat and a few quizzes...the winners will even get the chance to grab one of the "EFMC Goodiebox".

We look forward to meeting you all !

EFMC Young Scientists Network

"Crossing boundaries to Build Strong Networks for Early Career Researchers"

The **EFMC-Young Scientists Network** aims to **inspire**, **connect** and **facilitate** medicinal chemists and chemical biologists in their Early Career.

Registration is **free of charge** and open to all!

Our activities

Communication

- Interview of scientists
- Quizzes
- Photo Competition

Networking activities

- "Meet & Greet" events for young people
- Networking evenings at the EFMC-YMCS

Training activities

- "Soft-skills" workshops
- Career fairs
- Mentoring programme

Support to young scientists

- Travel grants to attend EFMC Events
- YSN Prize for best PhD/Post-doc
- Job & academic positions portal

Events & Meetings

- EFMC-YMCS (www.efmc-ymcs.org)
- EFMC-YSN MedChemBioOnline (www.efmc.info/efmc-ysn-medchembioonline)

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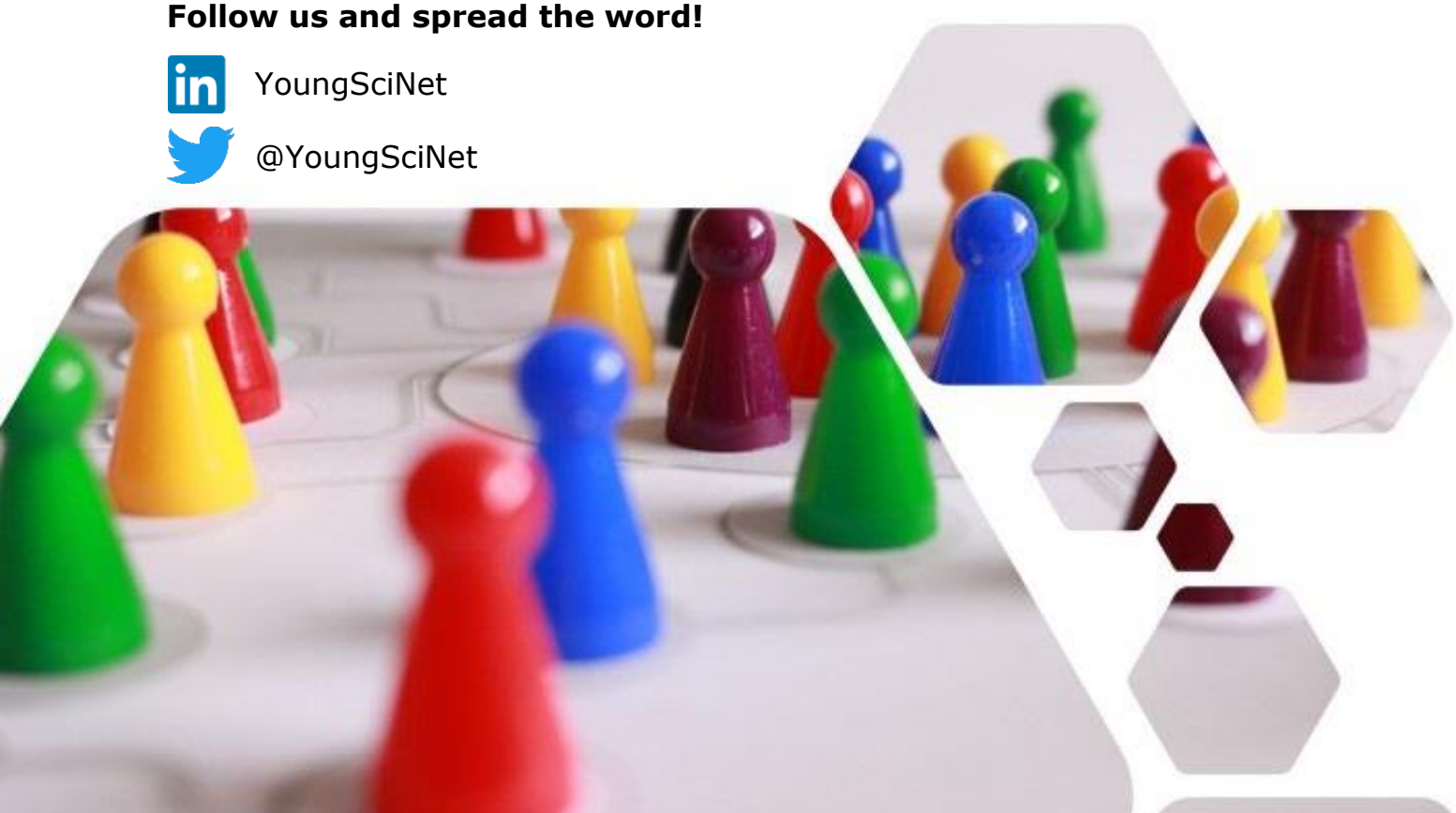

EFMC
European Federation
for Medicinal Chemistry
and Chemical Biology

EFMC is an independent association founded in 1969, representing 27 societies from 25 European countries, and more than **7500 scientists**.
It's main objective is to **advance the science of medicinal chemistry and chemical biology.**

Upcoming Events

EFMC-YSN MedChemBioOnline
Webinars mixing science, soft-skills training & round table discussions
www.efmc.info/efmc-ysn-medchembioonline

EFMC 16th Short Course on Medicinal Chemistry New Opportunities in GPCR Drug Discovery
Oegstgeest, The Netherlands | May 8-11, 2022

EFMC-ISMC 2022
XXVII EFMC International Symposium on Medicinal Chemistry
Nice, France | September 4-8, 2022

EFMC-YMCS 2022
9th EFMC Young Medicinal Chemists' Symposium
Nice, France | September 8-9, 2022

Awards

— The Nauta Pharmacochemistry Award for Medicinal Chemistry and Chemical Biology
— The "UCB-Ehrlich Award for Excellence in Medicinal Chemistry"
— Prous Institute - Overton and Meyer Award for New Technologies in Drug Discovery

Visit **www.efmc.info/awards** for more information

Prizes

— EFMC Prizes for Young Medicinal Chemists in Industry & Academia

Visit **www.efmc.info/prizes** for more information

EFMC-YSN

The Young Scientists Network

Building a strong network at an early stage in your career is crucial!
The aim of the EFMC-YSN is to **inspire, connect** and **provide opportunities** to medicinal chemists and chemical biologists in their Early Career.

Visit **www.efmc.info/ysn** for more information

Notes

Keynote Lectures
Biographies and Abstracts

Biographies of the Keynote Lecturers

Dr Werngard CZECHTIZKY(ASTRAZENECA, Mölndal, Sweden)
KL01 - Powerful New Tools for Augmented Medicinal Chemistry

Werngard Czechtizky is Head of Medicinal Chemistry for Respiratory, Inflammation and Autoimmunity (RIA) at AstraZeneca in Gothenburg, Sweden. She has a track record of delivery of clinical candidates and lead compounds across several therapeutic areas (CV, Diabetes, Pain, CNS, Inflammation and Respiratory). Werngard has continuously implemented state of the art technologies into Medicinal Chemistry. These include e.g. efficient integration of machine learning methods into drug discovery projects, setup of New Modalities Medicinal Chemistry capabilities, implementation of automated synthesis, purification and analytics facilities and efficient integration of compound synthesis with physchem & eADME profiling to accelerate DMTA cycles. Werngard is part of AZ's Global Chemistry Council, serves on scientific advisory boards of journals and conferences, and is co-/author of ca 80 publications and patents. She has studied at the Technical University of Graz, Austria, received a PhD from ETH Zürich and a postdoctoral training at Harvard University. Before joining AZ in 2017, she has been Head of Chemistry at Sanofi Frankfurt, Germany.

Dr Hasane RATNI (F. HOFFMANN-LA ROCHE LTD, Basel, Switzerland)
KL02 - Discovery of Risdiplam (Evrysdi): A Medicine for the Treatment of Spinal Muscular Atrophy

Dr H. Ratni is an Distinguished Scientist, Medicinal Chemistry, at F. Hoffmann-La Roche Ltd., Basel, Switzerland. He successfully, bring from early research to the clinic 6 molecules, one already successfully launched. He received his PhD at the University of Geneva and did a post-doc at Tokyo University before joining F. Hoffmann-La Roche Ltd in 2001. His research has mainly been devoted to the areas of neuroscience (e.g. V1a receptor antagonist, in human clinical trials, phase 3, for autism). In 2005, he participated in a secondment within the Roche group at Chugai Pharmaceutical Co. Ltd, Gotemba Japan. He was the chemistry discovery project leader of the SMN program for the treatment of spinal muscular atrophy, and inventor of Risdiplam (Evrysdi) approved by FDA in August 2020. His current focus is on gamma secretase modulator for Alzheimer disease. He is an author or co-author of more than 115 patents and publications and received the following awards: - 2014: Roche Leo Sternbach Award for Innovation in Chemistry. - 2016: Gold medal at the Roche Patent Inventor's recognition event. - 2019: Paper of the year award by the Society of Toxicology (DDTSS) - 2020: Roche CEO Award for Excellence - 2020: Senior Industrial Science Award by the Swiss Chemical Society (SCS)

Dr Nir LONDON (THE WEIZMANN INSTITUTE OF SCIENCE, Rehovot, Israel)
KL03 - Covalent Inhibitors - From Discovery to Functionalization

Dr Nir London completed his PhD in computational structural biology at the Hebrew University in 2012. He then pursued a post-doctroal fellowship with Brian Shoichet at UCSF where he developed a pioneering virtual screening platform for covalent inhibitor discovery. In 2015 Dr. London joined the Weizmann Institute of Science, where he is currently the Alan and Laraine Fischer Career Development Chair in the Dept. of Chemical and Structural Biology. Dr. London's lab is focused on covalent chemical biology and drug discovery and is developing new technologies to discover and functionalize covalently acting compounds. His honors include amongst others the Chorev award by the Israel Chemical Society, the Alon fellowship, and a BCRF-AACR Career Development Award.

POWERFUL NEW TOOLS FOR AUGMENTED MEDICINAL CHEMISTRY

Werngard Czechtizky

AstraZeneca, Pepparedsleden 1, 431 50 Mölndal, Sweden

Medicinal Chemistry is a very versatile scientific discipline and highly interconnected. It is permanently enhanced by new science and technologies, and requires a continuous development of all scientists in this rapidly evolving field of Drug Discovery. This presentation will paint a picture of how Medicinal Chemistry is embedded into Drug Discovery 2021, and intertwined with rapidly advancing early biology around omics, new types of biological targets, diverse target ID methods and chemical biology. It will further cover how Medicinal Chemistry is augmented by access to new synthetic modalities, protein structure prediction, machine learning/AI, physics-based drug design, and enriched by new methods in organic synthesis, high throughput experimentation, automation and new hit discovery methods.

DISCOVERY OF RISDIPLAM: A MEDICINE FOR THE TREATMENT OF SPINAL MUSCULAR ATROPHY

Hasane Ratni

F. HOFFMANN-LA ROCHE
PRBD-CM LEAD GENERATION - Medicinal Chemistry
Grenzacherstr. 127
4070 Basel
Switzerland

Spinal muscular atrophy (SMA) is an inherited disease that leads to loss of motor function and ambulation, and a reduced life expectancy. We have been working to develop orally-administrated, systemically-distributed small molecules to increase levels of functional SMN protein. Herein, we describe the discovery risdiplam that focused on thorough pharmacology, DMPK and safety characterization and optimization. This molecule is the world first approved small molecule RNA splicing modulator.

COVALENT INHIBITORS - FROM DISCOVERY TO FUNCTIONALIZATION

Nir London

Department of Chemical and Structural Biology
Weizmann Institute of Science
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Inhibitors and drugs that are able to form a covalent bond with their protein target has several advantages over traditional binders. They are attracting significant interest as underscored by FDA approvals of several rationally designed covalent drugs, such as Ibrutinib and Afatinib. My research team is focused on covalent ligand discovery and has developed methods including covalent virtual screening, covalent fragment screening, and computational redesign of reversible binders. We have now transitioned from discovery of covalent binders to their functionalization as for instance reversible covalent targeted degraders (PROTACs), and most recently the discovery of a new class of electrophiles that allows covalent binding triggered release of a specific cargo. I will share two stories: one on the discovery of a Pin1 covalent inhibitor, starting from a covalent fragment screen and going all the way to in vivo efficacy in various cancer models. And another on our recently discovered covalent ligand directed release (CoLDR) chemistry that allows the functionalization of covalent binders.

Notes

Oral Communications Abstracts

DESIGN AND SYNTHESIS OF NOVEL BENZOTHIAZOLE-PIPERAZINE PROPANAMIDE DERIVATIVES FOR MULTI-TARGETED APPROACH IN ALZHEIMER'S DISEASE TREATMENT

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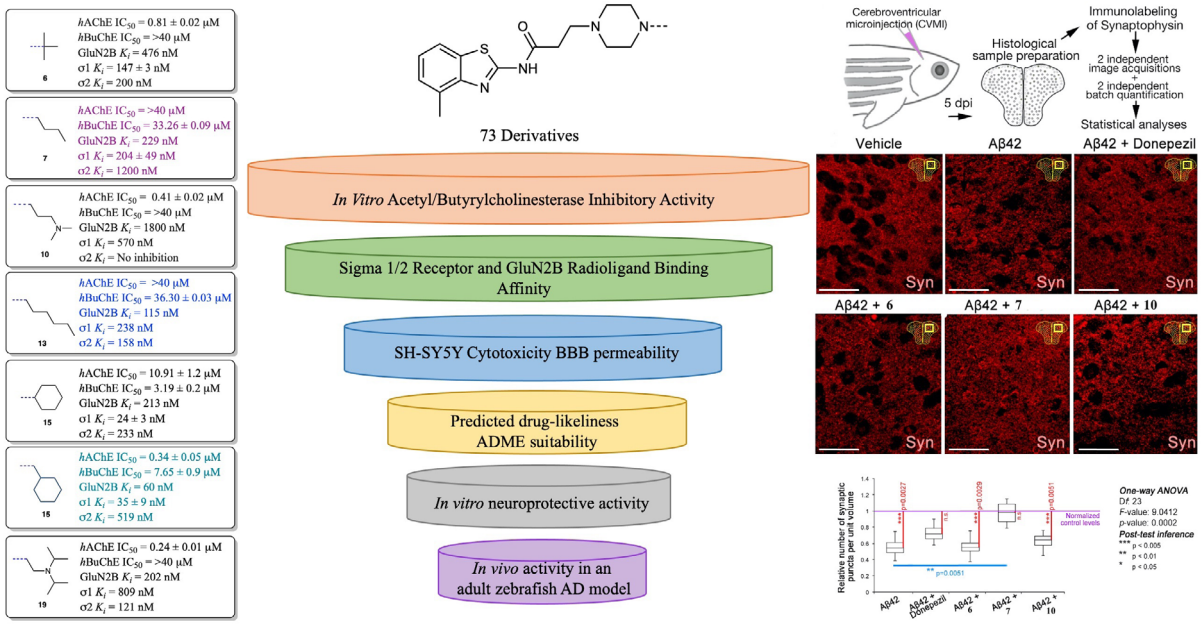
Alzheimer disease (AD) is a progressive neurodegenerative disorder and the most fatal type of dementia[1]. Drug discovery studies aimed to develop a disease modifying treatment which are based on individual targets ended up failure so far in clinical trials[2]. Nowadays, it has been accepted that the multifactorial nature of AD, requires multi-effective treatment[3]. In this regard, seventy-three novel compounds with benzothiazole ring attached to various piperazines with a propanamide linker were designed for the development of novel multi-targeted ligands that possess symptomatologic effect along with neuroprotective and disease modifying effect by binding to the key receptors involved. Firstly, seventy-three compounds were tested for their cholinesterase inhibitory activities. Twenty-five compounds found to display potent inhibitory activity (IC_{50} < 10 µM) were then tested for binding affinities over $\sigma_{1/2}$ receptors and NMDA GluN2B domain. Eight compounds that possessed potent activity in all selected targets were further subjected to *in vitro* neurotoxicity and *in vitro* blood-brain barrier permeability assays. Their ADME properties were suitable *in silico*. Compounds were then tested for their *in vitro* neuroprotective activity and their effect on synaptic density in an adult zebrafish AD model (figure). Lastly, molecular docking studies were performed for each target. Furthermore, eleven compounds were tested for their P2X7 receptor inhibitory activities. Derivatives with nitrogen bearing aromatic ring displayed good P2X7R inhibitory activity whereas hit compounds did not show any significant activity.



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DESIGN AND SYNTHESIS OF DUAL INHIBITORS OF DYRK1A/CLK1 KINASES INVOLVED IN NEURODEGENERATIVE DISEASES.

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Alzheimer's disease (AD) is the most common cause of dementia, and a neurodegenerative disease that affects nearly 50 million people worldwide. Despite the increasing development of solutions to fight AD symptoms, a major challenge for medicinal chemists is the development of efficient curative treatments. An innovative solution is the kinase inhibition, and it has been proven that over-expression of DYRK1A and CLK1 kinases is involved in neuronal degeneration pathway observed especially in Alzheimer's disease.[a] Our project takes part in a collaboration with European multidisciplinary teams to design new dual inhibitors of these two kinases.

Recently, our laboratory of medicinal chemistry has developed new families of heterocyclic molecules with high therapeutic interest. Imidazo[2,1-*b*][1,3,4]thiadiazole derivatives have found applications in oncology, infectiology or neurodegenerative diseases, but few functionalization methods were described.[b]

Consequently, we developed several methodologies to modulate regioselectively on the *C-2*, *C-5* and *C-6* positions of this scaffold.[c] The use of various reactions such as S_NAr, C-H arylation or palladium catalyzed cross coupling allowed us to increase the molecular diversity of suchderivatives. By modifying not only functionalization groups but also the scaffold, replaced by bioisosters, we developed new series of molecules to test their inhibitory activity on kinases.

Thanks to SAR studies conducted with our ANR partners, we designed selective and dual inhibitors of DYRK1A and CLK1 kinases, with IC_{50} in range of nM.

The synthesis of our original compounds, the promising results of biological tests and the perspectives of the project will be presented in this communication.

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TOWARDS THE DEVELOPMENT OF ACSL4 SELECTIVE INHIBITORS TO PREVENT FERROPTOSIS IN NEURODEGENERATIVE DISEASES

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Ferroptosis is a novel regulated cell death (RCD) pattern coined in 2012 following the study of antitumor agents selectively lethal to Ras-mutated cell lines.[1] In addition to its involvement in cancer, ferroptosis is associated with neurodegenerative diseases (NDD) such as Parkinson, Alzheimer and Huntington's diseases.[2,3,4,5,6] To prevent these pathologies, various researches have focused on designing synthetic ferroptosis inhibitors that can be classified into lipophilic radical-trapping antioxidants (RTA) or iron chelators.[7,8] Recent reports suggest that acyl-CoA synthetase long-chain family member 4 (ACSL4) is an important contributor to ferroptosis and is a sensitive indicator of pathophysiological ferroptosis.[9,10] Thus, ACSL4 inhibition is a promising therapeutic approach to prevent ferroptosis-related NDD. However, even if there are strong evidences of the role of ACSL4 in ferroptosis and a well-established link between ferroptosis and NDD, the involvement of ACSL4 in NDD is yet to demonstrate. In this context, our work focus on the development of ACSL4 selective and potent inhibitors to validate the role of the enzyme in NDD. To date, only few inhibitors of ACSL4 are reported and their lack of selectivity do not allow the validation of the role of ACSL4 in NDD.[11,12] This is the case of rosiglitazone (rosi), the most potent reported inhibitor (rACSL4 IC_{50} = 0.6 μM) which is also a peroxisome proliferator-activated receptor γ (PPARγ) agonist. To develop selective ACSL4 inhibitors, we firstly produced the enzyme and developed biophysical and biochemical methods to screen different chemical libraries, including a library of commercially available drugs, a fragment library and a library of PPARα/γ modulators. In total, we screened more than 2000 compounds in differential scanning fluorimetry (DSF) and identified 80 primary hits. We assessed those ligands in an activity assay and further validated them in microscale thermophoresis analysis (MST). We validated 45 molecules as ACSL4 inhibitors among the primary hits. To assess the selectivity of these hits for ACSL4, we evaluated them on ACSL3, the protein that shares the most sequence identity with the target. In an effort to optimize both activity and selectivity, we synthesized more than 60 hit analogues that we evaluated in activity and affinity.

This optimization permitted a five times increase in potency simultaneously with an improvement of the selectivity profile. Moreover, a preliminary cellular evaluation on dopaminergic neuron precursors under ferroptotic conditions demonstrated cytoprotective effects for the hit evaluated.

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SMALL ORGANIC MOLECULES TO TRIGGER THE ACTIVITY OF NK CELLS

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Natural killer (NK) cells provide rapid responses to viral-infected cells and play a critical role in tumor immunosurveillance by directly inducing the death of tumor cells. Instead of acting via antigen-specific receptors, lysis of tumor cells is mediated by alternative receptors, including NKp30.

Using computational tools, we designed a family of small organic molecules (SOMs) based on the structure of the NKp30 receptor [1, 2]. Synthetic, stability and overall binding score considerations were used to select a subfamily of ca. 15 compounds. From these, 10 completely characterized entities were tested in an MS-based binding assay using the extracellular portion of the receptor, which led to the identification of one lead compound.

Primary cultures of human NK cells were used to probe the stimulation of NK cell responses by the lead compound. EC50 values below 0.2 μM were found in TNF-α, IFN-γ and Granzyme B release assays. Co-cultures of NK cells and the tumor cell line HCT116 were used to determine the effect of the lead compound on the cytotoxicity of NK cells. NK cell cytotoxicity doubled upon treatment with 1.25 μM of lead compound, as compared to control incubations, presenting and EC50 value of ca. 0.15 μM.

Our lead compound was proven effective in activating the cytotoxic activity of NK cells, as demonstrated by the cytokine release and the tumor cell death assays. Further work aims to derivatize our ligands with tumor-targeting molecules to increase the specificity of the NK cell cytotoxic response.

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NOVEL HDAC6-SELECTIVE INHIBITOR FOR GLIOBLASTOMA TREATMENT

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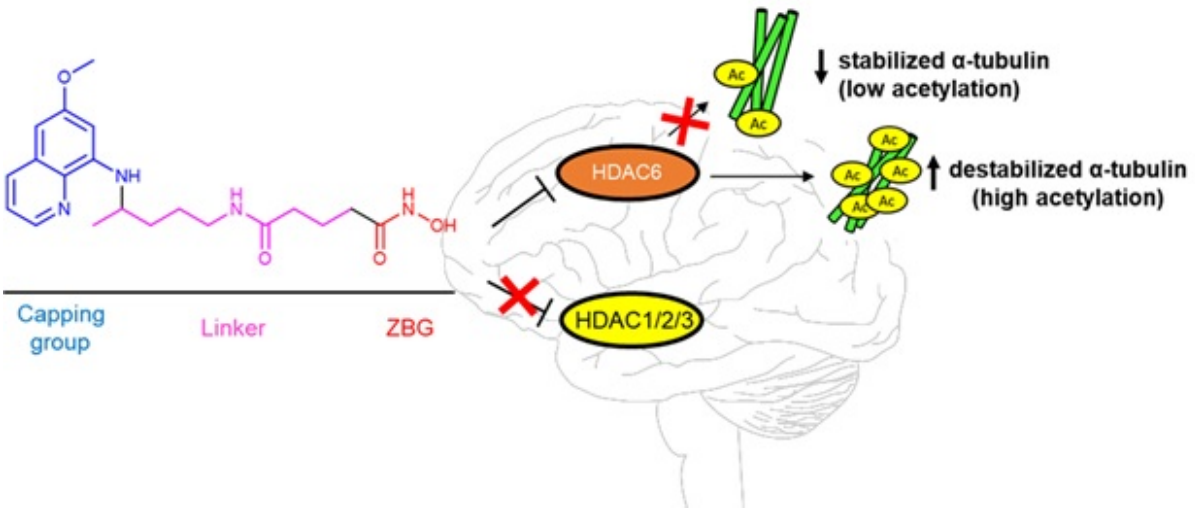
Glioblastoma multiforme is one of the deadliest types of cancer. Various approaches are being investigated and inhibition of histone deacetylases (HDACs) is showing promising results (1). However, since non-selective HDAC inhibition can cause serious side effects, scientists are focusing on the development of selective inhibitors. HDAC6 is a unique HDAC with a wider binding side expressed primarily in the cytoplasm. Since its targets are non-histone proteins, it has a role in cytoskeletal organisation and chaperone activities (2).

We have developed a novel HDAC6 selective inhibitor – sahaquine. Sahaquine is a primaquine derivative linked with a hydroxamic acid moiety via a glutaric acid linker. The quinoline ring of primaquine is the capping group that fits better into the wider active site of HDAC6. Sahaquine inhibited HDAC6 in nanomolar concentrations without affecting other HDACs in glioblastoma cells. Additionally, sahaquine inhibited glioblastoma cell invasion and decreased levels of EGFR and its downstream targets – phosphorylated AKT and phosphorylated ERK1/2. To investigate sahaquine's safety and metabolism, a matrix-assisted laser desorption/ionization-mass spectrometry imaging (MALDI MSI) method applied to zebrafish larvae was developed.



This work has been fully supported by the Croatian Science Foundation (IP-09-2014-1501), the Canadian Institute for Health Research (MOP-119425), and the Natural Sciences and Engineering Council of Canada (RGPIN 04994-15). The work of doctoral student M. Beus has been fully supported by the Young researcher's career development project – training of doctoral students of the Croatian Science Foundation founded by the European Union from the European Social Fund.

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DESIGN AND SYNTHESIS OF BRAIN PENETRANT P38-ALPHA MITOGEN-ACTIVATED PROTEIN KINASE INHIBITORS

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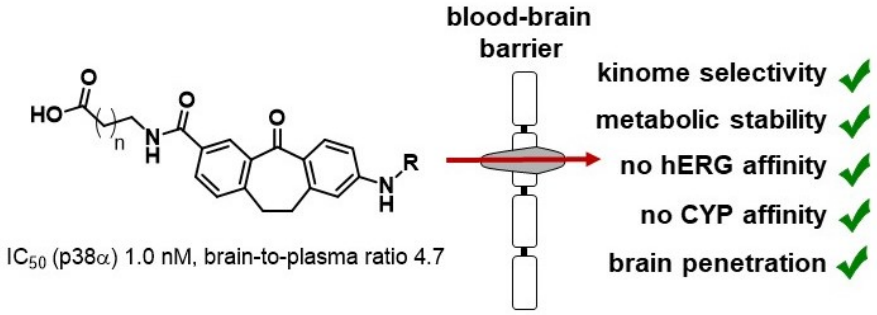
The p38α mitogen-activated protein (MAP) kinase is a serine/theronine kinase and involved in cell signaling cascades. This enzyme was originally identified as a drug target for chronic inflammatory diseases in the mid-1990s and plays a central role in modulation of production of various pro-inflammatory cytokines at both transcriptional and translational levels.[1] The p38α MAP kinase is also expressed in glia and neurons, and its phosphorylation may lead to synaptic dysfunction. Therefore, the p38α MAP kinase is also considered as a drug target for different central nervous system (CNS) disorders, especially, when associated with neuroinflammatory responses.[2-4]

As a potential therapeutic approach, we synthesized novel very potent, highly selective and metabolically stable Skepinone-based p38α MAP kinase inhibitors.[5] These inhibitors were optimized to cross the blood–brain barrier via either hydrophobic diffusion or amino acid transporters.



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METATACS: A STRATEGY FOR METASTASIS PREVENTION THROUGH TARGETED FASCIN DEGRADATION

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The lifetime risk to develop cancer is estimated to be around 50%, and – although survival rates have vastly improved over the last few decades – cancer remains the second leading cause of death worldwide.[1] ***Metastasis is the major contributor to cancer mortality***, and describes the ability of cancer cells to detach from the primary tumour, invade the body and colonise at a distal site. ***Fascin, a structural protein which is overexpressed in aggressive metastatic cancers with poor survival rates***, is integral to the formation of actin-rich protrusions (invadopodia) necessary for cancer cells to invade and migrate through tissue. Inhibition of fascin's actin-bundling activity has been shown to dramatically reduce tumour cell invasion and formation of metastases.[2]

Targeted protein degradation through PROTACs is a promising strategy that could overcome the challenges of ***drugging a structural protein and disrupting its protein–protein interaction*** encountered with traditional small molecule therapeutics. These bifunctional molecules are designed to bring fascin in close proximity with an E3 ligase, inducing ubiquitination of the pro-metastatic target, and tagging it for selective degradation by the proteasome. Recycling of the PROTAC and destruction of fascin would result in depletion of protein levels, likely eliciting a ***prolonged therapeutic response whilst minimising risk of side effects***.[3]

Given the difficulty of developing small molecule ligands for structural proteins, HaloPROTACs were chosen as ***proof-of-concept for selective fascin degradation*** **via** ***the PROTAC mechanism***. Instead of binding through a ligand, HaloPROTACs attach to HaloTag fusion proteins covalently.[4] Accordingly, a series of compounds was designed and synthesised (Figure 1a), and a HaloTag-fascin fusion protein was cloned and expressed in different cancer cell lines. Dose-response and time-course assays showed ***degradation of HaloTag-fascin by up to 80%*** with as little as 0.1 µM HaloPROTAC concentration. This decrease was observed as early as 4 hours after treatment, with ***sustained degradation beyond 72 hours*** (Figure 1b).

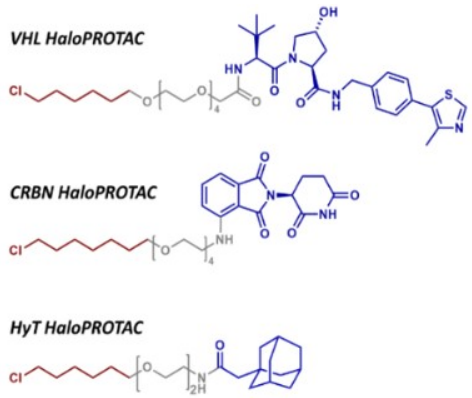
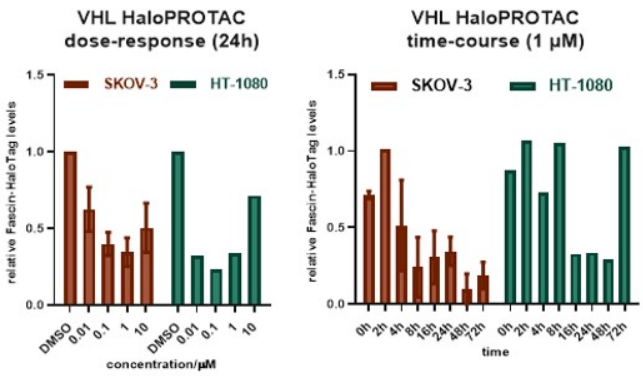


Figure 1: a) HaloPROTACs.



b) VHL HaloPROTAC dose-response and time-course assays.

The impact of selectively degrading endogenous fascin on invadopodia formation can be evaluated through functional and phenotypic assays. Failure to form the invasive structures in the absence of fascin could then inhibit cancer cell invasion, paving the way for the development of anti-***meta***static PRO***TACs*** (***MetaTacs***) able to ***sustainably reduce and prevent metastasis*** in cancer patients.

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STRUCTURE ELUCIDATION OF ARTIFICIAL, SELF-ASSEMBLING SQUALENE-CONJUGATES AND β-PEPTIDES

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Squalene-conjugates and β-peptides were investigated as two different types of self-associating systems. Structure elucidation was made by NMR spectroscopy, then the self-association was studied.

Ecdysteroids (posterone-2,3-acetonides) and their squalenoylated derivatives were investigated with two different linker regions. Complete ^{1}H and ^{13}C assignations were made, in case of the steroid moiety the diastereotopic groups were distinguished and the presence of the squalene-linker side chain was verified. The configuration of C=N double bond in oximes was also determined. It was a challenging task to differentiate the atoms/groups which are in very similar chemical environment. With the help of extreme high resolution HMBC and HSQC spectra by ultra-high magnetic field and band-selective pulse sequences the complete assignment was performed for these compounds. The self-association was investigated on supramolecular level, the self-assembly was found in form of nanomaterials in aqueous solution.

β-Peptide pentamers which are made from *trans*-[1*R*,2*R*]-2-aminocyclohexanecarboxylic acid (ACHC) residues and six different β-amino acids as 3rd unit were studied. Backbone protons were assigned, then the secondary structures were determined. In three peptides stable H14 helix was formed, in further three peptides less stable structures were identified. NOE-correlations, amide NH-ND exchange and ECD measurements were carried out for the investigation of the conformation. In case of the flexible β-alanine building block, [1*R*,2*R*,3*S*,4*S*]-*diexo* -3-aminobicyclo[2.2.1]hept-5-ene-2-carboxylic acid (ABHEC) and [1*R*,2*S*,3*R*,4*S*]-*diexo* -3-amino-7-oxabicyclo[2.2.1]hept-5-ene-2-carboxylic acid (AOBHEC) residues, stable helices were discovered. Differences were found for [1*S*,2*S*,3*R*,4*R*]-*diexo*-ABHEC, [1*S*,2*R*,3*S*,4*R*]-*diexo*-AOBHEC and *Z* -β-dehydroalanine as middle unit, stable self-organization could not be observed.

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* Authors contributed equally in this work

INTRACELLULAR RECEPTOR MODULATION: INTRACELLULAR LIGANDS FOR CHEMOKINE RECEPTORS

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CC Chemokine receptor 2 (CCR2) is a class A G protein-coupled receptor (GPCR), which plays key roles in the migration of immune cells during physiological or inflammatory responses. As such, CCR2 represents a potential drug target in a variety of inflammatory and immune diseases, such as atherosclerosis, multiple sclerosis and cancer. Many CCR2 antagonists have been developed over the years; yet, all of them have failed in clinical trials due to lack of efficacy mostly. In this regard, our crystal structure of CCR2 has provided evidence that chemokine receptors can also be targeted from an intracellular binding site.[1] These intracellular allosteric modulators possess many advantages over traditional 'orthosteric' antagonists, particularly their ability to inhibit the receptor in a non-competitive and insurmountable manner. Furthermore, the high conservation of such intracellular binding site among chemokine receptors provides an opportunity to design both selective and multitarget intracellular antagonists.

Based on the structure of known CCR2 intracellular ligands, we aimed to design 1) multitarget ligands, which inhibit multiple chemokine receptors; and 2) irreversible intracellular ligands for selective targeting of CCR2. We used a combination of radioligand binding, functional assays and *in silico* modeling to evaluate the binding affinity, functional activity and binding mode of the different intracellular derivatives. Overall, our medicinal chemistry approach allowed us to find several multitarget intracellular ligands for chemokine receptors CCR1, CCR2 and CCR5; as well as a selective, covalent ligand for CCR2. Furthermore, these compounds displayed an insurmountable mechanism of inhibition, which is highly relevant in inflammatory diseases characterized by elevated levels of endogenous chemokines. Overall, these ligands open up new avenues for targeting chemokine receptors from the intracellular side.

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A MATING MECHANISM TO GENERATE DIVERSITY FOR THE DARWINIAN SELECTION OF DNA-ENCODED SYNTHETIC MOLECULES

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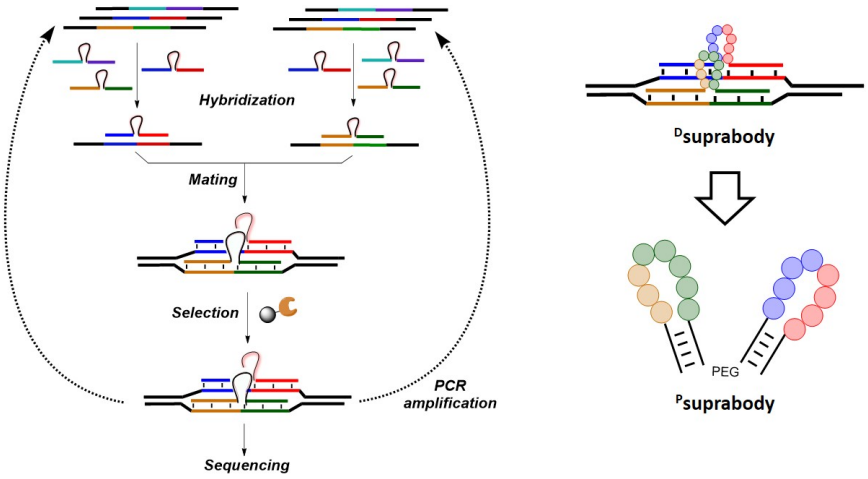

DNA-encoded library technologies empower the screening of synthetic molecules but have thus far not tapped into the power of Darwinian selection with iterative cycles of selection, amplification and diversification. Herein we report a simple strategy to rapidly assemble libraries of conformationally constrained peptides that are paired in a combinatorial fashion (suprabodies). We demonstrate that the pairing can be shuffled after each amplification cycle in a process akin to DNA shuffling or mating to regenerate diversity. The method has been validated with selections against streptavidin and applied to the discovery of PD-L1 binders. We further demonstrate that the binding of self-assembled suprabodies can be recapitulated by smaller (*ca*. 7 KDa) synthetic products that maintain the conformational constrain of the peptides.

TAMING MULTIPLE CONTIGUOUS CHIRAL CENTERS THROUGH DYNAMIC KINETIC RESOLUTION

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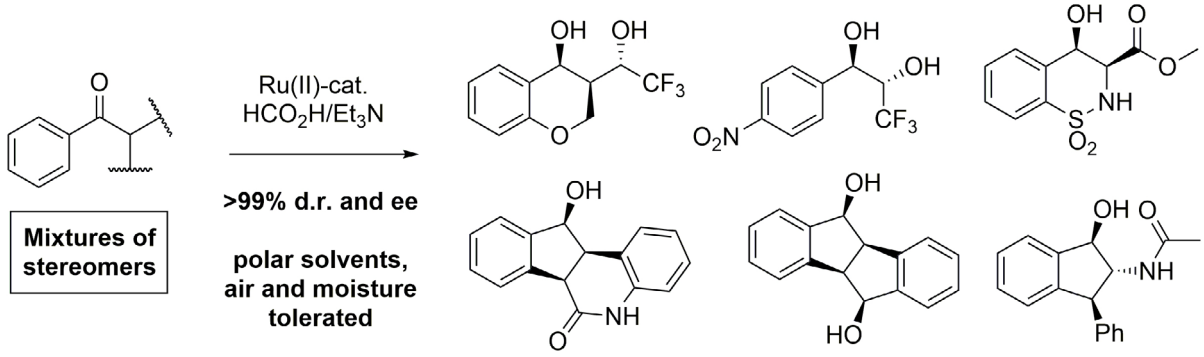
Bioactive molecules with three-dimensional scaffolds and multiple chiral centers are more successful in transition from discovery, through clinical trials, to drugs than their easily accessible flat competitors, because of the pinpoint on-target activity and better physical properties.[1]

Asymmetric transfer hydrogenation (ATH) of ketones using Noyori-Ikariya type ruthenium(II)-catalysts has proved as a well-behaved and user-friendly platform for the synthesis of complex chiral secondary alcohols, where up to four contiguous stereocenters can be controlled in a single chemical operation through dynamic kinetic resolution (DKR).[2] This type of catalysis is not sensitive to air and moisture, is compatible with polar solvents, and involves an easy workup, which are key merits for introduction of a new synthetic method into a med-chem setting.

Intriguing multi-chiral molecular architectures such as CF_3-substituted 1,2- and 1,3-diols,[3] enantiopure benzosultams,[4] nonplanar tetracyclic scaffolds,[5] and 1,2,3-trisubstituted indans,[5,6] are therefore available in few well understood synthetic steps from commercially available starting material. Substrate scope of DKR-ATH and basic mechanistic insights (to demystify the stereochemistries) will be presented as well as our endeavors to incorporate such chemical motifs into bioactive molecules with improved physical and ADME properties.



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MODULATORS OF COACTIVATOR-ASSOCIATED ARGININE METHYLTRANSFERASE 1 (CARM-1): THERE AND BACK AGAIN

Ciro Milite

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The methylation of arginine residues is a post-translational modification found in both nuclear and cytoplasmic proteins, which are involved in several different cellular processes, including transcriptional regulation, RNA metabolism, and DNA damage repair. Enzymes of the protein arginine *N*-methyltransferase (PRMT) family catalyse the transfer of a methyl group from the donor *S*-adenosyl-*l*-methionine (SAM or AdoMet) to the guanidinium side chain of arginine residues in the target protein. A few years ago, starting from **AMI-1** (the first inhibitor of PRMTs)[1] we identified **EML108**, which was characterized by an improved selectivity profile among methyltransferases and a good cellular activity.[2] Later, pursuing our efforts toward the identification of potent and selective PRMTs inhibitors, we replaced the hydroxynaphthalene moiety with the bioisosteric indole framework. Surprisingly, instead of new PRMT inhibitors, we identified a new class of CARM-1 activator able to increase the methylation of histone (H3) or nonhistone (PABP1) substrates both in in vitro and in cellular models.[3] Therefore, we moved back to our original scaffold **EML108** and, applying a multisubstrate adduct approach, we designed some new ligands of PRMTs. Firstly, we prepared some derivatives bearing a guanidine moiety connected to the naphthalene scaffold *via*a variable linker, then the scaffold was further functionalized with an adenosine moiety (Figure 1). With this multi-substrate-adduct approach we were able to identify new nanomolar selective inhibitors of the arginine methyltransferase CARM-1

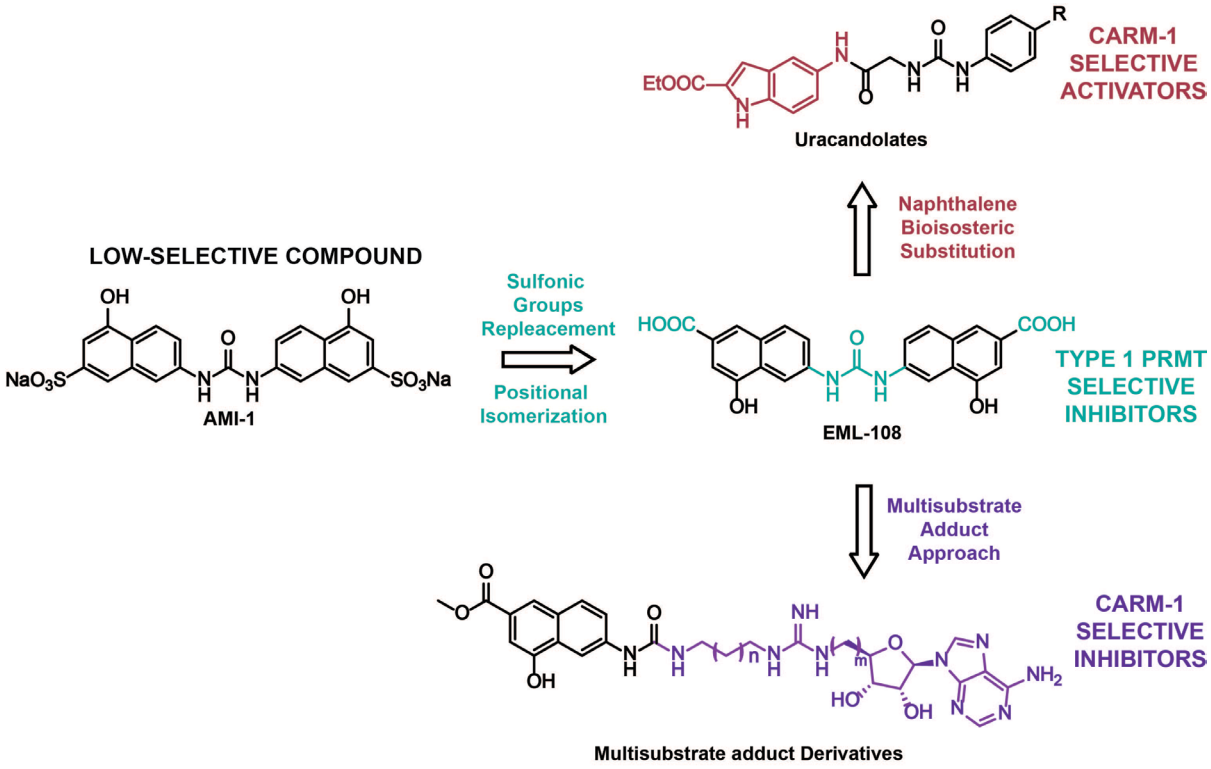

Figure 1. Approaches for the identification of CARM-1 modulators.

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HYDROXAMIC ACID-FUNCTIONALIZED PEPTIDE MICROARRAYS FOR THE STUDY OF ZN(II)-DEPENDENT HISTONE DEACETYLASES

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Peptide microarrays are excellent technologies for the elucidation of protein-protein interaction sites and the discovery of peptide ligands. One method for the preparation of peptide microarrays is SPOT synthesis, where minute amounts of peptide are synthesized on a cellulose membrane that is directly applied in biochemical assays. SPOT arrays have been used to study epigenetic proteins, as these interact with the tails of histones, which can be mimicked by the displayed peptides. Here, we introduce a hydroxamic acid-containing building block into SPOT arrays in order to trap the transient interaction of the histone tails with the Zn(II)-dependent histone deacetylase (HDAC) enzymes. This building block serves as surrogate of acetyl-lysine and therefore informs on the substrate requirements for individual HDACs as well as enables the discovery of potent inhibitors. The μSPOT technology, with which a single SPOT array can be copied onto hundreds of assay-ready coated slides, was used to generate interaction maps of the four nuclear class I HDACs against the four core histones. Introduction of additional amino acid modifications revealed key histone posttranslational modifications that disrupt HDAC binding. Subsequent functional assays confirmed the interference of histone 3 serine 10 phosphorylation (H3pS10) with deacetylation at lysine 9 (H3K9ac), and identified peptide inhibitors with nanomolar potency and diverse inhibitory profiles across the HDAC family. Most importantly, a potent inhibitor of class I HDACs and HDAC6 could be applied directly in a cellular assay to generate hyperacetylation of their known substrates: histone 3 and α-tubulin. We envision that similar functionalized microarrays could find broad application to identify isozyme-selective substrates and facilitate the development of isozyme- or protein complex-selective HDAC inhibitors and affinity probes.

CLEISTANOLATE ANALOGUES: SYNTHESIS, CITOTOXICITY AND SAR STUDY

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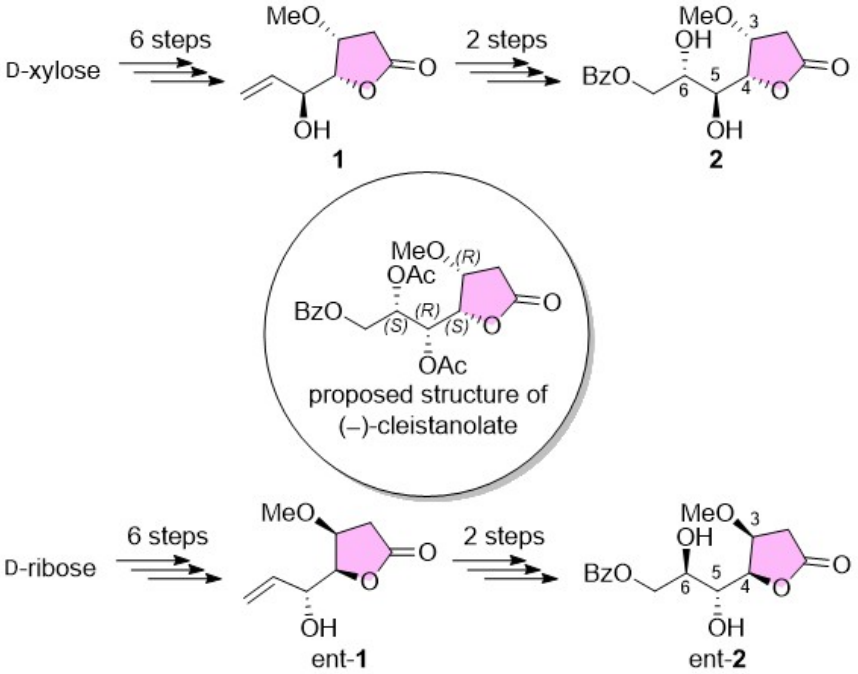
(−)-Cleistanolate is natural product isolated from methanol extract of leaves of *Cleistochlamys Kirkii* (Annonacae) in 2017.[1] Herein, we report the synthesis of (5*S*)-monobenzoylated analogue of proposed structure of (−)-cleistanolate (**2**) and its enantiomer (ent-**2**, Scheme 1.) starting from commercially available D-xylose and D-ribose via key chiral intermediates **1** and ent-**1**. Antitumour activity against panel of human tumour cell lines and one normal cell line will be presented. Additionally, the influence of some of stereocenters on antitumour activity will be discussed in detail.



Scheme 1. (5*S*)-monobenzoylaed cleistanolate analogue **2** (overall yield 5.72%) starting from D-xylose and its enantiomer ent-**2** (overall yield 3.14%) starting from D-ribose.

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DEEPSIBA: CHEMICAL STRUCTURE-BASED INFERENCE OF BIOLOGICAL ALTERATIONS USING DEEP LEARNING

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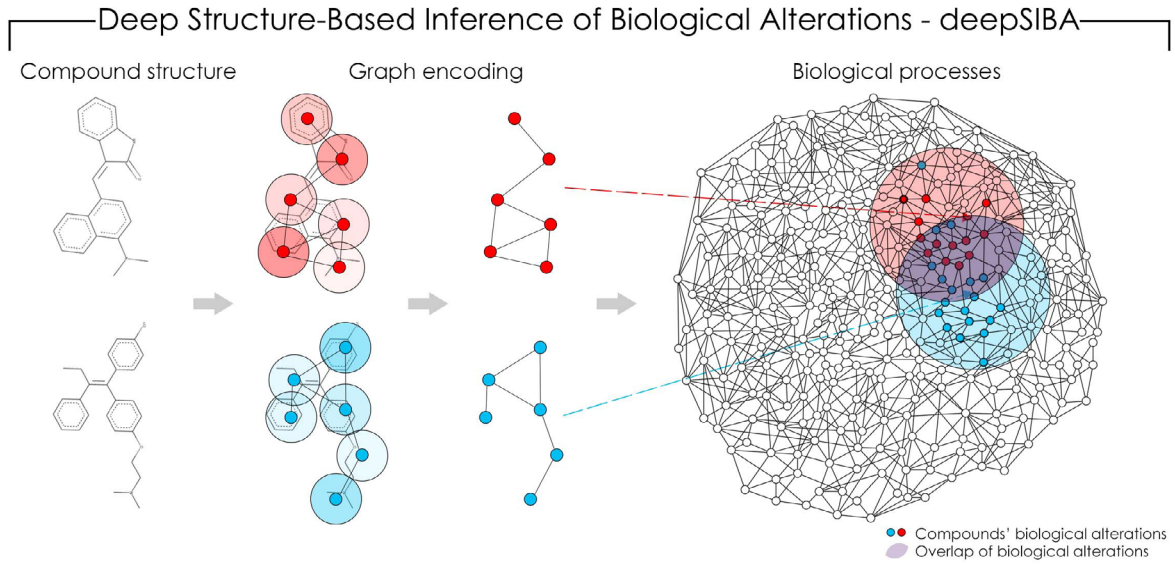
Predicting whether a chemical structure leads to a desired or adverse biological effect can have a significant impact for in-silico drug discovery. In this study, we developed a deep learning model where compound structures are represented as graphs and then linked to their biological footprint. To make this complex problem computationally tractable, compound differences were mapped to biological effect alterations using Siamese Graph Convolutional Neural Networks. The proposed model was able to encode molecular graph pairs and identify structurally dissimilar compounds that affect similar biological processes with high precision. Additionally, by utilizing deep ensembles to estimate uncertainty, we were able to provide reliable and accurate predictions for chemical structures that are very different from the ones used during training. Finally, we present a novel inference approach, where the trained models are used to estimate the signaling pathway signature of a compound perturbation, using only its chemical structure as input, and subsequently identify which substructures influenced the predicted pathways. As a use case, this approach was used to infer important substructures and affected signaling pathways of FDA-approved anticancer drugs.



ITH15004, A NOVEL BLOOD-BRAIN BARRIER-PERMEABLE P2X7 ANTAGONIST: FROM DRUG DESIGN TO PHARMACOLOGICAL APPLICATIONS

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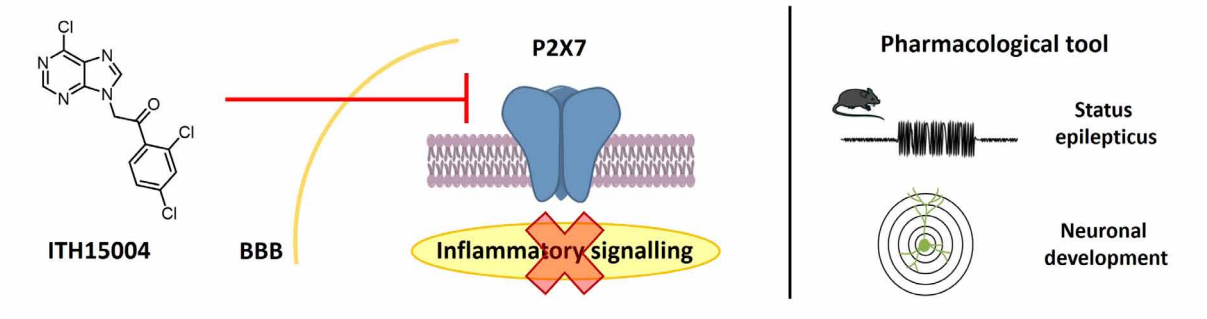
The purinergic P2X7 receptor is a ligand-gated ion channel activated by high concentrations of ATP as it occurs in cell damage and inflammatory conditions. P2X7 activation is involved in the development of several neurodegenerative, neurological, and psychiatric diseases [1]. In this work, we identified a novel blood-brain barrier (BBB)-permeable non-nucleotide purine P2X7 antagonist, potentially capable of acting in the central nervous system (CNS) [2].

After rational design supported by computational tools, a total of 31 compounds were synthesised. Their inhibitory activity was assessed by both cytosolic calcium measurements and YO-PRO-1 dye uptake in human P2X7-transfected HEK293 cells. *X. laevis* oocytes were employed to validate the screening results and define the selectivity of the active compounds by two-electrode voltage clamp. The anti-inflammatory activity of the novel derivatives was tested in mouse peritoneal macrophages by measuring P2X7-induced IL-1β release. Parallel artificial membrane permeability assay (PAMPA) was performed to evaluate compounds permeability profile. Additionally, P-glycoprotein (Pgp) ATPase activity in the presence of the most potent compounds was measured.

The arylpurinylethanone ITH15004 showed consistent, interspecies, and selective P2X7 inhibition. It is also expected to cross the BBB, without being expelled from the CNS by the Pgp, differently from the well-known P2X7 antagonist JNJ-47965567. These results demonstrate that non-nucleotide purine-based derivatives inhibit P2X7 and have a good permeability profile to act in the CNS. ITH15004 has been already employed as pharmacological tool in embryonic mouse neuron cultures for neuronal growth studies [3] and in a mouse model of epilepsy [4]. Further optimisation of ITH15004 is directed to increase its pharmacodynamic and kinetic properties and will help with the development of novel drugs for the treatment of neuroinflammatory-based CNS diseases.



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FAR-RED FLUORESCENT DNA BINDER ALLOWS HOST-PATHOGEN INTERACTION STUDIES OF MULTIDRUG-RESISTANT BACTERIA

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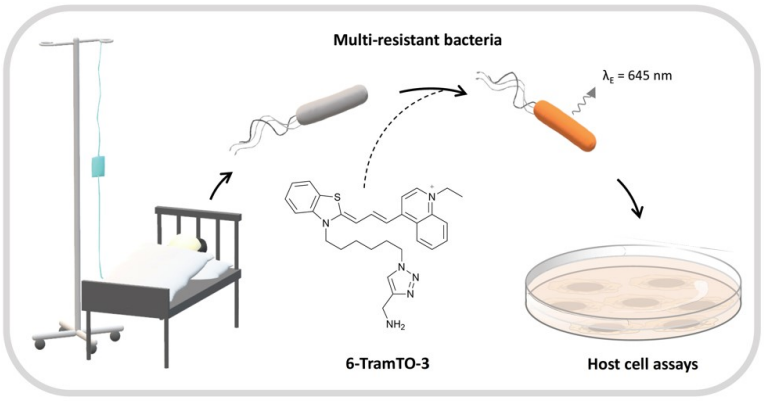

The excessive use of antibiotics has pushed the spread of multidrug-resistant bacteria at alarming rates.[1] As a consequence, incremental failure of antimicrobial treatment in patients leads to increased fatality rates due to the systematic spread of bacteria and organ failure. New research tools compatible with resistant bacteria, such as fluorescence labelling approaches, are urgently required. Detailed investigation of the disease–causing strategies of drug-resistant bacteria interacting with their hosts will assist rational drug design[2] and may eventually reduce fatality rates of infected patients.

In this context, we introduced a **chemical alternative to** the green fluorescent protein (**GFP**) technology for imagining living bacteria and enabling real-time infection studies. Unlike GFP methodology, our molecule **6-TramTO-3** allowed **fluorescent labelling of multidrug-resistant bacteria**. The novel far-red fluorescent nucleic acid stain efficiently labels bacteria **without affecting growth** and viability in contrast to known commercially available nucleic acid stains. Thus, in a proof-of-principle experiment we stained *Klebsiella pneumoniae*, a major threat to hospitalized patients, and for the first time deciphered divergent interaction strategies of antibiotic-resistant versus antibiotic-sensitive *Klebsiella* strains with immune cells.[3]

6-TramTO-3 serves an easy to use **off-the shelf reagent** suitable to study a variety of bacteria, which have been difficult to modify for fluorescent-protein expression, and apply them in host pathogen interaction studies to **understand the disease strategies** of antibiotic-resistant bacteria, an emerging worldwide threat.[3]

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NEW 5-ARYLIDENEIMIDAZOLONES IN THE ONGOING BATTLE AGAINST BACTERIAL AND CANCER MULTIDRUG RESISTANCE

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Multidrug resistance (MDR) is a serious and growing problem in the treatment of various diseases *e.g.* bacterial infections and cancer [1,2]. Therefore, searching for new drugs able to overcome MDR is an important task for medicinal chemists. Antibiotic adjuvants, which block one or more mechanisms of bacterial resistance, *e.g.* efflux pumps, PBP2a, β-lactamases, are a new therapeutic hope in battle against bacterial MDR [1]. Such compounds should not possess antibacterial activity in term to minimize the risk of developing secondary drug resistance. In case of MDR in cancer cells, new active "adjuvants" should be selective towards cancer cells and may possess intrinsic anticancer activity. One of frequently occurred MDR mechanisms is ABCB1 efflux pump, also called glycoprotein P (Pgp) [2]. Previous studies performed in the group of imidazolone derivatives proved their various biological activities including the inhibition of bacterial and cancer MDR mechanisms [3,4].

Hence, new series of 5-arylideneimidazolones, with amine fragment at position 2 or 2 and 3, were investigated. The new compounds were designed based on SAR analysis for previously found active compounds. Then, 3-6-step syntheses of the new series were conducted, which included: Knoevenagel condensation, S-methylation and reaction with amine, going with(out) Dimroth rearrangement, confirmed by crystallographic analyses for selected compounds. Final products were tested on their potency of antibiotic adjuvants in various susceptible and resistant Gram positive and Gram negative bacterial strains. Due to that, the assays for antibacterial activity and ability to decrease antibiotic MICs were carried out. Most of the synthesized products were tested in cancer cytotoxicity and rhodamine 123 accumulation assays in order to determine their ability to overcome cancer MDR. Moreover, an attempt was taken to define potential mechanism(s) of action, using *in silico* and *in vitro,* assays. Molecular modeling for interaction of selected compounds with PBP2a (bacterial target) and ABCB1 (cancer cell target) was conducted. As mechanistic studies *in vitro*, RTE assays in Gram negative bacterial strains as well as Pgp-Glo luminescence assay were used. The most promising compounds found in bacterial and/or cancer cell assays were tested in various *in silico* and *in vitro* ADMETox studies, including metabolic stability, hepatotoxicity, mutagenicity. Moreover, selected physicochemical properties, *i.e.* water solubility, lipophilicity, stability in acidic and basic conditions, were examined. SAR analyses were conducted.

In conclusion, 2-amine-5-arylideneimidazolones seem to be promising group in the ongoing battle against both bacterial and cancer MDR *via* either antibiotic adjuvant potential, or ABCB1 modulating activity. Recent studies proved also anticancer activity for the series, in some cases highly selective towards MDR cell line.

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PHOSPHORYLATION-INDUCING CHIMERIC SMALL MOLECULES

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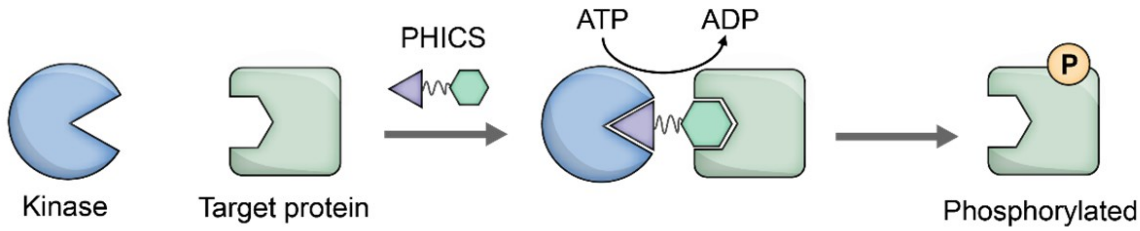

Several new classes of small molecules are emerging that endow new functions to enzymes via proximity-mediated effects. Protein phosphorylation profoundly influences their structures and functions, and kinase inhibitors have had a transformative impact on basic science and medicine. We hypothesize that small molecules that *induce* phosphorylation of any given protein-of-interest on-demand will also be useful in many scenarios. For example, such molecules can be used to trigger cell-signaling events or neo-phosphorylations that are not observed in a native cellular environment. Neo-phosphorylation can alter protein structure and function, evoke an immune response, or affect the protein's interaction with other biomolecules, particularly with RNA/DNA that have negatively-charged phosphodiester backbone. We describe a new class of bifunctional molecules termed phosphorylation-inducing chimeric small molecules (PHICS) formed by linking a kinase binder with a small-molecule binder of the target protein (*J. Am. Chem. Soc.* **2020**, 142, 14052). Using PHICS and AMP-activated protein kinase (AMPK) or protein kinase C (PKC), we have induced phosphorylation of BRD4, BTK, FKBP12 and ABL. Furthermore, PHICS induced a signaling-relevant phosphorylation of the target protein Bruton's tyrosine kinase (BTK) in cells. PHICS exhibited the hallmarks of a typical bifunctional molecule, including the hook effect, turnover, dependence on linker, isoform specificity, and dose- and temporal-control of phosphorylation. Beyond these studies, we have generated PHICS to effect tyrosine phosphorylation and developed kinase binders that induce phosphorylation at different stoichiometries on the target protein. We envision that PHICS-mediated native- or neo-phosphorylations will find utility in basic research and medicine.

Flash Poster Presentations Abstracts

ELECTROPHILIC WARHEADS FOR THE ASSESSMENT OF TRACTABLE CYSTEINES

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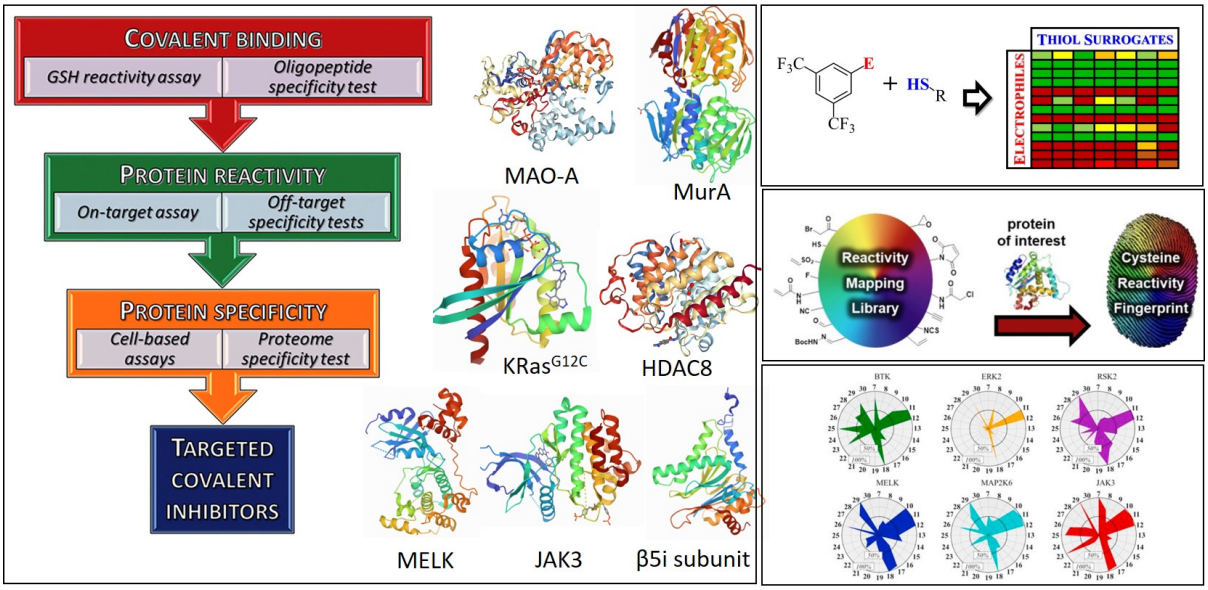
Targeted covalent inhibitors (TCIs) has been truly come pf age in chemical biology and medicinal chemistry. Here, we discuss the toolbox developed at our research group for the covalent fragment based characterization and target-specific optimization of cysteine-targeting electrophilic warheads.

In order to systematically explore and extend the potential warhead chemotypes, we developed a methodology for the detailed reactivity and selectivity analysis of large and chemically diverse covalent fragment libraries. We have evaluated several cysteine-derivatives and found *L*-glutathione (GSH) as the optimal surrogate for reaction kinetics providing thiol-reactivity and aqueous stability data [*Bioorg. Med. Chem.* **2020**, *28*, 115357.]. Then, the selectivity for the nucleophilic amino acid residues was mapped using a designed oligopeptide with multiple nucleophilic residues. With selected covalent fragments we demonstrated that warheads acting by different mechanisms have a significant impact on functional, enzyme family, species and protein specificities [*Eur. J. Med. Chem.* **2018**, *160*, 94.]. Next, a library of covalent fragments was compiled having the same core carrying a diverse set of warheads to characterize tractable cysteines in protein targets. The warhead library was screened on a variety of protein targets applying biochemical assays, Ellman's assay, LCMS/MS, ^{1}H, ^{15}N-HSQC and ^{19}F NMR. We have successfully revealed a new covalent mechanism of inhibition for MAO-A by binding to the noncatalytic C321 and C323, and contributed to the identification of accessible cysteines influencing the activity of HDAC8 [*ChemBioChem* **2020**, *22*, 743.]. Moreover, we have experimentally mapped the reactivity and accessibility of tractable cysteines in kinases leading to efficient covalent JAK3 inhibitors and the identification of MELK kinase as a possible covalent target [*Eur. J. Med. Chem.* **2020**, *207*, 112836.].

Overall, our results support that there is no universal warhead available for different targets. The required specificity of TCIs needs the optimization of both noncovalent and covalent interactions. Thus, the implementation of warhead profiling by the toolbox presented here might be a reasonable approach to facilitate TCI programs.



FROM ORTHOSTERIC AND ALLOSTERIC MODULATORS TO DUALSTERIC/BITOPIC LIGANDS: A NEW MOLECULAR ALLIANCE AT CB2 RECEPTOR

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G protein-coupled receptors (GPCRs) are characterized by low subtype selectivity and by multiple active states responsible of distinct functional outcomes following by the binding of both allosteric and orthosteric ligands (biased signaling). In order to engender subtype and functional selectivity, dualsteric/bitopic ligands have been developed by linking the orthosteric and allosteric pharmacophoric units using specific spacers. This strategy offers access to GPCR modulators with a unique receptor-subtype and signaling selectivity profile and, as a consequence, to drugs with fewer side effects. Focusing specifically to cannabinoid CB2 and CB1 receptors (CB2R and CB1R), our group already reported that the co-treatment of the dual target CB1R/CB2R orthosteric agonist **FM6b** (Figure 1) [1] with the CB2R positive allosteric modulator (PAM) **EC21a** (Figure 1) [2] improves the anti-inflammatory effect in the modulation of the release of pro- and anti-inflammatory cytokines in lipopolysaccharides (LPS)-activated mouse BV2 microglial cells, compared to the treatment of **FM6b** alone [3]. On the basis of these results, we synthesized a new class of compounds, **A1**-**A8**, potentially able to bind to both allosteric and orthosteric sites simultaneously linking the pharmacophoric portion of **EC21a** with that of **FM6b** (Figure 1). Among all the compounds of the series, **A1** (**FD22a**) (Figure 1) showed to be the most promising according to functional assays such as beta-arrestin, GTPgammaS and cAMP and *in vitro* tests regarding neuroinflammatory activity on BV2 cells. **A1** (**FD22a**) was then furtherly studied in the same model of neuroinflammation using HMC3, a microglial human cell line, and also *in vivo* in a murine model of neuropathic pain. **A1** (**FD22a**) paves the way to the bitopic modulation within the Endocannabinoid System being the first dualsteric/bitopic ligand of the CB2R.

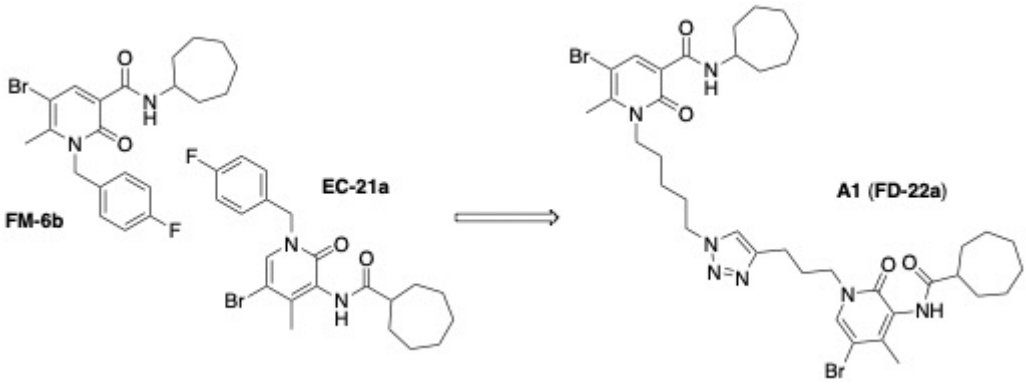

Figure 1. Chemical structure of **FM6b**, **EC21** and **A1 (FD22a)**.

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SYNTHESIS AND IN VITRO EVALUATION OF NOVEL G6PD INHIBITORS.

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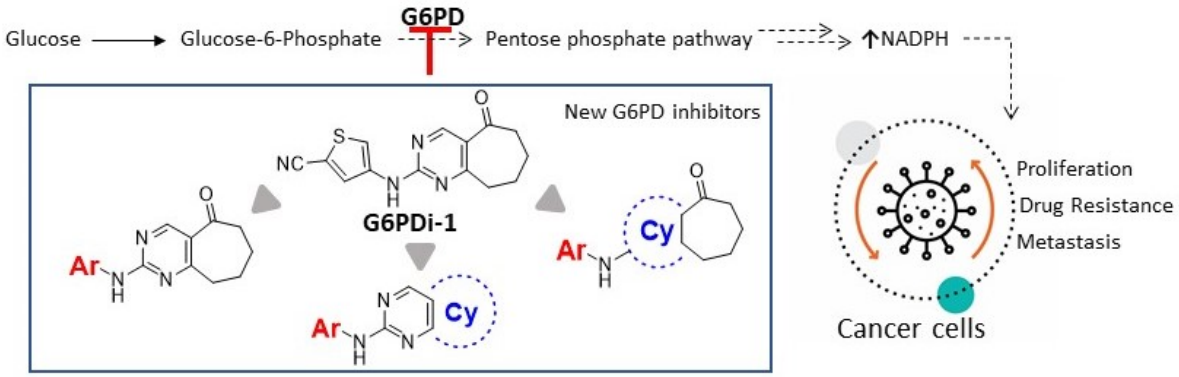
The pentose phosphate pathway (PPP) supports cancer cell survival and growth by generating pentose phosphate for the synthesis of nucleic acid and nicotinamide-adenine dinucleotide phosphate (NADPH), a molecule involved in reductive biosynthesis reactions (e.g. fatty acid synthesis) and redox homeostasis, both processes that promote cell survival under stress conditions. In cancer cells, PPP sustains malignant proliferation, angiogenesis, and protects from apoptosis [1]. Enhanced PPP activity and higher expression levels of the enzymes involved in the PPP metabolic pathway have been detected in invasive and aggressive cancers and correlated with the onset of chemoresistance [2]. The first and principal enzyme of PPP is glucose-6-phosphate dehydrogenase (G6PD) that produces NADPH. Aberrant activation of G6PD leads to enhanced cell proliferation and adaptation in many types of cancers. Based on this evidence, we developed a promising series of G6PD inhibitors designed accordingly to the structure of the G6PDi-1, a non-steroidal small molecule inhibitor of G6PD shown to effectively impair the oxidative branch of the PPP at micromolar concentration [3]. The results collected in ovarian cancer cells (SKOV3) show that these compounds decrease NAPDH cellular levels as low as 2 hours following treatment, starting from 0.1 μM. In line with this observation, the absolute flux of the oxidative branch of the PPP measured using radioactive CO_2 was significantly reduced by all the compounds tested and within a range similar to that of the G6PDi-1. These promising results warrant further study in *in vivo* preclinical models to assess their pharmacological and pharmacodynamical profile. However, they suggest that the small G6PD inhibitors synthesized represent an attractive tool for the design and synthesis of innovative drugs that could be used in cancer, particularly in those subsets characterized by enhanced PPP.



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RATIONAL DESIGN OF LIGHT-CONTROLLED BIOACTIVE COMPOUNDS FOR PHOTOPHARMACOLOGY

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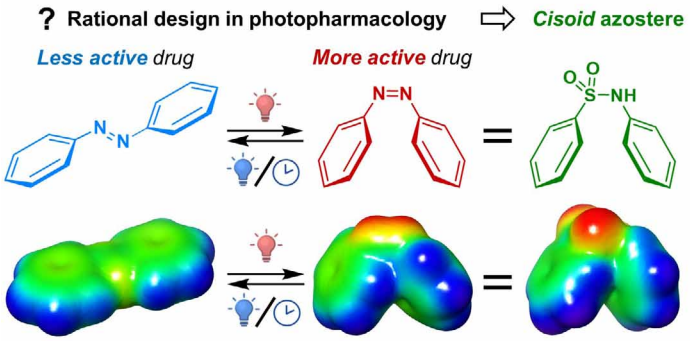
Photopharmacology employs light to control the bioactivity of drugs with exceptional bio-orthogonality and spatiotemporal precision, thus potentially increasing site- and organ-selectivity in pharmaceutical applications.[1–3] The incorporation of molecular photoswitches (such as azobenzenes) into drugs enables this non-invasive control, since their irradiation induces reversible changes in the structure and properties of the drug.[2] In most applications, photopharmacology aims to design photoswitchable drugs that are more active in the metastable state, i.e. “cis-on”[4] in the case of classical azobenzenes. In particular, if this property is combined with a large difference in potency between the photoisomers, it ensures effective control of biological processes.[5]

Here we present the description of general criteria for the rational design of cis-on photoswitchable drugs. To explore the requirements for molecular similarity with cis-azobenzene, we have analyzed the geometrical and electrostatic properties of two-atom-linked biaryl systems. The biaryl sulfonamide motif was selected as an example of this approach because of its bent geometry and favorable dipole moment, as well as its high incidence in medicinal chemistry. Azologization of cisoid substructures may provide a rich source of inspiration for photopharmacology and has the potential to guide the rational design of light-controlled bioactive compounds.



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SYNTHESIS, DEVELOPMENT AND EVALUATION OF A THERAPEUTIC PEPTIDE CONJUGATE TO PROTECT BIOMATERIALS FROM UNDESIRED IMMUNE ATTACK

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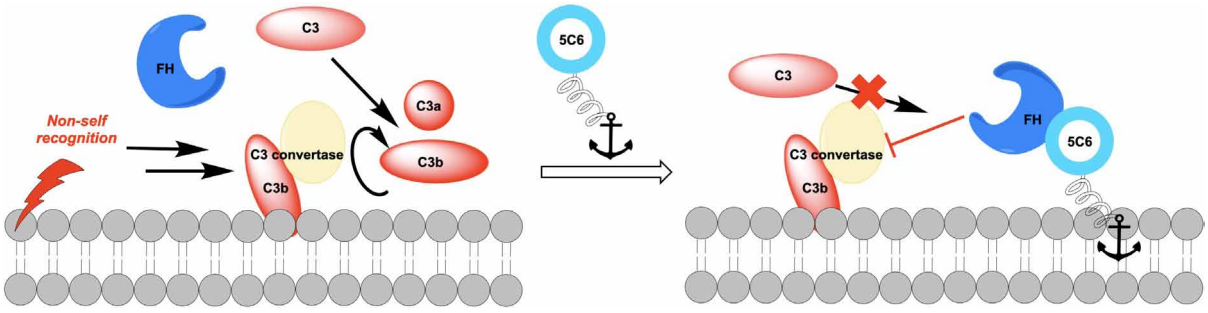
Although important progress has been made to protect biomaterials such as transplants, implants or liposomes from undesired host immune recognition and attack, several problems remain unsolved. One of these is the involvement of the complement system, the humoral part of innate immunity. It broadly and swiftly recognises non-self surfaces, leading to direct cell damage and induction of the adaptive and cellular innate immune system. In order to restrict complement activation to non-self or degenerated surfaces, the organism tightly controls complement through regulators in solution and on surfaces. One promising approach to protect biomaterials from immune attack, inspired by microbial immune evasion, is to specifically recruit these large molecular complement regulators with small to medium-sized molecules to surfaces and to prevent complement attack in situ.

Pursuing this idea, a disulphide-bridged cyclic peptide (5C6) was previously discovered by our group through phage display screening. 5C6 showed nanomolar binding affinity to the plasma-borne, major complement regulator Factor H (FH). Attractively, FH inhibits complement's central self-amplificatory C3 convertases where all three activation pathways converge. 5C6 could reduce complement activation when combined with appropriate tethering motifs by acting as a bridge between FH and model surfaces [1,2].

Based on this initial hit, comprehensive structure-activity relationship studies were conducted in which all residues having been identified for being important for the FH-5C6 interaction were investigated through replacement with commercially available or tailor-made building blocks. Furthermore, changes in the macrocycle size and efforts to replace the disulphide by a more bioinert functional group allowed us to further improve the properties of 5C6. Moreover, the ideal position for the tether conjugation was determined by ELISA and biophysical methods. Finally, functional tests in clinically relevant models are currently being undertaken to assess the translational significance of these findings.



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NEUROPROTECTIVE, NEUROTROPHIC AND ANTI-INFLAMMATORY ACTIVITY OF NEW SYNTHETIC DHEA NEUROTROPHIN MIMETIC DERIVATIVES

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Neurotrophins are a family of growth factors namely nerve growth factor (NGF), brain-derived growth factor (BDNF), neurotrophin-3 (NT3) and neurotrophin-4/5 (NT-4/5) that regulate proliferation, differentiation and survival of neural cells. Each neurotrophin binds to its respective high affinity Trk receptor (NGF to TrkA, BDNF and NT4 to TrkB, and NT3 to TrkC) and all neurotrophins, albeit with low affinity, to $p75^{NTR}$ receptor. Several studies highlight the relationship between neurodegeneration and changes in the expression of neurotrophins and/or their receptors. Neurosteroids play a central role in the control of survival, development and function of neurons while reduced levels in the brain have been associated with neurodegenerative diseases. Recent studies have shown that the well-known neurosteroid dehydroepiandrosterone (DHEA) presents neuroprotective effects and acts as a neurotrophic factor in the brain by interacting with the neurotrophin receptors TrkA and $p75^{NTR}$ [1]. Considering that DHEA is metabolized in humans into estrogens and androgens and long-term administration could increase the risk of cancer development, we embarked on the synthesis of new analogues aiming to improve the neuroprotective and antiapoptotic activity of the parent molecule, without the undesired hormonal side effects [2].
In the present work, the synthesis of chiral C17-spirocyclopropyl substituted DHEA derivatives will be described. The C17-spiro cyclopropyl moiety was decorated by a variety of pharmacophore groups through Horner-Emmons, Wittig, Cross-Coupling and Click reactions, while, hit-to-lead optimisation studies were performed on the most prominent first generation derivative. The new compounds were evaluated for their ability to activate TrkA and TrkB receptors in addition to the respective downstream signalling pathways. Furthermore, they were assessed for neuroprotection in PC12 and NIH-3T3^{TrkB} cells against serum-deprivation induced cell death. Selected agonists were evaluated for their ability to promote survival of mature neurons, as well as proliferation and differentiation of neural stem cells. In addition, the protective effects of the compounds on neuronal cells or neural stem cells against toxic Aβ oligomers was studied. Finally, the compounds' ability to attenuate the LPS-induced inflammatory responses in primary murine microglia was also examined.

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This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 765704 (www.euroneurotrophin.eu).

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INHIBITION OF THE PROTEIN–RNA INTERACTION OF LIN28 AND LET-7 WITH TRISUBSTITUTED PYRROLINONES

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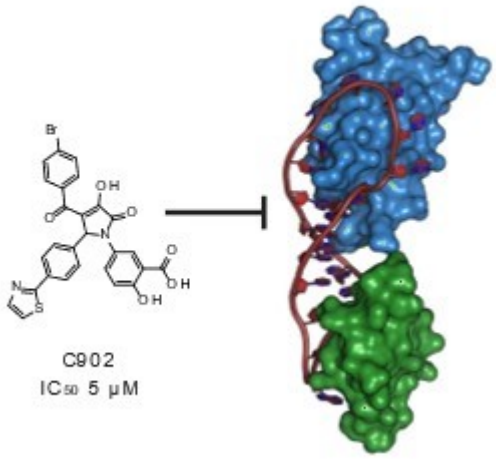
RNA binding proteins are emerging targets in chemical biology since they are involved in the regulation of biogenesis and metabolism of coding and non-coding RNAs. The RNA binding protein LIN28 plays a central role in promoting cellular pluripotency by modulating biogenesis of the miRNA *let-7*, which is a regulator of cell differentiation. Binding of LIN28 blocks maturation of the *let-7* miRNA and promotes degradation of precursor *let-7*. Importantly, dysregulation of the bistable switch formed by LIN28 and *let-7* is observed in human cancers and associated with poor prognosis. Thus, we are using different approaches to develop new small-molecule inhibitors to disrupt the disease-associated interaction of LIN28 and *let-7*. In one such approach, we performed a fluorescence polarization-based high throughput screening and identified a series of trisubstituted pyrrolinones as new inhibitors of LIN28–*let-7*. The inhibitory activity of the most potent compound (C902) was validated in an electrophoretic mobility shift assay and nano differential scanning fluorimetry assaying thermal stability. A cellular assay measuring levels of mature *let-7* in JAR cells showed an increased level of mature *let-7* upon treatment with C902. Furthermore, structure-activity relationship analysis revealed important structural features for LIN28–*let-7* inhibition.[1]



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NOVEL CLASS OF ANTINECROPTOTIC INHIBITORS: SYNTHETIC APPROACH AND IN VITRO ASSAYS

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Necroptosis is a regulated form of cell death that is associated with several inflammatory and degenerative diseases. The identification of necroptosis' pathway mediators, such as receptor interacting protein kinase-1 (RIPK1), and the prevalence of this type of cell death in critical human illnesses, led to an intensive, but yet unaccomplished, search for high quality necroptosis inhibitors.[1]

To find novel necroptosis inhibitors, a high-throughput cell-based phenotypic screening was performed in the iMed.ULisboa. A group of novel RIPK1 inhibitors**,** with a IC_{50} in the low micromolar range, were identified (Figure 1).[2]

Aiming to modulate the interaction with the RIKP1's binding pocket, a library of necroptosis inhibitors based on the hits, was prepared and studied in enzymatic and cell-based assays (Figure 1). The diversified groups introduced allowed the understanding of the structural requirements for activity and the development of potent RIPK1 and necroptosis inhibitors.

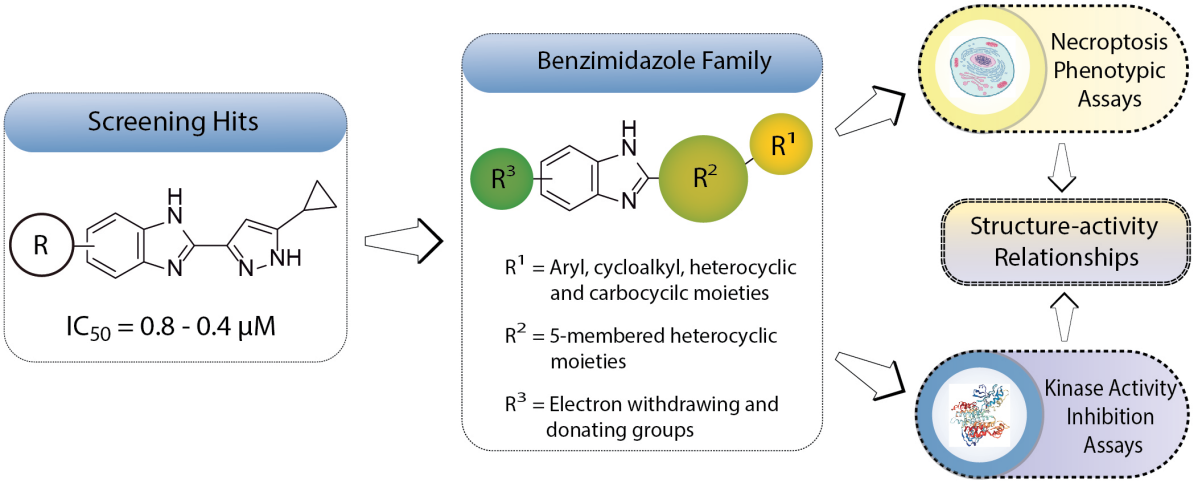

Figure 1: A library of necroptosis inhibitors, based on novel RIPK1 inhibitors, was synthesised and studied in enzymatic and *in vitro* assays leading to structure-activity relationships.

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AZAAURONES AS NOVEL CHEMOTYPES AGAINST MYCOBACTERIUM TUBERCULOSIS: SAR, ADME PROFILING AND PHOTO-SWITCHING PROPERTIES

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Tuberculosis (TB) is a deadly disease caused by a single infectious agent, *Mycobacterium tuberculosis* (*M.tb*). The complexity and duration of the treatment lead to misuse and low compliance by patients, increasing 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]
A family of azaaurone-based derivatives, from a chemical library developed in iMed.ULisboa, revealed to be active against *M.tb*, including multidrug- and extensively drug-resistant tuberculosis from clinical isolates, at a submicromolar level. [3] Despite the promising activities, this new scaffold displayed poor ADME properties. We now report the complete SAR exploration and ADME profiling of newly synthesized derivatives. Along with an enhanced metabolic stability and solubility, rings A and B as well as *N*-substitutions were extensively explored. (Figure 1) The double bond within the scaffold was also reduced to a single bond, generating a new family of saturated azaaurones. Furthermore, the *E* and *Z* isomers were isolated, allowing a differential study of each and revealing biological- and photo-conversion.

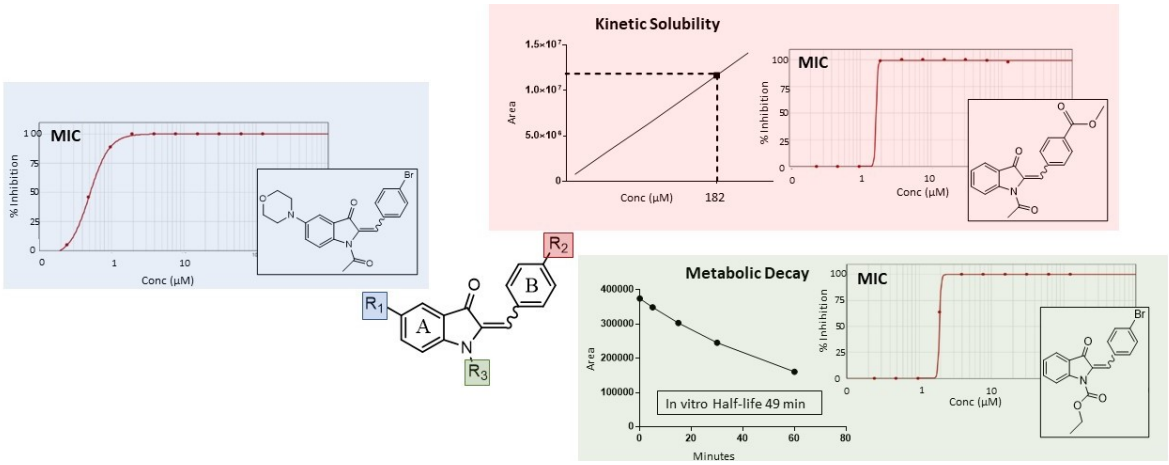

Figure 1 Azaaurones as potent antitubercular derivatives.

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COMPARATIVE STUDY OF CK1-CRBN AND CK2-CRBN COMPLEXES USING COMPUTATIONAL METHODS

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Casein Kinase 2 (CK2) is a validated target for the treatment of cancer and despite the efforts to selectively inhibit this kinase, only one compound, CX4945 (Silmitasertib®), has reached phase II clinical trials.[1] CK2 has been one of the drug targets of interest in our research group, for the past years where we have focused our efforts on the design and synthesis of dual-targeted inhibitors against CK2 and one of its phosphorylation targets.[2,3] Our aim is to use the knowledge we have on this field to design PROTACs for the selective degradation of CK2. PROTACs (PROteolysis TArgeting Chimaeras) are heterobifunctional molecules that mediate selective degradation of a protein of interest (POI) by recruiting a given E3 ligase. In the last few years, the requirement for the formation of a stable ternary complex (E3:PROTAC:POI) and the selection of a suitable linker for efficient degradation has acquired special relevance.[4] Despite the interest of CK2 for cancer treatment, to this date only one CK2-directed PROTAC has been described that is able to produce directed degradation by interaction with $CRL4^{CRBN}$.[5] The E3 ligase $CRL4^{CRBN}$ linked to thalidomide or one of its derivates (lenalidomide and pomalidomide) is capable of ubiquitinating CK1.[6] However, despite the high structural homology with CK2, surprisingly the latter is not a substrate of this E3 ligase.[7]

Here we attempt to analyze, by means of computational methods, the dynamic behavior of CK1-CRBN and CK1-Lenalidomide-CRBN complexes and compare them to that of the corresponding CK2-CRBN and CK2-Lenalidomide-CRBN complexes. Our final aim is to understand the structural differences and dynamic behaviors in both sets of complexes that will be exploited for the design of CK2 selective PROTACs.

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PROTAC TECHNOLOGY TO INVESTIGATE DYSREGULATION OF THE UBIQUITIN-PROTEASOME SYSTEM

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Background: The Ubiquitin-Proteasome System (UPS) consists of a multiple enzymatic step process with sequential action of ubiquitin-activating enzymes (E1), ubiquitin-conjugating enzymes (E2), and ubiquitin ligases (E3). The addition of polyubiquitin chains to a target protein serves as a recognition marker for its proteolytic degradation through the proteasome. Dysregulation of this system, such as the presence of non-functional E3 ubiquitin ligases, may result in oncogenic substrates overexpression.[1] In particular, the lack of VHL E3 ubiquitin ligase in von Hippel-Lindau (VHL) disease has shown to increase cell accumulation of HIF-1α and HIF-2α transcription factors, leading to angiogenesis and tumors' high vascularization.[2] Close to 60% of patients with VHL disease develop clear cell renal cell carcinoma (ccRCC) or renal cysts.[3]

Aim: The growing evidence of HIF-2α oncogenic role fostered the development of HIF-2α antagonists.[4] In this work, we aim at inducing HIF-2α knockdown by taking advantage of the promising PROTAC Technology.[5] Proteolysis Targeting Chimeras (PROTACs) are heterobifunctional molecules that specifically bind and bring into proximity a target protein and a given E3 ubiquitin ligase. Contrary to protein inhibition, PROTACs act as degraders by using the cell's protein degradation pathway to remove specifically labeled proteins.

Methods: We designed and synthesized the warhead of our degraders, responsible for engaging HIF-2α, starting from the structure of HIF-2α antagonists. In parallel, as the HIF-2α binding site is completely buried, we conducted molecular modeling studies to identify linker attachment points allowing the binding affinity retention. We selected polyethylene glycol (PEG) linkers to connect the warhead to the corresponding E3 ligase binder, responsible for recruiting CRBN and MDM2 E3 ligases.

Results and conclusion: PROTACs *in vitro* activity has been accessed by multiple functional assays in RCC cells lacking VHL. In particular, our degraders showed activity in the nanomolar range after 4h treatment by western blot. In perspective, we plan proteomic analysis to quantitatively study protein expression in cells and we consider performing *in vivo* studies. So far, we demonstrated that our first class of degraders targeting HIF-2α are effective in restoring dysregulation of the Ubiquitin-Proteasome System (UPS) due to the lack of VHL.

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TOWARDS PROTAC-MEDIATED DEGRADATION OF CBP/EP300

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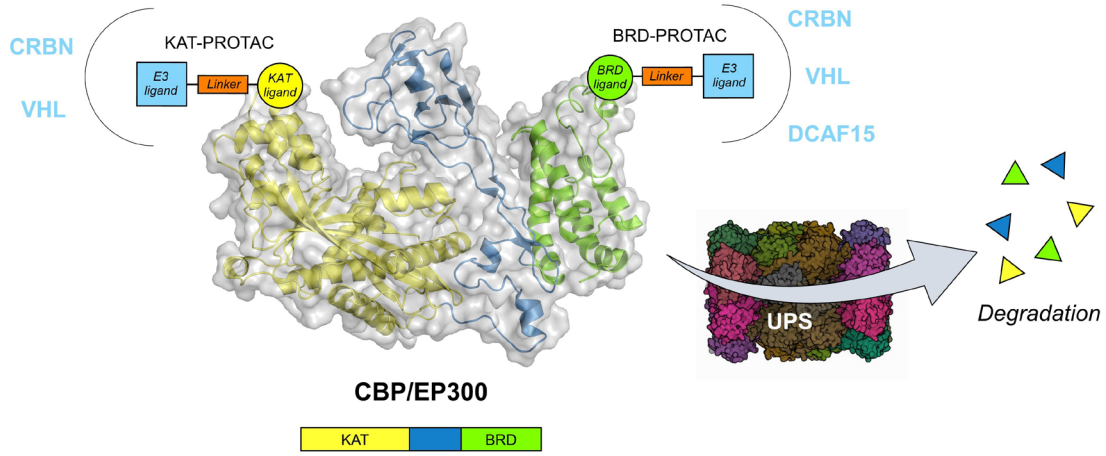
CREB Binding Protein (CBP) and EP300 are two proteins with a high sequence homology that participate in chromatin remodelling and act as co-activators of several transcription factors.[1] Their lysine acetyl transferase domain (KAT) and bromodomain (BRD), which respectively acetylate and bind acetylated lysine residues on histone tails and other proteins, are crucial for these functions.[2] CBP/EP300 are often overexpressed, mutated or dysregulated in cancer, making them extremely interesting targets for medicinal chemistry.[1,2,3]

After developing CBP/EP300 bromodomain-binding molecules with sub-micromolar potency and promising bioavailability,[4] we now aim to elucidate the contribution of CBP/EP300 to gene regulation by inducing their degradation through PROteolysis TArgeting Chimeras (PROTACs). PROTACs consist of a binder for a protein of interest (POI) connected to an E3 ligase ligand through a linker. With a catalytic mechanism, PROTACs promote the formation of a ternary complex with the POI and the E3 ligase leading to polyubiquitination of the POI and consequent proteasome-mediated degradation. In such way, PROTACs enable loss of function of the whole protein independently from the bound domain.[5]

Starting from the analysis of X-ray crystal structures of our in-house[4] and other published probes[6] in complex with CBP/EP300, we have designed and synthesised a series of bifunctional compounds with the ability to simultaneously bind either the BRD or KAT domain and a E3 ligase. Empirical exploration of several linkers and E3 ligands allowed optimisation of the probes' potency, cell permeability and target engagement. Binding affinity and ternary complex formation have been measured with biochemical assays while cellular target engagement, degradation and phenotypical effects were evaluated in multiple cell systems. In conclusion, we developed a library of compounds with some valid candidates to enable CBP/EP300 degradation as a powerful therapeutic alternative to classical protein inhibition.



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HIGH-AFFINITY GLYCOMIMETIC LIGANDS FOR HUMAN SIGLEC-8

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We present here the identification of glycomimetics with low μM affinity towards the human Siglec-8. Siglec-8 is an immunoglobulin-type lectin solely expressed on eosinophils and mast cells, and weakly on basophils. Many pathological conditions are associated with altered functions and/or numbers of these cells, among which allergic inflammation and asthma[1]. Despite the only partially known biological mechanism of action, the pharmacological importance of Siglec-8 has been demonstrated as eosinophil apoptosis and inhibition of mast cell degranulation could be achieved by means of anti-Siglec-8 monoclonal antibodies or synthetic glycopolymers decorated with Siglec-8 ligands[2]. However, no small molecules targeting Siglec-8 have been described so far. Such molecules could be useful to better elucidate the apoptotic cellular pathway and potentially provide a new pharmacological approach for eosinophil and mast cell associated diseases.

The glycan epitope recognized by Siglec-8 is the tetrasaccharide 6'-sulfo sialyl Lewis^x (6'S-sLe^x)[3]. While the sialic acid carboxylate and the sulfate group on the galactose are involved in two crucial salt bridges, fucose and glucosamine show minor contributions to binding. Therefore, not surprisingly, we discovered that the related disaccharide Neu5Ac-Gal6S represents the minimal binding epitope (Fig. 1). This disaccharide served as lead compound for our search of new ligands with improved affinity and drug-like properties. In addition, it has been recently reported that sulfonamide modifications at the 9-position of the sialic acid moiety lead to compounds with increased activity[4].

Applying different strategies, such as Gal6S replacement with non-carbohydrate moieties, bioisostere modifications, and extension of the glycerol side chain (Fig. 1), we synthesized a new series of glycomimetic structures. The best representative exhibits a low μM affinity, *i.e.* an almost 20-fold improved affinity compared to tetrasaccharide 6'S-sLe^x. ITC measurements revealed that binding of 6'S-sLe^x is punished with a substantial entropic penalty, whereas the disaccharide mimetics exhibit beneficial entropic and enthalpic contributions.

Our study made available potent small-molecule Siglec-8 antagonists, which can be used to further explored the biological role of Siglec-8.

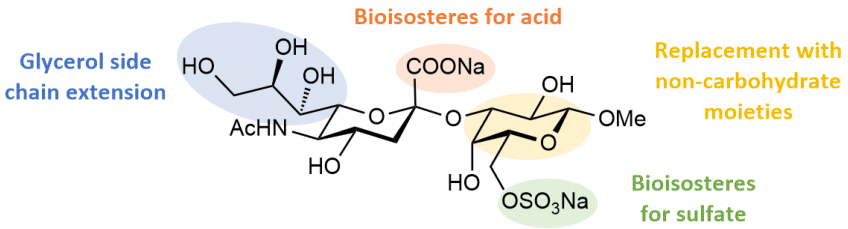

Figure 1. Chemical structure of Neu5Ac-Gal6S and the various modifications exploited for the discovery of high-affinity ligands.

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OPTIMIZATION OF COMPOUND METABOLIC STABILITY VIA SHAP

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Metabolic stability is very important parameter to be considered during the design of new compounds with potential biological activity. However, due to the great complexity of processes of xenobiotic tranformation occurring in the living organisms, prediction and in silico evaluation of metabolic stability is very difficult.

In the study, we developed a metodology for metabolic stability evaluation together with indication of structural features influencing this parameter. It is based on three machine learning algorithms (Naive Bayes, SVM, and trees) and uses predictive models constructed on metabolic stability data (expressed as half-lifetime) gathered in the ChEMBL database (human and rat data are considered). Compunds are represented using two key-based fingerprints: MACCSFP and Klekota&Roth Fingerprint. After prediction of compound half-lifetime, SHAP values are calculated. They inform about the contribution of particular structural moieties to the model output, which enables detection of substructures that can be important during compound metabolic stability optimization.

In order to enable the usage of methodology by wide community, we prepared a web service, which is available at https://metstab-shap.matinf.uj.edu.pl/. It enables detailed analysis of the results obtained on the ChEMBL data, as well as SHAP-based evaluation of compounds submitted by the user. The constructed tool can be of great help to medicinal chemists, assisting in the metabolic stabolity optimization of ligands.

Acknowledgments

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SYNTHESIS AND BIOLOGICAL EVALUATION OF NOVEL VARIOUSLY SUBSTITUTED 3-BENZYL-QUINOLIN-2(1H)-ONES AS POTENT AGONISTS OF THE GPR55 RECEPTOR

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GPR55 is a G protein-coupled receptor highly expressed in microglia cells that has recently attracted attention for its implication in neuroinflammatory processes, although its precise role is not yet fully understood. Initially included among the orphan receptors, it has been subsequently proposed as the type-3 cannabinoid receptor (CB_3 R), even if its categorization is still being studied. Recent evidences highlighted a neuroprotective effect mediated by GPR55 agonists in neural stem cells.[1] On the other hand, another recent study[2] suggested an anti-neuroinflammatory effect exerted by GPR55 antagonists/inverse agonists.[3]

Given the limited number of GPR55 modulators reported in literature, we focused our work on the development of novel and selective GPR55 ligands, which could be potentially useful as tool compounds to validate this receptor as a therapeutic target and to explore its physio-pathological role in neuroinflammation.

Starting from 3-benzylcoumarin derivatives recently reported as GPR55 antagonists/inverse agonists (general structure **A**, *figure 1*),[3] we designed and synthesized a novel series of 3-benzylquinolin-2(1*H*)-ones (general structure **B**, *figure 1*) in which the lactone group of the central coumarin scaffold was replaced by an amide moiety and including other structural modifications in the peripheral substitution pattern. Some of the new derivatives showed very high affinity (with K_i values in the low nanomolar range) for GPR55 and almost complete selectivity over CB_2 receptor. Interestingly, these structural changes shifted the functional activity to pure agonism, making some of the new compounds among the most potent GPR55 agonists developed to date. We also expanded the set by introducing additional modifications on the substituents at certain positions of the 3-benzylquinolin-2(1*H*)-one scaffold, in order to deepen the structure-activity relationships about this type of molecules and provide further information about the GPR55 binding site.

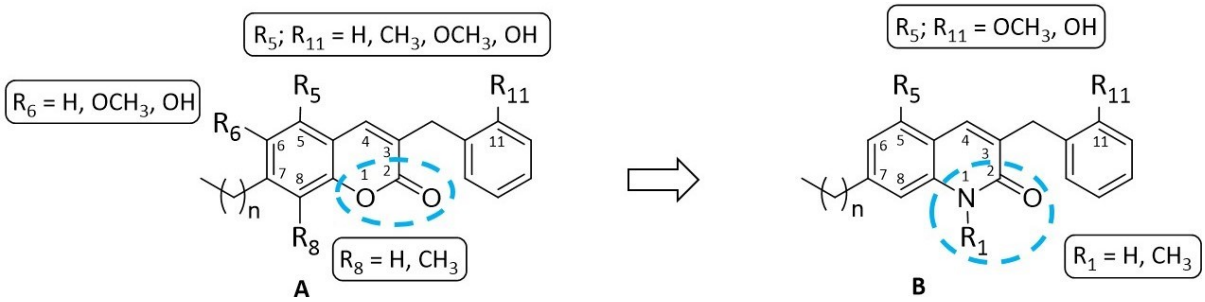

Figure 1. Structural modifications providing the novel series of 3-benzylquinolin-2(1*H*)-ones (**B**) from 3-benzylcoumarins (**A**).

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IN VIVO PHOTOCONTROL OF ADRENERGIC NEUROTRANSMISSION

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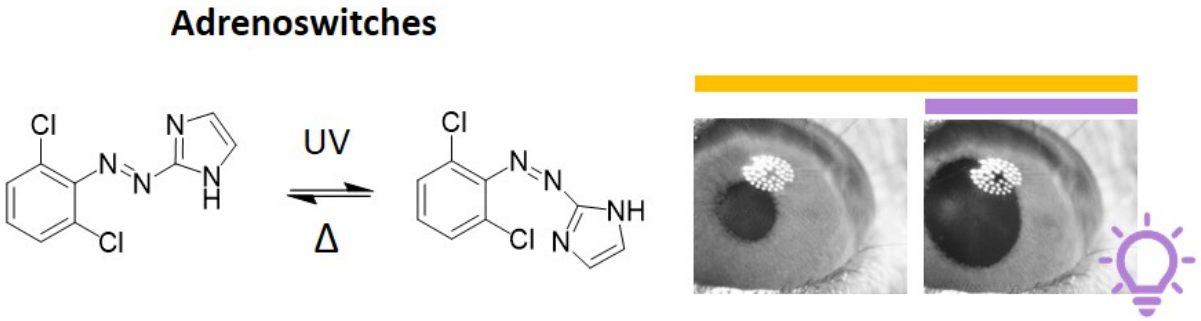
Adrenoceptors are ubiquitous and regulate most vital functions in the human body, including heart and respiratory rate, digestion, smooth-muscle contraction, gland secretion, and pupil diameter among others. In addition, adrenergic neurons firing from the locus coeruleus towards different areas of the central nervous system mediate alertness, responses to acute stress and danger, pain modulation, arousal, sleep-wake cycles, as well as neuroplasticity and cognitive behaviour. Despite the physiological relevance of adrenergic neurotransmission, molecular methods to precisely modulate the activity of endogenous adrenoceptor and to functionally dissect their pathways *in vivo* are not available.

Here we present a set of photochromic ligands, that we call adrenoswitches, to switch on and off adrenoceptor activity with high spatio-temporal resolution. Using a non-canonical azologization approach, we have designed novel arylazoheteroarene units that we have characterized *in vitro* and in two animal models (zebrafish locomotion and pupillary reflex in mice). The drug-like properties of these molecules, their efficacy and absence of acute toxicity in zebrafish larvae, and most remarkably the fact that specific adrenergic photomodulation was readily and reversibly achieved in the mammalian eye by topical application without formulation, all indicate that adrenoswitches could be a disruptive tool to dissect physiological adrenergic signaling and to develop safe and effective therapies. For example, photocontrol of adrenoceptors at specific locations might allow to single out individual adrenergic projections from the locus coeruleus, or to selectively decouple pupil tone from environmental illumination.



DESIGN AND SYNTHESIS OF NOVEL ANDROGEN RECEPTOR SPLICE VARIANT-7 PROTACS FOR THE TREATMENT OF CASTRATION-RESISTANT PROSTATE CANCER

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The field of proteolysis targeting chimeras (PROTACs) has been rapidly expanding over the last two decades thanks to its promise for the discovery and development of completely new therapeutic interventions focused on the elimination of disease-causing proteins.[1] PROTACs are hetero-bifunctional compounds that bind simultaneously to a protein of interest (POI) and an ubiquitin E3 ligase by means of two small molecules ligands connected by a chemical linker.[2] The forced close proximity of the target protein and the E3 ligase triggers the POI polyubiquitylation and thus its subsequent proteasomal-dependent degradation.[2] To date, PROTAC technology has been applied to multiple proteins in a variety of cancer types and other diseases.[1,2]

Herein, we decided to exploit the targeted proteasomal-dependent degradation of the androgen receptor splice variant 7 (AR-V7) in order to identify an innovative treatment for lethal prostate cancer. Indeed, although second-line antiandrogen therapy (SAT) is the standard of care in men with castration-resistant prostate cancer (CRPC), resistance inevitably occurs.[3] One of the major mechanism of resistance to SAT involves the emergence of androgen-receptor (AR) splice variants, such as AR-V7, which are constantly activated and lack the AR domains that are targeted by existing AR-directed therapeutics.[4]

In the present study, the design, synthesis, and *in vitro* biological characterization along with preliminary pharmacokinetic studies of a series of AR-V7 degraders will be presented.

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THE E3 PLATFORM: AN INVESTIGATION OF THE 3D POCKET SPACE FOR PROTAC DESIGN

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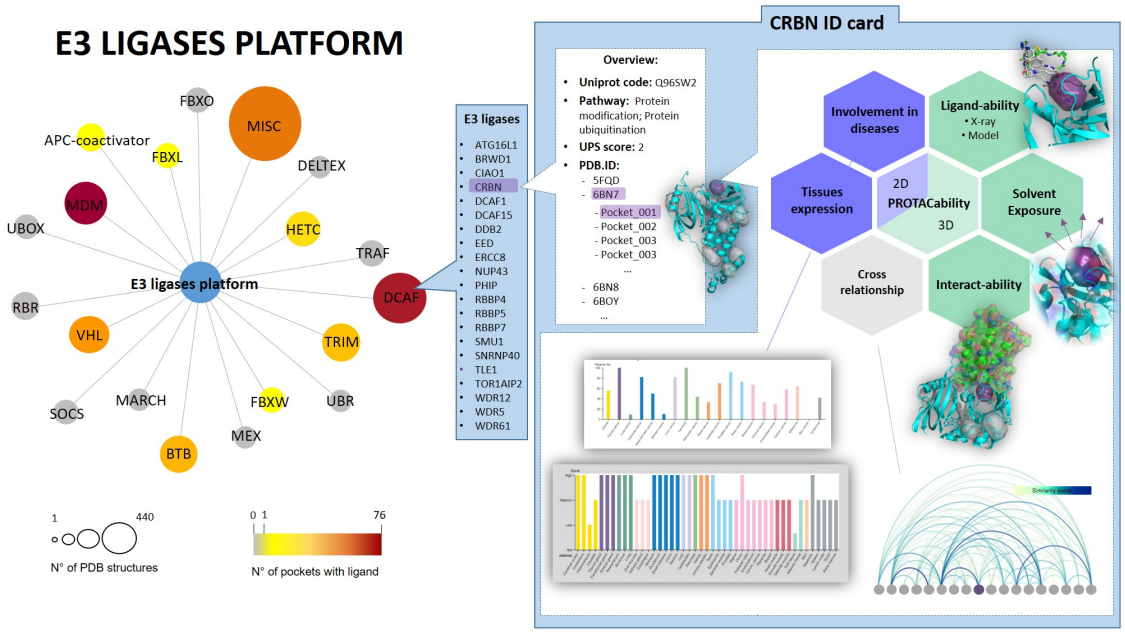
In recent years, targeted protein degradation through the ubiquitin-proteasome system (UPS), has emerged as a novel therapeutic strategy in drug discovery. The ubiquitin E3 ligase plays a key role in the ubiquitination process by dictating the specificity towards the target protein which is to be degraded. Exploiting the enormous potential of the E3 ligases, the PROTAC (Proteolysis-targeting chimeras) technology was born. This is based on the use of small molecules, specially designed, to promote the interaction between a specific E3 ligase and the desired target protein to be degraded. Although there are already many examples of well-functioning PROTACs, it should be noted that these exploit only 1% of the available E3 ligases. Indeed, about 10 (especially CRBN and VHL) of the 632 human E3 ligases have been used for the design of new PROTACs for targeted protein degradation.The aim of this study is to develop a navigable E3 ligases platform to increase knowledge about this class of proteins, enabling selection of new E3 ligases and new ligands for the design of new PROTACs which are more powerful and selective towards the desired target. In particular, the E3 ligases are represented via an identity card combining 2D and 3D descriptors. The former comprise collected meta-data such as the expression in tissues and involvement in pathologies of the human organism while the latter are generated with a computational approach in order to characterize all identified cavities in the analyzed E3 ligase X-ray structures and provide innovative information. To build the platform all E3 ligase structures available in the PDB were collected. For each structure, using the FLAP algorithm[1], all the pockets were identified and these were characterized with a score, named “PROTAC-ability score”, enabling the identification of pockets that are theoretically more predisposed than others, for the design of ad hoc PROTACs. This score was generated by combining three different calculated 3D descriptors and using ligases for which PROTACs are already known its reliability was confirmed. Moreover, other computational analysis were performed a) to find cross-relationships across the whole space of the E3 pocketome and b) to explore drug-repurposing opportunities by comparing them with known ‘liganded’ targets.



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A NOVEL COVALENT APPROACH FOR STABILISING 14-3-3σ PROTEIN-PROTEIN INTERACTIONS

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The 14-3-3 protein family play important roles in maintaining normal cell function through interaction with over 200 partner proteins which are involved in human diseases[1,2]. For this reason, 14-3-3 protein-protein interactions (PPIs) have enormous potential as drug targets. In particular, stabilisation of certain 14-3-3 PPIs can lead to considerable physiological effects. A limited number of 14-3-3 PPIs stabilisers have been reported so far with drawbacks in terms of synthetic tractability, drug-likeness or their poor selectivity towards the seven human 14-3-3 isoforms. Therefore, it is crucial to identify and develop novel 14-3-3 stabilisation mechanisms that may circumvent such drawbacks.

This poster will describe our approach of stabilising two distinct 14-3-3σ interactions with the tumour suppressor p53 and the estrogen receptor α (ERα) via covalent modification of 14-3-3σ. Notably, a clinically approved radioprotective prodrug called Amifostine is dephosphorylated in vivo to reveal its active form, the aminothiol WR-1065[3]. It has been shown that WR-1065 acts as a scavenger for reactive oxygen species[4] and it also modulates the activity of the transcription factors NFkB, AP-1 and p53 via covalent disulphide bond formation[5]. There is also compelling evidence that WR-1065 enhances wild-type p53 activity[6] and rescues the activity of p53 mutants by modulating protein conformation[7]. According to our biophysical data and high-resolution mass spectrometry (HRMS) experiments, WR-1065 specifically binds to 14-3-3σ by forming a disulphide bond with a specific cysteine residue on the human protein, stabilising its interaction with p53 and ERα. Furthermore, WR-1065 enhances the effect of the known natural small molecule stabiliser FC-A[8], which points to a novel mechanism of action. These findings were confirmed by crystal structure which provides a structural basis for p53/14-3-3σ stabilization and will allow the development of a library of potential new selective and more potent small-molecule stabilisers of this specific PPI.

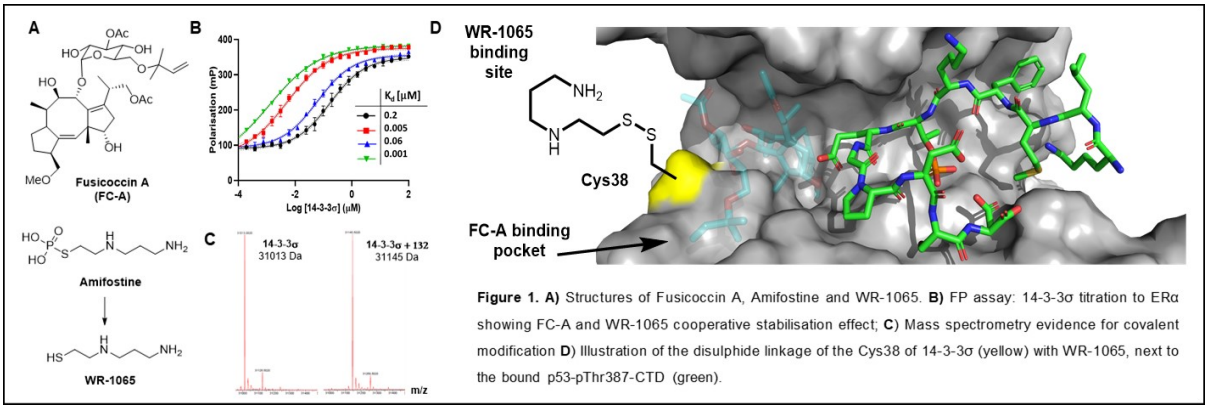

Figure 1. A) Structures of Fusicoccin A, Amifostine and WR-1065. **B)** FP assay: 14-3-3σ titration to ERα showing FC-A and WR-1065 cooperative stabilisation effect; **C**) Mass spectrometry evidence for covalent modification **D**) Illustration of the disulphide linkage of the Cys38 of 14-3-3σ (yellow) with WR-1065, next to the bound p53-pThr387-CTD (green).

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TARGETING MYELOID LEUKEMIAS USING HUMAN DIHYDROOROTATE DEHYDROGENASE INHIBITORS BASED ON 2-HYDROXYPYRAZOLO[1,5-a]PYRIDINE SCAFFOLD: OVERCOMING OF METABOLIC ISSUES

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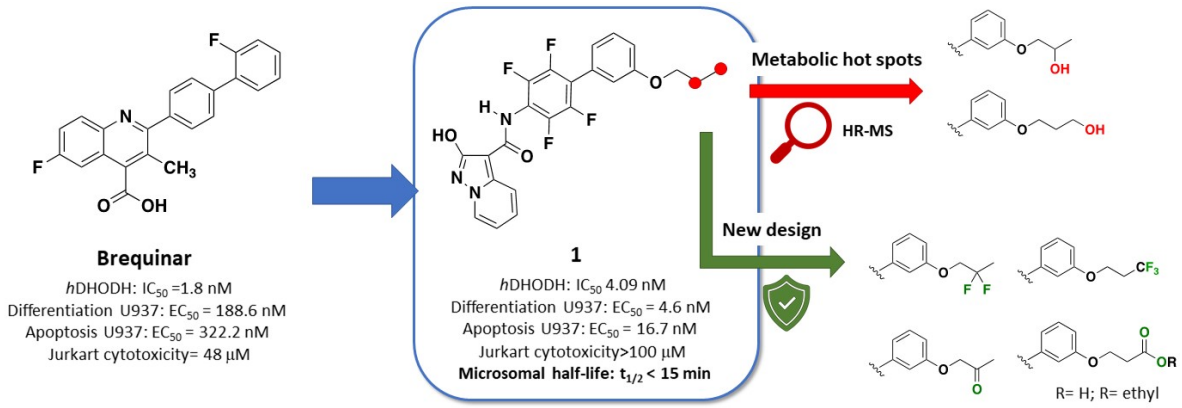
Human Dihydroorotate Dehydrogenase (*h*DHODH), a mitochondrial enzyme that plays a pivotal role in the *de novo* pyrimidine biosynthesis, has been associated to Acute Myelogenous Leukemia (AML), as *h*DHODH inhibitors are able to restore myeloid differentiation (1). In recent years, we designed potent *h*DHODH inhibitors applying a scaffold-hopping approach to *brequinar*'s structure (2). By investigating the lead compound's SAR, we recently discovered compound 1 (Figure), a candidate superior to brequinar in terms of *in vitro* potency. Unfortunately, compound 1 showed *in vitro* metabolic instability, as it soon undergoes to hydroxylation on alkoxy side chain by microsomal enzymes (3). In this occasion, we investigated on the metabolic hydroxylation site through the synthesis of the possible products of microsomal metabolism according to literature (4) and their comparison to compound 1' s own metabolite in high resolution mass spectrometry (HR-MS); then we designed a new generation of *h*DHODH inhibitors protected from metabolic oxidation on the alkoxy side chain. Design, synthesis, *in vitro*metabolism and biological characterization of the new developed compounds are here described and discussed.



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Notes

Posters
Abstracts

METAL CHELATING COMPOUNDS TARGETING SIGMA RECEPTORS AND POTENTIAL THERAPEUTIC EXPLOITATIONS

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Metals are fundamental to regulate many biological processes and their unbalance is correlated with different pathologies such as neurodegeneration and cancer. In Alzheimer disease, iron, copper and zinc ions have been found in high concentration in amyloid plaques and neurofibrillary tangles[1]. Moreover, high density of iron can decrease furin activity, producing stimulation of β secretase, rather than α secretase and increase APP translation [2,3]. Iron metabolism has been also studied in Huntington disease, where an uncorrected iron homeostasis regulation has been found[4]. In Parkinson disease high levels of iron have been detected in substantia nigra, temporal cortex and globus pallidus[5]. Metals play an important role also in cancer. Cancer cells are characterized by higher metabolism and DNA synthesis because of their faster growth and replication. Because of the crucial role of iron in enzymatic complexes involved in these mechanisms, alteration of iron homeostasis impacts on cancer proliferation. In our continuous effort to offer new strategies to treat such dramatic and complex pathologies, we matched antineurodegenerative and antiproliferative activities of Sigma receptors ligands together with metal chelation, which represents an ancillary activity able to improve and extend efficacy of classic Sigma receptors ligands. Sigma-1 Receptor is a chaperone protein localized at the mitochondrial-associated Endoplasmic Reticulum (ER) membranes (MAM) in association with the binding immunoglobulin protein (BiP) in a resting state. Upon agonist stimulation or Ca^{2+} depletion from ER, Sigma-1 receptors dissociate from BiP and stabilize Inositol 1,4,5-trisphosphate receptor type 3 (IP3R3) at MAM, increasing Ca^{2+} transfer from ER into mitochondria and facilitating adenosine triphosphate (ATP) production[6]. Recently, many studies proved the ability of Sigma-1 receptor to exert neuroprotective effects through different mechanisms such as intracellular Ca^{2+} regulation, prevention of oxidative stress and anti-apoptotic effects. Conversely, Sigma-2 receptor, even if lesser known than the Sigma-1 subtype, has a deeper importance in cancer disease. Indeed, Sigma-2 receptor can reduce ER stress[7] through control of Ca^{2+} release. This effect is possible because of interaction with IP3 and ryanodine receptors, activation of caspase and mitochondrial superoxide production[8] which can lead to cell death. With the aim of combining the Sigma receptors mediated cell death pathways with metal chelation effects we produced four different classes of ligands: Thiosemicarbazones, Pyridin/Pyran-ones, Salicylamides and Isoquinolinones. Structural differences among the four classes provided interesting Structure-Affinity Relationships (SAfiR) and some ligands showed good affinity for Sigma receptors and chelating properties.

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DESIGN AND SYNTHESIS OF NOVEL SELENOESTERS AND SELENOCYANATES FOR THE TREATMENT OF CHAGAS DISEASE

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Chagas disease, caused by the parasite *Trypanosoma cruzi* (*T. cruzi*) is one of the most prevalent tropical neglected diseases and causes high mortality and morbidity in endemic countries. Current treatments for this disease, nifurtimox, and benznidazole, are ineffective in the chronic phase and produce severe adverse effects. Therefore, novel therapies are urgently required. The trace element selenium (Se) has an important role in human health, due to its antioxidant, antiinflammatory, and pro-immune properties. Recently, novel Se-containing compounds with *in vitro* activity against *T. cruzi* have been described (1). Among them, selenocyanate derivatives showed the best results, with IC_{50} values in the nanomolar range and high selectivity indexes. Therefore, in this work, we have designed and synthesized a series of novel selenocyanates with potential activity against *T. cruzi*. The molecules also include a group selenoester as a strategy to potentiate the effect and introduce structural variability. The novel compounds were tested for their anti-*T. cruzi* activity against epimastigotes and their cytotoxicity was evaluated in Vero cells to establish their selectivity indexes. Furthermore, their *in vitro* activity against promastigotes of Leishmania major was also established.

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STUDY ON THE ANTIOXIDANT AND ANTINEOPLASTIC ACTIVITIES OF QUATERNIZED BENZIMIDAZOLE ARYLHYDRAZONES

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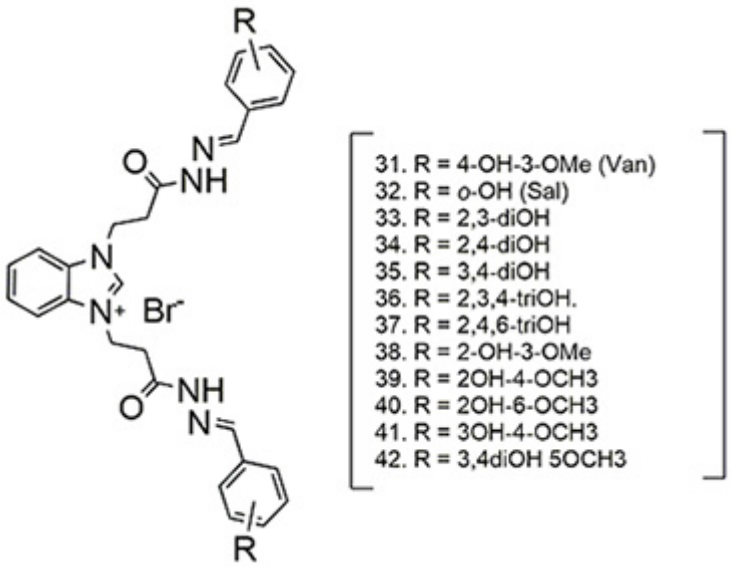
The excessive concentration of reactive oxygen species (ROS) disrupts the existing homeostatic balance and hinders the antioxidant capacity of the organism to inhibit the free radical mediated processes which inevitably leads to oxidative stress considered to be the common underlying mechanism leading to damage of proteins, cellular dysfunction and demise. This is related to serious pathological conditions, such as cancer and neurodegenerative disorders.The introduction of versatile pharmacophores in one molecule could lead to the generation of benzimidazole architectures interacting with biological targets in two different mechanisms or by demonstrating synergistic activity. In our previous studies[1] we have demonstrated that N,N'-disubstituted 2-iminobenzimidazoles possess cytotoxicity against different cancer cell lines and are capable of exhibiting antioxidant action. Herein we present the synthesis of dialkylated benzimidazole compounds containing a quaternary N-atom and hydroxy and methoxy aryllhydrazone moieties with residues of substituted that were synthesized using phase-transfer catalysis. Their antioxidant activity was assessed spectrophotometrically using the stable free radical ABTS and their protective effect against iron-induced oxidative damage was evaluated on biologically important molecules, lecithin and deoxyribose. The in vitro cytotoxicity of selected compounds was tested in concentrations ranging between 0.4 and 200 μM on a panel of tumorigenic (malignant melanoma – A-375, epidermoid carcinoma of the skin – A-431) and non-tumorigenic (normal human keratinocytes – HaCaT, normal mouse fibroblasts – CCL-1, normal transformed human embryonic kidney cells – HEK293) cell lines following ISO 10993-5, Annex C. The compounds were cytotoxic to the tumor cell lines in concentrations between 3.125 and 150 μM, wherein two of the compounds showed a lower antineoplastic activity (median inhibitory concentrations between 25 and 200 μM) then the third one (median inhibitory concentrations between 3.125 and 25 μM).



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COUPLING INTERRUPTED FISCHER AND MULTICOMPONENT UGI-JOULLIE' TO CHASE CHEMICAL DIVERSITY: FROM BATCH TO SUSTAINABLE FLOW SYNTHESIS OF PEPTIDOMIMETICS.

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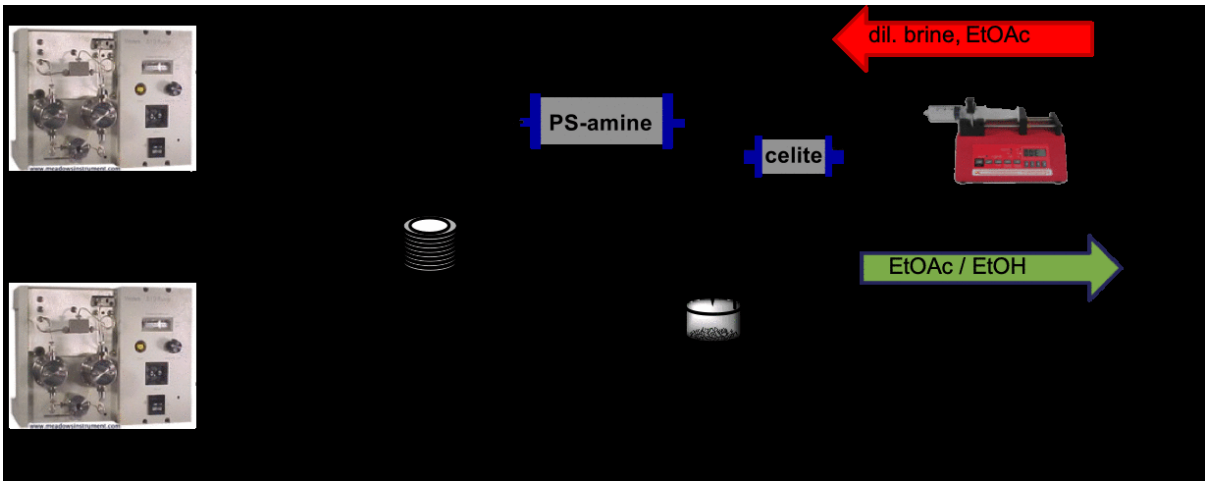
Organic synthesis is an enabling science with immense impact in many areas of research, including modern medicine and biology. The need for sustainable and green research to reduce the global carbon footprint leads to the emerging importance of "green chemistry"[1]. Among all new technologies, flow chemistry [2] in one of most important in this way. Flow chemistry can be applied to privileged scaffolds.[3] We report the application of this technology-based greener synthesis approach to the formation of privileged (spiro)indolenine and (spiro)indoline scaffolds [4]. Our flow chemistry protocol for the synthesis of 3,3-disubstituted indolenines is chemically based on interrupted Fischer indolisation reactions. The telescoped approach allowed generation of a library of indolenines and indolines with limited solvent consumption for both reactions and work-up procedures, and required minimum operator input. This newly developed protocol also displays the potential to turn into an effective coupling point for additional flow reactions for multistep syntheses.



Having development a reliable and scalable green and sustainable route to indolenines and indolines we decided to explore the use of indolenines as substrates of peptidomimetic frameworks through a multicomponent reaction.[5] The MCRs symbolize a strategy to reach eco-compatibility and eco-sustainability in the modern chemistry. Isocyanide-based MCRs (iMCR) represent the vertebral column of MCR's chemistry. We synthetized a library of compounds in batch mode to shift in flow approaches exploring the rapid library generation.

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DESIGN AND SYNTHESIS OF THE FIRST DUAL ACTIVATORS OF NEUROTROPHIN AND SEROTONIN 5-HT4 RECEPTORS, THE GAME-CHANGERS IN THE BATTLE AGAINST NEURODEGENERATIVE DISORDERS

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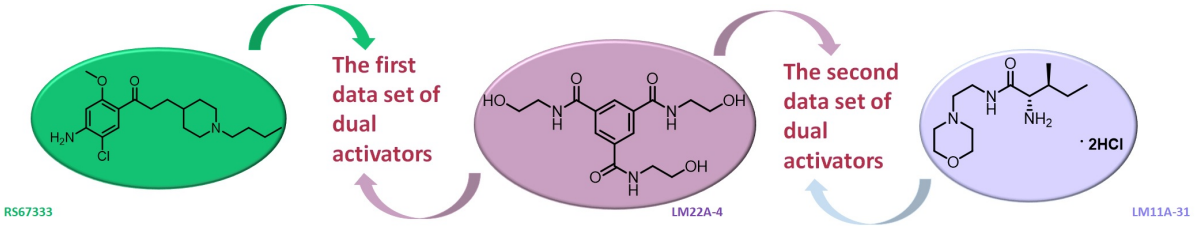
Numerous studies have been published about the implication of the neurotrophin tyrosine kinase receptor - TrkB in the pathogenesis of several neurodegenerative conditions[1]. Brain-derived neurotrophic factor (BDNF) and neurotrophin-4/5 (NT-4/5) activate the TrkB receptor with high potency and specificity, promoting neuronal survival, differentiation and synaptic function. On the other side, activation of the p75 neurotrophin receptor (a member of the tumour necrosis factor receptor family) can activate several signalling cascades. The TRAF6 (TNF Receptor Associated Factor 6) cascade which is inducing cell death and the RIP2 (receptor-interacting protein 2) cascade that propagates cell survival[2]. Based on all these findings we developed two strategies in order to design and synthesize small molecules, able to prevent neuronal death and to increase neuroregeneration. The first strategy was to use the main structural characteristics of LM22A-4[3], a known activator of the TrkB receptor, and modify it in order to obtain the compounds which will be not only ligands for TrkB receptor but also act as partial 5-HT$_4$ receptor agonists. There are evidences that the partial 5-HT$_4$ receptor agonist (RS67333) can increase the concentration of BDNF[4]. The second strategy was to modify LM11A-31[5], a molecule that is able to activate a specific cascade of the p75 neurotrophin receptor, inducing cell survival, and to merge it with TrkB activator, LM22A-4. As a result of our study, we have developed two new datasets of small molecules, potential TrkB/5-HT$_4$ receptors ligands and TrkB/p75 receptors ligands, which will be used for further biological research and hit to lead optimisation studies.



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SYNTHESIS OF NOVEL 5(6)-METHYL-2-AMINO-BENZIMIDAZOLYL HYDRAZONES AS PROMISING ANTICANCER AGENTS

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The potential of the benzimidazole architecture and its structural modification allows development of many clinically useful antineoplastic compounds. The 2-aminobenzimidazolyl pharmacophore is a key scaffold of many clinically useful chemotherapeutic drugs [1]. Moreover, combining 2-aminobenzimidazole heterocycle and hydrazine fragments has been studied and shown high activity against different cell lines. Some 2-imino-benzimidazole hydrazones were synthesized and possessed high cytotoxicity against MDA-MB-231, HT-29, HeLa and Hep G2-cell lines [2].

Microtubules are the key components of the cytoskeleton of α, β-tubulin. Three major binding sites on microtubules have been identified - taxane, vinca alkaloid and colchicine binding sites, although compounds targeting the colchicine-binding site have drawn the interest for their high anticancer potency. Some benzimidazole derivatives such as Flubendazole could selectively bind with β-tubulin and inhibit the microtubule polymerization [3]. A series of benzimidazole-2-urea derivatives were synthesized and studied their tubulin polymerization [4].

In the present study, we report the synthesis of a series of 5(6)-methyl-1H-benzimidazol-2-yl hydrazones and evaluation of their cytotoxic activity on MCF-7 (ER-positive breast adenocarcinoma) and AR-230 (chronic myeloid leukemia) cell lines. The 5(6)-methyl-2,3-dihidroxyphenyl benzimidazole-2-yl hydrazone showed the most pronounced toxic effect on the both cells. Moreover, the effect of the studied hydrazones on tubulin polymerization was evaluated *in vitro* by using highly purified bovine tubulin and compared to nocodazole and paclitaxel as reference.

Acknowledgments: Thanks are due to the National Science Fund of Bulgaria, Contract КП-06-Н39/4, for financial support of this work.

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DEVELOPMENT OF P53 ACTIVATORS TO TARGET COLORECTAL CANCER: HIT OPTIMIZATION OF TRYPTOPHANOL-DERIVED ISOINDOLINONES

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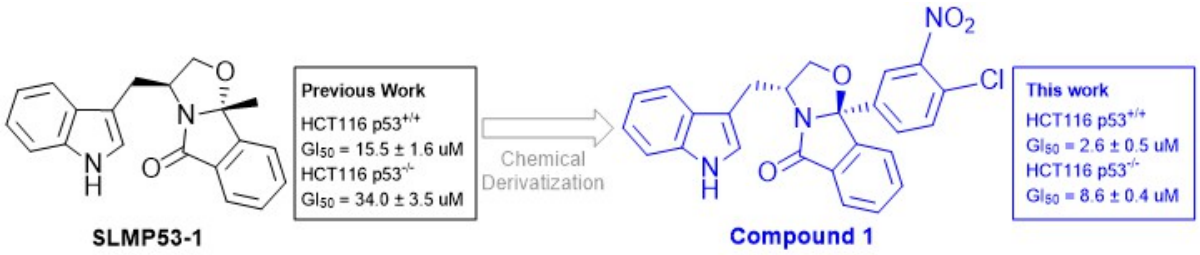
Colorectal cancer (CRC) is the third most recurrent cancer worldwide and often diagnosed in advanced clinical stage. Poor therapeutic options are available for its treatment, with surgery, neoadjuvant radiotherapy and adjuvant chemotherapy the ones with highest rate of success. The tumor suppressor p53 is a protein expressed in all types of human cancers. In 53% of CRC cases, the TP53 gene undergoes hemizygous loss and patients with mutated p53 gene gain multidrug resistance leading to therapy failure[1]. p53, in its wild-type status, is also found inactivated by its principal transcriptional targets from the murine double minute protein family (MDMs), MDM2 and MDMX. Hence, tackle full reactivation of protein p53 represents an appealing anticancer strategy[2]. In this area of research, we have identified a small molecule (SLMP53-1), reactivator of wild-type and mutant p53, with promising *in vitro* and *in vivo* p53-dependent antitumor activity in CRC[3]. In this communication, we will present our most recent results in the hit optimization of SLMP53-1, including the identification and synthesis of the Phase I metabolites identified for the most promising derivatives. The target compounds were evaluated as potential anticancer agents, showing promising anti-proliferative activities in human colorectal carcinoma HCT116 cells. From this process, we obtained a compound six-fold more active, and also more selective for HCT116 cells expressing p53 over cells without p53, and with low toxicity in normal cells (Figure 1)[4].



Acknowledgments

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NOVEL ACTIVITY-BASED PROBES AND NANOMOLAR PEPTIDOMIMETIC INHIBITORS AGAINST THE MAIN PROTEASE OF SARS-CoV-2

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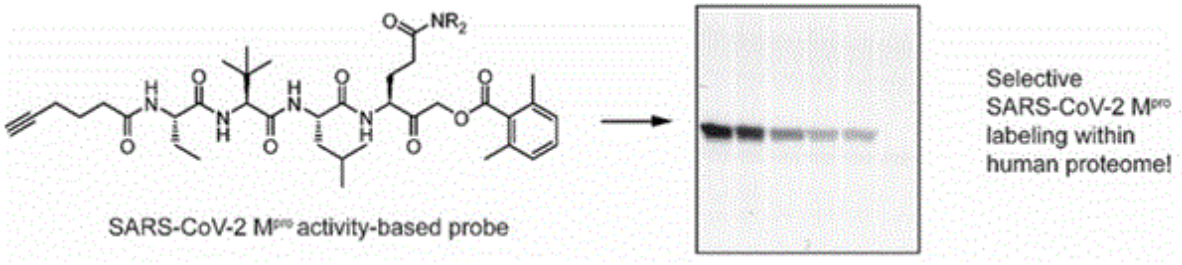
The global pandemic caused by SARS-CoV-2 calls for the fast development of antiviral drugs against this particular coronavirus. Chemical tools to facilitate inhibitor discovery as well as detection of target engagement by hit or lead compounds from high-throughput screens are therefore in urgent need. We here report novel, selective activity-based probes that enable detection of the SARS-CoV-2 main protease. The probes are based on acyloxymethyl ketone reactive electrophiles combined with a peptide sequence including unnatural amino acids that targets the nonprimed site of the main protease substrate-binding cleft. They are the first activity-based probes for the main protease of coronaviruses and display target labeling within a human proteome without background[1]. We expect that these reagents will be useful in the drug-development pipeline, not only for the current SARS-CoV-2, but also for other coronaviruses. Proof of this is our probes have enabled the discovery of a novel class of peptidomimetic inhibitors, which display nanomolar potencies against the SARS-CoV-2 main protease. Here we report the structures and antiviral activity of our new class of inhibitors and dwell into their binding mode thanks to the co-crystal structure of our most potent inhibitor with Mpro[2].



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2) Unpublished results

EVALUATION OF [1,2]OXAZOLE DERIVATIVES IN LYMPHOMA MODELS

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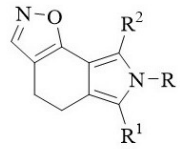
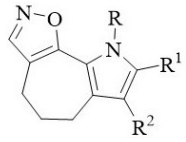
Non-Hodgkin lymphoma (NHL) is one of the most common haematological malignancy in the world, with more than 30 distinct subtypes divided into aggressive and indolent. Anti-tubulin agents are widely used in the treatment of lymphoma both alone and in combination chemotherapy regimens such as ABVD and R-CHOP.[1] The isoxazole moiety is a valuable chemical feature for the design of new tubulin-binding agents, hence we synthesized a class of new [1,2]oxazole derivatives for the treatment of different NHL subtypes.

All compounds were screened in the NCI's panel of 60 human cancer cell lines and some of them showed potent activity with GI_{50} values reaching the nanomolar level.[2,3] They were further tested in four lymphoma histotypes: germinal center B-cell and activated diffuse large B cell lymphoma (GCB-DLBCL and ABC-DLBCL), marginal zone lymphoma (MZL) and mantle cell lymphoma (MCL). Cell proliferation was measured with the MTT test after 72 h treatment. Compounds were pre-screened at the dose of 1 μM in SU-DHL-10, HBL1, VL51 and MINO cell lines. Those with percentage of proliferating cells down to 60% proceeded to screenings with a wider range of concentrations (0.15–10 μM). Several derivatives showed anti-proliferative activity with IC_{50} values between the low micromolar and the nanomolar range. The most potent derivatives, SIX2-G and SIX13-U, reached nanomolar activity in the majority of cell lines. Studies on the mechanism of action revealed strong inhibition of tubulin assembly and colchicine binding.

Structure-activity relationships (SAR) suggested that N-methoxybenzyl substitution at the pyrrole nitrogen plays an important role in the modulation of activity. In particular, methoxy groups in position 3,4 and/or 5 are relevant. Ring expansion in the tricyclic system did not affect the activity. However, the presence of a cyclohexyl ring and condensation of the pyrrole moiety in position [5,4-*e*] lead to very potent compounds which deserve further evaluation.



SIX



DGIX

Table 1. IC_{50} (μM) values, inhibition of tubulin assembly and colchicine binding of selected compounds

CPD	VL51	MINO	HBL1	SU-DHL-10	Inhibition of tubulin assembly (IC_{50})	% inhibition of colchicine binding
SIX13	0.27	0.23	0.25	0.28	2.1±0.2	77±0.5
SIX2-G	**0.12**	**0.07**	**0.08**	**0.07**	**2.3±0.3**	**80±0.6**
SIX13-O	0.25	0.23	0.27	0.26	1.7±0.2	72±2
SIX13-S	0.27	0.37	0.47	0.5	3.2±0.1	34±4
SIX13-U	**0.1**	**0.07**	**0.09**	**0.1**	**1.7±0.06**	**57±2**
DGIX7	0.25	0.25	0.3	0.25	2.6±0.03	62±2
DGIX8	0.5	0.6	0.9	0.6	4.6±0.8	25±0.01
DGIX14	0.2	0.4	0.6	0.3	3.2±0.06	38±0.7

In conclusion, [1,2]oxazole derivatives are very promising anti-proliferative agents with activity in different lymphoma histotypes, among which MINO cell line (MCL) is the most sensitive. Results will be discussed.

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LOCKED ANALOGUES OF L-ISOSERINE AS POTENTIAL SUBTYPE-SELECTIVE GAT3 INHIBITORS

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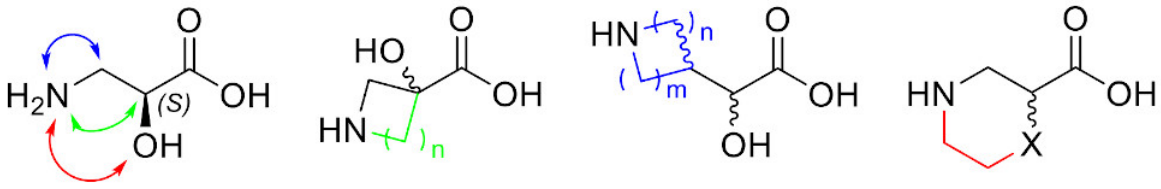
In the brain, extrasynaptic γ-aminobutyric acid (GABA) maintains a persistent "tonic" inhibition through the activation of GABA-A receptors. Extrasynaptic GABA levels are mainly regulated by the astrocytic GABA reuptake transporters (GATs) GAT3 and BGT1.[1] During an ischemic stroke, the surface expression of GAT3 is reduced, causing increased tonic inhibition.[2] Augmented extrasynaptic levels of GABA leads to neuroprotection in the acute phase, but impairs the formation of new structural and functional circuits required for recovery in the chronic phase. Oppositely, reduced tonic inhibition after a stroke facilitates functional recovery.[3]

In 2017, Lie et al. demonstrated that post-stroke delayed administration of L-isoserine, a moderately selective GAT3 substrate inhibitor, increases GAT3 expression, reduces tonic inhibition and facilitates post-stroke functional recovery in mice models.[2]

Therefore, L-isoserine is a useful scaffold for the investigation of the SARs that underlie GAT3 inhibition and subtype-selectivity. Particularly, this part of the project is dedicated to the identification of locked analogue of L-isoserine with increased GAT3 potency and selectivity, that will represent a unique platform for further investigation of the SARs.



Upon assessment of GAT3 inhibition and subtype-selectivity in the [^{3}H]GABA competition uptake assay, the most promising compounds will be evaluated in the FLIPR membrane potential assay to define their activity as substrate or non-substrate inhibitors and further evaluated for in-vivo activity in post-stroke animal models.

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BENZOTHIAZOLYLUREA-BASED 17β-HSD10 INHIBITORS – DESIGN, SYNTHESIS, IN VITRO AND IN VIVO EVALUATION

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There is a well-documented connection between Alzheimer's disease (AD) and mitochondrial dysfunction. One of mitochondrial enzymes affected in AD is 17β-hydroxysteroid dehydrogenase type 10 (17β-HSD10), also known as amyloid-β binding alcohol dehydrogenase (ABAD). Importantly, it has been shown that inhibition of this enzyme is beneficial in AD and also protects against amyloid-β toxicity [1–3]. We have developed several series of benzothiazolyl urea compounds in order to find novel 17β-HSD10 inhibitors applicable for pre-clinical studies [4,5].

Compounds were prepared and screened in an enzymatic assay to determine their inhibitory ability towards purified 17β-HSD10. For active compounds were determined IC_{50} values and inhibition kinetics. Selected compounds were tested in the cellular assay using fluorescent substrate CHANA. Two most promising compounds were selected for bioavailability study when they were administered to mice or rats by i.v. injection or p.o. gavage and compared to experimental inhibitor AG18051.

More than 100 novel compounds were designed and synthesized. All compounds were evaluated for 17β-HSD10 inhibitory ability *in vitro*, where several compounds showed promising inhibitory activity in both enzymatic (IC_{50}< 2 μM) and cellular (EC_{50}< 5 μM) assays. The novel compounds were found to be uncompetitive inhibitors of 17β-HSD10 with selectivity towards the enzyme-substrate complex. The experimental inhibitor AG18051, as well as the two novel leads, showed, however, only minimal CNS exposure after p.o. administration to rats.

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DESIGN AND SYNTHESIS OF 3,5-SUBSTITUTED 1,2,4-OXADIAZOLES AS CATALYTIC INHIBITORS OF HUMAN DNA TOPOISOMERASE IIA

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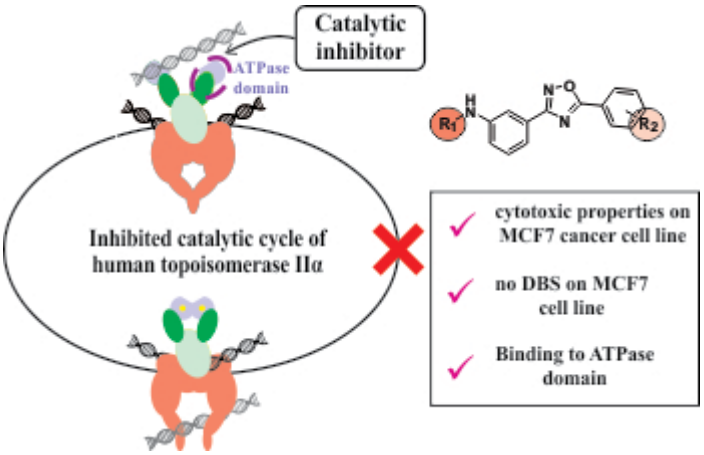
DNA topoisomerases are enzymes that catalyse topological changes of the DNA molecule and act as important validated anticancer targets in chemotherapy [1]. Clinically used inhibitors of type II topoisomerases, topo II poisons, such as etoposide and doxorubicin, although efficient, suffer from severe side effects such as cardiotoxicity and induction of secondary malignancies. This occurs mainly because they induce the formation of DNA double strands breaks (DSB) [2]. In our research we are investigating a new catalytic inhibition mechanism of human topo IIα that could prevent the occurrence of severe side effects associated with topo II poisons [3].

We designed, synthesized, and evaluated compounds from the class of 3,5-substituted 1,2,4-oxadiazoles that act as catalytic inhibitors of topo IIα. By rigidization of initial oxadiazole derivatives we aimed to enhance their interactions with the targeted topo IIα ATP binding site. Obtained compounds inhibited topo IIα as catalytic inhibitors and displayed binding to the isolated ATPase domain. Proposed binding mode of a new inhibitor was evaluated by classical molecular dynamics simulations and derived dynophore models served to evaluate the components of molecular recognition. Selected compounds were cytotoxic on the MCF-7 breast cancer cell line and did not induce DSB, indicating a different mechanism of cytotoxicity compared to topo II poisons also at the cellular level. 3,5-substituted 1,2,4-oxadiazoles appear to be promising compounds for further anticancer development [4].



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TARGETING A PATHOGENIC LECTIN: DESIGN, SYNTHESIS AND EVALUATION OF BC2L-C INHIBITORS

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Multi-drug resistant (MDR) pathogens have become a high-profile threat to public health. In particular, opportunistic MDR pathogens such as *Burkholderia cenocepacia* are responsible for healthcare-associated infections and increase mortality, especially for patients admitted with cystic fibrosis or immuno-compromising conditions. As other opportunistic Gram-negative bacteria, this pathogen establishes virulence and biofilms through lectin-mediated adhesion. The "anti-adhesion" therapy is a tactic devised to fight against this mechanic: by inhibiting the lectin-mediated adhesion of bacteria to host tissues, the infectious process is blocked at its initial stage without leading to selective pressure and resistance.

B. cenocepacia's BC2L-C is a *superlectin* featuring two distinct lectin domains, and thus displays dual carbohydrate specificity.[1,2] Consequently, BC2L-C is believed to enable cross-linking of B. cenocepacia to human epithelial cells during pulmonary infection and "Cepacia Syndrome": rapid decline of respiratory function leading to sepsis and high mortality. The interactions between BC2L-C's N-terminal and human histo-blood group epitopes is particularly interesting to target for inhibition.[1] As an anti-adhesion tactic, we aim to design glycomimetic inhibitors of BC2L-C-Nter.

Here, we report the extensive structural study of the targeted interaction, which led to 3 crystal structures in complex with human oligosaccharides (**Figure 1A**).[3] In order to design BC2L-C-Nter antagonists, computational study of the structures identified ligandable pockets adjacent to the main binding site (**Figure 1A**). In turn, fragment screening provided a small library of ligand candidates (**Figure 1B**), later validated through crystallography.[4] We further report a successful campaign of modular synthesis towards a glycomimetic panel of C- and N- fucosides (**Figure 1C**). Evaluation of the generated structures includes STD-NMR, SPR and ITC experiments against target BC2L-C-Nt. The leading inhibitor provided satisfactory affinity and resulted in a crystal structure of the antagonist/lectin complex (**Figure 1D**). With these results, a second generation of BC2L-C-Nt inhibitors can be designed. Our latest results will be described in the communication.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 765581.

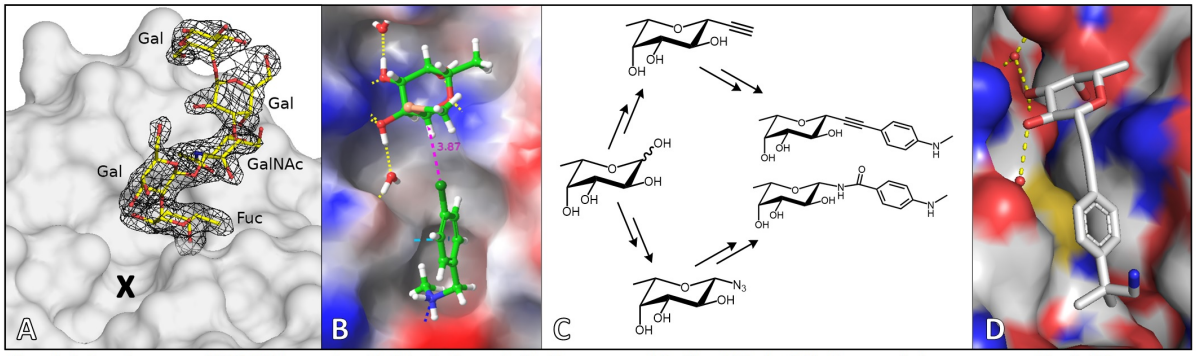

Figure 1. A: Crystal structure of BC2L-C-Nt in complex with Globo H oligosaccharide, **X** represents a vicinal ligandable site. **B:** Binding pose of a fragment screened for site **X** in presence of the monosaccharide. **C:** Modular synthetic strategy towards glycomimetic C- and N-fucosides. **D:** Crystal structure of BC2L-C-Nt in complex with a C-fucoside inhibitor.

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DESIGN AND SYNTHESIS OF NOVEL RNA BINDERS FOR THERAPEUTIC APPLICATIONS

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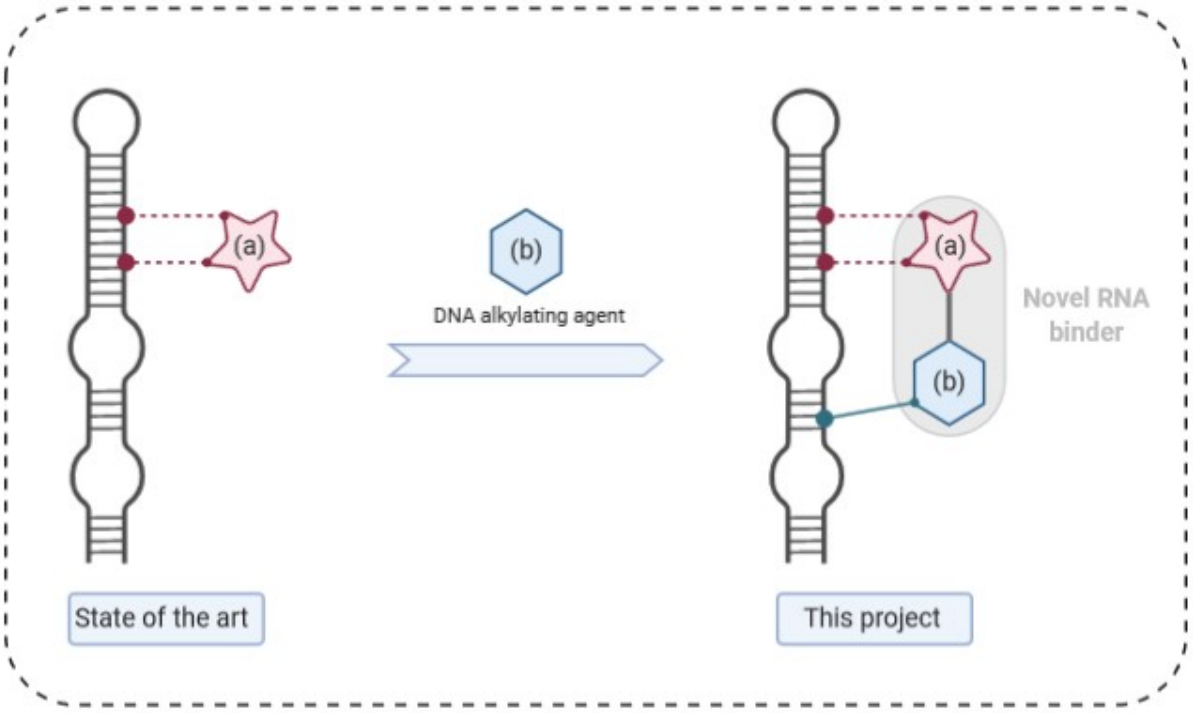
The design and synthesis of nucleic acids ligands recently became a major issue in the medicinal chemistry field of research. In particular, RNA is one of the most intriguing and promising biological target for the discovery of innovative drugs in a large number of pathologies[1]. Various biologically relevant RNAs that could serve as drug targets have already been identified such as mammalian microRNA (for anticancer therapies), bacterial RNA (discovery of new antibiotics) and viral RNAs[2]. Given that some of the reported RNA ligands still lack selectivity, large efforts to develop specific binders recently succeeded with the FDA approval of Risdiplam (Evrysdi^{TM}, Roche) as a mRNA splicing modifier against spinal muscular atrophy[3]. This, together with a large number of marketed antibiotics binding prokaryotic ribosomal RNA inhibiting protein synthesis in bacteria, proves that ligand specifically interacting with their target could represent an extremely promising therapeutic strategy.

The aim of the present work is to take advantage of the irreversible binding mode of some DNA alkylating agent, used in several cancers treatment, for the design of novel RNA ligands bearing the ability to bind the target in a covalent manner. With this strategy, we aim to design more selective and efficient ligands composed of (a) a well-known RNA ligand used as a driver to interact with the target and (b) an alkylating agent scaffold to strongly bind the target (figure 1). In this context, we decided to target both oncogenic miRNAs for anticancer applications and prokaryotic RNA for innovative antibacterial approaches. The stability and biological activity of the synthesized compounds will be evaluated against these different types of RNA targets.



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PRECISION DRUGS: A COVALENT STRATEGY TO MINIMIZE SIDE EFFECTS OF PI3K INHIBITOR CANCER THERAPY

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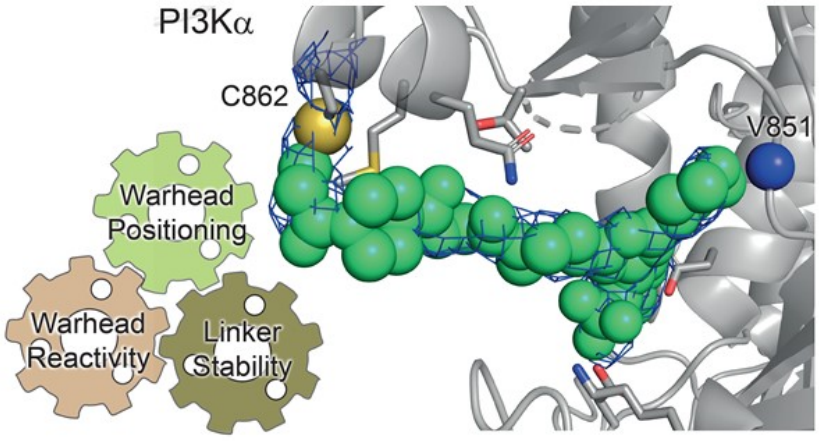
Inhibitors of the phosphatidylinositol 3-kinase (PI3K) – protein kinase B (PKB/Akt) - mechanistic target of rapamycin (mTOR) axis are considered as valuable assets in cancer therapy. A considerable effort has been dedicated to the development of drugs targeting the PI3K-mTOR axis[1-4], and some of them are currently evaluated in preclinical and clinical studies.

Herein we present a strategy to convert a phase II clinical candidate, a pan-PI3K inhibitor (PQR309, bimiralisib) [1,5], into highly selective PI3Kα-covalent inhibitors aiming to minimize the on-target metabolic side effects of PI3K inhibitor cancer therapy. We exploited a rational approach to increase target selectivity by covalently targeting a PI3Kα non-conserved nucleophilic amino acid side chain, namely Cys862. A reactive moiety, so called warhead, was introduced into a chemically modified bimiralisib.

A combination of warhead activity design, proximity and orientation allows a tight control of reversible inhibitor binding and isoform selective covalent binding. To avoid off-target reactions, we have set up a method to quantitatively evaluate warheads' reactivity and optimize for selective Cys862 modification. An extensive Structure Activity Relationship (SAR) study was performed and a wide range of linear and restricted rotation linkers introduced. A comprehensive understanding of the kinetics of irreversible inhibition allowed to interpret SAR and identify compounds with optimal k_{inact} (maximum potential rate of inactivation). X-ray crystallography and mass spectrometry experiments validated the covalent modification of Cys862. Our pilot compounds exceed specificity and potency over an experimental dimethyl-substituted enone, CNX-1351[6]. Moreover, they are metabolically stable in rat liver microsomes and outperform the rapidly metabolized CNX-1351.

Our strategy to investigate and tune warheads' reactivity represents a major step forward in the rational design of covalent chemical tools, overcoming the serendipity in the discovery of irreversible compounds. Moreover, we provide highly selective chemical tools to dissect PI3K isoform signaling in physiology and disease. A clarification of the role of the different PI3K isoforms in insulin signaling allows to address the challenges in isoform selectivity and to develop PI3K inhibitors showing ideal isoform specificity.



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SYNTHESIS, BIOLOGICAL EVALUATION, AND IN SILICO MODELLING OF N-SUBSTITUTED QUINOXALINE-2-CARBOXAMIDES.

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Despite the established treatments, tuberculosis remains an alarming threat to public health according to WHO.[1] Novel agents are needed to overcome the increasing rates of resistance and perhaps achieve eradication. As part of our long-term research on pyrazine derivatives, we prepared a series of N-substituted quinoxaline-2-carboxamides and evaluated their in vitro antitubercular activity. Several quinoxaline derivatives were found in the literature to possess antitubercular activity.[2] Quinoxaline-2-carboxylic acid was activated by oxalyl chloride and reacted with different anilines or benzylamines in the presence of pyridine at room temperature, overnight with stirring, and then obtained crudes were purified with flash chromatography. In addition to activity assessment, final compounds were screened for their in vitro cytotoxicity on HepG2 liver cancer cell lines. In vitro activity against MtbH37Ra (represented by MIC) ranged between 3.91–500 μg/mL, with most compounds having moderate to good activities (MIC < 15.625 μg/mL). One compound was identified during in vitro cytotoxicity assay as a potential selective cytotoxic agent and was therefore further investigated for cytotoxic actitivies.

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DRUGLIKE OXINDOLE-LACTAM HYBRIDS AS SELECTIVE BUTYTYLCHOLINESTERASE INHIBITORS

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Butyrylcholinesterase (BuChE) is gaining relevance as a therapeutic target for Alzheimer's disease (AD) over the past few decades. With increasing evidence that this enzyme is present at increased levels in patients with severe AD, while acetylcholinesterase (AChE) tends to be depleted, targeting selectively BuChE might be a successful strategy for the therapeutics of severe cases of AD. Up until now, therapeutic options available in the market are mostly selective to AChE, with only rivastigmine working as a dual inhibitor, and therefore they play a major role in early to moderate cases of AD, a disease with growing socio-economic impact in modern societies [1].

Over recent years, several isatin-derived compounds were described as possessing great BuChE inhibition activity [2]. In addition, multiple compounds bearing lactams in their scaffold also display relevant cholinesterase inhibition activity [3]. Our goal was to combine the oxindole and the lactam scaffolds in a single molecule, in a molecular hybridization approach. With multicomponent reactions (MCRs) emerging as one of the most versatile tools to access structural diversity, we decided to design our compounds exploring the recently reported Ugi four-center three-component reaction (U4c3CR) reported by Silvani and co-workers [4]. We successfully synthesized 17 β-lactam-oxindole hybrids (13 of them new) and 14 new γ-lactam-oxindole derivatives.

The bioactivity of these compounds was evaluated using eel AChE and equine BuChE. Of the synthesized compounds, only one exhibited moderate activity against AChE, while 15 out of the 31 compounds exhibited some degree of BuChE inhibition. Furthermore, three of the hybrids exhibited IC_{50} below 10 μM, with the best result achieved being as low as 1.75 μM. A clear trend showed that γ-lactam-oxindole hybrids tend to be more active than their β-lactam-oxindole hybrid counterpart. Further studies performed using a web-based free tool, SwissADME [5], showed the druglikeness of these compounds, as they are compliant with five of the most relevant druglikeness filters (Lipinski, Ghose, Veber, Egan and Muegge). These findings open the door for the discovery of new drug candidates for the treatment of AD, targeting selectively BuChE, using MCRs.

Acknowledgements

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UNTIE THE SKEIN: DECIPHERING THE MECHANISMS GOVERNING THE INTERACTION OF DRUGS WITH MUCUS USING A BIOSIMILAR MUCUS MODEL

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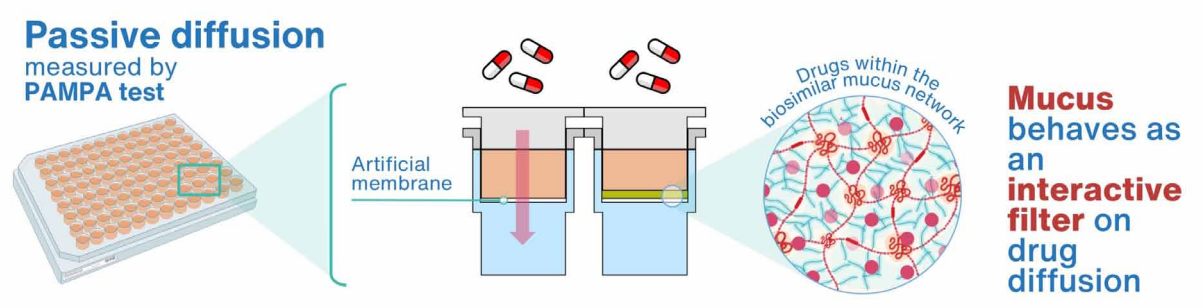
Mucus covers the wet epithelia of the human body ensuring protection against air pollutants or pathogens. Drugs administered by oral or pulmonary routes have to overcome the mucosal layer to be absorbed and to exert the therapeutic effect. However, mucus can represent a strong barrier to tackle even for drugs, especially in those pathological conditions where mucus is overproduced [1]. Despite the critical role played by mucus on drug absorption, very little is known about the molecular properties that promote the interaction of drugs with mucus. In addition, there are no standardized mucus models to be employed in the early drug discovery processes for the screening of potential drug candidates.

We have developed a biosimilar mucus model that mimics a pathological mucus [2]. A natural polysaccharide was used to reproduce the viscoelastic behavior while the composition was mimicked by adding mucin which is the main glycoprotein forming mucus. The mucus model was coupled to phospholipid membrane-permeable supports (PAMPA) to recreate an artificial mucosal surface which was suddenly used to study the diffusion of 45 commercially available drugs.

The mucus model not only represented a physical barrier, but it really behaved as an interactive filter. Different structures related differently to mucus. The diffusion of the majority of the tested drugs was either reduced or remained unvaried. For some compounds, the diffusion was even enhanced in presence of mucus. This observation could change the perception of mucus from a mere barrier to a dynamic modulator of drug absorption.

Since drug development is characterized by a high rate of failure, the availability of a mucus platform could help to reduce at an early drug discovery stage the number of poor performers that reach preclinical trials.



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THE ANTIBACTERIAL ACTIVITY OF PEPTIDE DENDRIMERS AND POLYMYXIN B INCREASES SHARPLY ABOVE pH 7.4

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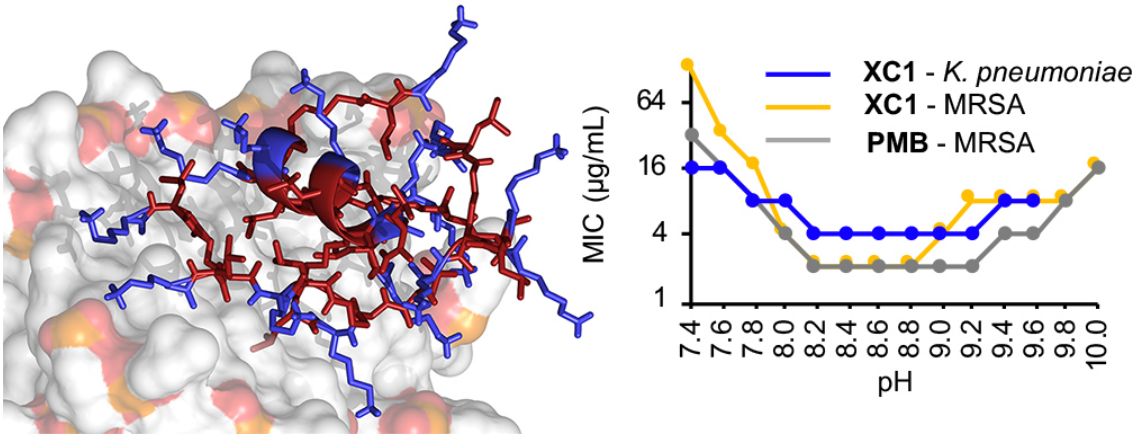
Recently we reported antimicrobial peptide dendrimer (AMPD) **G3KL** and **T7** with potent activities against *P. aeruginosa* and *A. baumannii*, two of the most problematic antibiotic-resistant nosocomial pathogens[1][2]. In our efforts to develop new AMPDs against Gram-negative bacteria, we investigated their activity at acidic and basic pH, which correspond to the conditions of the site of bacterial infections on skin or biofilms and chronic wounds respectively. Removing the eight low pKa amino termini by substituting the N-terminal lysine residues with aminohexanoic acid in our reference dendrimer **G3KL** provided the modified peptide dendrimer **XC1** with a broader pH-activity range. Furthermore, we discovered that raising the pH to 8.0 reveals strong activities against *Klebsiella pneumoniae* and methicillin at pH 7.4, an effect also observed with polymyxin B and tentatively assigned to stronger binding to the bacteria at higher pH as observed with a fluorescence labeled dendrimer analog. This work has been published in *Chemical Communication*.[3]



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PROTAC-KLING PERMEABILITY AND CELL-SELECTIVITY ISSUES USING AN ANTIBODY-MEDIATED APPROACH

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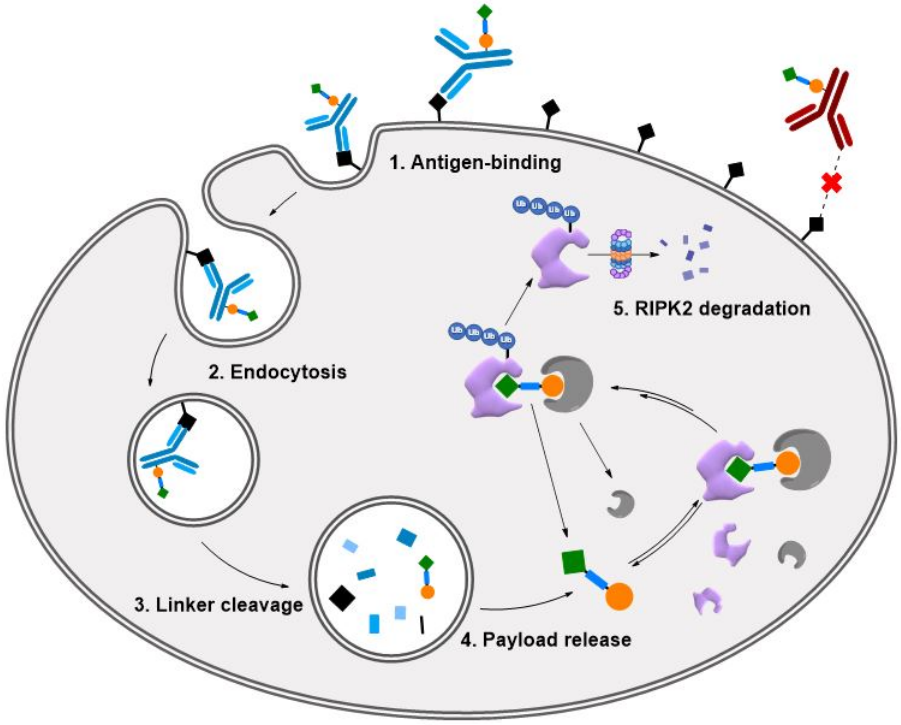
Proteolytic targeting chimeras (PROTACs) have emerged as a promising therapeutic strategy for targeted protein degradation, whereby the ubiquitin-proteasome pathway is exploited to degrade a protein of interest.[1] However, a major limitation of PROTACs is their large molecular weight resulting in poor cell permeability and poor pharmacokinetic properties.[2] One potential approach to overcome this is by forming an antibody-drug conjugate (ADC) for the antibody-mediated delivery of the PROTAC. This method of delivery would not only circumvent the issues outlined, but also allow for cell and tissue specificity to be achieved.

The design and synthesis of an anti-HER2 antibody/RIPK2 PROTAC conjugate is reported, using a disulfide rebridging dibromopyridazinedione conjugation warhead and a valine-citrulline PABC cleavable linker.[3] This ADC showed selective RIPK2 degradation in HER2+ breast cancer cell lines, whilst the equivalent anti-IL4 ADC showed no degradation. HER2- cell lines remained unaffected for both ADCs. This demonstrates the cell-specific targeted degradation of RIPK2 through the antibody-mediated delivery of a PROTAC molecule. Successful application of this approach could facilitate protein degradation with reduced toxicity profiles, lower dosage and novel PROTAC structures.



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MINIATURISED SAMPLE COLLECTION FOR THE ANALYSIS OF METHADONE IN HEROIN ADDICTION THERAPIES

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Opioid addiction, and in particular heroin addiction, is one the most widespread and worrying healthcare issues of modern society. The use of illicit opioids causes every year several health problems and the prevalence of death is relatively high. This issue becomes even more relevant considering the spread of new synthetic opioids on the black market. The most common pharmacological therapy makes use of heroin substitutes, such as methadone (MTD) within methadone maintenance treatments (MMT). However, MTD is an opioid derivative and its misuse can cause several side effects and health problems. For this reason, the monitoring of MMT patients is a crucial step in the evaluation of therapies. Classic bioanalysis is performed on blood plasma samples, with inherent and well-known drawbacks in particular due low subject compliance related to sample collection by venepuncture. The assessment of innovative sampling strategies such as miniaturised dried sample collection could increase patient compliance and bring other advantages like the increase of analyte stability and the possibility of storage and transport without the need of controlled temperature [1]. In this research, an original capillary-based whole blood microsampling methodology for the monitoring of patients undergoing MMT has been developed. The approach allows to prevent sample manipulation and increase patient compliance thanks to the possibility of collecting four blood microsample replicates directly from a single fingerprick [2]. In order to test the performance of the proposed approach, and original HPLC-ED (coulometric) method has been developed: the methodology was fully validated with satisfactory results in terms of precision (RSD < 11.5%) and sensitivity (LOQ=6 ng/mL). After validation, the analytical strategy was applied for the assessment of MTD blood levels in patients undergoing MMT. The obtained results are in good agreement with those obtained from a reference plasma LC-MS/MS method, thus demonstrating the effectiveness and the promising applicative potential of the proposed advanced microsampling and pretreatment approach, as a useful alternative tool for monitoring MTD use and investigate possible abuse of subjects undergoing opioid addiction therapies.

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NOVEL GLUTAMINYL CYCLASE INHIBITORS DISCOVERED BY MOLECULAR MODELING STUDIES

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The Glutaminyl cyclase (QC) activity is a post-translational event that involves the intramolecular cyclization of N-terminal glutamine or glutamate residues into the pyroglutamate (pE) residue of certain peptides and proteins by releasing either ammonia or a water molecule [1,2]. In addition to physiological functions, evidence demonstrates that human QCs have a crucial role in pathological processes in diverse diseases such as Alzheimer's disease (AD) [3], inflammatory [4], and cancer diseases [5]. Thus, efforts in discovering effective QC inhibitors have been pursued in recent years [6-7]. The goal of this research project is to discover new small molecules that effectively inhibit QC. The project was conducted by a customized molecular modeling protocol (Figure 1), including studies on QC binding pocket hot spots, druggability, and pharmacophore modeling followed by molecular docking-based virtual screening. Pharmacophore models were designed to accurately screen several drug-like compound databases. The retrieved hits were subjected to molecular docking and *in silico* filtered to predict pharmacokinetic properties. Then, 93 high-scoring compounds structurally distinct from known QC inhibitors were selected for a coupled-enzyme screening assay (adapted from [8]), which resulted in novel hit-compounds for this relevant therapeutic target. These may represent a starting point for further structural refinement.

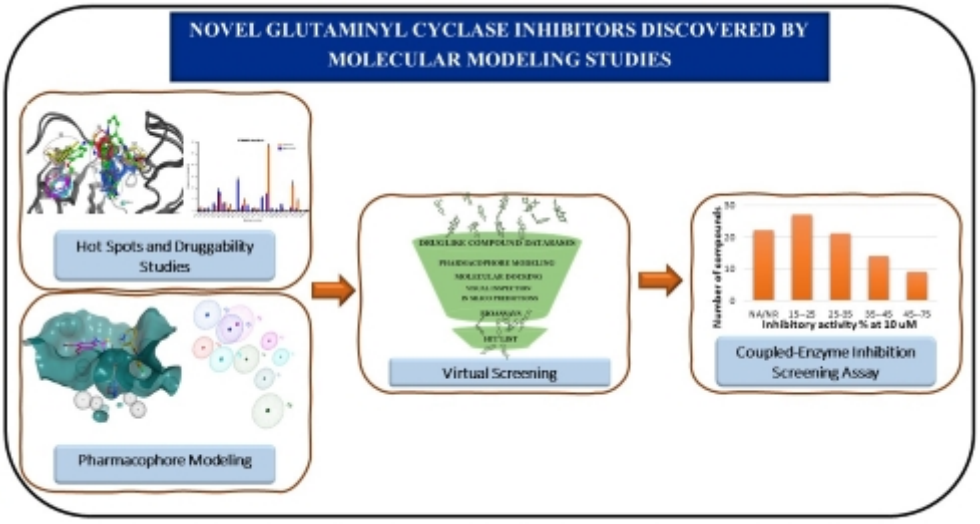

Figure 1. The virtual screening workflow applied to the identification of novel Glutaminyl Cyclase inhibitors.

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DISCOVERY OF AN EFFECTIVE DUAL αvβ6/αvβ8 INTEGRIN LIGAND AS A HERPES SIMPLEX VIRUS-1 ENTRY INHIBITOR

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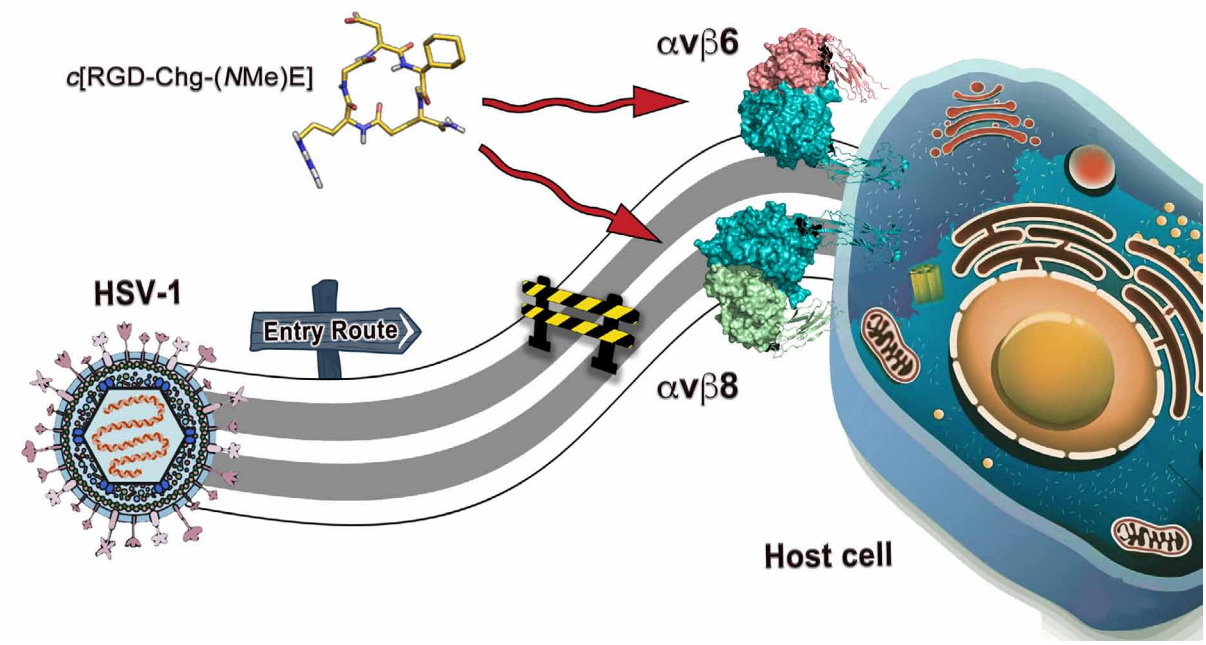
Herpes simplex virus (HSV) are widespread human pathogens which commonly causes recurrent infections of the skin, mouth, lips, eyes, and genitals. The HSV cell entry-fusion is a multistep process orchestrated by four essential glycoproteins, gD, gH/gL, and gB [1], which exploits the nectin-1 and HVEM (herpesvirus entry mediator) receptors to penetrate host cells. In addition to these, αvβ6 and αvβ8 Arg-Gly-Asp (RGD) integrins has recently come to the limelight as interchangeable co-receptors for the cellular penetration of HSV-1. In fact, a consistent drop in the infectivity of this virus has been obtained by contemporary inhibiting αvβ6 and αvβ8 either by cell exposure to subtype-selective monoclonal antibodies (mAbs) or through siRNA transfection. [2] In this work, we focused on a more affordable pharmaceutical approach, based on the design of small RGD-containing cyclic pentapeptides. We started this campaign from our recently developed αvβ6-selective peptide [RGD-Chg-E]-$CONH_2$ (**1**) [3], which was submitted to a systematic *N*-methylation with the aim to increase its affinity also toward αvβ8. Thus, a small library of *N*-methylated derivatives of **1** was synthesized and one of them, namely [RGD-Chg-(NMe)E]-$CONH_2$ (**6**), resulted in a potent dual αvβ6/αvβ8 binder. Extensive *in cell* evaluations demonstrated the capability of **6** to effectively impair HSV-1 cellular penetration through an integrin-dependent mechanism, prompting its further development as a new anti-HSV agent. Furthermore, a NMR/molecular modeling combined approach was employed to rationalize the renewed selectivity profile of **6** and to provide novel valuable hints for the design of RGD integrin ligands with the desired subtype specificity.



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BIOCHEMICAL ASSAY AND CAPILLARY ELECTROPHORESIS TO EXPLORE FRAGMENTS AGAINST COAGULATION FACTOR XIIa

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The fragment-based lead discovery (FBLD) approach is now widely incorporated into medicinal chemistry programs, both in industries and academia. The fragments explore more efficiently the protein chemical space. However, they are weaker binders with dissociation constants in the hundreds µM to low mM range. To observe such weak affinity for the target, mM concentrations are generally used during screening, leading to an important contribution of the low-level impurities present in the samples. Moreover, each screening technology has its potential pitfalls. Knowing them is the first step to avoid wasting time and resources on false positives.

In our laboratory, the ongoing medicinal chemistry project is against coagulation factor XIIa, a S1A serine protease implicated in coagulation, inflammation, and immunity. The potential indications of FXIIa inhibitors include artificial surface-induced thrombosis, hereditary angioedema, Alzheimer's disease, and multiple sclerosis [1]. Our team previously developed 3-carboxamido-benzopyrans [2]. Encouraging results demonstrate that the compounds are anticoagulants and are quite selective for the contact phase pathway [2c]. To support the modulations of the 3-carboxamido coumarins and to develop new chemical scaffolds, we decided to apply a FBLD strategy.

The initial fragment screening was performed by a validated biochemical assay. An affinity capillary electrophoresis method counter-screened a small set of positively charged fragments and highlighted a major source of false positives in our initial screening technology [3]. Further characterization of the active fragments was carried out by Yonetani-Theorell analysis.

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SYNTHESIS AND BIOLOGICAL EVALUATION OF PHENYLDIAZENYL SULFONAMIDES AS AROMATASE INHIBITORS

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Aromatase cytochrome P450 (CYP) enzyme complex is responsible for estrogen biosynthesis and disorder of aromatase levels can initiate several pathological conditions. Postmenopausal estrogen-dependent breast cancer is the most common female cancer. The exploration of new aromatase inhibitors represents an important approach for the identification of novel therapeutical treatments of breast cancer.[1] The inhibition of estrogen biosynthesis by aromatase inhibitors (AIs) constitutes one of the principal therapies for postmenopausal estrogen-dependent breast cancer.[2]

In this respect, a series of sulfonamides with phenyldiazenyl bioisoster of stilbene moiety were designed, synthesized and tested, assuming that these two pharmacophores could be effective for the design of new AIs (Figure 1).

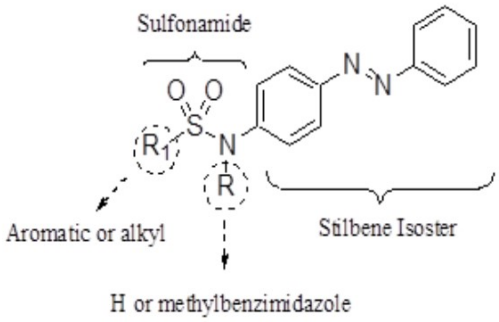

Figure 1. Analogue-based design of new stilbene sulfonamide derivatives as AIs.

Some of these new synthesized compounds showed an aromatase inhibition in the micromolar range and were also evaluated *in vitro* on the human breast cancer cell line MCF7 by MTT assay, cytotoxicity assay (LDH release), cell cycle analysis and apoptosis, revealing a dose-dependent inhibition profile. In particular, a phenyldiazenyl sulfonamide derivative displayed the best reduction in terms of metabolic activity and an anti-proliferative effect on MCF7 cells, being blocked in the G1/S phase checkpoint. The obtained results allow considering this compound as an interesting lead for the development of a new class of non-steroidal aromatase inhibitors.

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KTGS-GUIDED DISCOVERY OF INSULIN-DEGRADING ENZYME INHIBITORS : A STRUCTURE-ADME PROPERTIES STUDY

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Insulin-Degrading Enzyme (IDE), a 110 kDa zinc metalloprotease, was first discovered in 1949 for its ability to degrade insulin but yet remaining poorly understood. It is involved in the clearance of numerous physiological peptides such as insulin, glucagon or amyloid-β. Moreover, IDE acts not only as a protease but also has a chaperone-like activity. With its multi-functional activity, IDE seems to be at the crossroad of several biological pathways.[(a)] The X-ray structure of IDE was reported by Tang *et al.* in 2006 and revealed unusual structural features such as a large catalytic chamber and an exosite involved in the recognition and positioning of the substrate (Figure 1). [(b)] In order to deeply understand the multiple roles of IDE, by using the kinetic target-guided synthesis, the molecule BDM_44768 was discovered as a potent IDE inhibitor.[(c,d)] Thanks to our synthesis effort to afford more than 140 analogues of BDM_44768, we were able to optimise the pharmacological profile of this chemical series in terms of IDE inhibition and ADME properties. These studies provide us good probes to explore IDE's functions.

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XANTHONES: MULTIDRUG RESISTANCE CIRCUMVENTION AND INHIBITION OF VIRULENCE MECHANISMS OF BACTERIA

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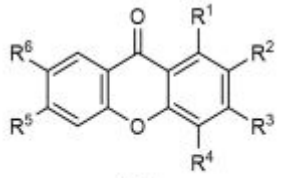
The overexpression of efflux pumps in bacteria is a common mechanism of antimicrobial resistance. Through this event, bacteria can ensure that the intracellular concentration of antibiotics is not enough to harm them. Efforts have been put towards the discovery of compounds that can successfully inhibit these pumps and at the same time be administered in combination with antibiotics and, therefore, restore their full activity. [1]

Xanthones are tricyclic privileged structures, whose scaffold allows for several chemical substitutions, leading to compounds with different activities. [2] In fact, this family of compounds has already been described as modulators of P-glycoprotein, an efflux pump present in eukaryotic cells. [3] In this study we investigated the potential of a library of 20 xanthones (**Figure 1**) to inhibit bacterial efflux pumps. The compounds were evaluated *in silico* for their potential to interact with the AcrAB-TolC efflux system, present in Gram-negative bacteria, and in a homology model of the efflux pump NorA, relevant in Gram-positive bacteria. Afterwards, xanthone derivatives were evaluated for their antibacterial activity in a strain of *Salmonella enterica* serovar Typhimurium SL1344 and in *Staphylococcus aureus* 272123, by a real-time ethidium bromide accumulation assay which led to conclusions on their activity as efflux inhibitors.



1 - 20

R = -OH, OCH_3, Cl, CH_3, CHO...

Figure 1 General structures of the xanthones used in this study.

The inhibition of biofilm formation and quorum sensing, mechanisms of adhesion and virulence, respectively, and related to efflux pumps, were also evaluated. The most promising xanthones were then evaluated for their cytotoxicity in mouse embryonic fibroblast cell line (NIH/3T3). One xanthone derivative arose as a promising efflux pump inhibitor with no antibacterial activity or cytotoxicity for the tested cell line. To the best of our knowledge, this is the first time that a xanthone derivative is described as an inhibitor of bacterial efflux pumps.

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NOVEL FUSED PYRAZOLE-DIAZEPINONE DERIVATIVES: SYNTHESIS AND CYTOTOXIC STUDIES

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1,4-Diazepinones are a class of privileged structures that have been receiving much attention because of diverse biological activities and their use as active ingredients in pharmaceuticals, such as Alprazolam or Anthramycin [1]. Dibenzodiazepine compounds are also interesting as allosteric inhibitors as for instance EGFR, PAK1 or CHK1 [2]. Natural as well as synthetic diazepine and diazepinone derivatives show wide range of bioactivities and still appear as target compounds of biological interest.

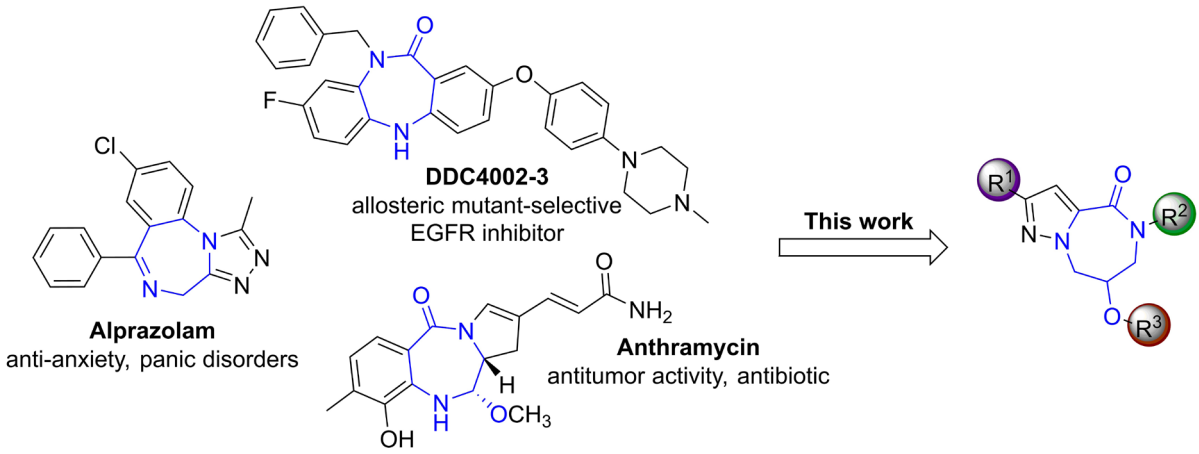

Fig. 1. Biologically relevant 1,4-diazepine derivatives

Fused azaheterocycles play important role as synthetic low molecular weight building blocks. The most studied are the ones fused with five- or six- membered heterocycles [3]. Such condensed systems distinguish various bioactivities – antidepressant, anxiolytic, antiviral, anticancer, etc. Up to date, however, there are very few systems with diazepinone ring fused to pyrazole.

Following literature review on pyrazole-containing pharmacophores and recent investigations on 1,4-diazepinone derivatives, in the present study the aim was to synthesize novel pyrazole derivatives carrying diazepinone structural unit and to evaluate their cytotoxicity. In short, commercially available acetophenones were used in the preparation of pyrazole core-possessing building blocks which were further elaborated into pyrazole-diazepinone target compounds. The structures of novel heterocyclic systems were elucidated by ^{1}H, ^{13}C and ^{15}N nuclear magnetic resonance spectroscopy and HRMS investigations. Cytotoxic activity was evaluated against human cancer cell lines K-562 and MCF7.

Acknowledgments

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NOVEL QUATERNARY AMMONIUM FLUOROQUINOLONE-BASED ANTIBACTERIALS: SYNTHESIS, ANTIMICROBIAL EVALUATION, AND DOCKING STUDIES

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Antimicrobial resistance was identified by the WHO as one of the three greatest threats to human health, a particularly serious problem for patients whose immune system is compromised. Persistent pathogens lead to higher health care costs due to more expensive drugs and extended hospital stays. Since a single drug is not always able to adequately control the illness, a combination of medicines with different pharmacotherapeutic profiles may be required. Hybrid drugs are molecules intended to act at multiple targets. Interestingly, the fluoroquinolone drugs linked to other antibacterial agents represent the most comprehensively described hybrid compounds [1].

Recently, in our laboratory, the fluorescent fluoroquinolone hybrid compounds featuring fused quaternary quinolone-triazolinium moiety have been developed [2-4]. Novel derivatives exhibited antibacterial activity against various pathogens, including biofilm-forming *Pseudomonas aeruginosa*, featured delayed antibiotic resistance development, caused a defect in DNA decatenation, and were potent DNA gyrase inhibitors comparable to the reference drug, ciprofloxacin.

This project aimed to evaluate biological activity of new antibacterials incorporating a fluoroquinolone drug and a quaternary ammonium compound to confirm the hypothesis that a new class of hybrid agents exhibits an unique dual mechanism of action: destabilization of bacterial membrane structures due to the presence of quaternary nitrogen atom and inhibition of bacterial type II topoisomerases elicited by fluoroqinolone portion.

Novel fluoroquinolone derivatives were design and synthesized by exhaustive alkylation to give compounds incorporating permanent positive charge on the nitrogen atom. The products were characterized by NMR, IR, MS, X-ray crystallography, and elemental analysis. Subsequently, the obtained derivatives were screened *in vitro* for antimicrobial activity against a panel of Gram-positive and Gram-negative bacterial strains. The most active compounds exhibited promising antibacterial action in the low micro- and nanomolar range, especially towards pathogens from the ESKAPE group. Molecular docking experiments revealed that all the synthesized compounds are able to interact in the fluoroquinolone-binding mode at active sites of bacterial type II topoisomerases.

The Project was financed by the Polish National Agency for Academic Exchange as part of the Bekker Scholarship Programme and Medical University of Gdańsk subsidies.

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NOVEL BIOCHEMICAL TOOLS TO ANALYSE RESISTANCE TO ANTIBIOTICS THROUGH A NEW QABP APPROACH

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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.[2] There has been an increasing interest in developing methods to profile enzyme activity, identify new therapeutic targets and biomarkers, and understanding their molecular mechanisms.

Quenched activity-based probes (qABP) are compounds that contain a fluorophore (F) and a quencher (Q) moiety covalently tagging active enzymes but not their inactive form. In this particular case, qABP only show fluorescence when is linked to the enzyme, working as a mechanism-based (suicide) inhibitor (Figure 1).[3]

This work started with the development and optimization of the synthetic methodology to obtain several beta-lactam-based qABP (different linkers, quenchers and fluorophores were used in the new molecules). Fluorescence studies of the compounds were performed to evaluate the quantum fluorescence yield (QY) before and after ring-opening in the presence of nucleophiles and beta-lactamases, obtaining final QY up to 92%. Gel-based proteomic studies measuring the fluorescence and the activity of qABPs against beta-lactamase and detection of beta-lactamase in biological matrices are reported.

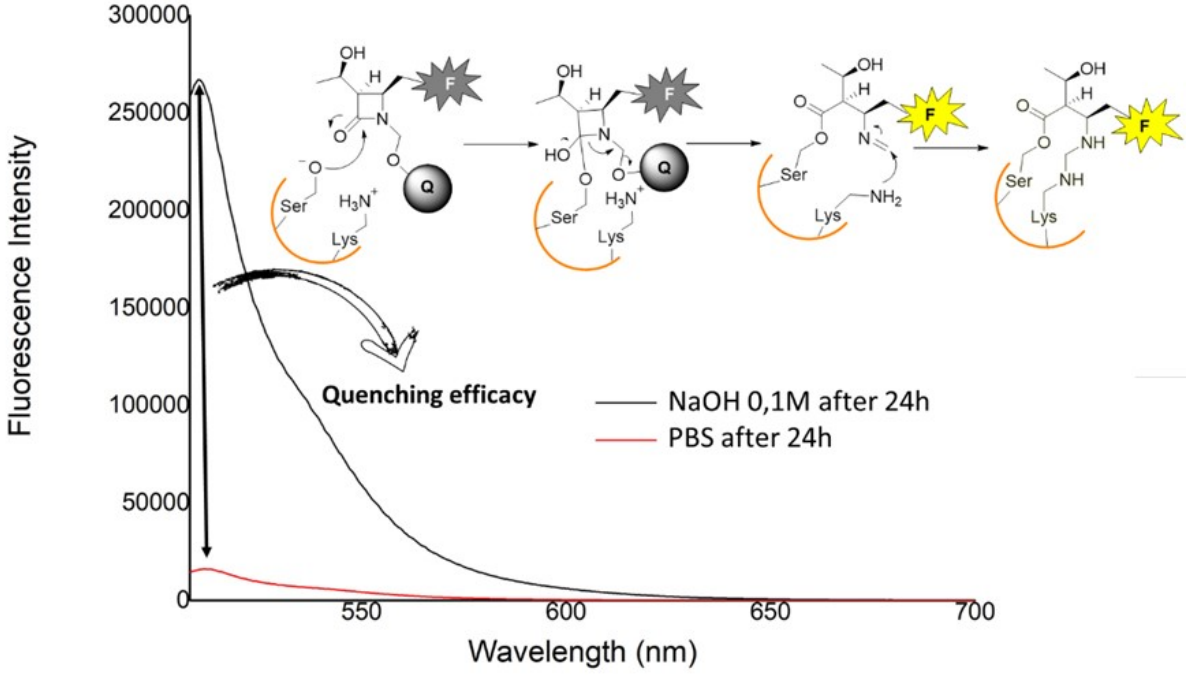

Figure 1 - qABP targeting beta-lactamase mechanism and fluorescence spectrum of qABP before and after reaction with NaOH.

Acknowledgments: Thanks to FCT through by the funding grants SFRH/BD/137459/2018 and PTDC/BBB-BEP/2463/2014

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ORTHOSTERIC/ALLOSTERIC BITOPIC LIGANDS: OPPORTUNITIES FOR FUNCTIONAL SELECTIVITY AT THE CB2R

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It is acknowledged that G protein-coupled receptors (GPCR), assume multiple active states that can be preferentially stabilized by orthosteric ligands or allosteric modulators, thus giving rise to pathway-biased signaling. One of the most promising strategies to expand the repertoire of signaling-selective GPCR activators consists of dualsteric/bitopic agents, which are hybrid compounds composed of orthosteric and allosteric pharmacophoric units. This approach is associated with several advantages over monovalent targeting strategies, including an increased affinity or selectivity, a bias in signaling pathway activation, and reduced off-target activity and therapeutic resistance.
Here, we describe the design, synthesis, and biological evaluation of bitopic ligands at cannabinoid receptor type 2 (CB_2R) of general structures **A** and **B** (Figure 1). Given the expression of CB_2R in microglial cells, astrocytes, and even some neuron subpopulations, this receptor type may be a clinically promising target for the control of brain damage in neurodegenerative disorders.
The bitopic ligands **A** and **B** were obtained linking, through different linkers, the pharmacophoric portion of the CB_2R positive allosteric modulator (PAM), previously identified by our research group[1], with that inspired by the class of the 1,8-naphthyridin-2(1*H*)-one-3-carboxamide derivatives, previously identified as CB_2R orthosteric agonists with a remarkable affinity[2].
Our results showed that most of these compounds displayed a significant bias towards the activation of the cAMP pathway over the recruitment of β-arrestin. Moreover, the best compounds were also evaluated for their modulation on cytokine production using the human microglial HMC3 cell line.

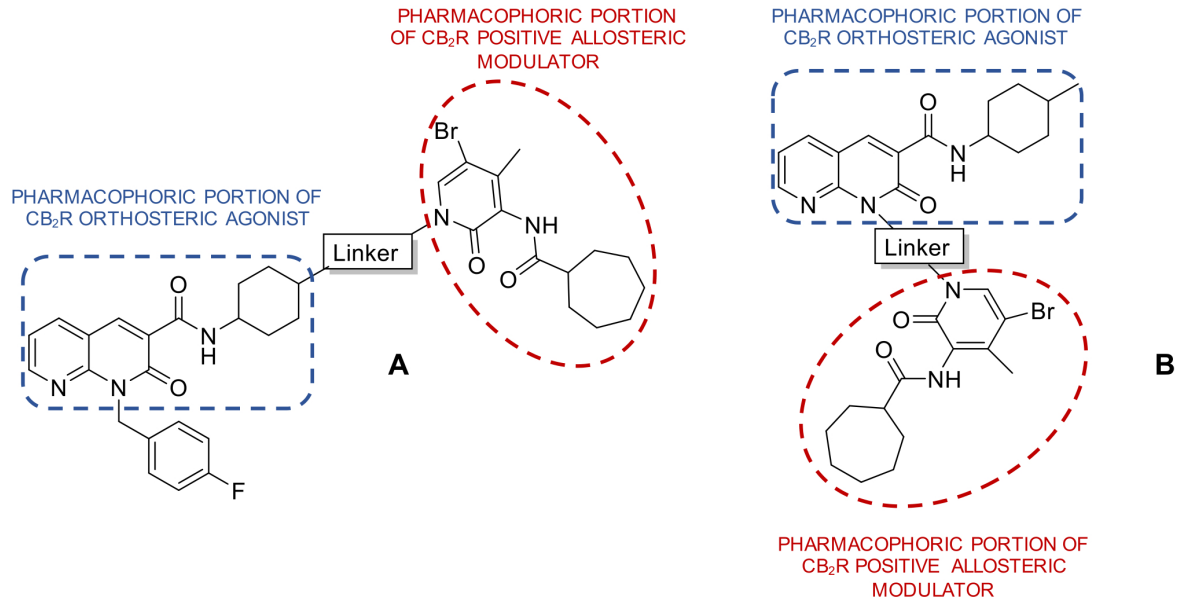

Figure 1. Rational design for the obtainment of potential CB_2R dualsteric/bitopic ligands **A** and **B**.

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NEW BIOCONJUGABLE NICOTINAMIDE PHOSPHORIBOSYLTRANSFERASE (NAMPT) INHIBITORS BEARING A FURAN MOIETY

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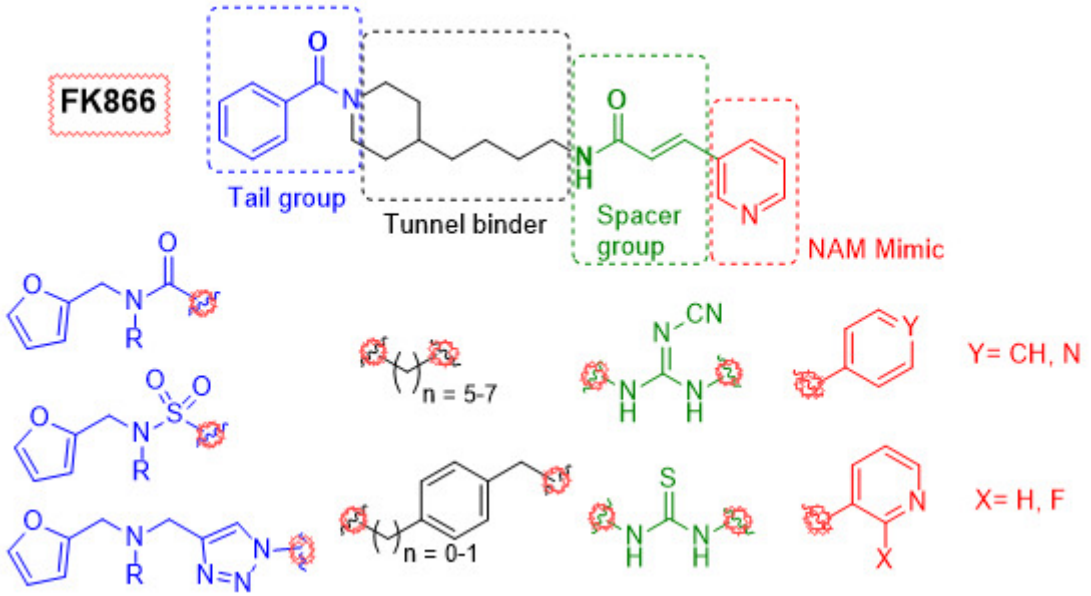
Nicotinamide phosphoribosyltransferase (NAMPT) catalyzes the rate-limiting step in the biosynthesis of NAD. It can be intracellular and extracellular. Intracellular NAMPT is involved in important biological processes such as energy metabolism in cells, in sirtuin function, DNA repair machinery, intracellular oxidative stress.[1]

The very first described NAMPT inhibitor is FK866. It leads cancer cells to apoptosis *via* intracellular NAD and subsequent ATP depletion. Despite its potent antiproliferative activity both *in vitro* and *in vivo*, it failed in human cancer trials due to the lack of anti-tumor benefit and side-effects. Therefore, several analogues have been described in the last two decades and two of them have reached clinical phase recently.[1]

In this communication, we present the synthesis and biological evaluation of a chemical library of new NAMPT inhibitors incorporating a furan moiety that has shown excellent anticancer activity in several cancer cell lines. Thus, antiproliferative assays towards solid and hematologic cancer cell lines, have allowed the identification of subnanomolar NAMPT inhibitors. The relevance of the furyl moiety is highlighted by the incorporation of these inhibitors into drug delivery systems through a heteronorbornadiene linker.[2,3] Structure-activity relationships, enzyme inhibition assays, *in vitro* and *in vivo* studies will be also discussed.



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IN SEARCH OF ORALLY BIOAVAILABLE PROTACS: MONITORING SOLUBILITY

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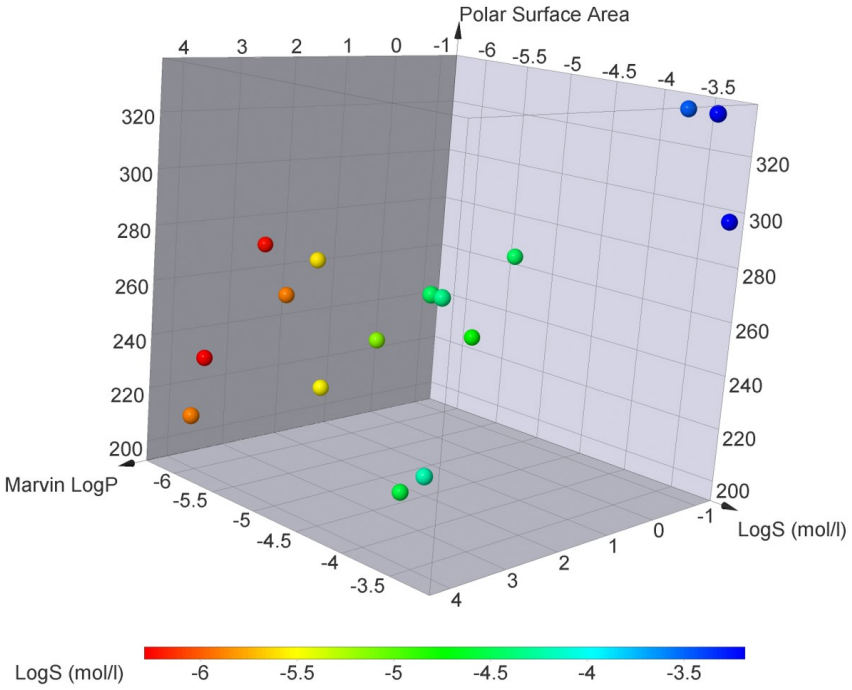
PROTACs are beyond-Rule-of-5 (bRo5) molecules with promising pharmacodynamic characteristics which are exponentially gaining interest in the market. However, their large and flexible structure can be responsible for drug metabolism and pharmacokinetics (DMPK) limitations that can hinder oral dosing.[1] Therefore, it is crucial to study their *in vitro* ADME descriptors (solubility, permeability, etc.) to improve their profile based on molecular properties.[2] Due to the lack of experimental solubility datasets for PROTACs, we decided to monitor thermodynamic solubility for a series of PROTACs and building blocks in aqueous environment at 25ºC and neutral pH. Samples were submitted to shake-flask method, filtered, and quantified by HPLC-UV. Experimental values were compared to computational solubility predictors (e.g. SwissADME, ADMETlab, etc.) and correlated to molecular properties (e.g. PSA, LogP, etc.) using *in silico* tools. Moreover, the relationship between the PROTACs and the building blocks was assessed.



GRAPHICAL ABSTRACT: IN SEARCH OF ORALLY BIOAVAILABLE PROTACS: MONITORING SOLUBILITY

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BENZOTHIADIAZOLE DERIVATIVES ENDOWED WITH STAT3 INHIBITION

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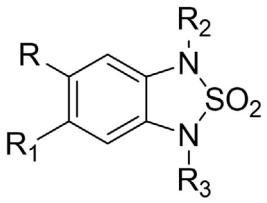
Signal Transducer and Activator of Transcription 3 (STAT3) is a latent cytoplasmic protein over-expressed in various cancer cell lines[1,2]. As a part of our ongoing research focused on compounds showing STAT3 SH2 domain inhibiting activity[3,4], by a virtual screening approach, we identified 5,6-dimethyl-1*H*,3*H* -2,1,3-benzothiadiazole-2,2-dioxide (**1**) as potential inhibitor. Several derivatives were synthesized (Figure 1) and tested. Since compound **1** exhibited the most interesting activity (IC_{50} = 15.8 ± 0.6 μM by AlphaScreen-based assay), we decided to investigate the mechanism of its activity by liquid chromatography, MS and UV studies, discovering compound **1** unexpected interaction also with cysteine residues[5].



R = H, Cl, CH_3, NO_2, etc.
R_1 = H, Br, CH_3
R_2, R_3 = H, CH_3, CH_2Ph

Figure 1 . Benzothiadiazole-2,2-dioxide derivatives set

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ATP, S-NITROSYLATION AND GLYCINE: A THREE-WAY REGULATION MECHANISM MODULATING THE ACTIVITY OF SERINE RACEMASE

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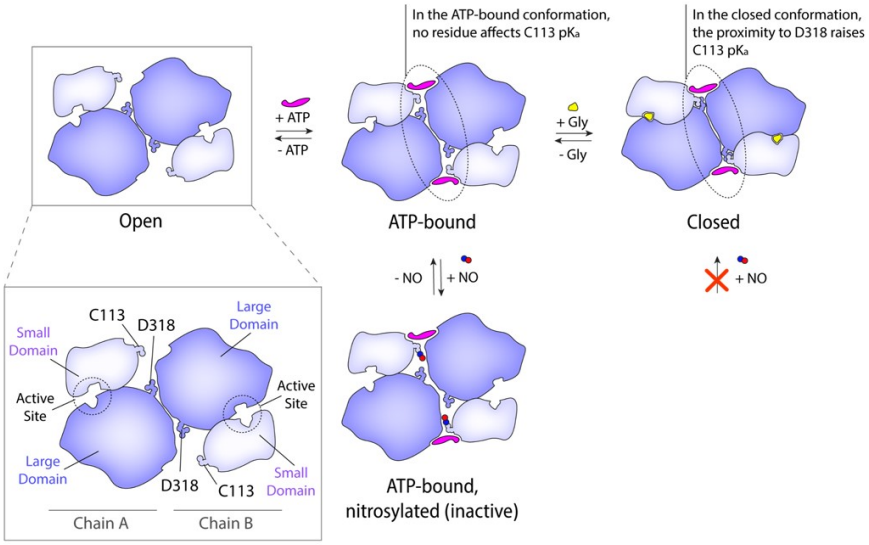
D-serine is a neuromodulator that co-activates NMDA glutamatergic receptors, produced in several tissues and mainly in cerebral regions. D-serine is synthesized by serine racemase (SR), a pyridoxal 5'-phosphate (PLP)-dependent homodimeric enzyme that catalyzes the reversible racemization of L-serine to D-serine and the irreversible deamination of L- and D-serine to pyruvate and ammonia. D-serine has been demonstrated to be involved in amyotrophic lateral sclerosis (ALS) pathogenesis. Indeed, the deletion of the gene encoding SR slowed down the disease progression in ALS mouse models and protected against cerebral ischemia and excitotoxicity. Therefore, SR is an interesting target for the development of innovative ALS treatments.

It was found that SR is regulated by S-nitrosylation at Cys113, with a 7-fold reduction of the enzyme activity. To get insights into the mechanism of regulation, we investigated the correlation between S-nitrosylation and SR conformations stabilized by ATP, a positive allosteric effector of SR, and glycine, a physiological inhibitor of SR, by integrated experimental and theoretical approaches. We demonstrated that S-nitrosylation-mediated negative regulation occurs through the stabilization of an open, less-active conformation of the enzyme. The reaction of SR with either NO or nitroso donors is conformation-dependent and occurs only in the conformation stabilized by ATP, in which the e-amino group of Lys114 acts as a base towards the thiol group of Cys113. In the closed conformation stabilized by glycine, the side chain of Lys114 moves away from that of Cys113, while the carboxyl side-chain group of Asp318 moves significantly closer, increasing the thiol pK_a and preventing the reaction. We concluded that ATP binding, glycine binding, and S-nitrosylation constitute a three-way regulation mechanism for the tight control of SR activity.



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KINETIC EVALUATION OF SULFUR(VI) FLUORIDE REACTIVE ELECTROPHILES FOR APPLICATION TO CHEMICAL PROBES

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Expanding the range of biological systems that can be investigated and targeted by chemical biology approaches is becoming increasingly crucial to the development of novel therapeutics. Reactive functionalities capture specific compound-target interactions, so can be leveraged as tools to facilitate the discovery and development of new medicines.[1,2] Established functionalities, including photoreactive and cysteine-reactive moieties, hold limitations that result in limited applicability across the proteome. Sulfur(VI) fluorides have emerged as attractive reactive moieties, due to the ability to target numerous nucleophilic amino acids and achieve quantitative modification, thus expanding the druggable proteome.[3] Sulfur(VI) fluorides (S^{VI}-F) are inherently electrophilic and so are susceptible to hydrolysis, forming an inactive sulfonic acid species. Thus, it is crucial to strike a balance between stability and reactivity of S^{VI}-F moieties prior to application.

Here we present a biologically relevant, three-part approach to the evaluation of S^{VI}-F species as reactive functionalities, and develop understanding of the parameters that influence the modification of targets by reactive tools (Figure **1**).

[image]

Our workflow to optimise S^{VI}-F functionalities integrates the assessment of aqueous stability and reactivity, kinetic modification and performance in chemoproteomic studies. Our solution studies revealed a vast range of S^{VI}-F stability and reactivity, thus reinforcing the importance of profiling, prior to application. Further, computational LUMO energy calculations displayed excellent correlation, enabling the future prediction of S^{VI}-F reactivity. To achieve biologically-relevant insight, the S^{VI}-F moieties were appended to small molecule binders for anchorage within protein targets. The use of intact protein LCMS permitted crosslinking studies and kinetic profiling to explore the complex interplay between the recognition and covalent modification events. We further demonstrated the cell-compatibility of S^{VI}-F moieties and conducted chemoproteomic studies with S^{VI}-F-substituted kinase probes in live cells. Assessment of the proteome-wide engagement by the probes revealed the significant impact of S^{VI}-F reactivity, while multiple probes exhibited remarkable selectivity for specific protein targets.

Based on these studies it is envisaged that S^{VI}-F functionalities will be a valuable addition to the reactive tools strategy. The use of S^{VI}-F functionalities expands the ligandable proteome and thus assists the development of novel medicines.

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DESIGN, SYNTHESIS, BIOCHEMICAL ACTIVITY EVALUATION, AND STRUCTURE-ACTIVITY RELATIONSHIPS OF NEW STEROIDAL AROMATASE INHIBITORS. THE CASE OF C-RING SUBSTITUTED STEROIDS

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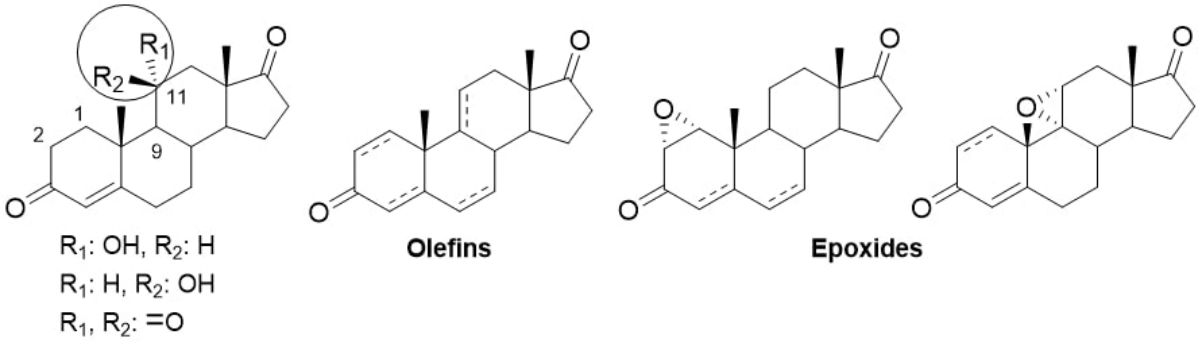
Epidemiological studies from 2020, estimated that breast cancer is the one with the highest incidence and mortality rates in women (1). About 80% of this type of cancers are estrogen dependent. Thus, one of the therapeutic strategies to address this disease is the use of drugs that reduce the levels of circulating estrogens. One type of these drugs are the aromatase inhibitors (AIs), which block the conversion of androgens to estrogens by the aromatase enzyme. AIs undoubtedly have a major role in the treatment of breast cancer, in all its stages. However, there are only three AIs in clinical use, and they have several associated side effects, which cannot be overlooked, namely the progressive loss of bone density (osteoporosis) with increased risk of bone fractures and bone pain, as well as cardiovascular disorders. In addition, another serious problem associated with AIs is the development of resistance, which consequently leads to cancer re-growth and tumor progression. For all these reasons, research to discover new AIs to increase the options for the treatment of breast cancer is very relevant. Our group traditionally investigates in the medicinal chemistry and cell biology/biochemistry fields related with steroidal AIs, mainly exploring the A, B and D-ring modifications, and discovering new molecules with potent anti-aromatase activity (2,3). In this work, it is our intention to go further in this research by designing, synthesizing, and studying C-ring substituted steroidal AIs. Based on these considerations, we have studied some steroids functionalized at C-11 position with an α or β hydroxyl group and with a carbonyl group. Steroidal olefins and the respective epoxides at C-9, C-11 were also studied (Figure1). It was found that the carbonyl group at C-11 is more beneficial for aromatase inhibition than the hydroxyl group, and the C-ring epoxides were more potent than the C-ring olefins, leading to the discovery of a very strong aromatase inhibitor, with an IC_{50} of 0.011 μM (the steroidal AI in clinical use, Exemestane, presents an IC_{50} of 0.050 μM). In another approach, the anti-aromatase activity of steroidal 1,2-epoxides and steroidal 1,2-olefins was compared. It was found that, in this case, the A-ring epoxides were less potent than A-ring olefins. Overall, this work can contribute for the discovery of new lead compounds which can be translated to new drugs for the treatment of hormone-dependent breast cancer.



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SUSTAINABLE SYNTHESIS OF PHOTOSENSITIVE POLYMERIC MATERIALS FOR ANTIMICROBIAL APPLICATIONS

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In the last decade, there has been a selective adaptation of microorganisms to drugs, which led to great enhancement of multi-drug resistant strains (MDRS). Nosocomial respiratory tract infections caused by MDRS are a huge global healthcare problem and engage economical concerns, since they contribute to mortality of the most vulnerable individuals and require their long-term stays in hospitals.[1] Therefore, there is an urgent need to develop new antimicrobial entities/materials capable of killing microorganisms in hospital surfaces and medical devices such as endotracheal tubes used in mechanical ventilation.[2] To this effect, photodynamic inactivation (PDI) of microorganisms, which combines a photosensitizer molecule, molecular oxygen (O_2), and a light source of appropriate wavelength, has been studied as an alternative to conventional antimicrobials. Recent reports have been described in the literature concerning the immobilization of photosensitizers onto polymeric materials, including the functionalization of polyvinyl chloride (PVC) with curcumin[2] and porphyrins.[3] Herein, we present our recent achievements regarding the development of photosensitive polymeric materials, through two different approaches (Figure 1). The first approach consists on the adsorption of curcumin in conventional PVC films, while the second consists on the synthesis of green polycarbonate materials[4], through CO_2 addition reactions to epoxides, followed by covalent immobilization of tetrapyrrolic macrocycle photosensitizers. Antimicrobial *in vitro* photodynamic inactivation studies will be presented for the different photosensitive materials in order to assess and select the best material and immobilization approach for future development of efficient photosensitive medical devices such as endotracheal tubes.

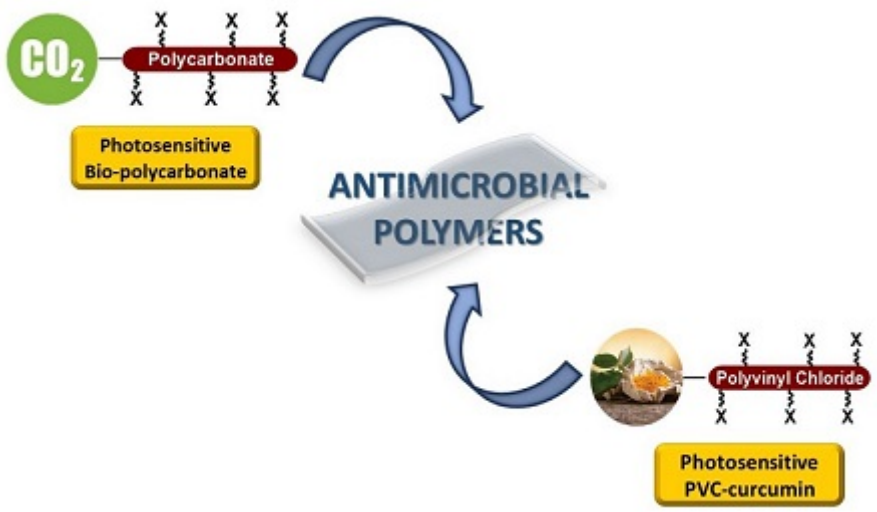

Figure 1: Two different approaches for preparation of photosensitive antimicrobial polymeric materials.

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5-HT6 RECEPTOR ANTAGONISTS AND CHOLINESTERASE INHIBITORS WITH ANTIOXIDANT PROPERTIES AS NEW MULTIFUNCTIONAL ANTI-ALZHEIMER'S LIGANDS

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Alzheimer's disease (AD) is the most common form of dementia with a constantly growing number of cases. The neurodegenerative changes are caused by misfolding, aggregation, and accumulation of certain proteins in brain tissues which lead to the death of cholinergic, serotonergic, and other neuron populations, and thus to cognitive and behavioral impairments [1]. Additionally, metal dyshomeostasis and oxidative stress contribute to the development of neurodegeneration. Due to this complex pathology, the discovery of disease-modifying pharmacotherapy is a huge challenge. Currently available first-line drugs, acetyl- and/or butyrylcholinesterase inhibitors, increase levels of acetylcholine in the brain and temporarily reduce symptoms and slightly delay the progress of the disease. Other interesting biological targets in the search for AD treatment are the serotonin 5-HT $_6$ receptors (5-HT$_6$R) [2]. Their pharmacological blockade not only enhances cholinergic neurotransmission but also has positive behavioral effects due to the antidepressant and anxiolytic activity. To address the complex AD pathology, a multifunctional ligands approach, where one molecule interacts with at least two biological targets, prevails in the search for new drugs.

In our research, we focused on the development of multifunctional ligands - 5-HT$_6$R antagonists and cholinesterase inhibitors. We designed them by combining pharmacophore fragments of 5-HT$_6$R antagonists with well-known cholinesterase inhibitor – donepezil (Fig.1). The results of *in vitro* studies (Ellman assay and radioligand binding assay) revealed ligands with nanomolar activity towards human butyrylcholinesterase and human 5-HT$_6$R. Further examination of their antioxidant activity showed their moderate ability to scavenge the pre-formed radical in the ABTS assay. Finally, two ligands were found to be selective Fe^{2+} chelators. Due to the complementarity of the selected biological targets and the multidirectional activity of several new compounds, we propose them as an excellent starting point for further development and optimization.

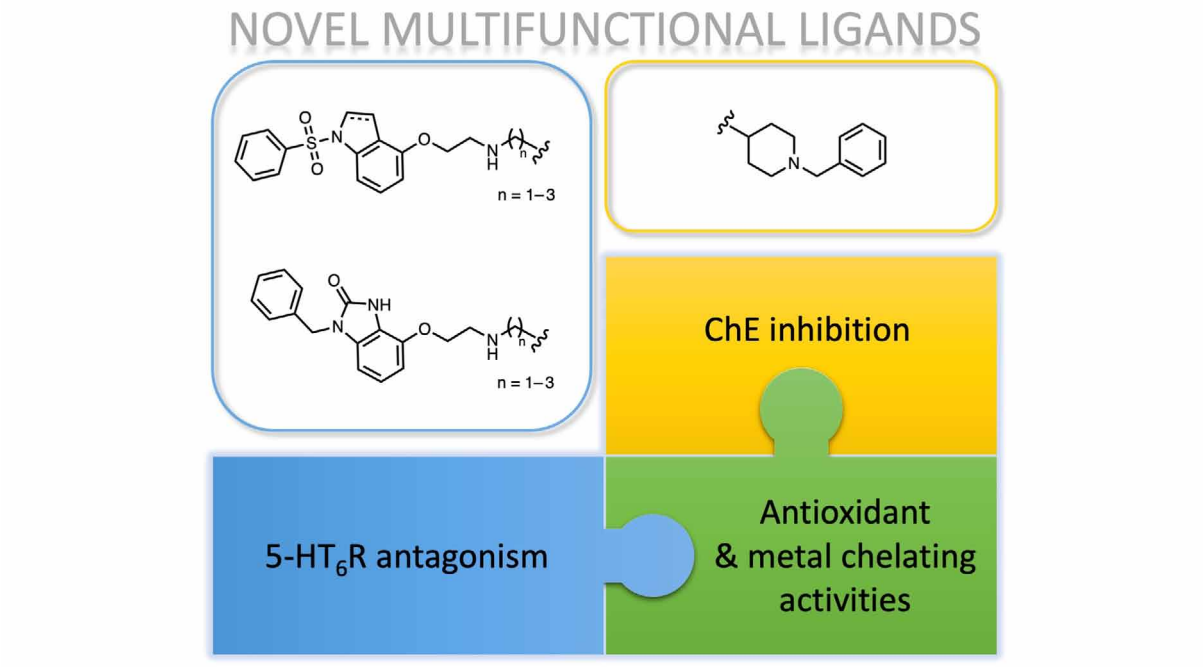

Figure 1. Multifunctional ligands combining pharmacophore fragments of 5-HT$_6$R antagonists with well-known cholinesterase inhibitor – donepezil.

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THE DEVELOPMENT OF A SUB/SUPERCRITICAL FLUID CHROMATOGRAPHY BASED PURIFICATION METHOD FOR PEPTIDES.

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Peptide drugs are essential components of the pharmaceutical industry with a multiplicity of therapeutic properties, such as being anti-hypertensive, anti-microbial, anti-diabetic, and anti-cancer. These molecules are similar in physiological structure and function to the body's endogenous signalling molecules and are therefore ideal candidates for the development of the next generation of drugs. However, the purification of these peptides can be problematic due to poor solubility and stability, which often results in low peptide yields. Peptides are traditionally purified *via* RP-HPLC methods, which are tedious and employ harsh solvents that generate harmful waste to the environment. There is a growing need for more cost-effective and sustainable purification methods of these biologics. SFC can provide a greener peptide purification approach with more environmentally friendly mobile phases such as CO_2 and methanol, which can easily be recycled with minimal environmental impact. Currently, there is limited knowledge regarding the SFC purification of peptides. Herein, this study investigated SFC methods to purify a tetrapeptide (LYLV), octapeptide (DRVYIHPF), and nonapeptide (LYLVCGERG) on commercially available columns at an analytical scale. The 2' ethyl pyridine column proved to be optimal based on its reproducibility, peak shapes, efficient separations, and retention factors with peptide recoveries ranging from 80-102%. The run times were reduced to 13 minutes, as opposed to the traditional RP-HPLC methods of 50 minutes, thus making this SFC method an efficient, greener, and more cost-effective approach for the purification of these peptides.

Keywords: Sub/supercritical fluid chromatography (SFC); PFP column; diol-HILIC column; 2' ethyl pyridine column; tetrapeptide [insulin β chain peptide (15-18)]; octapeptide [angiotensin II]; nonapeptide [insulin β chain peptide (15-23)].

NOVEL HEPATITIS B CAPSID ASSEMBLY MODULATORS

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Although an effective prophylactic vaccine against Hepatitis B Virus (HBV) infection is available since the 1980s, chronic infection by HBV remains a major public health problem with almost 300 million of people infected worldwide.[1] Approximately 20 to 30% of individuals with Chronic Hepatitis B (CHB) eventually develop cirrhosis, liver failure, or hepatocellular carcinoma. Currently, there are two main treatment options. The standard of care is treatment with a nucleos(t)ide analogue, which is generally given lifelong and is well tolerated. In some parts of the world or for specific populations, finite treatment with pegylated interferon is also used, but this has significant side effects. As a consequence, the development of novel anti-HBV drugs having a distinct mechanism of action remains crucial.
Capsid Assembly Modulators (CAMs) are small molecules that interfere with the capsid assembly, a key step in virus production, by interacting with the HBV core protein.[2] Recently, many capsid assembly modulators with novel scaffolds have been reported, including heteroarylpyrimidines[3] and sulfamoylbenzamides[4] some of which have been advanced into clinical trials.

The present communication discloses the structures and corresponding synthesis, antiviral properties and preliminary ADME profile of novel Capsid Assembly Modulators.[5]

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STRUCTURAL FINE-TUNING OF DESMURAMYLPEPTIDE NOD2 AGONISTS DEFINES THEIR IN VIVO ADJUVANT ACTIVITY

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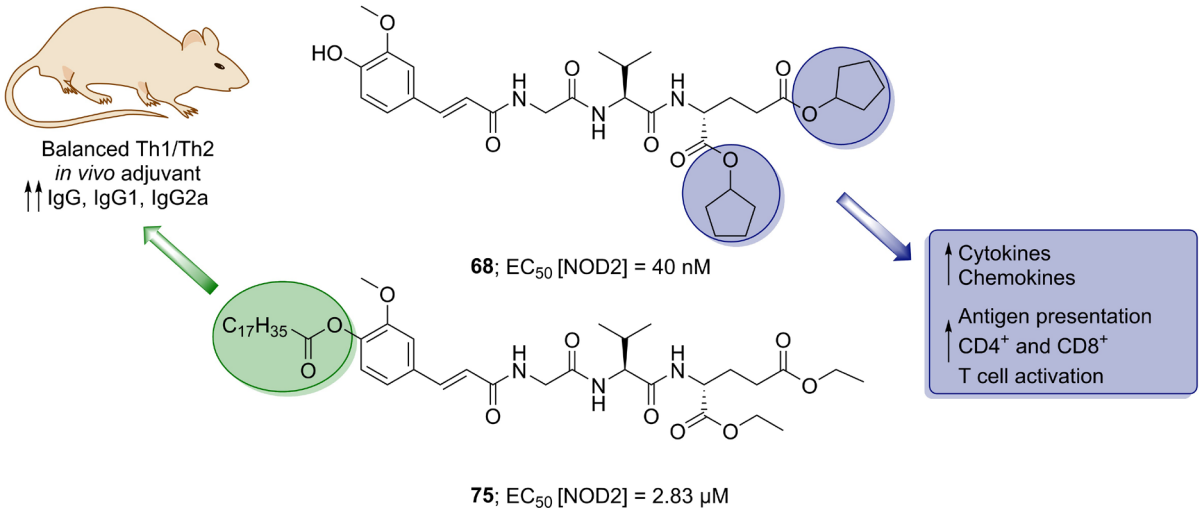

Nucleotide-binding oligomerization-domain-containing protein 2 (NOD2) is a well characterized innate immune receptor in the pattern recognition receptor superfamily. It is responsible for detecting and responding to fragments of bacterial peptidoglycan, with muramyl dipeptide (MDP) known as the smallest peptidoglycan molecular signature still capable of activating NOD2.[1] Activation of NOD2 results in a wide range of innate and adaptive immune responses, which make NOD2 an attractive target for the development of novel vaccine adjuvants and cancer immunotherapeutics.[2,3]

The discovery that the full glycopeptide scaffold is not mandatory for NOD2 agonism led to the concept of desmuramylpeptides, a class of compounds that lack the carbohydrate moiety of MDP. In our study, which was recently published in the Journal of Medicinal Chemistry,[4] we report on the design, synthesis and biological evaluation of a series of novel desmuramylpeptides, which are based on the structures of promising NOD2 agonists previously reported by our group.[5] Several compounds emerged as potent nanomolar *in vitro* NOD2 agonists, with immunostimulatory effects on peripheral blood mononuclear cells at the protein and transcriptional levels. These compounds also augmented the dendritic-cell-mediated activation of T cells in an antigen presentation assay and enhanced the cytotoxic activity of peripheral blood mononuclear cells against malignant cells. Importantly, a C_{18} lipophilic tail was identified as a pivotal structural element that confers *in vivo* adjuvant activity in conjunction with a liposomal delivery system. Accordingly, liposome-encapsulated desmuramylpeptides showed promising adjuvant activity in a mouse model of adjuvanticity, surpassing that of MDP, while achieving a more balanced Th1/Th2 immune response, thus highlighting their potential as vaccine adjuvants.

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DYNOPHORE-BASED APPROACH TO INHIBITOR DESIGN: DEVELOPMENT OF HUMAN DNA TOPOISOMERASE IIA CATALYTIC INHIBITORS

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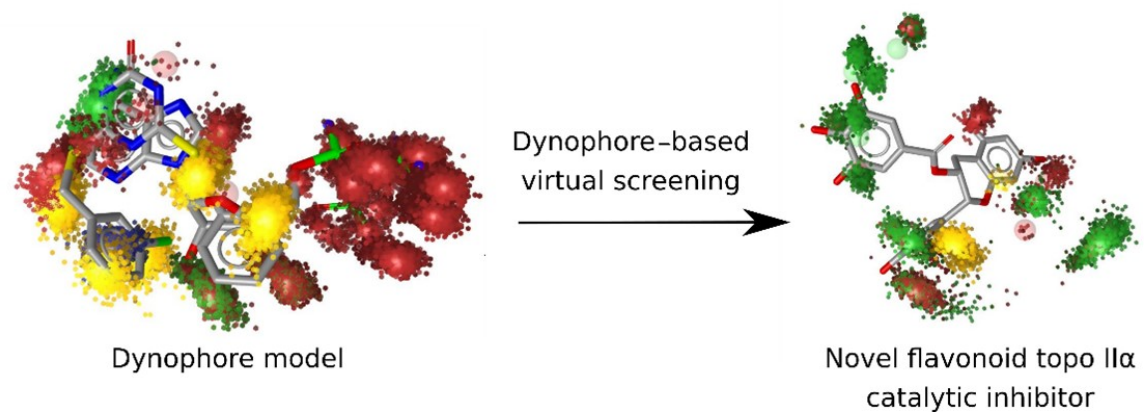
DNA topoisomerases represent a diverse family of complex molecular motors that catalyze the topological changes of the DNA molecule and are established targets of chemotherapy [1]. Clinically used inhibitors of human type II DNA topoisomerases, topo II poisons, such as etoposide and doxorubicin, possess severe side effects such as cardiotoxicity and induction of secondary malignancies. These occur mainly because such compounds induce formation of DNA double strands breaks (DSB). To steer away for these unfavorable attributes, we are investigating a new alternative catalytic topo IIα inhibition paradigm developing molecules that would bining to its ATP binding site. [2,3].



In this study, we utilized a human DNA topo IIα as a model target to outline a dynophore-based approach to catalytic inhibitor design. Based on MD simulations of a known catalytic inhibitor [4] and a native ATP ligand we derived a joint dynophore model that supplemented the structure based-pharmacophore information with a dynamic component. Subsequently derived pharmacophore models were employed in a virtual screening campaign using a library of natural compounds. Experimental evaluation returned 5 flavonoid compounds, possessing promising topo IIα catalytic inhibition. Binding studies also suggested the interaction with the ATPase domain. The proposed binding modes of hit compounds were investigated in extensive MD simulations, essential dynamics and MM-PBSA free energy calculations to reconnect the obtained results to the initial dynophore-based screening model. Besides, a new design strategy that incorporates a dynamic component of molecular recognition, the discovered flavonoids also comprise new hit compounds acting via an alternative inhibition mechanism on this establish anticancer target.

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ELUCIDATING CELL DEATH MECHANISMS OF NOVEL HYBRIDS DERIVED FROM NATURAL PRODUCTS

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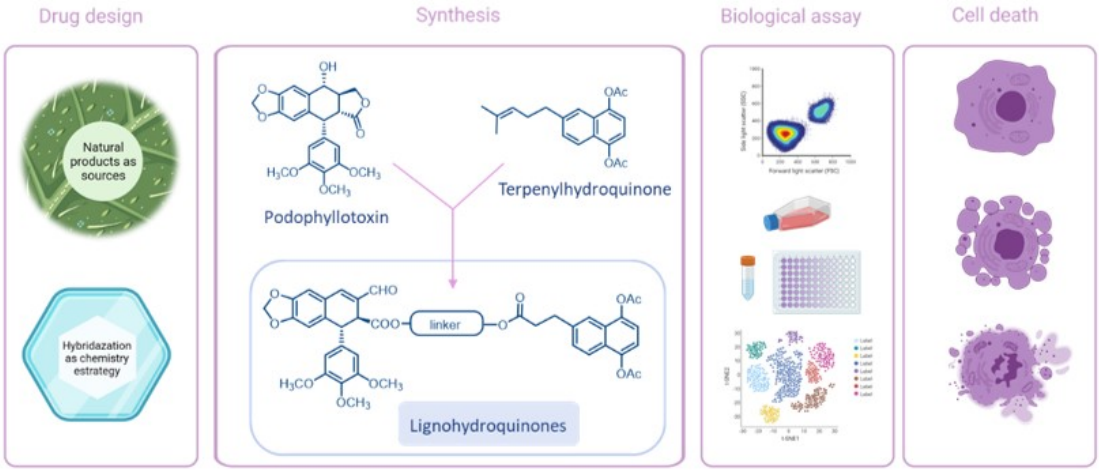
Hybridization is a widely used strategy in rational drug discovery. The conjugation of different active molecules can provide an unlimited number of combinations, which could greatly increase the molecular chemo-diversity[1]. In this sense, different kinds of natural products with varied structure, origin or mechanism of action have been combined to produce new bioactive entities. The synergistic or additive effect of those chemical entities is a promising approach in case of cancer therapy to avoid resistances or discover new applications for future treatments[2]. Here, we explore the cell death mechanisms of a new family of antitumoral compounds derived from podophyllotoxin and terpenylquinones. Both precursors showed different mechanisms of action, podophyllic aldehyde arrests cell cycle in G2/M at μM concentration[3] whereas terpenylhydroquinones are supposed to present a potent pro-oxidant effect that induces cell death[4]. Combination of those natural product derivatives is expected to have a synergistic effect that would improve the cytotoxicity and selectivity of the hybrid compounds. In this report, we explore the activity of new hybrids named “lignohydroquinones” describing biological tests that have been carried out to know the cytotoxic potency and to explore possible mechanisms of action of the new set of hybrids.



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BISCYCLOLIGNANS: A FAMILY OF CYTOTOXIC CONJUGATES DERIVED FROM THE NATURAL PRODUCT PODOPHYLLOTOXIN AS DUAL INHIBITORS.

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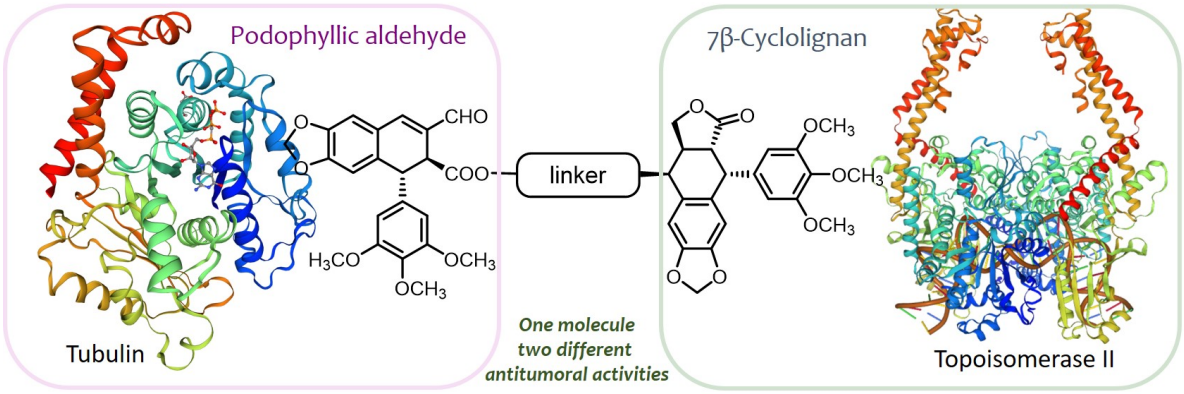
Podophyllotoxin is an approved antiviral cyclolignan, isolated from *Podophyllum* sp., that inhibits the tubulin polymerization, preventing the formation of microtubules through interaction at the colchicine binding site[1]. It also has cytotoxic properties and structural changes performed on podophyllotoxin led to etoposide, which was approved as an anti-tumoral drug, although acting by a different mechanism of action, etoposide is an inhibitor of DNA-topoisomerase II[2]; this change is related to the chemical changes performed on the cyclolignan skeleton. Several other chemical transformations performed by our group on the podophyllotoxin skeleton had led to compounds with interesting cytotoxic results. This is the case of the podophyllic aldehyde, a non-lactonic derivative with improved cytotoxicity and selectivity towards certain tumor cell lines[3]. It shares the same mechanism of action as podophyllotoxin, that is, the inhibition of tubulin polymerization. Considering the different pharmacological activities derived from the podophyllotoxin skeleton and using the hybridization as a drug discovery technology, in this communication, we report the synthesis and the *in silico* evaluation of a new family of hybrids presenting two cyclolignan units, combining the inhibition of tubulin and topoisomerase II. Based on successful previous reports[4,5], obtention of fragment precursors from the natural product podophyllotoxin, design and synthesis of novel conjugates will be display together with preliminary results of biological activity.



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BENZIMIDAZOLYL CARBAMATES AS ANTITUMOR AGENTS ACTIVE ON HEAD AND NECK SQUAMOUS CARCINOMA CELL LINES

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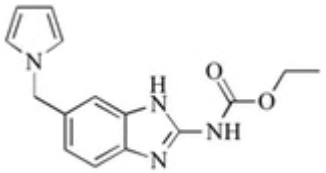
Benzimidazole and pyrroles nucleus are known to have a remarkable biological activity. Benzimidazole structures are also known to inhibit some kinases involved in carcinogenesis, such as c-KIT, ABL, B-RAF [1-2]. Years ago, we designed and synthetized a pyrrolylmethylbenzimidazole carbamate named RDS 60 [3] (figure 1). NIH tested this compound for its antitumor activity against a panel of tumor cell lines and it was found very active. Nowadays we have resumed the studies on this compound with the aim to widen the assays on more specific and aggressive tumors not tested at that time from NIH, and to elucidate its mechanism of action.



RDS 60

RDS 60 has been shown to inhibit proliferation on both CAL 27 and FaDu cell lines of Head and Neck squamous carcinoma cell at 1 μM after 48h of treatment, not inducing significant effects on a culture of healthy human fibroblasts at the same concentrations. It was found to induce apoptosis since its ability to cause the cleavage the PARP-1 marker, confirmed with WB analysis. RDS 60 also prevents the cells from organizing regular mitotic spindles and completing mitosis correctly by arresting them in the G_2/M phase thanks to the binding to β-tubulin.

Considering these new results and that tubulin binding agents have been recognized as valid antineoplastic agents for forty years [4], we decided to design and synthesize new analogues of RDS 60.

RDS 60 was synthesized constructing the pyrrole by a Clauson-Kaas reaction [3]. This only leads to pyrroles unsubstitued on α and β positions. The new congeners have been conceived through an alkylation with the proper benzyl chloride of pyrrole derivatives obtained by TosMIC reaction from the proper Michael acceptor.

The first alkylating agent was supposed to be 3,4-dinitrobenzyl chloride. However, the instability of this chloride in the alkylation conditions, pushed us to use different benzyl chlorides as reagents. Hence, we tried with 4-amino-3-nitro-benzyl chloride but the alkylation led to the required pyrroles in very low yields, because of formation of side products. Then, we used a protected form, 4-(Cbz-amino)-3-nitro-benzyl chloride and the alkylation with this compound gave alkylated pyrroles in good yields.

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ONE RING TO RULE THEM ALL: THE INDOLE IS INDISPENSABLE FOR ANTITRYPANOSOMAL PAULLONES

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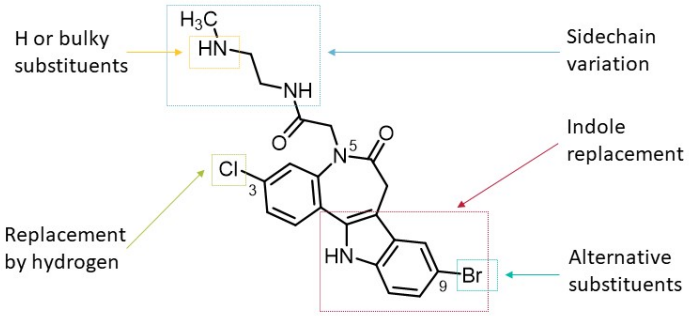
Parasites from the genera *Trypanosoma* and *Leishmania*[1] cause certain neglected tropical diseases. As treatment is scarce, new strategies to combat these diseases are required, including chemotherapeutic approaches. In our approach, the molecular target is a vital enzyme of the parasites' unique trypanothione-based thiol redox metabolism: the trypanothione synthetase (TryS)[2]. Previous work has shown that N^5-substituted paullones like MOL2008 display antitrypanosomal activity as well as TryS inhibition[3].

We aimed to investigate how the paullone scaffold can be modified to improve activity, selectivity and aqueous solubility. The novel compounds' design is partly based on molecular docking studies on TryS homology models. Different series of compounds were obtained with significant modifications, including replacement of the indole and variation of the N^5 side chain. Minor changes included substitutions in the 3- and 9-position of the paullone scaffold and the amine functional group in the sidechain. We tested the activity of the synthesised compounds against the infective stage of *Trypanosoma brucei brucei* and recombinant TryS from *T. brucei*. On the one hand, alternative 9-substituents and bulkier residues in the sidechain led to sustained antitrypanosomal activity. On the other hand, major modifications such as molecule downsizing negatively impacted the biological activity, rendering the indole moiety indispensable for a compound to be active.



MOL2008 (an N^5-substituted 3-chlorokenpaullone)
IC_{50} (*Li*TryS) = 0.15 ± 0.06 µM
EC_{50} (*T. b. brucei*) = 4.3 ± 0.7 µM

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SYNTHESIS, OPTIMIZATION, AND BIOLOGICAL EVALUATION OF NEW 4-OXO-1,4-DIHYDROQUINOLIN-3-ADAMTILAMIDES DERIVATES FOR THE DEVELOPMENT OF CB2 FLUORESCENT COMPOUNDS.

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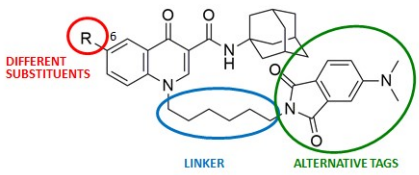
Cannabinoid receptor subtype 2 (CB2R), together with the subtype 1 (CB1R), belongs to the EndoCannabinoid System (ECS). CB2R, firstly known as "peripheral cannabinoid receptor", has been found to be overexpressed in different type of cancers and in the onset of neuroinflammation. CB2R up-regulation on microglial cells has been widely demonstrated, suggesting CB2R as useful target in the early diagnosis and treatment of neurodegenerative diseases. Therefore, CB2R modulation has triggered a great interest both in the therapeutic and diagnostic field.

In order to investigate the role of CB2 in pathological conditions and measure its expression, several CB2R fluorescent probes were synthesized, as green and safe diagnostic tools, as an alternative to the classic radioligands, commonly employed to study this target.

For these reasons, we recently developed as CB2R fluoligand, compound **1** (Figure 1), bearing a quinolone core (responsible for CB2R affinity and selectivity) linked by an hexamethylene spacer to the 4-*N*,*N* -dimethylaminophthalimide (as green emitting moiety). Compound **1** showed a good pharmacodynamic profile towards CB2R (K_i = 130 nM) and a good selectivity towards the same target as devoid of affinity towards the CB1R subtype (14 %@ 1μM).

With the aim to improve the pharmacodynamic properties of compound **1**, we proceeded with its optimization, through three types of structural modifications: i) insertion of substituents on the quinolone core to improve CB2R affinity; ii) modifications of the length of the spacer linking the fluorescent tag to the quinolone scaffold; iii) introduction of fluorescent tags alternative to the phthalimide nucleus with an emission spectrum shifted towards longer wavelengths (Red and NIR regions) to have greater versatility for other fluorescence techniques such as BRET. The compounds resulting from this study were tested for their affinity and selectivity towards CB2R, as well as for their fluorescent properties, providing interesting insights into CB2R fluorescent ligands structural requirements. Molecular docking simulations complemented the experimental findings providing a molecular rationale behind the observed CB2R affinities, hence paving the way for a rational design of new and better performing fluorescent probes.



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MASS SPECTROMETRY FRAGMENT SCREENING TO IDENTIFY SELCETIVE APOBEC3 LIGANDS

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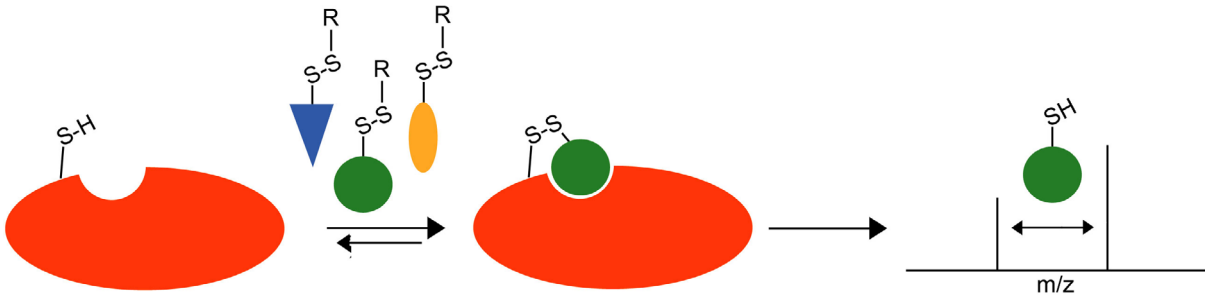
Apolipoprotein B mRNA-editing enzyme, catalytic polypeptide-like 3 (APOBEC3) is a family of seven single-stranded (ss)DNA cytosine-to-uracil deaminase enzymes (A3A/B/C/D/F/G/H) that function to clear foreign DNA from cells, such as in the event of viral infection. Additionally, APOBEC3-catalyzed deamination has been associated with cancer genome mutations that contribute to tumor metastasis and the evolution of resistance to cancer therapies, signaling this protein family as an important therapeutic target. Previous work by the Harki laboratory and collaborators have identified inhibitors of APOBEC3 proteins that are modestly potent, but inactive in cells. The goal of this project is to develop a mass spectrometry (MS)-based ligand binding assay to utilize in screens of molecules with electrophilic handles to identify selective ligands for A3A, A3B, and A3G. The ligands that are identified from screening will be characterized and optimized leveraging medicinal chemistry, structural biology, computational chemistry, and biochemical assays to yield novel APOBEC3-specific inhibitors.

We have developed a whole protein MS-based binding assay to screen for APOBEC3 ligands that identifies covalently-bound fragments by a shift in mass. The covalently-bound fragments are relatively quantified as the percent of ligand bound based on sum intensities of the adducted and non-adducted peaks. An initial pilot screen has been performed against the c-terminal domain of A3G(ctd), which is the only APOBEC3 protein that contains a native cysteine near the active site, using a small library (n=35) of fragments containing reversible electrophiles. Two compounds were identified from this screen as potential ligands for further examination. Additional protein chemistry work to install non-native cysteines in A3A for structure-function studies and subsequent electrophile screens will be presented.



PHOTOSWITCHING MOLECULES FOR THE SPATIOTEMPORAL TARGETING OF CANCER STEM CELLS WITH LIGHT

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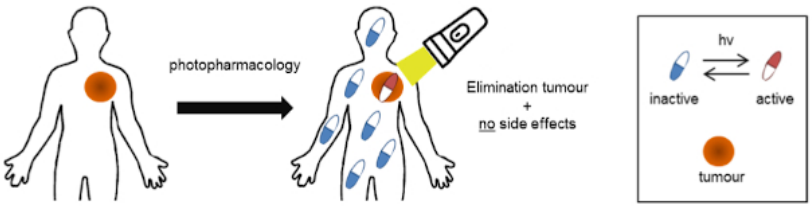
Cancer treatment still represents a major challenge, with continuous increase in incidence, morbidity and relapse. It is now accepted that resistance to conventional chemotherapy is often caused by a small population of cells that can self-renew and differentiate into the cells that constitute the bulk of the tumour, designated cancer stem cells (CSCs).[1] CSCs can adopt a quiescent state, which is not affected by standard anti-proliferative chemotherapy. Since the CSC concept was first demonstrated,[2] the field of CSCs has seen an enormous advance, and to date CSCs have been identified, isolated and characterised from various human cancers. However, paradoxically **approaches to avoid tumour relapse caused by CSCs are scarce**.

The lack of selectivity of small molecule drugs, which often leads to side effects, is caused by the inability to control their biological activity in time and space. This remains a major issue in the care of cancer patients amongst others. **Photopharmacology aims to use light as an external non-invasive element to control drug activity**, allowing for the activation of drugs with high spatial and temporal precision.[3] The design of light-responsive molecules is based on photochromism, where a light-regulated moiety is incorporated into a drug, leading to changes in polarity, geometry, and end-to- end distance upon isomerisation. These changes can be designed to alter its biological activity.

Given the similarity of CSCs to normal stem cells, targeting only the former is a big challenge. However, doing so is essential for a treatment that is well-tolerated. We believe that this challenge can be overcome by designing **drugs that can be activated only in the tumour area by external light**.



We will present our first proof of concept results. We have synthesised histone deacetylase inhibitors (HDACis) that change their conformation under illumination with different wavelengths. We have photocharacterised them to determine the optimal switching conditions and half-time, and tested them in enzymatic and cellular assays. We have optimised them to be activable under visible light and to increase the activity difference between dark vs light conformations. These constitute the first reported examples of photoactivable molecules targeted to eliminate CSCs with external spatiotemporal control.

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A PHENOTYPIC SCREEN IDENTIFIES A SMALL MOLECULE THAT INDUCES DIFFERENTIATION OF AML CELLS IN VITRO AND SHOWS ANTI-TUMOUR EFFECTS IN VIVO

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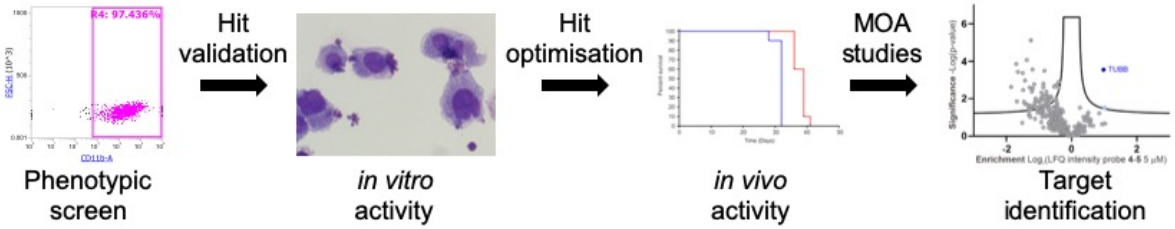
Acute myeloid leukemia (AML) is the most aggressive type of blood cancer, and the second most common leukemia in adults. AML arises from genetic mutation(s) which cause an arrest of differentiation at the progenitor or precursor stage, blocking production of downstream blood lineages.[1] The standard of care for many years has been intensive cytotoxic chemotherapy, which often results in numerous side effects and low rates of complete remission. An alternative approach that has gained much interest in the recent years is to relieve the differentiation block of AML cells, instead of directly inducing apoptosis.[2] Such therapies are expected to be more effective and less toxic. However, the reported examples are targeted to specific mutations and are only effective to narrow patient populations.

Our goal was to identify novel small molecules that induce differentiation to AML cells regardless of their genetic status. For this purpose, we developed a robust *in vitro* screening assay which we utilised to perform a pilot screen of over 1,000 small molecules. The screen was followed by a validation strategy which identified a range of structurally distinct hits that could differentiate AML cells of several subtypes. An extensive medicinal chemistry program to improve the properties of these compounds delivered highly potent molecules with favorable physicochemical properties, enabling the progression to *in vivo* studies.

One of the compound classes proved to decrease tumour volume and increase survival in a xenograft model of AML. Subsequent intensive mechanism of actions studies, including RNA sequencing and chemoproteomics, identified the biological target driving the observed differentiation effect.



This work identified novel small molecules able to induce differentiation in wider patient populations, which have the potential to be more effective and better tolerated than current agents. It also proposed an unknown role of the identified target towards the induction of differentiation in AML.

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NEW FIDAXOMICIN ANTIBIOTICS: COMBINING METABOLIC ENGINEERING AND SEMISYNTHESIS

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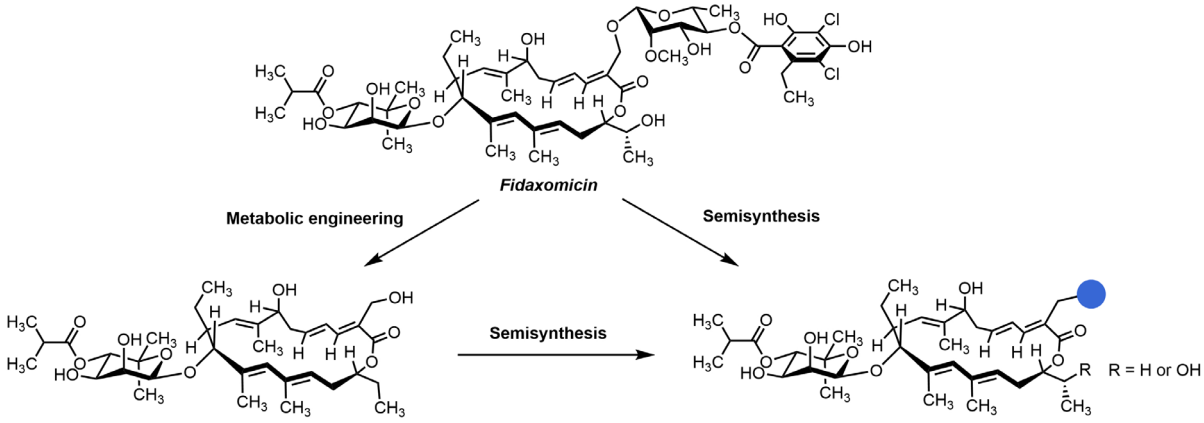
Fidaxomicin (tiacumicin B, lipiarmycin A3)[1] is a glycosylated macrocyclic antibiotic with potent activity against various Gram-positive bacteria through inhibition of the RNA-polymerase (RNAP).[2] In 2015 our research group accomplished the first total synthesis of this complex natural product.[3,4] Fidaxomicin is marketed to treat *Clostridium difficile* infections in the gut. Moreover, its antibacterial activity against resistant strains of *Mycobacterium tuberculosis* and *Staphylococcus aureus* is of great interest, as these strains still pose a global problem.[5,6]



However, treatment of these systemic infections with fidaxomicin is prevented by its low water solubility. The central goal of this study was the synthesis of new fidaxomicin derivatives with reduced structural complexity that retain or improve their antibiotic activities, while improving other parameters like solubility. Our investigation pursued two strategies: the isolation of shunt metabolites from a mutant fidaxomicin producing strain followed by their chemical modification and semisynthesis of new antibiotics by chemical modification of the natural product itself. The antibiotic activity of derivatives was rationalised by modelling their binding to bacterial RNAP.

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DESIGN OF NOVEL PYRIMIDINE DERIVATIVES AS BACE-1 INHIBITORS BY MOLECULAR DOCKING

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Dementia causes changes progressively in the cognitive, functional and behavior of the person (1). Alzheimer's disease (AD) is one of the most common types of dementia and a destructive neurodegenerative disease (2,3). Aβ in amyloid plaques causes neurodegeneration which explains its relationship with Alzheimer's (8). The major pathophysiology of AD are amyloid plaques formed by amyloid-β (Aβ) and intraneuronal neurofibrillary tangles formed by the accumulation of hyperphosphorylated tau proteins (2,3,4). Also, amyloid-β (Aβ) is formed because of the split of amyloid-β protein precursor (AβPP) by β-secretase (BACE-1) and then by γ-secretase. As a result of some researches, a relationship was found between BACE1 activity and Aβ accumulation in the brain. This association leads to a notable increase in the pathological production of Aβ by the increase in BACE-1 activity (5). With the discovery of BACE-1, it was investigated that compounds such as atabecestat, elenbecestat and umibecestat can be effective in AD but they have failed to supply clinical consequences for patients suffering cognitive damages (6). Verubecestat tried in the treatment of mild to moderate Alzheimer's disease was not beneficial and increased side effects (7). Design and development of novel BACE-1 enzyme inhibitors can reduce AD by preventing amyloid plaque formation and present a new approach to the treatment. For this reason in this study, we aimed to examine the active site of BACE-1 through molecules which have ongoing studies in clinical phases by molecular docking and design novel pyrimidine derivatives which will be effective in Alzheimer's disease by provide inhibition of BACE-1.

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TWO NOVEL O-METHYL ANALOGUES OF (–) GONIOFUFURONE: SYNTHESIS, BIOLOGICAL EVALUATION AND SAR ANALYSIS

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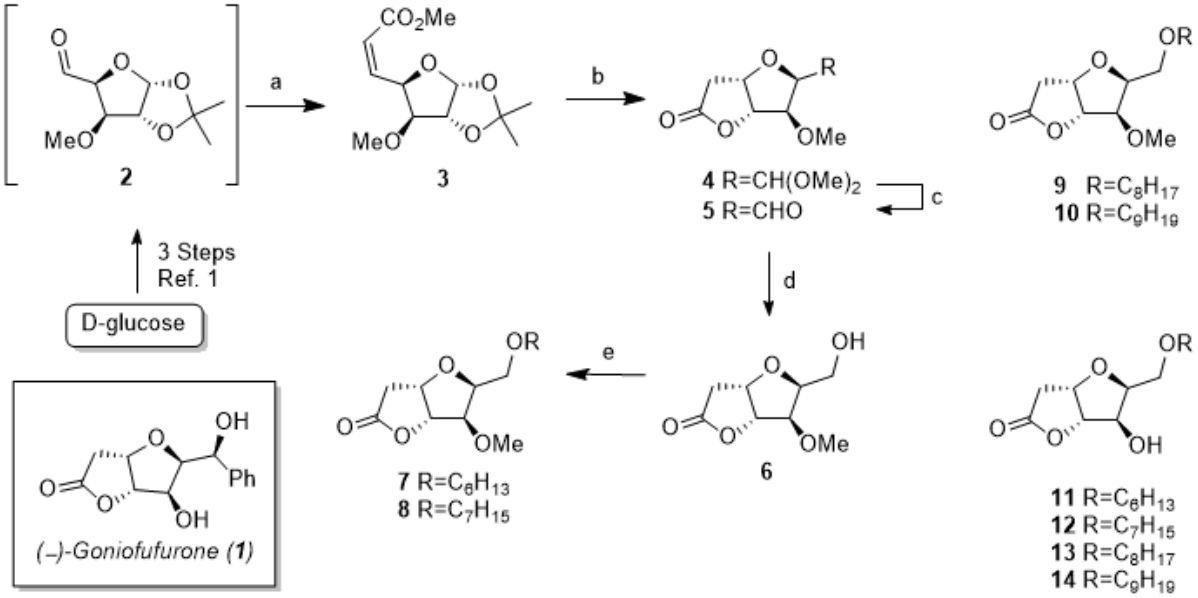
(–)-Goniofufurone (**1**) is the opposite enantiomer of the naturally occurring (+)-goniofufurone and both compounds show significant antitumour activity. In order to obtain a more potent compound, the synthesis of two novel *O*-methyl analogues of (–)-goniofufurone starting from D-glucose was performed (*Scheme* 1)[1]. The results of antiproliferative activity of **7** and **8** against a number of human tumour cell lines will be presented. Structure-activity relationship (SAR) of **1**, new analogues and few analogues (**9–14**) previously synthesized in our laboratory will be presented in detail.



Scheme 1. (a) $Ph_3P{:}CHCO_2Me$, MeOH, 0°C, 0.5 h then rt, 1.5 h; (b) 2.5% H_2SO_4/MeOH, reflux, 2 h, $NaHCO_3$, rt, 1 h, (c) 9:1 TFA/H_2O, rt, 1 h; (d) $NaBH_4$, MeOH, rt, 1.5 h; (e) $C_6H_{13}Br$for **7**, $C_7H_{15}Br$ for **8**, Ag_2O, AgOTf, Et_2O, reflux.

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ANALYSIS OF BINDING AFFINITY BETWEEN ANTIPLATELET DRUG DIPYRIDAMOLE AND HUMAN SERUM ALPHA-1 ACID GLYCOPROTEIN UTILIZING MICROSCALE THERMOPHORESIS

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An understanding of the interaction of various drugs with plasma proteins is essential for understanding their systemic pharmacology and toxicology. Thus, information concerning the effects of the acute phase responses on the ligand binding ability of plasma can be used to optimize drug administration protocols in clinical practice. Alpha-1 acid glycoprotein (AGP) has been reported to be a major plasma protein that strongly binds basic drugs such as dipyridamole, drug that is easily synthesized from 5-nitroorotic acid (1-2). In this study, microscale thermophoresis (MST) is used as an analytical technique for determining binding affinity between antiplatelet drug dipyridamole and AGP. MST is a powerful technique in quantitation of binding events based on the movement of molecules in microscopic temperature gradient. The concentration of AGP in plasma can significantly increase in various diseases (cancer, inflammatory disease) or following trauma (burns, surgery). Changes in AGP concentration could potentially alter the free fraction of drugs in plasma or at their target sites and eventually affect their pharmacokinetic disposition and pharmacological action. Given that an increase number of drugs have been shown to bind preferentially to AGP, a better understanding of this unique interaction may provide great benefit for drug discovery and development (3). Knowing the change in drug binding affinity to AGP as well as knowing the change in different medical situations can improve the therapeutic and/or toxicological outcome of treatment in dealing with a large number of different drugs.

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ACTIVATION MECHANISM OF CALCIUM PUMP SERCA BY SUBSTANCE CDN1163 – HYPOTHESIS AND THEORETICAL PREDICTION

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The calcium ATPase from sarcoplasmic reticulum belongs to the P type ATPases, which represent a large group of membrane proteins. P type ATPases use energy from ATP hydrolysis for transfer of cations or phospholipids via biological membranes. With providing a calcium transport from cytosol to the lumen of sarcoplasmic reticulum, SERCA has two primary functions in cell, the induction of muscle relaxation by reducing calcium levels in cytosol and the maintaining the stock of calcium in sarcoplasmic reticulum, necessary for the next muscle contraction. Disrupted SERCA function was observed in the framework of many chronical diseases (diabetes, cardiovascular diseases, neurodegenerative disorders, etc.). Substances capable of increasing SERCA activity are therefore potentially drugs.

SERCA is composed of 3 cytoplasmic domains, N (nucleotide) – in which nucleotide binding occurs, P (phosphorylation) – containing the phosphorylation place and from A (activator) domain – which mediates the conformational changes in the transmembrane region that are necessary for the binding of calcium ions. It also contains 10 transmembrane helices, providing two sites for the binding of calcium ions, and small luminal loops.

The synthetic substance CDN1163 was obtained on the basis of virtual screening of databases of available substances[1]. Many beneficial effects of this substance have been already documented, although the mechanism of its effect on SERCA has not been precisely determined yet. It is assumed, that it acts by an allosteric mechanism.

Our aim was to determine the probable binding sites of CDN1163 on SERCA1a protein by means of molecular modeling methods. According to the theoretical prediction with YASARA software, the compound can bind in the cytoplasmic part as well as at the interface of the cytoplasmic and transmembrane part. The results will be verified experimentally in further investigation.

This work was supported by projects: APVV-15-0455 and VEGA 2/0127/18.

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FREE RADICALS FUNCTION-BASED SCREENING OF COMPOUNDS WITH NEUROPROTECTIVE POTENTIAL

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Free radical production plays an important role in aging and neurodegenerative disorders [1]. Much evidence has been accumulated about the participation of free radicals in the pathogenesis of these disorders. Oxidative and nitrosative stress includes mitochondrial dysfunction, protein and DNA damage, and α-synuclein aggregation, or changes in calcium signaling [2].

The presented research focuses on identifying newly synthesized compounds [3] of a neuroprotective nature based on the action of free radicals. For this purpose we have conducted initial screening assays.

The experiments were carried out on the mouse hippocampal cell line (HT-22). The conducted studies have shown that, apart from the increase in neuronal proliferation, the compounds do not generate the expression of intracellular calcium and oxidative stress.

Summarizing, the obtained preliminary results are promising for the future development of treatment for neurodegenerative diseases.

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IN SILICO INSIGHTS TOWARDS RATIONALIZATION OF HEDGEHOG RECEPTOR PATCHED1 MULTI-DRUG EFFLUX INHIBITION

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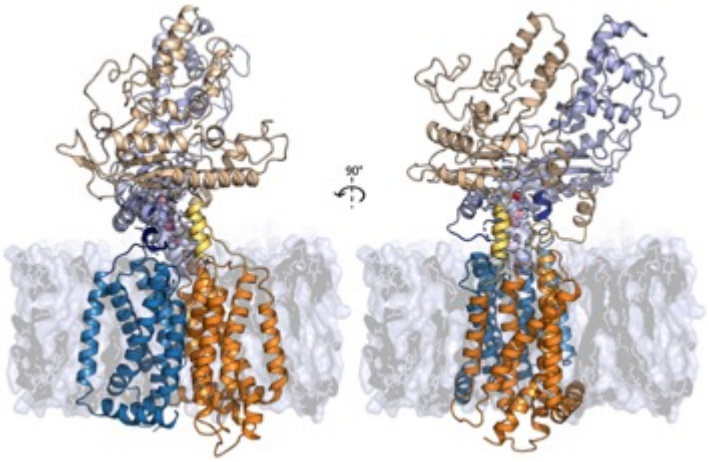
The Hedgehog receptor Patched1 (PTCH1), physiologically involved in embryogenesis and tissue regeneration in adults, has been discovered to be a multidrug transporter that carries cholesterol and chemotherapeutic agents outside cells, thus contributing to chemotherapy resistance of cancer cells[1,2]. PTCH1 is over-expressed in many recurrent and metastatic cancers such as lung, breast, prostate, colon and melanoma. Furthermore, the proton-driven drug transport activity of PTCH1 is limited to cancer cells due to the "reversed pH gradient" given by the enhanced glucose utilization. This reversed pH gradient is also considered a hallmark of malignant cancers. Altogether, these specificities make PTCH1 a particularly relevant and highly specific new therapeutic target.

A screening of marine sponge extracts led to the discovery of the very first compound able to inhibit the efflux activity of PTCH1 and overcome chemotherapy resistance: Panicein A Hydroquinone (PAH)[3]. Further studies showed that PAH, when administered in combination with standard therapeutics, such as doxorubicin or vemurafenib, is able to increase the drug cytotoxicity in melanoma cells *in vitro* and *in vivo* without undesirable side effects, thus representing a promising novel therapeutic strategy[4].

By means of different computational techniques this study aims at shedding light over the mechanism by which PAH binds to PTCH1 and inhibits sterols and chemotherapeutics transport. We first performed a thorough characterization and druggability analysis of the main putative substrate binding pockets identified from available cryo-electron microscopy structures. Further, a set of PAH analogues, which showed different *in vitro* activity, were submitted to microsecond-long all-atom MD simulations in water solution. Analysis of the conformational landscape of the compounds was performed by evaluation of different dynamical descriptors. Finally, a binding mode prediction through a blind ensemble docking methodology enabled to rationalize the interaction between PTCH1 and PAH and derivatives in terms of their intrinsic physico-chemical properties[5]. The obtained results will guide future drug design with the aim to synthesize compounds that will more potently inhibit PTCH1 drug efflux activity and increase standard treatment efficacy against resistant cancer cells.



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STYRYLQUINAZOLINE DERIVATIVES AS PROMISING ANTICANCER DRUGS FOR GLIOBLASTOMA TREATMENT

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Glioblastoma multiforme (GBM) is the most common cancer among primary tumors of the brain and central nervous system, accounting for as much as half of all glioblastomas. The median survival with GBM is 12-15 months, and the 5-year survival rate remains below 5% despite intensive treatments. One of the possibilities to improve the therapeutic potential of and increase the arsenal of applied cancer therapies is to design and introduce novel tyrosine kinase inhibitors. Tyrosine kinases mediate cellular signal transduction by participating in and controlling many critical cellular processes, such as proliferation, growth, migration, metabolism, and cell death. Precise regulation of these processes through kinases determines the proper functioning of cells throughout the body. It is worth emphasizing at this point that in the case of many cancer, their function is disturbed – by mutations or excessive activity so that they become promising targets for anticancer therapies. [1][2]

Recently, we have focused on searching for novel derivatives that could become potential drugs in the fight against GBM. Our team has developed and synthesized an extensive library of styrylquinazoline (SQ) derivatives for several years, from which promising inhibitors were selected for further research for GBM.[3][4] The cytotoxicity of SQ derivatives was performed using MTS tests on human glioblastoma cancer cell lines: DKMG/EGFRvIII, U-87, and U-251. In general, tested compounds exhibited very high activity against tested GBM cells (IC_{50} below 2 μM). In the next step, we focused on a deeper investigation of SQ's molecular mechanism of action. First, we investigated the influence of tested compounds on cell cycle progression and apoptosis induction. The results showed that the SQ may cause a strong cell cycle arrest in the G2/M phase and partially in G0/G1 phase. This phenomenon could be explained by the effect of sulfonic SQ on the polymerization of tubulin, which may cause the observed cytotoxic effects and mitosis inhibition.[5] In addition, the impact of SQ on the EGFR/mTOR signaling pathway was determined using the western blot technique. A change in the expression of the protein level of this pathway: EGFR, mTOR, and AKT, was observed. Finally, we investigated the possibility of using novel SQ derivatives in combination therapy with the known EGFR inhibitors: osimertinib and afatinib. The analysis of the results revealed several synergistic interactions between the tested compounds.

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SELEC-PROTACS: NOVEL CANCER TARGETING PROTEIN DEGRADERS

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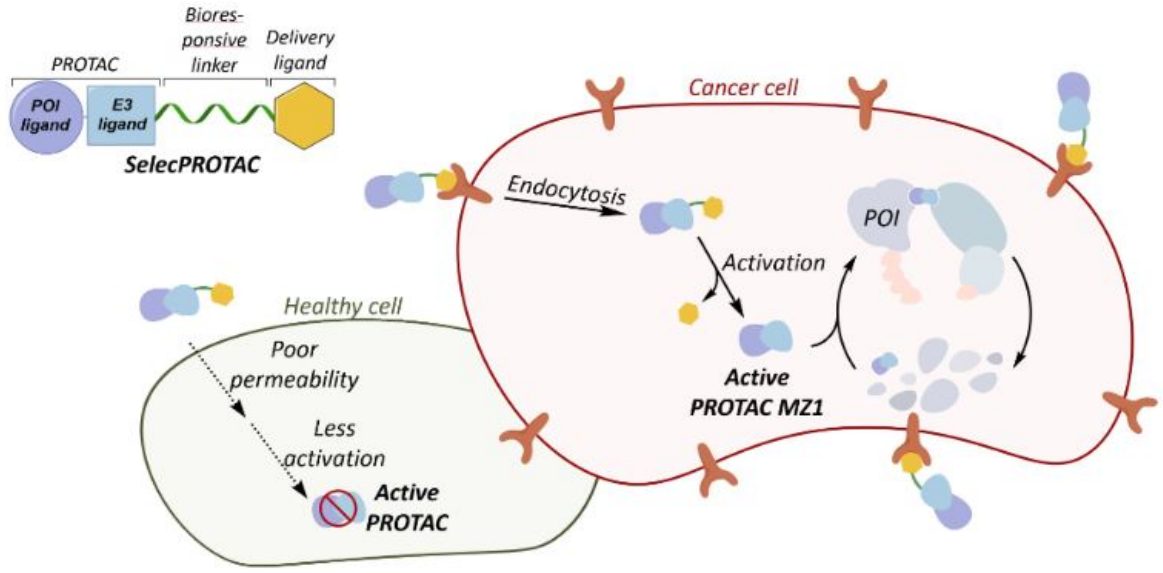
PROteolysis TArgeting Chimeras (PROTACs) are bifunctional probes capable of hijacking the cells' natural proteasomal degradation machinery to completely degrade the protein of interest (POI). PROTACs have enabled the targeting of many proteins so far considered undruggable by a classical occupancy-based inhibition mechanism, thereby expanding therapeutic prospects for various pathologies including cancer.[1]

Despite PROTACs being an efficient catalytic method to degrade malfunctioning proteins in cancer cells, many of their targets are also expressed in healthy cells, where they play an essential role in the cells' functioning. Hence, on-site side effects are one of the major drawbacks of PROTACs, due to the lack of selectivity between healthy and cancerous cells. [2]

To tackle these issues, this work has focused on developing cancer cell selective and bioresponsive degraders called SelecPROTACs. These small-molecule prodrugs have been designed to capitalize on some of the key characteristics of cancer cells to ensure targeted delivery and PROTAC release only upon reaching the cancerous environment.[3]

We have synthesized proof-of-concept probes based on the BRD4 PROTAC MZ1[4], bearing a cancer-cell favoring delivery ligand appended through a bioresponsive cleavable linker, to enable cancer cell specific activation. These inactive prodrugs have sown to release the active PROTAC via the expected mechanism and have comparable POI degradation ability to the parent PROTAC in cancerous cells. As desired, these prodrugs show reduced degradation activity in non-cancerous cells, owing to decreased uptake or drug release.

However, a negative control analogue with a non-cleavable linker also maintains some limited biological activity, leading us to question the stability of the key connection sites and the linker on the molecule. Hence, future efforts will be focused on modifying linkage mechanism for enhanced stability in biological conditions and to further improve the spatio-temporal control and selectivity of the release at the site of interest.



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DESIGN AND SYNTHESIS OF THE FIRST INDOLE-BASED BLOCKERS OF PANX-1 CHANNEL

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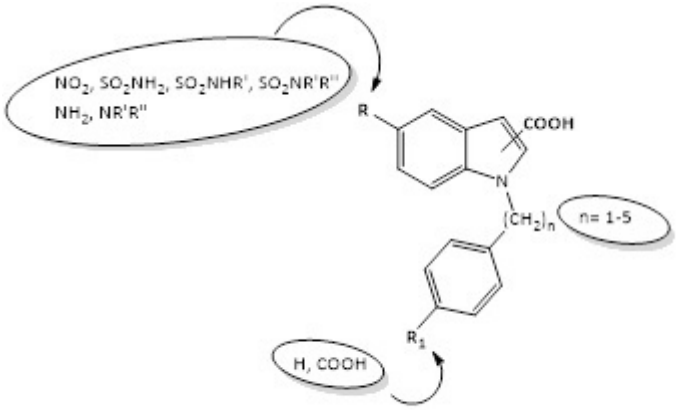
Pannexins (Panxs), first identified in 2000, are a family of proteins expressed throughout the body and forming non-junctional plasma membrane channels, which connect the intra- and the extra-cellular spaces.[1,2] Till now, three isoforms of Panxs have been identified, named Panx1, Panx2, and Panx3; Panx1, whose heptameric structure was recently elucidated,[3] is the most abundant and the only one to be well characterized.[4] Different mechanisms have been identified for the activation of Panxs channels such as the increase of particular cations concentration, proteolytic cleavage by caspase 3 or caspase 7, and mechanical stimulation.[5] Once activated, these channels allow both ions (mainly chloride) and ATP permeability, the latter responsible for the regulation of the activity of other receptors, such as the purinergic P2XR and P2YR subtypes. Different studies have highlighted an up-regulation and over-expression of Pannexin channels in several disorders such as neuropathic pain, certain types of cancer, Parkinson's and Alzheimer's diseases.[5] The involvement of Pannexins in a variety of pathological processes makes these proteins a very attractive therapeutic target. Our interest in the study of Panx-1 blockers was triggered by the fact that a specific class of Panx-1 inhibitors has not yet been discovered and all compounds used so far for the study of this channel have a different primary target such as the anti-gout drug Probenecid or Carbenoxolone, or alternatively are no drugs, as for example the food dye Brilliant Blue FCF or 5-Nitro-2-(3-phenylpropylamino)benzoic acid (NPPB). As first approach to this new research, we have selected from our library some simple scaffolds, whose synthesis and elaboration were fast and easily reproducible. Among all the poly(hetero)cycles analyzed, we have identified the indole scaffold as the most suitable for the synthesis of Panx-1 inhibitors. We elaborated this nucleus by inserting the characteristic groups of NPPB and of the drug Probenecid, such as the nitro group, the sulfonamide function variously substituted, one or more carboxylic groups, as well as other chemical moieties. All the new products have been tested using the two electrode Voltage Clamp technique on *Xenopus Laevis*oocytes expressing Panx1 channels exogenously. Moreover, two selected compounds were tested in vivo in a model of neuropathic pain and finally, molecular modeling studies were performed.



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NEW ANTI-ALZHEIMER BIOLOGICAL ACTIVE HYBRIDS COMBINING ENONE AND PYRIMIDINE SCAFFOLDS

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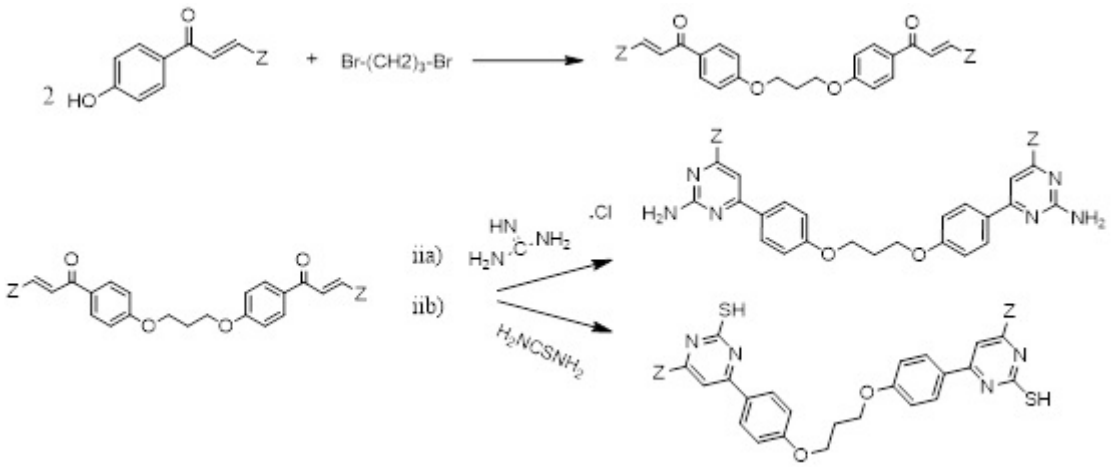
Epidemiological and experimental studies have already established the functional relationship between Alzheimer disease, oxidative stress and inflammation. [1, 2, 3] The reason that creates AD and other dementias is not fully discovered as well as the links among all these paths. Thus, we attempted to clarify these relationships and introduce new therapeutic agents. Multitarget therapeutic strategy can be used to inhibit two or more biological targets, acting on an enzyme and/or a receptor, or affect an ion channel and a transporter. [4] Therefore it is evident that the treatment of Alzheimer Disease (AD) could benefit from the use of multipotent drugs that present free radical scavenging, anti-inflammatory and AChE inhibitory activity. Chalcones are biogenetic precursors of flavonoids in higher plants displaying a wide variety of pharmacological properties. They are well known intermediates for the synthesis of various heterocyclic compounds. Cyclization of chalcones, leading to thiazines, pyrimidines, pyridazines, attracted a developing research increase within the heterocyclic chemistry for several years due to their quick accessibility and the broad spectrum of biological activities. In the present study it has been synthesized a series of new pleiotropic compounds which have multiple biological activities that uses the enone moiety as basic scaffold. [3, 4]

Using computer aided drug design and previous biological data from known chalcones,flavonoid derivatives and pyrimidines we designed a series of new bis-chalcones and their corresponding bis-pyrimidines with possible inhibition on achetylocholinasterase and lipoxygenase as well as antioxidant activity.

Chemistry: [3, 4, 5, 6]



The compounds were tested *in vitro* for their ability to: a) inhibit in vitro AchE, b) inhibit lipid peroxidation of linoleic acid, c) inhibit soybean lipoxygenase and d) interact with DPPH. The results were discussed in terms of structural characteristics and physicochemical properties of the molecules.

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THIAZOLIDINEDIONE "MAGIC BULLETS" SIMULTANEOUSLY TARGETING PPARγ AND HDACS: DESIGN, SYNTHESIS, AND INVESTIGATIONS OF THEIR IN VITRO AND IN VIVO ANTITUMOR EFFECTS

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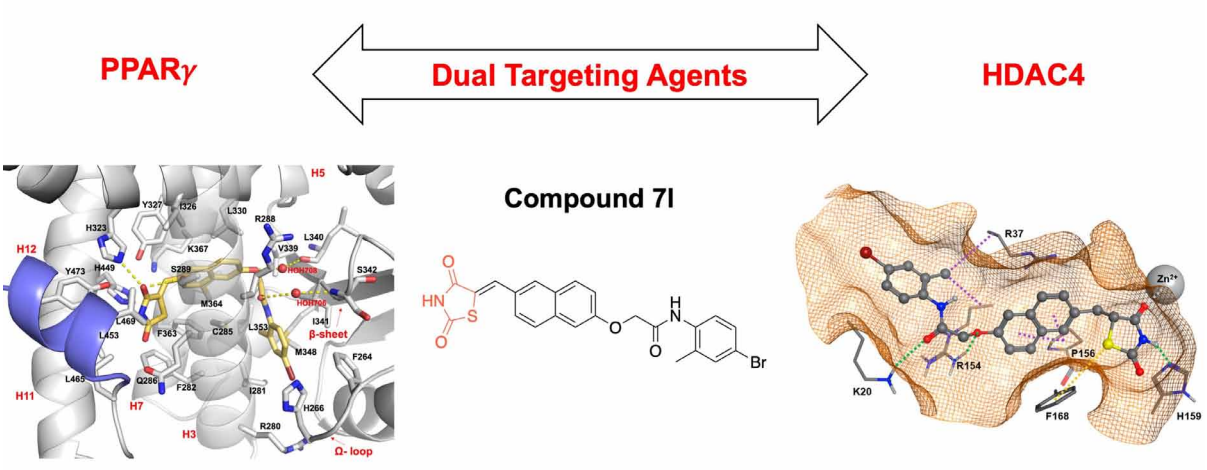
Monotargeting anticancer agents suffer from unexpected resistance and target nonspecificity concerns due to tumor heterogeneity. To overcome these concerns, an alternative strategy of multitargeting can be applied to hit multiple cancer hallmarks and achieve the desired pharmacological efficiency with reduced detrimental effects such as drug–drug interactions, unforeseen side-effects, and poor patient compliance [1]. A recent focus of anticancer drug discovery has been directed to epigenetic targets, such as the histone deacetylases (HDACs), that are aberrantly expressed in a variety of solid and hematopoietic malignancies [2]. In addition, the subtype γ of the peroxisome proliferator-activated receptors (PPARs) is vital to cancer cell growth regulation [3]. Reasonably, our combined treatment with HDAC inhibitors and PPARγ agonists has displayed potential antitumor effects, increasing the cytotoxicity effects in a synergistic/additive manner against different cancer cell lines and resulting in the arrest of proliferation and increased apoptosis. Our work involves the rational design and synthesis of 25 novel TZD-based naphthylidene derivatives that can simultaneously target PPARγ and HDAC. Six out of 25 compounds acted as dual-targeting agents with submicromolar potencies. Compound **7i** and **7l** were the most potent dual targeting agents, with EC_{50} values toward PPARγ 0.245 μM and 0.359 μM and IC_{50} values against HDAC4 of 1.1 μM and 0.55 μM, respectively. Additionally, compounds **7c** and **7i** were cytotoxic to CCRF-CEM cells (CC_{50} = 2.8 and 9.6 μM, respectively), induced apoptosis, and caused DNA fragmentation. Furthermore, compound **7c** modulated the expression of c-Myc, cleaved caspase-3, and caused in vivo tumor regression in CCRF-CEM tumor xenografts. Thus, this study provides a basis for the rational design of dual/multitargeting agents that could be developed further as anticancer therapeutics.



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DIFFERENT DRUG DESIGN APPROACHES TO TACKLE O-GLCNAC TRANSFERASE INHIBITION.

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O-GlcNAc transferase (OGT) is an essential enzyme that catalyzes the transfer of N-acetylglucosamine (GlcNAc) onto Serine and Threonine protein residues. This dynamic post-translational modification occurs on hundreds of cellular targets, and its impairment is linked with severe human pathologies such as Diabetes and Cancer. Unfortunately, the role of OGT in these pathogenic processes is still not completely understood. Hence, selective and cell-permeable OGT inhibitors are strongly needed to further study the O-GlcNAc cycle and validate OGT as a therapeutic target.

This communication will cover our recent efforts in exploring the chemical space of OGT inhibitors by combining different drug design approaches.

Structure-based virtual screening of fragment-like and drug-like libraries led to the identification of novel uridine mimetic scaffolds that target the enzyme's active site. As an alternative strategy, the presence of new, potentially druggable pockets in the protein structure was investigated.

The molecular modelling results served as the basis for the rational design and synthesis of new OGT inhibitors that exhibited IC_{50} values in the micromolar range.

ENHANCING BINDING OF SPIROPYRAZOLINE OXINDOLES TO MDMS BY IN SILICO HIT-TO-LEAD OPTIMIZATION

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The p53 tumor suppressor protein is involved in several biological mechanisms that assure the integrity of the human genome. However, in all types of human cancers, the p53 tumor suppressor function is inactivated by mutation or gene deletion or by overexpression of negative regulators such as MDM2 and/or MDMX. In the latter case, great efforts have been done to develop p53-MDM2 protein-protein interaction (PPI) inhibitors which some have achieved clinical trials. Still, these efforts were not enough for a full reactivation of p53. Later, the negative regulator MDMX has also emerged as an important target. Both MDM2 and MDMX interact with p53 by four amino acids, Phe19, Leu22, Trp23, and Leu26. Though, the structural and conformational differences between the regulators hinder the use of p53-MDM2 PPI inhibitors as efficient p53-MDMX PPI inhibitors. For this reason, the discovery of potent small molecules acting as dual p53-MDM2/X PPI inhibitors is still a challenge. For a full reactivation of p53, development of drugs that disrupt the interaction of p53 with both negative regulators is still an essential matter[1].

Our research team has been working on the development and optimization of indole-based compounds to obtain dual p53-MDM2/X PPI inhibitors[2,3]. Previously, we have identified a spiropyrazoline oxindole derivative that induces apoptosis and cell cycle arrest at G0/G1 phase, upregulates p53 steady-state levels, and leads to a decrease of MDM2 levels. However, the compound did not bind to MDM2 in competitive binding assays[2]. Moreover, molecular docking studies have revealed that this derivative did not have important interactions necessary to bind to MDM2. For this reason, we have been focusing our efforts on the development of new spiropyrazoline oxindole derivatives that target both MDM2 and MDMX. In this communication, we report the construction of virtual libraries of spiropyrazoline oxindoles derivatives by adding fragments to the scaffold to obtain dual p53-MDM2/X PPIs inhibitors by structure-based virtual screening of these libraries over the MDM2/X structures. Moreover, the *hit* compounds identified *in silico* screening were further optimized and 30 new molecules were synthesized and evaluated in cancer cell lines and competitive binding assays for MDM2 and MDMX.

This work was supported by national funds through FCT - Fundação para a Ciência e a Tecnologia, I.P., under the project PTDC/QUI-QOR/29664/2017, iMed.ULisboa (UIDB/04138/2020) and fellowships SFRH/BD/137544/2018 (E. A. Lopes) and SFRH/BD/117931/2016 (M. Espadinha).

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SYNTHESIS OF MATRIX METALLOPROTEINASE-2 (MMP-2) FLUORESCENT INHIBITORS FOR CANCER PROGNOSIS

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Matrix metalloproteinases constitute a family of zinc-dependent endopeptidases that participate in the breakdown of connective tissue.[1] Overexpression of these enzymes is found in different diseases. Among all MMPs, we focus on MMP-2 since they are related to cancer progression and are overexpressed in bladder, breast, colon, lung, prostate, and gastric cancers.[2,3] Therefore, the level of MMP-2 can be used as an incidence rate for cancer prognosis and treatment.

In previous studies developed in our research group, potent and selective inhibitors of MMP-2 were synthesized. These compounds combine a Zinc Binding Group (ZBG) able to chelate the Zn atom present in the active site and a subunit designed to interact with the S1' pocket responsible for the selectivity.[4]

In the present work, we propose the incorporation of fluorescent ligands to the inhibitors described above without compromising their affinity for MMP-2. The final objective is to obtain selective MMP-2 probes for the diagnosis, monitoring of tumours and their application in targeted therapies (Figure 1).

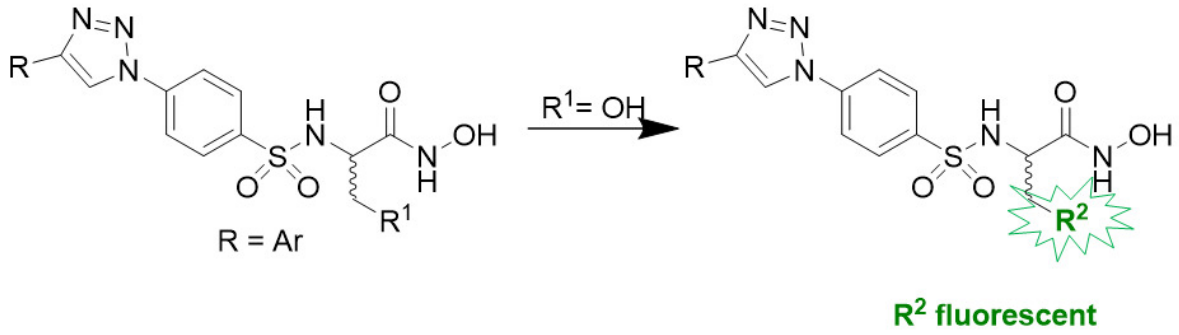

Figure 1. Design of potential MMP-2 fluorescent probes

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SYNTHESIS, MOLECULAR STRUCTURE CHARACTERIZATION AND CYTOTOXICITY STUDY ON A SERIES OF 2-ARYL-[1,3]THIAZOLO[3,2- a]BENZIMIDAZOL-3(2H)-ONES CONTAINING NITROARYL AND NITROHETEROARYL MOIETIES

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The nitro group is unique functional group in pharmacy and medicine. Much of the effort of medical chemistry has focused on the use of nitro compounds for anticancer agents [1]. These drugs exert their cytotoxic effects through a variety of mechanisms, such as inhibition of topoisomerase, histone deacetylase, also by alkylation of DNA or by inhibition of tubulin polymerization. They are applied in different areas due to their ability to undergo bioreduction [2]. The nitro group is reduced in organism by special enzymes – nitroreductases [2]. As a result of this process nitro radical anions and further cytotoxic agents are formed in hipoxide environment.

With the aim of developing some new thiazolo[3,2- a]benzimidazol-3(2H)-ones able to exert selective cytotoxic effect in hypoxic conditions, we have synthesized a series of derivative containing nitroaryl and nitroheteroaryl moieties. The compounds were obtained from 2-mercaptobenzimidazole, chloroacetic acid and relevant benzaldehydes, using two methods of synthesis. The first two-step reaction pathway is carried with isolation of the corresponding benzimidazolethiazolone as intermediate product. The second one is one-pot synthesis and affords directly the 2-aryl-[1,3]thiazolo[3,2- a]benzimidazol-3(2H)-ones. In this way, we have obtained products containing nitro group both in the benzimidazole fragment and the 2-aryl moiety.

The molecular structure was characterized by spectral and theoretical methods. The theoretical spinal density and electron affinity were calculated in order to evaluate the propensity of the compounds to nitro reduction. The potential ability for transport in human organism and bioavailability was estimated based on physic-chemical parameters such as lipophilicity, molecular volume, number of hydrogen bond acceptors and donor, rotatable bonds.

The in vitro cytotoxicity of the represented compounds was tested in concentrations ranging between 0.4 and 200 μM on a panel of tumorigenic (malignant melanoma – A-375, epidermoid carcinoma of the skin – A-431) and non-tumorigenic (normal human keratinocytes – HaCaT, normal mouse fibroblasts – CCL-1, normal transformed human embryonic kidney cells – HEK293) cell lines following ISO 10993-5, Annex C. The compounds were cytotoxic to the tumor cell lines in concentrations between 3.125 and 150 μM.

Acknowledgments: This work was supported financially by the National Science Fund of Bulgaria, Contract КП-06-Н39/4.

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SATURATION TRANSFER DIFFERENCE NMR (STD NMR) AS USEFUL TOOL FOR THE CHARACTERIZATION OF MULTIMERIC PYRROLIDINE IMINOSUGAR BINDING TO GH1 β-GLUCOSIDASES

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Iminosugars are biologically active compounds well known as glycosidase inhibitors.[1] The display of these molecules in a multivalent fashion can afford better inhibitors in terms of potency and selectivity.[2] The elucidation of the chemical basis accounting for the interaction of inhibitors of glycosidases of therapeutic interest is the key aspect for the design of selective inhibitors that could be used as pharmacological chaperones to treat diseases caused by deficiency of these enzymes.[3]

Bacterial enzymes are in general easy to manipulate and may serve as models to analyze structural aspects related to homologous human proteins. In this work we report the synthesis of multimeric pyrrolidine iminosugars and their biological evaluation towards the GH1 β-glucosidases A and B isolated from *Paenibacillus polymyxa* (BglA and BglB). Saturation Transfer Difference NMR (STD NMR) experiments have been carried out in order to unveil the detailed characterization of ligand binding in the enzyme-inhibitor complexes in solution. This study could be helpful in the accurate design of potent inhibitors of the homologous human enzymes.

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METHYLHYDROXYLAMMONIUM DERIVATIVES - LYSINE-TARGETED IRREVERSIBLE INHIBITORS FOR THE ANTI-VIRULENCE BACTERIAL TARGET TYPE I DEHYDROQUINASE

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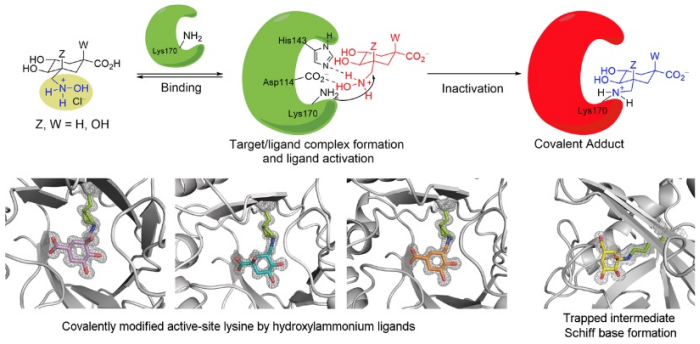
In recent years, irreversible inhibitors have gained momentum in the drug development pipeline with numerous clinical candidates under study and more than 50 FDA-approved drugs.[1] Their enormous advantages, as well as the more detailed knowledge available today on the real risks associated with these types of compounds, explain its increasing presence in the drug discovery programs.[2] Nonetheless, the design of this type of compounds is still a challenge, because they must combine selectivity (target affinity) and reactivity (covalent modification) in a single chemical entity, thus enabling inhibitory potency without triggering off-target effects. In recent years, a great deal of effort has been devoted to the development of ligands bearing functional groups, often referred to as latent electrophiles, that become activated towards covalent modification upon binding. Most of them are meant to modify cysteine residues, whereas the available latent electrophiles for selective lysine residues modification remains sparse. Herein we report the first example of a methylhydroxylammonium derivative that causes a specific covalent modification of an active-site and a sterically inaccessible lysine residue of the type I dehydroquinase enzyme (DHQ1), which is a recognized target for the development of new anti-virulence agents.[3] DHQ1 is present in pathogenic bacteria such as *Salmonella typhi* and *Staphylococcus aureus*. These first-in-class compounds **1–3** proved to cause the irreversible inhibition of the DHQ1 enzyme from *S. typhi* and *S. aureus* by covalent modification of the catalytic Lys170/Lys160 through a stable amine bond, which was demonstrated by resolution by X-ray crystallography at 1.08–1.25 Å of the corresponding crystal structures of the enzyme/ligand adducts.[4] A two-dimensional QM/MM umbrella sampling simulation study of the covalent modification mechanism suggested that the direct displacement by nucleophilic attack of the catalytic lysine residue with the release of hydroxylammonium would be plausible. These studies might open up new opportunities for the development of novel lysine-targeted irreversible inhibitors bearing a methylhydroxylammonium moiety as a latent electrophile.



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A PATIENT-CENTRIC MINIATURISED SAMPLING AND ANALYSIS APPROACH FOR THE EVALUATION OF CNS DRUGS FOR TDM PURPOSES

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Antipsychotic-based therapies within psychiatric regimens require a careful and frequent evaluation of drugs and active metabolites blood concentrations. To this purpose, microsampling can lead to significant benefits when compared to classic in-tube routine sampling and analysis, for both bioanalytical laboratories and clinical decision-making, with the perspective of drug therapy personalisation in a patient-centric view [1]. Microsampling approaches not only meet the needs of patients and clinicians, but also allow the development of miniaturised protocols that can be easily implemented in automated systems and sustainable analytical procedures thanks to the reduction of both sample volume and waste solvents [2].

To this aim, an innovative and miniaturised sample collection procedure was proposed in this research work for the accurate sampling of microvolumes of whole blood directly from a fingerprick in a minimally invasive way. In order to develop a novel microfluidic technology, 3D microstructures were designed ad hoc for whole blood microsampling and realised by 3D printing. The miniaturised channels based on microfluidic properties were carefully drawn to allow the accurate collection of minute blood volumes ranging from 5 to 10 μL. The miniaturised platform was extensively optimised regarding all the parameters involved in the sampling step, such as collection time and accuracy, to evaluate possible under- or over-sampling, as well as the influence of blood haematocrit (and thus blood density). In addition, microsample drying time and storage under different conditions of temperature and humidity were taken into consideration and carefully tested. Then, a fast and straightforward pretreatment procedure coupled to a sensitive LC-UV-MS/MS method was developed and validated, in order to obtain a high-throughput, fully automatable strategy for the evaluation of central nervous system (CNS) drugs for therapeutic drug monitoring (TDM) purposes. The novel miniaturised approach based on 3D microfluidic structures showed promising results in terms of sampling accuracy with a SD 78%, precision (RSD <5ng/mL). These data proved how the proposed workflow could lead to reliable quantitative results for the accurate determination of drugs and metabolites in biological samples. This microsampling strategy therefore represents a valid tool for the frequent and minimally invasive collection of whole blood, being an effective patient-friendly alternative to traditional invasive withdrawal, with the perspective of encouraging home- and self- sampling procedures.

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ANTIPLASMODIAL ACTIVITY OF NOVEL AMIDE-TYPE HARMICINES

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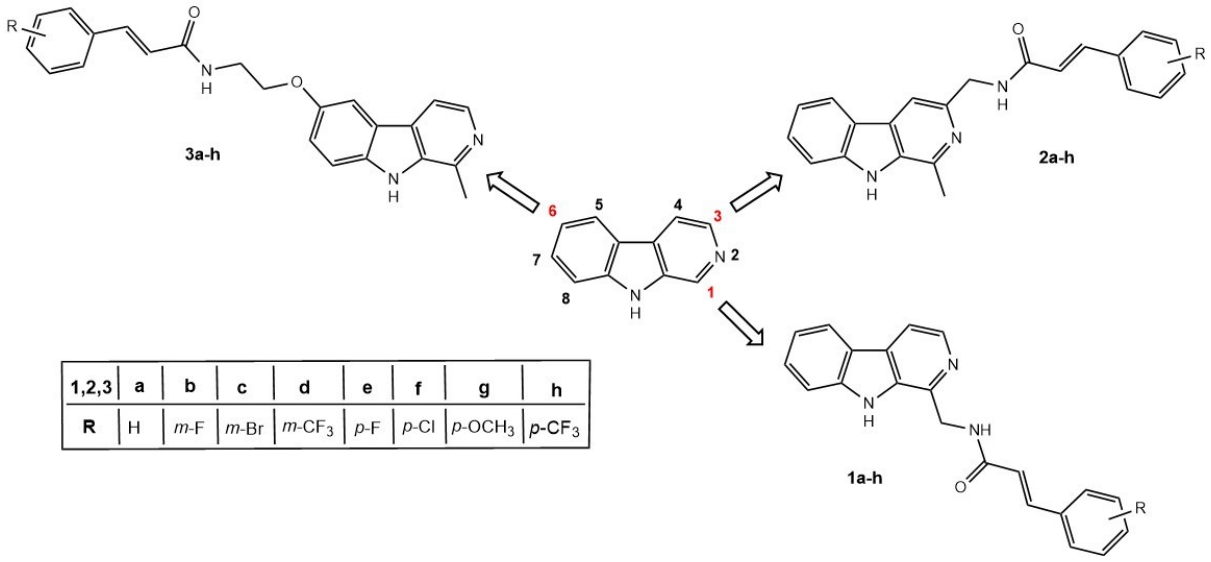
Malaria is one of the most prevalent human infectious diseases caused by the parasites of the genus *Plasmodium* (1). Spread of the multidrug resistant *Plasmodium* strains, particularly *P. falciparum* in Southeast Asia, urges the discovery of agents with novel mechanisms of action (2). Previously, we synthesized amide-type harmicines, *i.e.* hybrid compounds consisting of β-carboline alkaloid harmine and cinnamic acid derivatives, which exerted significant antiplasmodial activity (3). Therefore, we prepared novel amide-type harmicines at the positions C-1, C-3 and O-6 of harmine's β-carboline core, **1a**-**h**, **2a**-**h** and **3a**-**h**, respectively (Figure). Here we disclose the results of the *in vitro* screening of their blood schizonticidal activities against two strains of *P. falciparum* (chloroquine-sensitive strain, *Pf*3D7, and chloroquine-resistant strain, *Pf*Dd2).



1,2,3	a	b	c	d	e	f	g	h
R	H	*m*-F	*m*-Br	*m*-CF_3	*p*-F	*p*-Cl	*p*-OCH_3	*p*-CF_3

Figure. Amide-type harmicines prepared at C-1, C-3 and O-6 of the β-carboline ring.

The analysis of the obtained results has shown that the antiplasmodial activities decreased according to the pattern: **3 > 2 > 1**. Harmicines **2** and **3** displayed antiplasmodial activities in low micromolar and submicromolar concentrations, whereas harmicines **1** were inactive at the highest concentration tested. Notably, compound **3d**, *m*-trifluoromethylcinnamic acid derivative, exhibited the highest antiplasmodial activities with IC_{50} values 0.12 ± 0.02 μM and 0.32 ± 0.08 μM for *Pf*3D7 and *Pf*Dd2 strains, respectively.

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DESIGN AND SYNTHESIS OF MANNICH BASE-TYPE DERIVATIVES CONTAINING IMIDAZOLE AND BENZIMIDAZOLE AS LEAD COMPOUNDS FOR DRUG DISCOVERY IN CHAGAS DISEASE

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The protozoan parasite *Trypanosoma cruzi* is the causative agent of Chagas Disease, the most important parasitic infection in Latin America. The only treatments currently available are nitro-derivative drugs that are characterised by high toxicity and limited efficacy. Therefore, there is an urgent need for more effective, less toxic therapeutic agents. We have previously identified the potential for Mannich [1,2] base derivatives as novel inhibitors of this parasite. To further explore this family of compounds, we synthesized a panel of 69 new analogues, based on multi-parametric structure-activity relationships, which allowed optimization of both anti-parasitic activity, physicochemical parameters and ADME properties. Additionally, we optimized our *in vitro* screening approaches against all three developmental forms of the parasite, allowing us to discard the least effective and trypanostatic derivatives at an early stage. We ultimately identified derivative **3c**, which demonstrated excellent trypanocidal properties; both its druggability and low-cost production make this compound a promising candidate for the preclinical, *in vivo* assays of the Chagas disease drug-discovery pipeline.

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COPPER (II) BROMIDE AS EFFICIENT CATALYST FOR SILYL TO BISARYLMETHYL ETHERS INTERCONVERSION (TRANSPROTECTION)

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Organic synthesis of complex molecular structures is still heavily relying on selective protection and deprotection of functional groups.[1,2]Selectivity and compatibility in these critical steps are often at the heart of a successful synthesis,[2,3]and it is not so rare in total synthesis that a protecting group has to be changed for another one, more compatible with the next steps of the synthesis.[3]This is usually performed by deprotection and reprotection. Transprotection, converting one protecting group to another, usually from a different orthogonal set, in a single step would be a better choice.[2]However, straightforward methods for such transprotection are surprisingly scarce.[2,4]Developing new conditions for the protection and deprotection of alcohols recently led us to introduce diarylmethyl ethers as protecting groups using palladium(II) salts[5]and more recently copper(II) bromide[6]as catalysts. During our investigations, we demonstrated that copper(II) bromide induced the formation of diarylmethyl cations. we have further expanded the scope of syntheticapplications of copper salts in organic chemistry, demonstratingthat transprotection from silyl to diarylmethyl ethers can beachieved in good to high yields with $CuBr_2$as catalyst at room temperature.With very mild conditions, with a wide tolerance to otherprotecting groups, this new interconversion of silyl to diarylmethylprotecting groups will find applications in organic synthesis, especially in the total synthesis of natural products.

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ONCOFAP: A NEW PAN-TUMORAL PLATFORM TARGETING FIBROBLAST ACTIVATION PROTEIN

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Conventional cancer chemotherapy relies on the administration of cytotoxic compounds which rapidly kill dividing cells and that do not selectively accumulate in tumors, thus leading to dose-limiting toxicity (1). Cancer-specific monoclonal antibodies are now used in the clinic as tumor targeting vehicles of potent cytotoxic drugs (2), with the generation of Antibody-Drug Conjugates (ADCs). This approach has led to the marketing authorization of nine antibody-drug conjugates (3).

The use of antibodies as targeting delivery vehicles present some intrinsic limitations such as slow and heterogeneous extravasation in solid tumors, high cost-of-goods and risk of immunogenicity (4). Small organic ligands which selectively bind with high affinity to tumor-associated antigens are increasingly applied as targeting delivery vehicles of small payloads such as radionuclides (5), drugs (6). and fluorophores (7). to tumor sites. In principle, the use of small ligands for targeting applications offers several advantages compared to intact immunoglobulins, including superior penetration of solid neoplastic lesions (8), lower immunogenicity (4) and reduced cost-of-goods (9). Low molecular weight compounds may reach their target *in vivo* in a matter of seconds, thanks to rapid extravasation after intravenous administration.

Fibroblast Activation Protein (FAP) has recently emerged as a tumor-associated antigen with abundant and selective expression in the majority of human solid malignancies. Here we describe the development of OncoFAP, an ultra-high affinity small organic ligand of Fibroblast Activation Protein (FAP), which enabled the generation of a pan-tumoral targeting platform.

The portability of OncoFAP allowed us to generate various fluorescent, radiolabeled and cytotoxic derivatives of OncoFAP to study the biodistribution properties, the tumor targeting performances and the therapeutic activity in preclinical models of renal cell carcinoma.

The sub-nanomolar affinity constant of OncoFAP towards human FAP and its cross-reactivity against the murine isoform allows a sharp and clear translation from preclinical tumor models to clinical applications.

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OPTIMIZATION OF A NOVEL FAST ACTING TRANSMISSION BLOCKING ANTIMALARIAL AGENT

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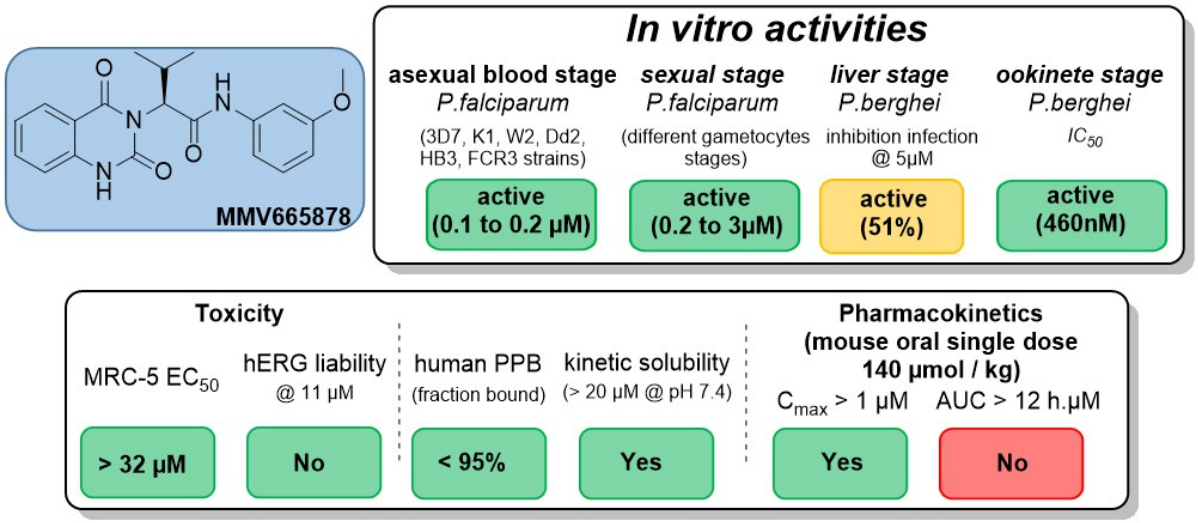
Despite significant progress in the control of malaria with a net reduction of morbidity and mortality over the past years, it remains as one of the deadliest infectious diseases in the world. New drugs with broad therapeutic potential and novel modes of action to overcome emerging drug resistances are urgently needed. Key features of the next-generation antimalarial, termed single-exposure radical cure and prophylaxis (SERCaP), have been rationalized and resulted in the recommendation of a series of Target Candidate Profiles (TCPs). Notably, TCP1 requires rapid elimination of the initial parasite burden, at least as fast as chloroquine.
In this context, the quinazolinedione MMV665878 with its antimalarial activities against multiple life stages of Plasmodium, fast acting and transmission blocking activities, has great potential to deliver useful drugs for malaria parasite eradication. Indeed, it displays potent parasite growth inhibitory activities on erythrocytic stages, but modest activity on liver stages,1 activities on early ring blood stages inducing greater metabolic perturbation than artemisinin2 and an initial cytocidal activity greater than chloroquine but slower than dihydroartemisinin.3 It also inhibits mature stage V gametocytes, potentially preventing P. falciparum transmission to mosquitoes.4 More recently, it has been described as a potent red blood cells invasion inhibitor.5 Moreover, this quinazolinedione-based scaffold shows remarkable selectivity window with a low toxicity for human cells and no cardiotoxicity risk. However pharmacokinetic issues are encountered and include moderate overall exposure and/or modest bioavailability,1 issue probably caused by rapid metabolism and elimination.



Herein, we report our progress towards the optimization of this quinazolinedione-based antimalarial series.

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PHOTODEGRADATION OF PHARMACEUTICAL CONTAMINANTS USING PHTHALOCYANINE–GRAFTED TITANIUM DIOXIDE NANOPARTICLES

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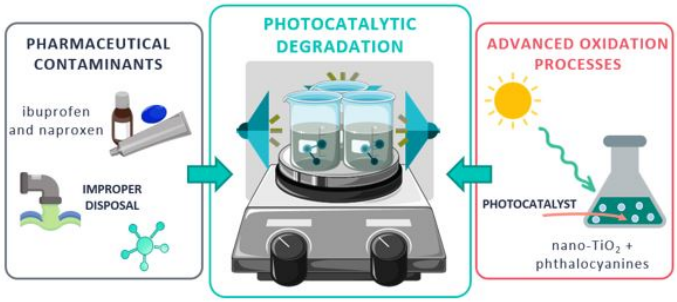
Frequent use of pharmaceuticals and their improper disposal increasingly contribute to environmental pollution (1). Ibuprofen and naproxen, commonly used nonsteroidal anti-inflammatory drugs, are usually not completely removed in the wastewater treatment process (2,3). To address this problem, we attempted to develop new photocatalysts that would enable an effective and sustainable photocatalytic method for water remediation (4).

For this purpose, either copper(II) phthalocyanine (CuPc) or zinc(II) phthalocyanine (ZnPc) were deposited on titanium dioxide nanoparticles (40 nm), yielding two types of photocatalytic composites: CuPc@TiO_2 and ZnPc@TiO_2. Briefly, TiO_2 nanoparticles were suspended in a Pc solution and after the adsorption-desorption equilibrium was reached, the solvent was evaporated and the resulting blue powder was dried. The photocatalytic experiment was carried out to assess the materials' feasibility of photodecomposition of either naproxen or ibuprofen. The photocatalytic material was suspended in ibuprofen or naproxen aqueous solution and the mixture was irradiated under continuous stirring with UV light (λ_{max} = 365 nm). The samples were collected and HPLC-MS/MS was used to determine the concentration of the drugs throughout the experiment duration.

Photocatalytic degradation of both ibuprofen and naproxen occurred according to the first-order kinetics. 92% of ibuprofen was removed after six hours of the experiment with CuPc@TiO_2 used as the photocatalyst. In the case of naproxen ZnPc@TiO_2 displayed the highest effectiveness with 94% removal of the drug within one hour of the experiment. To evaluate the sustainability of the developed material, the recyclability of the photocatalysts was also tested in a series of consecutive experiments in which no decrease in photoremediation efficiency was observed. Moreover, dose-dependent mutagenicity Ames Test was performed for both materials, CuPc@TiO_2 and ZnPc@TiO_2. The results indicated that our photocatalytic materials do not act as mutagenic agents towards the microorganisms present in the photoremediated sewage.

This study was supported by the National Science Centre, Poland, under grant number 2016/21/B/NZ9/00783.



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SYNTHESIS OF SPIROCYCLIC-DHEA DERIVATIVES AS NEUROTROPHIN MIMETICS

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Neuro**t**rophins (**NT**s) compose a small family of Neurotrophic Growth Factors constituted of four orthologues, associated by structure and function: Nerve Growth Factor (NGF), Brain-Derived Growth Factor (BDNF), Neurotrophin-3 (NT-3) and Neurotrophin-4 (NT-4, also known as NT-5 and NT-4/5). These large proteins after being secreted, both centrally and peripherally, bind with high affinity and selectivity to the **T**yrosine **R**eceptor **K**inases (TrkA, TrkB and TrkC), to produce powerful neuroprotective and neurogenic effects. On the other hand, the **P**an **N**euro**t**rophin **R**eceptor **75** (**p75^{NTR}**) is also bound by NTs with low affinity and by their immature forms (**pro-n**euro**t**rophins, **pro-NT**s) with high affinity, generally resulting in cell death. This small and general introduction is just the tip of the iceberg of the complex equilibrium of Neurotrophin signaling. Based on these premises, many pharmacological strategies were attempted, but an optimal solution is yet to be identified. Due to their polypeptidic nature, in fact, NTs are not suitable for therapeutic use and small druggable molecules, mimicking neurotrophins's beneficial actions are highly desirable.[1]

Following the discovery that the endogenous steroid precursor **De**h**y**dro**e**pi**a**ndrosterone (**DHEA**) activates the neurotrophin receptor TrkA exerting an activity that is both neuroprotective[2] and anti-neuroinflammatory[3], our group synthesized a small library of variously substituted dehydroepiandrosterone derivatives. The 17-spiro-epoxy derivative BNN27 showed anti-apoptotic and neuroprotective activity, resulting from the selective activation of the TrkA receptor.[4-6] BNN27, in contrast to DHEA, not only possesses improved affinity for TrkA, but does not exhibit hormonal side effects.

As a continuation of our studies on steroidal neurotrophin mimetics, we embarked on the synthesis of C17-spiro-DHEA derivatives bearing five or six-membered rings substituted with a variety of pharmacophore groups in order to probe the stereoelectronic requirements for optimum neurotrophic/neuroprotective/neurogenic activity. The new derivatives were evaluated for their agonistic activity for TrkA and TrkB receptors as well as for their neuroprotective properties on appropriate cellular models. Moreover, their effect on inflammatory responses was tested using microglia cells.

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PLGA NANOPARTICLES: A SUCCESSFUL APPROACH FOR THE PENETRATION OF SMALL MOLECULES WITH THERAPEUTIC POTENTIAL IN THE CENTRAL NERVOUS SYSTEM

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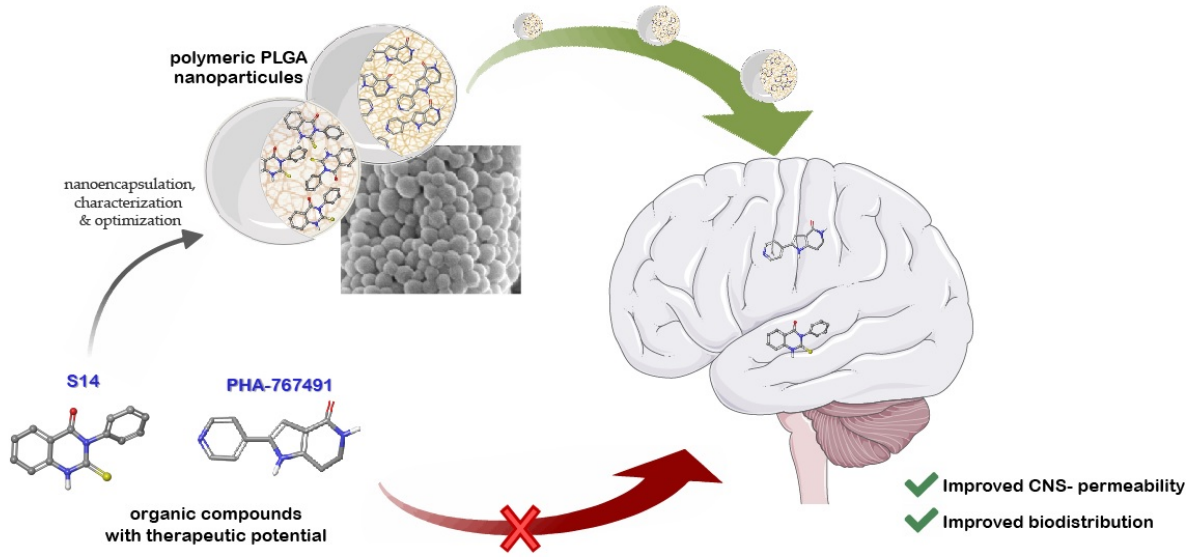
One of the main hurdles in the design and development of drugs for neurodegenerative diseases is the penetrance to the central nervous system (CNS), protected by the blood-brain barrier. Recently, polymeric nanoparticles have been used as new formulations to target specific organs and produce controlled release of certain drugs. In this work, we describe poly(lactic-co-glycolic acid) (PLGA) based polymeric nanoparticles loaded with two interesting drugs: S14 and PHA-767491.

Phenyl-2-thioxo-(1H)-quinazolin-4-one, called S14, is a PDE7 inhibitor that has shown very promising results to treat Parkinson's disease[1]. Encapsulation of this drug inside PLGA-PVA nanoparticles resulted in improved CNS penetrance and biodistribution in mice[2].

1,5,6,7-Tetrahydro-2-(4-pyridinyl)-4H-pyrrolo[3,2-c]pyridin-4-one, also known as PHA-747691, is a CDC-7 inhibitor which could have potential in the treatment of amyotrophic lateral sclerosis, since this kinase has recently been proposed as a new therapeutic target for this fatal disease[3]. Preparation of PLGA-PVA nanoparticles has shown, not only improvement in the predicted CNS permeability but also reduced levels of hyperphosphorylated TDP-43 protein compared to the free drug[4].



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DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION OF NEW RNA LIGANDS TARGETING miR-210: MODULATION OF THE CIRCADIAN CLOCK FOR CANCER CHEMOTHERAPY

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Disruption of the circadian clock is associated with a variety of human pathologies, including cancer, and the expression of several clock genes is perturbed in many tumors.[1] The aberrant clock gene expression in tumors likely plays a causal role in the development of cancer and the survival of tumor cells. For instance, several epidemiological studies indicated that the incidence of breast cancer is higher among women who spent more years and hours per week working nightshifts. These observations suggest the hypothesis that pharmacological modulation of clock-related proteins may be an effective anticancer strategy.

Recently, Dr. Grimaldi reported the identification of a close connection between the circadian clock and MAX/MNT transcription networks. This study revealed a crucial role of the MAX transcriptional repressor (MNT) in regulating the activity of the molecular clock by repressing the expression of circadian genes, which expression is driven by E-box sequences within their promoters. Notably, the expression of MNT under diverse conditions, such as hypoxia and cancer, appears regulated by miR-210.[2] The laboratory of Dr. Duca (ICN) has a successful experience in the design of multimodal small molecules targeting miRNAs. More specifically, various series of compounds have been designed and synthesized to target oncogenic microRNAs precursors in a selective manner and showed very promising biological results during intracellular studies.[3,4]

To this end, the main objective of this project and the collaboration between Grimaldi and Duca is to identify a novel pharmacological approach that modulates the circadian activity through the targeting of the miR-210/MNT axis. On the one hand, the molecules generated will represent a valuable pharmacological tool for studying the role of miR-210 in circadian clock regulation. On the other hand, these molecules will provide suitable chemical scaffolds for the development of innovative clock modulators for treating circadian-related pathologies. Indeed, the anticancer activity of the miR-210 inhibitors will be also assessed.

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SYNTHESIS OF HARMIRINES – NOVEL 1- AND 3-SUBSTITUTED HARMINE-COUMARIN HYBRIDS

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The emergence of cancer drug resistance poses a significant global public health challenge leading to cancer remission. Thus, there remains a constant need for new effective anticancer agents. Harmine belongs to a class of naturally occurring β-carbolines – pharmacologically active alkaloids having a broad spectrum of antitumor activity through diverse mechanisms [1]. Similarly, coumarins, compounds widely distributed in plants, also exert anticancer activity [2]. Therefore, β-carboline alkaloids, as well as coumarins, might present lead compounds in the field of drug discovery. To this end, we have prepared harmirines, new hybrid molecules consisting of both pharmacophores, linked by a 1,2,3-triazole. The novel series of harmirines possesses substituents at positions 1 or 3 of the β-carboline core.

To prepare the title compounds, we have synthesized coumarin-based terminal alkynes **1a-d** and harmine-based azides **2** and **3**. Alkynes were prepared in a one-step reaction by the treatment of hydroxycoumarins with propargyl bromide in the presence of cesium carbonate. The preparation of harmine-based azides **2** and **3** at positions 1 and 3 of the β-carboline ring was previously described by our research group [3]. Synthesized alkynes and azides were the starting compounds for the Cu(I)-catalyzed azide-alkyne cycloaddition resulting in two types of hybrids: 1- and 3-substituted harmirines **4a**-**d** and **5a**-**d**, respectively. Harmirines **5a-d** were prepared using Cu(II)-acetate precatalyst in methanol. On the other hand, due to the fewer byproducts and easier purification, sodium ascorbate was a reducing agent of choice for the generation of Cu(I) from $CuSO_4 \times 5H_2O$ in the synthesis of harmirines **4a-d**. The scheme outlines the general route leading to the title compounds (Figure). The structures of newly prepared compounds **4** and **5** were confirmed by standard methods (IR, ^1H, ^{13}C NMR, MS). The evaluation of their antiproliferative activity is in progress.

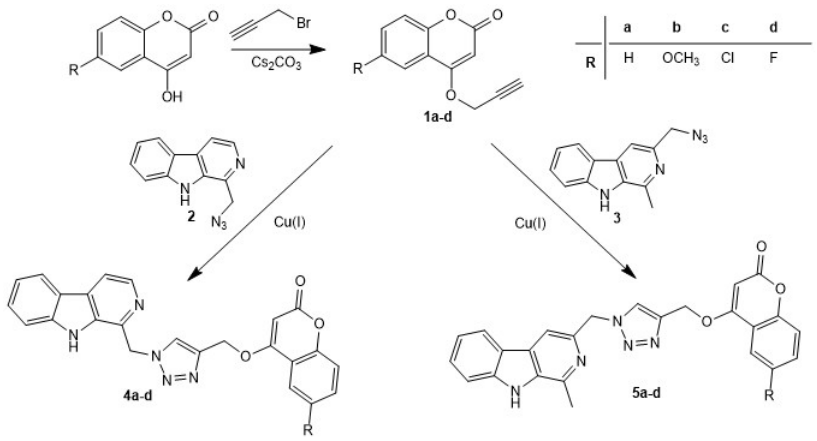

Figure. Synthesis of novel harmirines

This work was fully supported by the Croatian Science Foundation under the project number UIP-2017-05-5160.

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IDENTIFICATION OF NOVEL SIRT1 ACTIVATORS ENDOWED WITH CARDIOPROTECTIVE PROFILE

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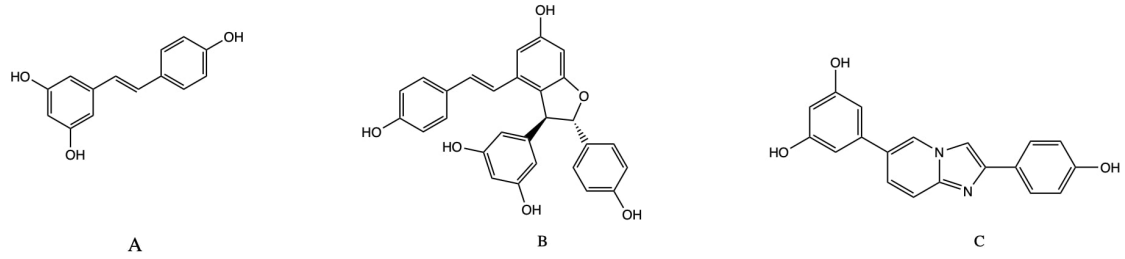
Sirtuins (SIRTs) are $NADP^+$-dependent proteins belonging to the superfamily of histone deacetylase (HDAC). [1] They are located in the nucleus (SIRT1, 6, 7), cytosol (SIRT2) and mitochondria (SIRT3-5) [2, 3] and play a key role in regulating cell homeostasis.

Among the different Sirtuin isoforms, SIRT1 has been the first one to be discovered and still is the most investigated one. Being involved in cell metabolism, survival and senescence, it is acknowledged as a privileged drug target for the treatment of several pathologies. Therefore, compounds able to inhibit SIRT1 could play a role in cancer and inflammation, while derivatives able to activate it could be mainly used as therapeutic agents against cardiovascular disorders and age-related syndromes.

As regards the latter, both natural products, like resveratrol (**A**) and its dimer *trans*-ε-viniferin (**B**) (Figure 1), and synthetic derivatives have been described as effective compounds. However, despite the *in vitro* efficacy, they are generally characterized by unfavorable pharmacokinetic properties that preclude their practical use, thus highlighting the need of novel and more versatile derivatives.

Here we describe a novel class of SIRT1 activators, bearing the imidazo[1,2-*a*]pyridine scaffold, obtained by combining chemical synthesis and *in silico* and *in vitro* studies.

GP44 (**C**, Figure 1), characterized by a poly-hydroxylated substitution pattern, turned out to be the best SIRT1 activator of the series. Investigated in an *ex vivo* study performed on isolated and perfused rat hearts, submitted to ischemia/reperfusion (I/R) periods, it also proved to have cardioprotective activity, thus opening up a novel and promising beginning of the development of epigenetic cardioprotective agents.



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EFFECT OF RING-SUBSTITUTION ON LIPOPHILICITY IN SERIES OF META- AND PARA-(TRIFLUOROMETHYL)CINNAMANILIDES

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Two series of ring-substituted anilides of 3-(trifluoromethyl)cinnamic and 4-(trifluoromethyl)cinnamic acids were synthesized and characterized. Lipophilicity is essential for the drug bioavailability at the site of action because it affects solubility and permeability through membranes. Therefore hydro-lipophilic properties are one of the most important physicochemical characteristics of bioactive compounds.[1,2] High lipophilicity of drugs favors hydrophobic binding and may increase affinity to the target receptor. On the other hand, high lipophilicity may be associated with undesirable drug properties. In addition, highly lipophilic agents are targets of biotransformation enzymes, resulting in extensive and unpredictable metabolism.[3,4] These new bioactive agents were analyzed using the reversed-phase high performance liquid chromatography (RP-HPLC) to measure lipophilicity. The procedure was performed under isocratic conditions with methanol as an organic modifier in the mobile phase using an end-capped non-polar C18 stationary reversed-phase column. The compounds of both series multisubstituted by trifluoromethyl moieties were the most lipophilic, while only fluorinated and/or polyfluorinated derivatives showed the lowest lipophilicity. The data collected in this study will be used to evaluate the QSPR and QSAR of the studied compounds.

This study was supported by a grant project of the Comenius University in Bratislava, Slovakia (UK/228/2021) and by the Slovak Research and Development Agency (APVV-17-0373).

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SYNTHESIS OF TRIAZOLE-TYPE HARMICENES

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Malaria, a life-threatening infectious disease, affects more than 100 countries, putting half of the world's population in danger.[1] As resistance of the malaria parasite to the known antimalarial drugs rises, there is a crucial need for new and effective antimalarial agents.[2] To this end, we employed molecular hybridization of two bioactive scaffolds,[3] a popular approach in the discovery of molecules with improved properties, in the synthesis of harmicenes. Harmicenes represent hybrids comprised of harmine, a β-carboline alkaloid with confirmed antimalarial activity, and ferrocene, an organometallic compound that enhances antimalarial properties. Cu(I) catalyzed azide-alkyne cycloaddition, *i.e.* "click" chemistry, was selected as a method of choice for the preparation of two series of harmicenes, **1a**-**e** and **2a**-**e**, in the positions C-1, C-3, O-6, O-7 and N-9 of the β-carboline ring. Compounds **1** were obtained by reacting β-carboline-based alkyne with ferrocene-based azide, while harmicenes **2** were synthesized in the reaction of β-carboline-based azides and ethynylferrocene (Figure 1.). "Click" reactions proceeded smoothly with Na ascorbate and $CuSO_4 \times 5H_2O$ as a source of Cu(I) ions, in DMF/H_2O mixture (1:1), giving hybrids in good to excellent yields.

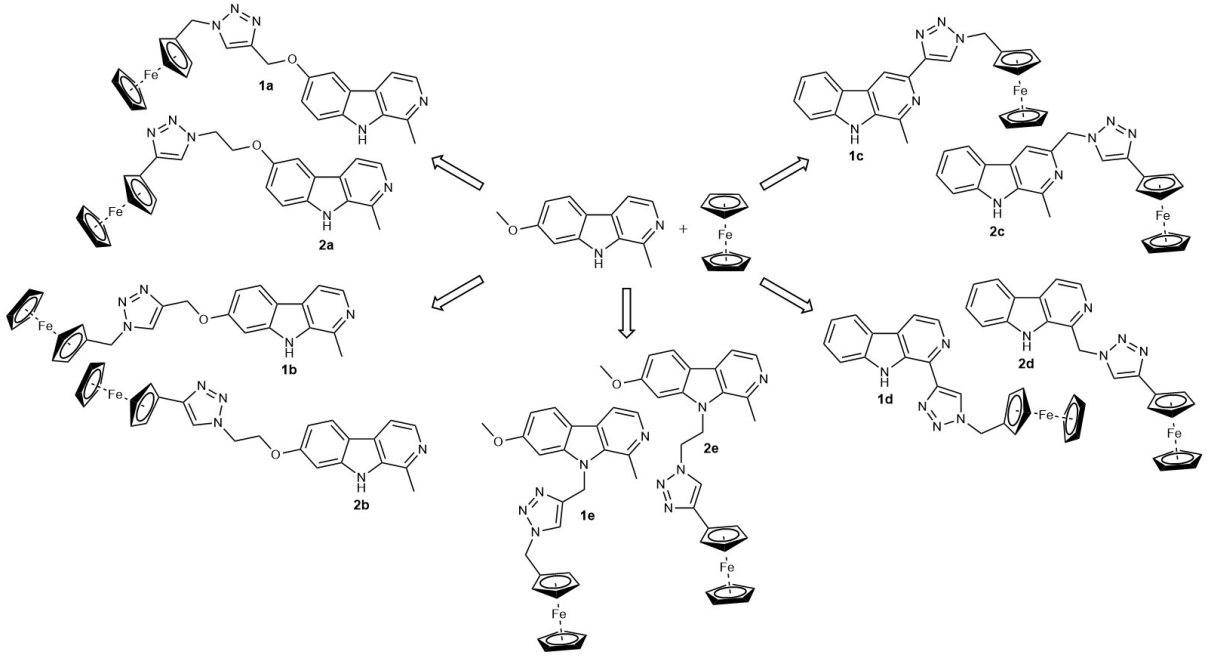

Figure 1. Synthesis of harmicenes **1a-e** and **2a-e** using "click" chemistry approach.

Structures of novel harmicenes were confirmed by ^1H and ^{13}C NMR, IR and MS. Evaluation of their antiplasmodial activity and cytotoxicity is in progress.

This work was fully supported by the Croatian Science Foundation under the project number UIP-2017-05-5160.

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REDOX ACTIVE OR THIOL REACTIVE? RAPID SCREENS FOR IDENTIFYING NUISANCE COMPOUNDS

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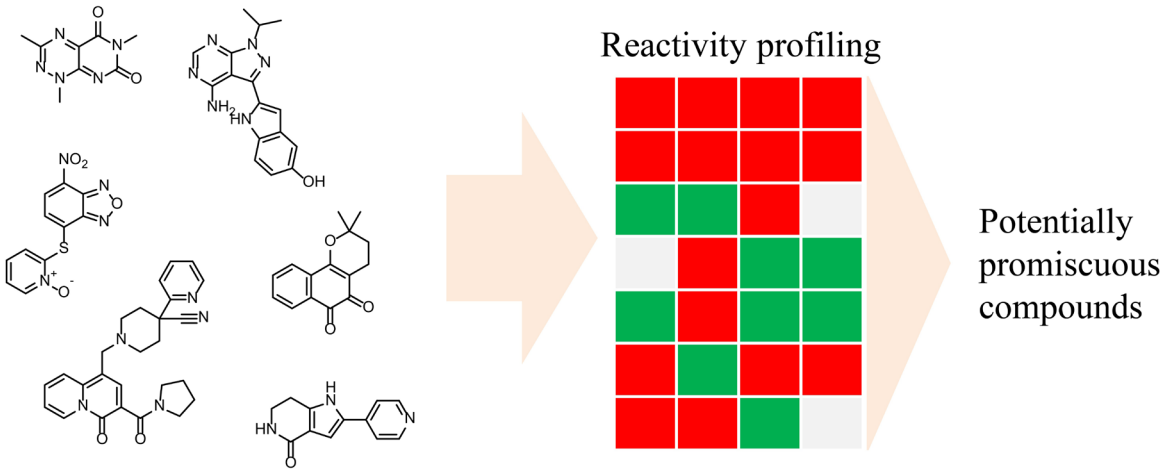
The identification of false positive hits is a crucial step in the early stages of drug discovery to avoid wasteful use of resources. Moreover, poor tool compounds in biological studies can generate false information about biochemical processes. The underlying promiscuous mechanisms of interaction with biological targets include aggregation, reactivity with protein residues, redox activity, interference of the assay system (PAINS), poor stability or solubility issues.

There are well established methods for the detection of aggregators, and many interfering compounds can be filtered before screening, e.g., using PAINS filters (1). We present guidelines for the detection of less obvious promiscuous mechanisms, namely redox activity and thiol reactivity. After a thorough literature review, selected assays were optimized and tested with 10 chemically distinct positive control compounds. Then we have further expanded our set of compounds that can be used as positive controls. Assay setups are quick and can be performed at low cost. They are orthogonal and capable of detecting multiple interference mechanisms, i.e. the horseradish peroxidase–phenol red assay detects H_2O_2 generation; the probe H_2DCFDA is sensitive to ROS; resazurin reacts with free radicals; and the Ellman's reagent detects covalent reactivity with thioles. Assay conditions have been optimized to improve robustness and sensitivity.

A library of 99 chemical probes and bioactive compounds was used to demonstrate the capabilities of our assay cascade. We have identified new types of compounds that were not previously known to be redox active. Even well prepared, filtered and manually curated compound libraries can still contain nuisance compounds that can only be identified experimentally.



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DEVELOPMENT OF A COUNTINOUS-FLOW PHOTOREDOX REACTOR AND ITS APPLICATION TOWARD THE EXTENSION OF A COMPREHENSIVE FRAGMENT LIBRARY

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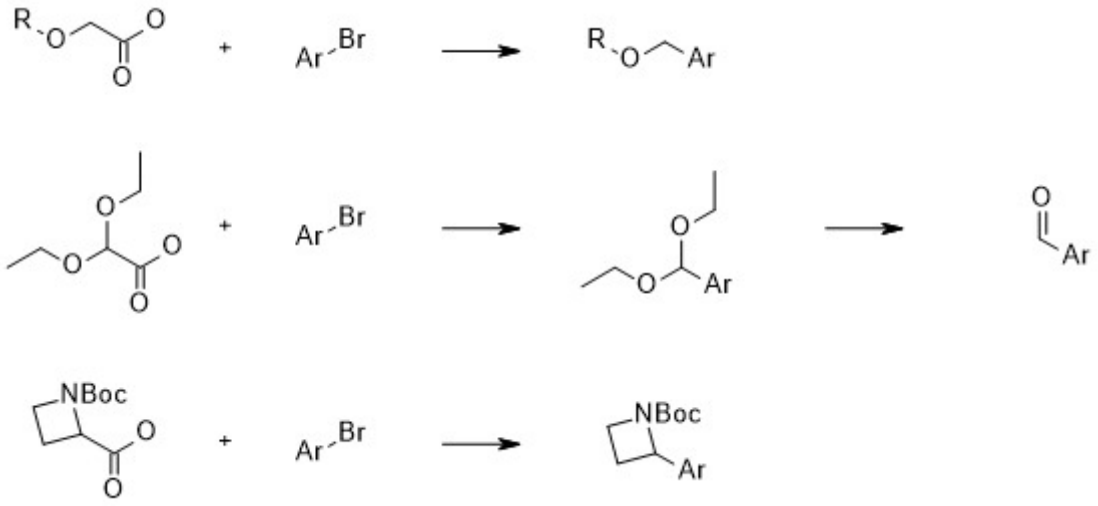
In the past few years, photochemical transformations have seen a countinous development to achiveve new organic pathways to drugs and their precursors. The countinous-flow photoredox chemistry's great efficency comes from better mass- and light transfer and even a better environment-friendly option as compared to its batch alternative.[1] Consequently, photochemical reactions can be accelerated substantially (from hours/days in batch to seconds/mins in flow) and lower photocatalyst loadings are often feasible. This reduction in reaction time minimizes potential byproduct formation and increases the productivity of the photochemical process.

At BioBlocks we have developed a countinous-flow photoredox reactor with a preparative HPLC sample loop that allows to carry out small scale reactions sequentially. The LED source was also made variable to change the intensity of the light as well as the wavelength.

The presentation describes our recent results on photoredox iridium-nickel dual catalysed decarboxylative coupling of aliphatic carboxylic acids with aryl halides using a countinous-flow photoredox reactor. The investigated reactions are shown in Scheme 1.

The method was amenable to produce fragment molecules or their precursors that were excellent addition to BioBlocks' comprehensive fragment library (CFL).



Scheme 1

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SYNTHESIS OF NEW FLUORESCENT CHEMICAL PROBES TARGETING BACTERIAL EFFLUX TO EARLY DETECT AND FIGHT THE FIRST BARRIER IN BACTERIA ANTIBIOTIC RESISTANCE

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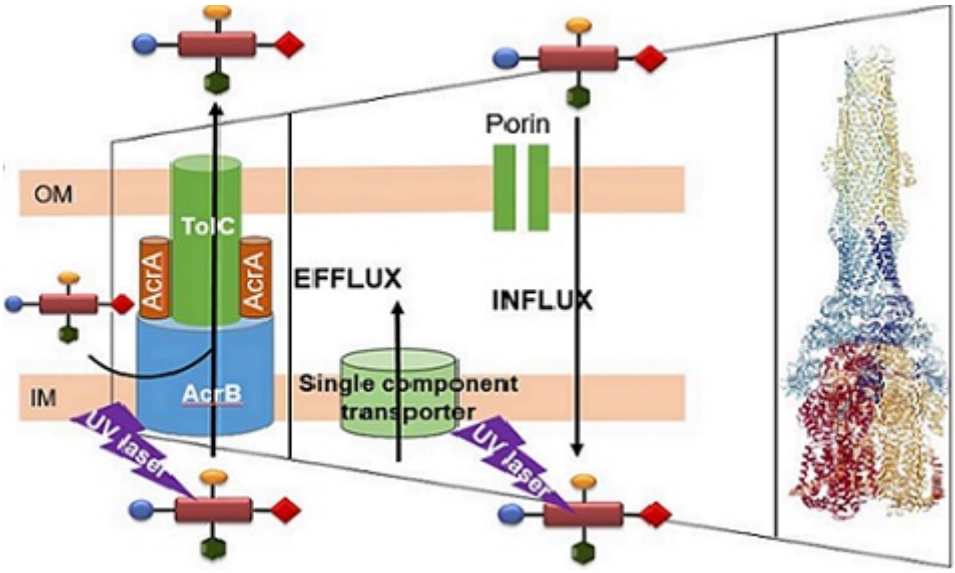
Antimicrobial resistance (AMR) is one of the more serious problem of Public Health. The 2014 WHO's global report [1] and the J. O'Neill's more recent [2] outlined worrying levels of AMR worldwide involving therapeutic failure especially in Gram-negative bacterial diseases. Resistance is a natural response of microorganisms to counteract pharmacological effects of active agents. Lots of mechanisms as membrane impermeability, enzymatic and intracellular target alterations contribute to the Multi-Drug Resistance (MDR) phenotypes. Furthermore, efflux overexpression is a major early-stage trigger in the MDR setting up. Resistance-Nodulation-Cell-Division (RND) efflux pump superfamily constitutes a tripartite protein system which is the resistance first line in Gram-negative bacteria [3]. They can extrude structurally different substances outside the cell space; hence antibiotics struggle to reach effective intracellular concentrations. Thanks to the spectrofluorometric method developed by our team [4], we are working on the use of new natural product derivatives with high and linear fluorescent signal with their intracellular concentration by a functionalized Fluorophore-based approach AcrAB-TolC Gram-negative producer strains. Their efflux substrate properties are useful reference to detect the capacity of antibiotics to cross membranes and to accumulate in bacteria especially the ones which express MDR resistance. These compounds must not have cytotoxic effects to minimize their resistance impact. Starting from a screening of chemical libraries of natural products and by a drug design approach, pharmacophores and their positions were identified on the fluorescent scaffold. Then, Structure-Activity Relationship studies allowed us to highlight physicochemical properties responsible not only for a high fluorescence intensity but also for substrate efflux-pump features. These results will guide a real-time diagnosis trial design allowing antibiotic drug accumulation monitoring, identification, minimization, and prevention in the early appearance of efflux bacterial resistance in clinic [5] while decreasing patient exposure.



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FRAGMENT-BASED APPROACH TO DRUG DESIGN ON GLYCOSYLTRANSFERASE WAAG FROM ESCHERICHIA COLI

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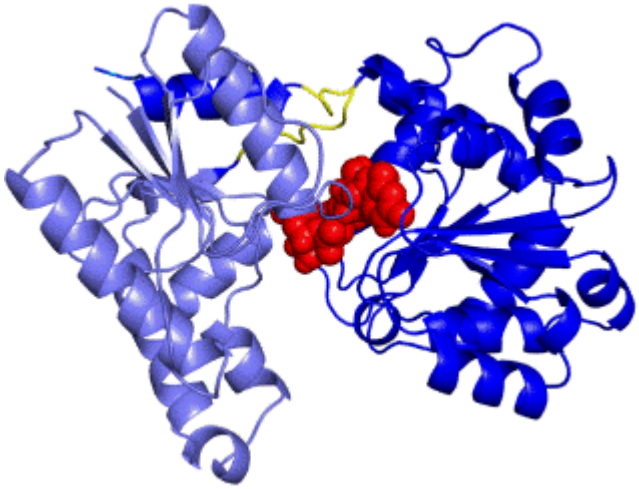
Multi-drug resistant (MDR) strains of pathogenic gram-negative bacteria are currently a major concern for public health. They cause gastroenteritis, urinary tract, blood and central nervous system infections. [1] Moreover, certain strains of *Escherichia coli* have easily spread in the community. A good way to contrast intrinsic resistance in *E. coli* is the possibility to inhibit WaaG, a glucosyltransferase which catalyses the transfer of an α-D-glucosyl portion from UDP-Glc onto l-*glycero*-d-*manno*-heptose-II, contributing in the synthesis of the core region of the lipopolysaccharide (LPS). A consequence of its inhibition is the destabilization of the outer membrane of *E. coli* by interfering with LPS core phosphorylation and results in truncated LPS immediately after the inner core heptose residues. This perturbation changes the chemical structure of the LPS and makes *E. coli* more susceptible to different classes of antibiotics. Previous computational molecular docking and experimental NMR studies elucidated the binding of natural ligands to WaaG. Some starting fragments (Maybridge fragment library) were utilized for some preliminary docking studies. [36] The first fragment (A1) showed to inhibit WaaG (IC_{50} = 1.0 mM), which opened for new structural modifications in order to design potent inhibitors. [37] Then, a fragment-based drug discovery (FBDD) process included the selection of different fragments (Library A). Molecular docking studies identified the most interesting scaffolds and functional groups for a better affinity with the UDP-binding pocket of WaaG. One of the most promising fragments from library A was compound **A4** (2-amino-4-phenylthiazole). Investigating the best-docked pose of compound **A4**, it showed binding in the uridine sub-pocket and the fragment orientation is highly similar to the uridine scaffold of UDP. The thiazole ring is positioned in the ribose region of UDP. The amino group of these fragments points in the same direction as ribose O3' in UDP. Ligand **A4** was tested via STD NMR experiments, exhibiting a good protein-ligand affinity profile. **A4** showed more than 70% STD signal. The experiment was repeated with UDP, as a binding competition assay, where **A4** still gave a significant signal (> 40% STD signal), confirming the importance of the ligand in term of binding affinity to WaaG active site. The figure shows the conformation of $UDP\text{-}Glc^{2-}$ in WaaG binding pocket, derived from U2F deposited co-crystallized (PDB code 2iw1).



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POLYPHARMACOLOGICAL INTERVENTIONS FOR NEURODEGENERATIVE DISEASES: DRUG DESIGN AND IN VITRO PROFILING OF NEW UROLITHIN DERIVATIVES

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Neurodegenerative diseases (NDDs) represent a set of neurological disorders with heterogeneous clinical and pathological expressions, but united by the progressive and irreversible loss of neuronal function. It is well established that the development and progression of NDDs result from several pathological mechanisms, including proteostasis collapse, neurotransmitter depletion, oxidative stress, neuroinflammation, and metal ion dyshomeostasis (1). In this context, the use of a polypharmacological approach against neurodegeneration is a promising strategy since it can provide beneficial effects by acting simultaneously on multiple targets. Among several drug design strategies lately developed, the Multi-Target Directed Ligand (MTDL) strategy has shown the most promising results; not by chance, most of drug candidates currently engaged in clinical trials have been obtained with this strategy (2). Urolithins are a class of coumarin-based metabolites of ellagic acid produced *in vivo* by gut microbiota. They have a wide range of biological effects, including the modulation of autophagy, and metal chelating and anti-aggregative properties (3). Using the MTDL approach, we developed a new class of urolithin derivatives by merging the hydroxydibenzopyran-6-one core with pharmacophores endowed with antioxidant, metal chelating or anticholinesterases activities. Then, we evaluated their autophagic induction, their inhibitory activity towards monoamine oxidases A and B (MAO-A and MAO-B, respectively) and cholinesterases, and their ADME-Tox properties.

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A NEW PRO-APOPTOTIC THERAPY FOR ACUTE MYELOID LEUKAEMIA USING MEDS433, A POTENT HUMAN DIHYDROOROTATE DEHYDROGENASE INHIBITOR

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In acute myeloid leukaemia (AML), blasts lose their ability to differentiate into mature cells and undergo apoptosis. Accordingly, a proapoptotic and differentiating therapy (arsenic and all trans retinoic acid, ATRA) has dramatically improved survival in acute promyelocytic leukaemia; however, such combination therapy is not available for other AML subtypes. While, in 2016, inhibition of dihydroorotate dehydrogenase (DHODH), a key enzyme of the pyrimidine biosynthesis, was found to induce differentiation in several AML models. In fact, brequinar (BRQ) was utilized in vivo studies.[1]We are optimising *h*DHODH inhibitors to improve potency and drug-like proprieties. Moreover, we would like to evaluate how different parameters such as, pK_a, LogD$^{7.4}$ of different carboxylic acid bioisosteres can influence *in vitro* and *in vivo* studies. The main objective is to identify the best inhibitor suitable for use in *in vivo* studies on AML animal model.

In this work we will present a new generation of *h*DHODH inhibitors able to reach the enzymatic BRQ inhibition potency levels. Our data showed that MEDS433, the best of two series, induced apoptosis in multiple AML cell lines, not only because of differentiation, but also directly. Its combination with antileukemic agents further increased the apoptotic rate, but when experiments were performed in the presence of physiological uridine concentrations, results were less impressive. Conversely, the combination of MEDS433 with dipyridamole induced metabolic lethality and differentiation in all AML cell lines; this extraordinary synergism was confirmed on AML primary cells with different genetic backgrounds and was unaffected by physiological uridine concentrations, predicting in human activity. [3] Finally, our preliminary results from *in vivo* experiments showed that *i*) MEDS433 wasn't toxic on Balb/c mice after 5 weeks of intraperitoneal administration at two different doses 10 and 25 mg/Kg and during acute toxicity experiment was not toxic ad dose of 1 g/Kg; *ii*) the half-life was limited to 3-4 hours and *iii*) MEDS433 had a good antileukemic activity (approximately 50% reduction of the tumour volume compared with control, after 18 days of treatment in THP1-xenograft models obtained from NSG mice). Theoretical design, modeling, synthesis, SAR, X-ray crystallographic data, biological assays, Drug-Like proprieties, pharmacokinetic studies, and *in vivo* evaluations on AML models will be here presented and discussed.

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NEW MULTITARGET THERANOSTIC COMPOUNDS AGAINST ALZHEIMER´S DISEASE

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Alzheimer´s Disease (AD), the most prevalent type of dementia, affects more than 40 million people worldwide[1]. It is a neurodegenerative disease that limits the ability to carry out daily life tasks and causes cognitive impairment. AD´s aetiology is not fully understood, but various alterations are known to be involved, like amyloid beta peptide and hyperphosphorylated tau protein deposition, an increase in oxidative stress and neuroinflammation, mitochondrial and calcium homeostasis dysfunction and an imbalance in the glutamatergic and cholinergic tone.

The drugs used at present for the treatment of AD don´t cure the disease, for they only achieve a temporary amelioration of symptoms. Diagnosis of AD is also a problem, and nowadays the disease can only be confirmed in *post mortem* studies. Consequently, a new approach is needed in the treatment and diagnosis of AD. Multitarget drugs are of great value in multifactorial diseases like AD. In addition, theranostic compounds provide therapy and diagnostic information at one time, allowing assessment of the molecule activity, the organism response and the pharmacokinetics. This is interesting for research purposes and future clinical applications related to personalised medicine, and plays a special role in current AD research[2].

Related to this, our research group focuses on styrylquinolines as potential drug candidates against AD due to their promising properties[3]. In a recent PhD thesis work[4], a novel family of these molecules was presented with remarkable features, such as: inhibition of tau aggregation, neuroprotective and antioxidant activity, while exhibiting beta amyloid detection in the near-infrared range. In this work, we describe the synthesis and characterization of new styrylquinoline derivatives bearing dicarbonyl moieties at the quinoline C-6 position as well as their boron complexes.

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TARGETING TRPV1 SOFTLY: SYNTHESIS AND DEVELOPMENT OF CAPSAICINOID MODULATORS WITH IN VIVO EFFICACY IN MOUSE MODELS OF SKIN DISEASES

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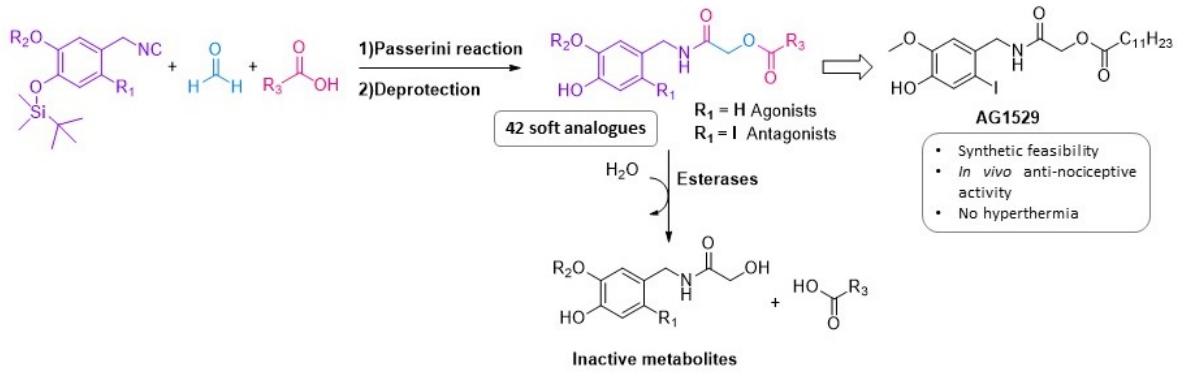
Transient receptor potential vanilloid 1 (TRPV1) channel is an ion channel that plays a crucial role in the pathogenesis of skin disorders, especially related to inflammation and pruritus.[1] Over the last decades, capsaicin and other agonists have been developed for topical application, but their important side effects, *i.e.* the initial burning sensation, frequent erythema reactions and skin carcinogenesis related to the long residence time in the skin, have always hampered the use of these compounds. Besides agonists, several topical antagonists have been discovered[2] but their clinical development has been undermined by hyperthermia induction and promotion of skin cancer.

In our work, 42 capsaicinoid soft analogues have been synthesized and developed.[3] These soft drugs are able to undergo topical deactivation by the hydrolyzing activity of skin esterases after having played their topical effect.[4] The implanting of an ester group in the lipophilic side chain of capsaicinoids using the Passerini multicomponent reaction affords both agonists and antagonists with good TRPV1 modulating activity. At the same time, these soft analogues are susceptible to *in situ* topical deactivation, avoiding the side effects related to the long-term residence in the skin and to the systemic distribution of the compounds. Among the antagonists, compound **AG1529** potently and competitively blocked capsaicin-evoked activation of hTRPV1, mildly affected pH and voltage-induced activation, and did not alter heat responses. Moreover, the compound abolished histaminergic and inflammatory sensitization of TRPV1 in nociceptors. Finally, topical application of **AG1529** dose-dependently attenuated histaminergic pruritus in mice, without increasing body temperature. Taken together, these pre-clinical results substantiate this capsaicin-based soft antagonist as a promising candidate for the treatment of psoriatic pruritus.



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PROTAC-MEDIATED INACTIVATION OF E3 LIGASES: FROM TOOLS TO CANCER TREATMENT

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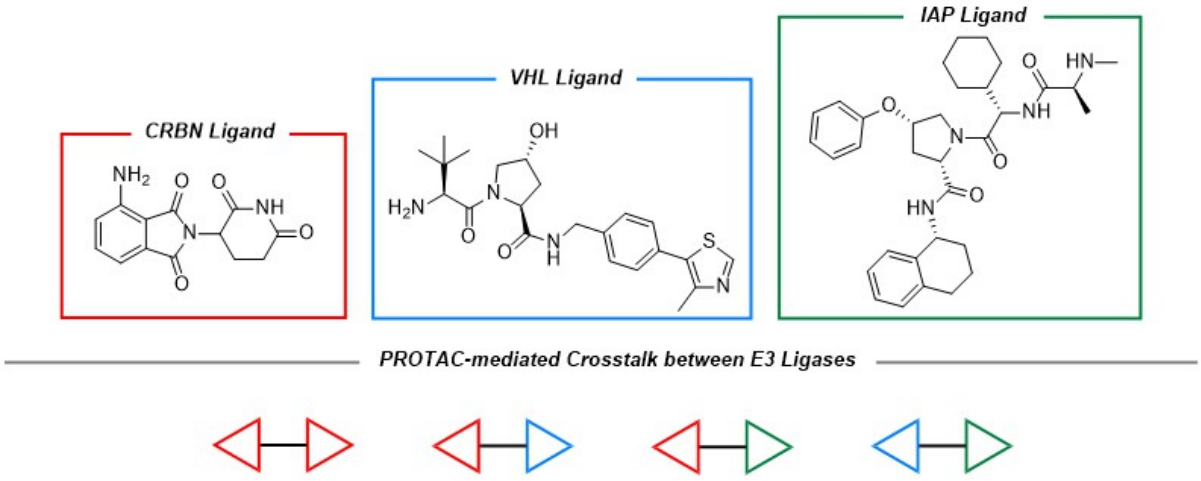
Targeted protein degradation is a rapidly growing field in drug discovery. In addition to their emerging role in the PROTAC technology, E3 ligases represent attractive therapeutic targets.[1] However, the development of E3 inhibitors is complicated by the absence of a catalytic domain.

To investigate whether a ligase itself can be targeted for degradation by PROTACs, we developed homodimeric PROTACs that caused a self-directed inactivation of cereblon (CRBN) *via* ternary complex formation, subsequent ubiquitination, and degradation.[2] Such homo-PROTACs might be a considerable leap forward to unravel the biological complexities of the E3 ligase CRBN and to provide additional insights into the mechanism of CRBN-targeting drugs. However, homodimers were hampered by the self-limiting reduction of the E3 ligase component and neomorphic activities on the transcription factors IKZF1 and IKZF3. A superior set of compounds was assembled from a CRBN and a Von Hippel-Lindau (VHL) ligand. For the first time, PROTAC-induced heterodimerization of two E3 ligases with unidirectional ubiquitination and efficient degradation of CRBN was described.[3]

Inspired by these results, our PROTAC-mediated depletion strategy was applied to therapeutically relevant E3 ligases. A combinatorial library of IAP/CRBN/VHL-based PROTACs was designed, synthesized, and investigated for their capability to target different BIRC domains.[4] The entire set of PROTACs spans pan-IAP degraders, XIAP-selective compounds, as well as dual-active entities. The homolog-selective modulation of degradation targets was not yet possible with small-molecule IAP inhibitors. Furthermore, our bivalent platform could potentially be used for targeting the E3 ligase Keap1, which was recently found to be upregulated in relapsed multiple myeloma patients. [5]



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NOVEL DERIVATIVES OF THE LEAD STRUCTURE D2AAK3 AS POTENTIAL ANTIPSYCHOTICS - SYNTHESIS AND AFFINITY STUDIES

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In the previous studies aimed at searching for novel compounds with antipsychotic properties, structure-based virtual screening was conducted [1]. Among found dopamine D_2 receptor antagonists, the compound D2AAK3 with 115 nM affinity for D_2 receptor was identified. It shows nanomolar or low micromolar affinity also for D_1, D_3, 5-HT_{1A}, 5-HT_{2A} and 5-HT_7 receptors. Interactions of D2AAK3 with its molecular targets at the molecular level were studied *in silico* using molecular modeling methods. Behavioral studies performed for D2AAK3 revealed that it decreases amphetamine-induced hyperactivity measured as spontaneous locomotor activity in mice, improves memory consolidation after acute treatment in passive avoidance test and exhibits anxiogenic activity 30 minutes after acute treatment in mice in elevated plus maze (this effect was reversed 60 minutes after administration of D2AAK3) [2]. In the light of above outcomes, D2AAK3 may be considered as a promising starting point for further optimization toward obtaining molecules with more beneficial receptor profile as for potential antipsychotics. A series of derivatives has been synthesized. The designed modifications of the lead structure included the exchange of substituent at piperazine moiety and elongation of the alkyl linker. The obtained compounds were tested in radioligand binding assays in order to evaluate their affinities for main molecular targets in schizophrenia. These results will be next complemented with behavioral studies.

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STRUCTURAL ANALOGUES OF METHYLPHENIDATE AS PARKINSON'S DISEASE-MODIFYING AGENTS

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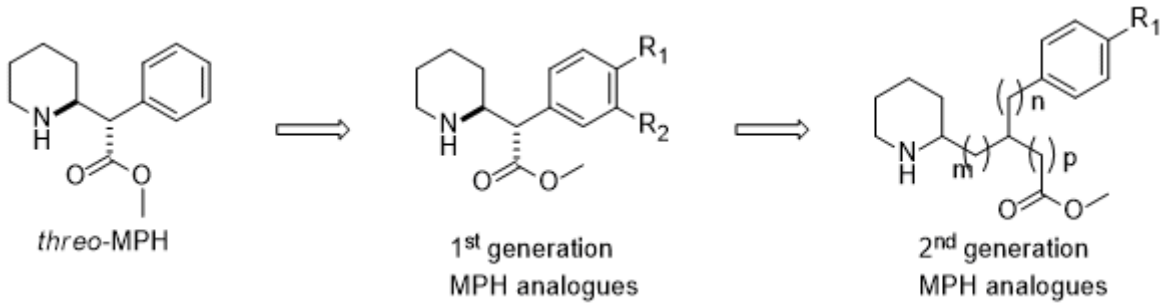
Parkinson's Disease (PD) is the second most common neurodegenerative movement disorder in the world today. Lewy bodies are cytosolic inclusions and significant pathological hallmarks of the disease and are composed of fibrillary aggregates of α-Synuclein (α-Syn) and Synapsin III (Syn-III). α-Syn is one of the most studied presynaptic protein and its misfolded forms are the main constituents of amyloid aggregates. [1,2]

Syn-III only recently arose as a crucial modulator of α-Syn aggregation and toxicity, in addition to its role as controller in vesicle trafficking, fusion and binding. Moreover, both Syn-III and α-Syn have been found to act as key regulators of nigrostriatal dopamine release and they cooperatively exert this function. [2-4]

Threo-Methylphenidate (MPH, Figure 1), a monoamine reuptake inhibitor clinically approved for the treatment of attention deficits and hyperactivity disorder (ADHD) and for counteracting freezing of gait in advanced PD, was recently found to stimulate an α-Syn/Syn-III-mediated locomotor response. Moreover, this MPH action is lost when Syn-III gene is silenced, suggesting that this protein is the target for MPH and that this activity is not related to DA transporter inhibition. [4]



We recently developed a "first generation" series of MPH analogs, showing punctual modifications on the aromatic moiety of MPH. [5] Among these derivatives, PK1 was found to stimulate *in vitro* the functional interaction between α-Syn and Syn-III, driving synaptic vesicle mobilization and reducing the size of α-Syn aggregates, 200 folds more efficiently that MPH. [6] In addition, we found that an acute PK1 i.p. treatment in human α-Syn transgenic mice was able to induce a motor recovery that resulted significantly improved when compared to what observed following MPH administration [6].

Following these observations, we have developed a computational-guided "second generation" series of compounds, changing the distance between the three main functional groups of the lead compound PK1 and evaluating novel modifications on the aromatic moiety which could reduce off-target effects. Among them, PK7 and PK12 were found to significantly stimulate Syn-III/ α-Syn complex, similarly to PK1. [6]

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BENZODIOXANE-BENZAMIDE FtsZ INHIBITORS: SYNTHESIS OF NEW DERIVATIVES AND THEIR BIOPHYSICAL AND BIOCHEMICAL EVALUATION

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The bacterial cell division is a complex process, leading to the scission of the parent cell in two daughter cells, genetically identical and having similar size. The achievement of a correct division is regulated by several bacterial proteins. This fine control involves the correct localization of the division septum as well as the ring constriction, that finally allows the physical separation [1]. Among these crucial proteins, FtsZ has been universally recognized as the main actor [2] and, consequently, as a potential and useful target for the obtainment of new antimicrobials. Indeed, FtsZ polymerizes at the centre of the cell into a circular structure (named Z-ring) which recruits all the downstream proteins that lead to cell division.

In the last years, we developed a huge series of benzamide inhibitors having high antimicrobial activity, which proved to be related to the inhibition of FtsZ [3,4].

In this poster, we present the synthesis of the recent derivatives, together with the results of the biophysical and biochemical characterization of the interaction with FtsZ. In particular, we determined FtsZ polymerization/depolymerization properties by fluorescence anisotropy and we estimated FtsZ polymers size by analytical ultracentrifugation. We also gathered information, using microscopy techniques, on how our compounds influence the morphology of the FtsZ polymers. In particular, we studied FtsZ polymerization in dilute solution and in the presence of macromolecular crowding agents, which mimic the crowded environment of the cytoplasm.

These analyses showed a correlation between the *in vivo* potency of the compounds and the level of *in vitro* FtsZ inhibition, allowing us to validate the target and to trace the path to future investigations.

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SELENOPHENO[3,2-C]- AND SELENOPHENO[2,3-C]- COUMARINS AND QUINOLINONES: SYNTHESIS, INHIBITION OF PROLIFERATION OF CANCER CELL LINES AND MDR PREVENTION

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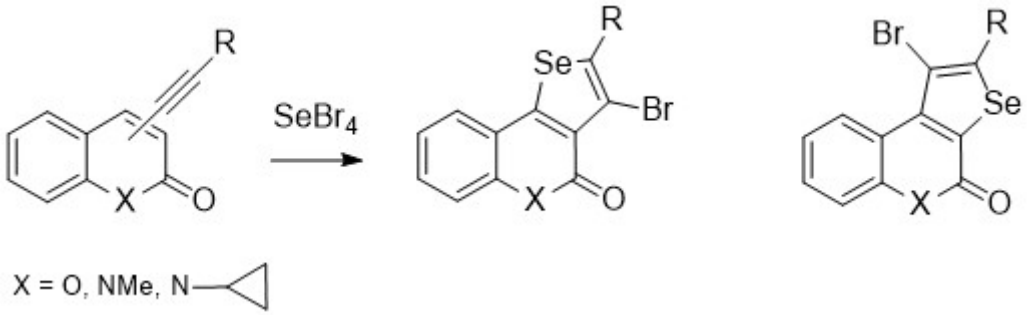
Cancer is the second leading cause of death worldwide. Coumarin and quinolinone systems are found in many natural compounds and present a wide range of biological activities. Naturally occurring furocoumarins are used in medicine (*Psoralen, Angelicin, Methoxsalen, Imperatorin*) mainly for skin diseases treatment. However, *in vitro* studies have demonstrated that furocoumarins can inhibit the growth of various types of cancer cells including breast cancer and non-small cell lung cancer [1]. It was reported that the derivatives of *N* -methylfuro[3,2-*c*]quinolone inhibit the growth of melanoma and gastrointestinal tumors [2]. Chemically synthesized anticancer drugs often cause multidrug resistance (MDR) of tumors, that is one of the most complicated clinical problems, it restricts the effectiveness of chemotherapy and increase patients mortality. Combination of drugs is one of the reported strategies to overcome MDR [3]. Previously, we described the synthesis of substituted selenopheno[2,3-*c*]- and [3,2-*c*]-coumarins and quinolinones and scientifically proved their ability to promote cancer cell apoptosis [4]. In current work it was found that the addition of selenophenoquinolinones in IC_{20} concentration impressively overcomes MDR of doxorubicin and mitoxanthrone on doxorubicin and mitoxanthrone resistant uterus sarcoma cell lines, while selenophenocoumarins had no effect. Our approach for the synthesis of selenophenocoumarins and selenophenoquinolinones involves reaction of corresponding ethynylheterocycles with *in situ* generated selenium tetrabromide.



Our results confirm selenophenoquinolinones as prospective scaffold for the development of non-toxic MDR preventing agents.

This work was supported by the PostDoc Latvia project Nr.1.1.1.2/VIAA/2/18/375 "Discovery of PARP inhibitors to overcome multidrug resistance"

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DESIGN, SYNTHESIS, AND EVALUATION OF COLCHICINE-SITE TUBULIN LIGANDS FOR CANCER THERAPY: A SELECTIVE CYTOTOXIC EFFECT

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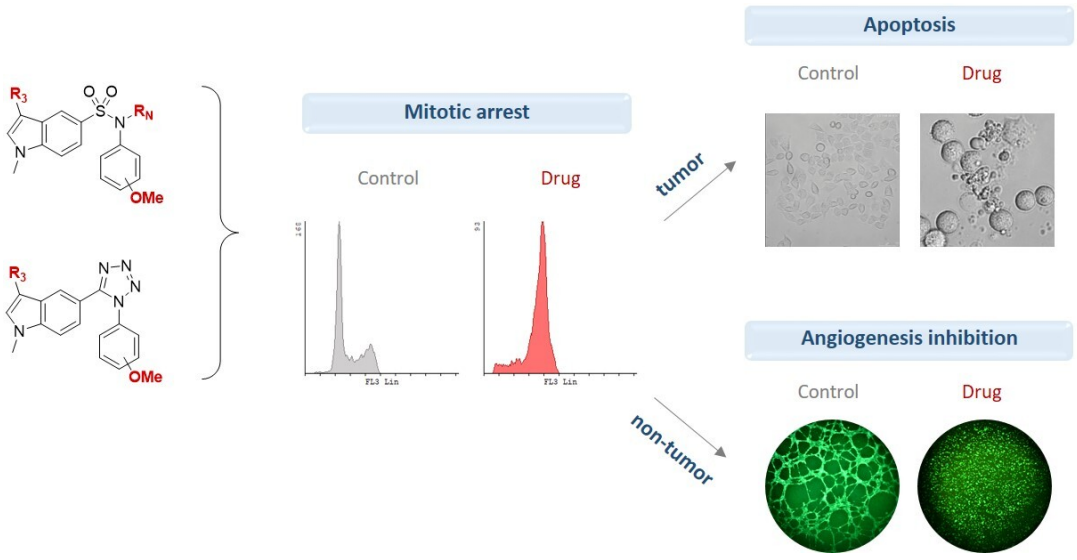
Cancer accounts for about 1 in 6 deaths globally and, despite the massive R&D investment, still constitutes a major public health issue. One of the most widespread drugs in cancer chemotherapy is paclitaxel, a tubulin-binding drug that acts as a microtubule-stabilizing agent. Paclitaxel and other taxanes usually face handicaps related to on- and off-target toxicity, acquired resistance, and pharmacokinetic problems meaning that there is room for further improvement.

In this work, we have created a library of compounds aimed to bind at the colchicine site in tubulin, which is a well-validated target in cancer treatment. The colchicine site embraces much lower molecular weight compounds that could tackle pharmacokinetic problems. Taking into account the requisites for proper binding, we conducted the design and subsequent synthesis of over fifty compounds with a sulfonamide group or a tetrazole moiety linking two aromatic rings: indole and poly-methoxylated benzene. We have explored substitutions at the indole 3-position, the methoxyphenyl ring, and the sulfonamide group, and selected six compounds after testing their anti-proliferative activity *in vitro*. The lead compounds displayed IC_{50} values in the nanomolar range against tumor cells (e.g. cervix, colorectal, and gastric carcinomas, leukemia, multiple myeloma), non-tumor cell lines, and primary cells, and are not affected by mainstream resistance mechanisms.

We have characterized the molecular target of the compounds employing biochemical assays, immunofluorescence analysis, and competition experiments. Our results indicate that these lead compounds behave as antimitotic agents, but they exert a different mechanism of action regarding tumor cells versus non-tumorigenic cells. i. On the one hand, we have found that after the mitotic arrest induced by these compounds, tumor cells enter apoptosis and die. This does not happen to non-tumor cells. ii. On the other hand, the compounds inhibit the formation of capillary-like structures in non-tumor endothelial cells. These results indicate that our indole-based compounds may be promising drug candidates for cancer chemotherapy, especially for the treatment of solid tumors. The cytotoxic effect against tumor cells could be backed up by the complementary inhibition of angiogenesis, thus hindering tumor growth. This work provides broad input for future research regarding the determinants that dictate different cell fates in distinct cell lines.



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EQATA: EQUITABLE ACCESS TO QUALITY ANTIBIOTIC THERAPIES IN AFRICA

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Antibiotic treatable infections are still a global health and mortality burden, causing 5.7 million deaths annually, the majority of which occur in low- and middle-income countries (LMICS). In Africa, mortality rate from lower respiratory infections and diarrhoeal diseases outnumbers that from HIV/AIDS, TB and malaria combined,[1,2]and rising antimicrobial resistance will further increase the mortality rates associated with bacterial diseases. CCDEP identified a number of barriers to treating infectious diseases LMICS face which include low antibiotic affordability and unreliable supply chains, which in combination with poor quality control, counterfeit pharmaceuticals entering the market, and poor stewardship lead to widespread misuse and overuse of antibiotics, further compounding the emergence of antimicrobial resistance. [1]

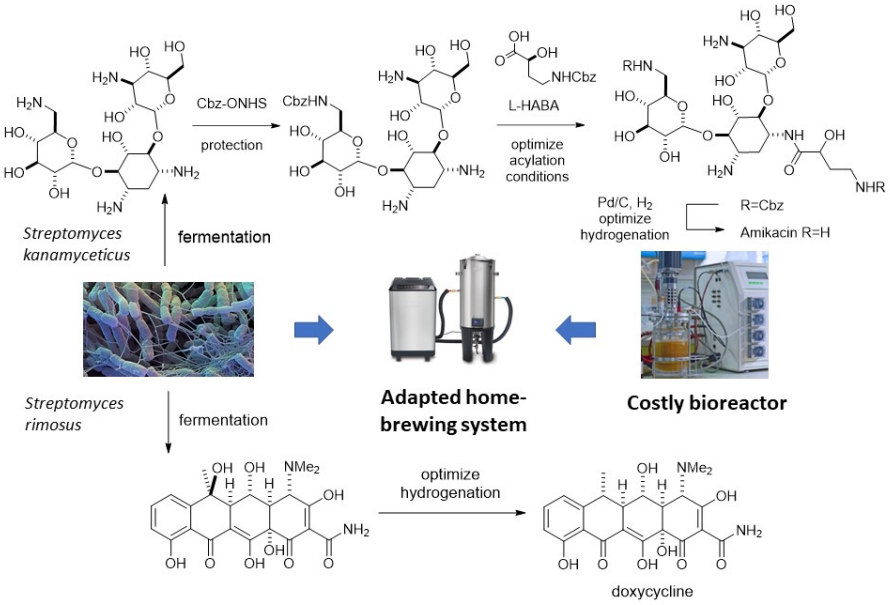
To tackle these challenges, two strategies have been proposed:

Sustainable and supply chain-safeguarded fermentative production of Access antibiotics

A robust yet simple and cost-efficient fermentation rig constructed from an adapted commercially available home brewing system will be developed for the fermentative production of precursors to two Access antibiotics[3] - **amikacin** and **doxycycline**, followed by optimization of their semi-synthesis to be cost-efficient and suitable for semi-industrial scale.

Drug discovery capacity building via community-driven discovery of novel bioactive molecules

In collaboration with our partners with Kenya, Tanzania and Nigeria, a citizen science-driven **microbial discovery** project will be launched. Run in collaboration with Tiny Earth,[4] the project will involve community-driven sampling soil and water and screening for presence of microorganisms producing novel, promising bioactive molecules.



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SMALL MOLECULE DRUG CONJUGATES: IN VIVO QUANTIFICATION OF RELEASED PAYLOAD AND LINKER OPTIMIZATION

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Traditional cancer chemotherapy consists in the administration of cytotoxic drugs which do not selectively accumulate at the site of disease, thus leading to dose-limiting off-target toxicities. The conjugation to tumor-specific ligands like antibodies and small molecules represents a promising strategy for the improvement of the therapeutic index of potent cytotoxic drugs and leads to the generation of Antibody Drug Conjugates (ADCs) (Gerber H.P. et al., Nat. Prod. Rep., 2013, 30; 625) and Small Molecule Drug Conjugates (SMDCs) (Srinivasarao M. et al., Nat. Rev. Drug Discov., 2015; 14, 203) Small molecules are endowed with better pharmacokinetic properties compared to antibodies displaying an increased and faster tumor penetration, reduced cost-of-goods and lower immunogenicity (Cazzamalli S. et al, J. Am. Chem. Soc., 2018; 140, 1617). SMDCs are pro-drugs composed by (a) a small organic ligand directed against a tumor-associated protein antigen, (b) a linker designed to be stable in circulation and to be efficiently cleaved only at the site of disease and (c) a highly potent cytotoxic payload. Since the efficacy and toxicity of ADCs and SMDCs are strictly related to the amount of the payload released at the site of disease and in off-target organs, it is essential to generate methods to quantify the amount of intact pro-drugs and free cytotoxic drug which can be found at different time points in the tumor, in healthy structures and in circulation (Bennett G., Mol. Cancer. Ther., 2020; 19, 1385). We present here the validation of a methodology based on mass spectrometry for the quantification of SMDCs and free drugs in biological samples. We then present the application of this methodology to the determination of the biodistribution of non-internalizing SMDC products targeting CAIX and FAP.

DISCOVERY OF 4-IODOPYRIMIDINE AS A POTENT AND SELECTIVE COVALENT INHIBITOR AND ACTIVITY-BASED PROBE FOR THE FAMILY OF MACROPHAGE MIGRATION INHIBITORY FACTOR (MIF)

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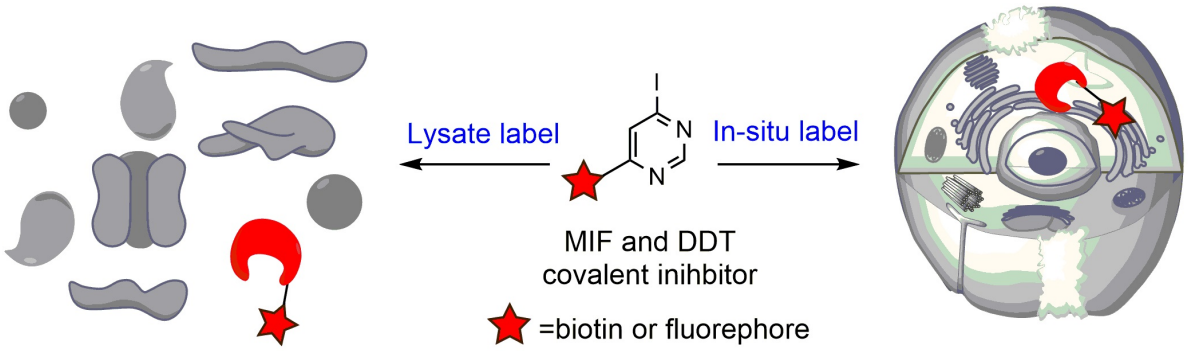
Electron-deficient halogenated (hetero)aryls are a group of reactive fragments that have been employed to covalently and unspecifically label cysteine on proteins via a nucleophilic aromatic substitution. 4-Iodo-6-phenylpyrimidine (4-IPP) is a covalent inhibitor for both macrophage migration inhibitory factor (MIF) and D-dopachrome tautomerase (DDT) in the MIF family. More importantly, 4-IPP also exhibits inhibition of MIF-related proliferative and proinflammatory activity in cells and animal models. However, the proteome-wide selectivity of 4-IPP is elusive. In our study, we synthesized a series of analogs of 4-IPP to explore the structure-activity relationships. To construct a probe for protein detection and enrichment, one of the most potent derivative was tagged with fluorephore or biotin, which did not weaken the potency of it. In the experiments with purified proteins, the probe react with both MIF and DDT in a dose- and time-dependent manner, and the reacted residue was 1-proline. By specifying the incubation time and using a MIF-selective blocker molecule, we can selectively label MIF or DDT with our probe. In a more complex cell lysate labeling assay, the probe exhibited a surprising selectivity on a band around 15 kDa, which contains both MIF and DDT. Of note, the probe was capable to localizing intercellular MIF and DDT in A549 and HeLa cells. Therefore, our study not only demonstrates the selectivity of 4-IPP derivatives, but also provides a powerful tool for MIF and DDT research.



3D-QSAR STUDIES FOR A SERIES OF 1,3,4-OXADIAZOL-2-ONE DERIVATIVES.

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The fatty acid amide hydrolase is an enzyme involved in the metabolism of the endocannabinoid system's ligands. Recently, there was a significant increase in the interest in this protein and other compounds affecting neurotransmission within the mentioned system. Several studies suggest that ligands working through this mechanism may be useful in the pharmacotherapy of several currently untreatable diseases e.g. schizophrenia, chronic pain, and depression [1-4]. One way to design selective and potent FAAH inhibitors is to thoughtfully examine the structure-activity relationship among a series of compounds with experimentally determined inhibitory activity.

Therefore, this work aimed to construct 3D-QSAR models for a series of FAAH inhibitors containing 1,3,4-oxadiazol-2-one moiety. The obtained models were characterized by good statistical parameters: CoMFA Q^2= 0.61, R^2=0.98; CoMSIA Q^2=0.64, R^2=0.93. The CoMFA model field contributions were 54% and 46% for steric and electrostatic fields, respectively. In the CoMSIA model, steric, electrostatic, hydrogen bond donor, and hydrogen acceptor properties were equal to 24%, 35%, 18%, and 23%, respectively. These models were validated applying the leave-one-out technique, the 7-element test set (CoMFA $r^2_{test\text{-}set}$= 0.91; CoMSIA $r^2_{test\text{-}set}$= 0.91), a progressive scrambling test, and an external validation criteria developed by Golbraikh and Tropsha (CoMFA r^2_0=0.98, k=0.95; CoMSIA r^2_0=0.98, k=0.89). As the statistical significance of the obtained model was confirmed, the CoMFA and CoMSIA field calculation results were mapped onto the enzyme binding site.

We believe that promising results obtained in this study will contribute to a better understanding of the structure-activity relationship among FAAH inhibitors and assist in designing novel, more potent compounds [5].

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IDENTIFICATION OF INDOLE-BASED SMALL MOLECULES AS ACTIVATORS OF INSULIN DEGRADING ENZYME

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Insulin degrading enzyme (IDE) is a zinc metalloprotease first characterised in 1949 for the insulin degradation, it is also responsible for the degradation of many and various substrates such amylin, glucagon, atrial natriuretic peptide, beta-amyloid peptide. IDE modulation can be a new strategy to treat both, type 2 diabetes (T2DM) and Alzheimer's disease (AD) by using, respectively, IDE inhibitors to reduce the insulin degradation and enhance its activity or IDE activator to improve the degradation of pathogenic beta-amyloid peptide. Here we describe the discovery of a new series of IDE small molecule activator by high throughput screening (HTS) and its pharmacomodulation.

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DISCOVERY OF hERAP2 MODULATORS VIA HIGH-THROUGHPUT SCREENING

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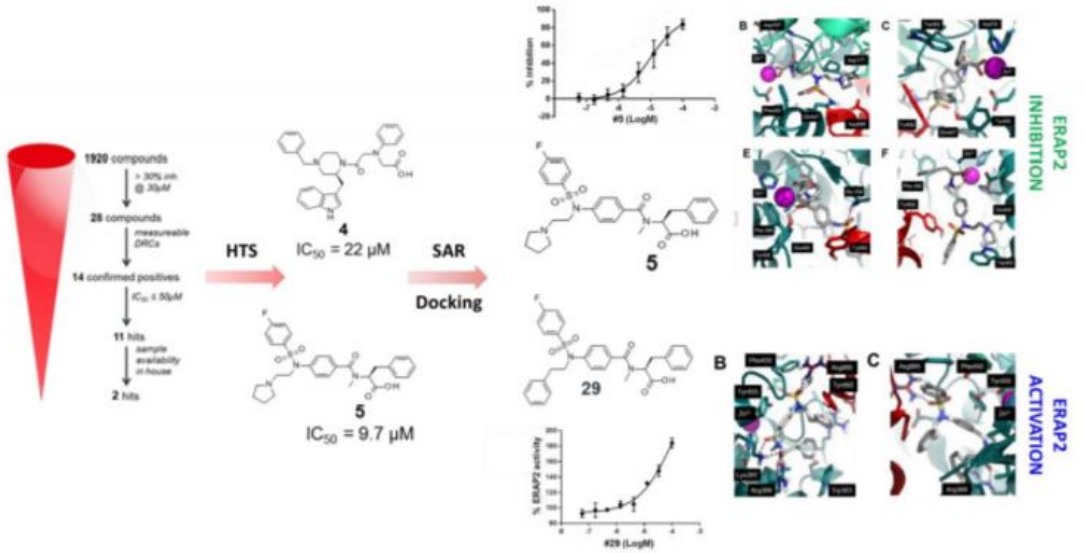
Endoplasmic reticulum aminopeptidase 2 (ERAP2), together with ERAP1 (50% homology), are responsible for antigenic peptides processing inside the ER and further addressing to MHC-I. Recently, ERAP2 has emerged as a promising pharmacological target through the validation of its role in several pathologies, among which autoinflammatory diseases and cancer. [1-2] While some selective ERAP1 modulators had already been disclosed, ERAP2 selective modulators remain highly desirable. A successful screening of a 1920-compound in-house library, that had been designed to target metalloenzymes, allowed us to identify the 2 promising hits **(4)** and **(5)**. Subsequent structure-activity relationships led to the discovery of selective ERAP2 inhibitors and, more surprisingly, to the 1st substrate depending ERAP2 activators. Docking studies revealed that both series exhibit undescribed binding modes.[3]



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MACROCYCLES IN DRUG DISCOVERY : A PROMISING SCAFFOLD FOR ENHANCING TARGET SELECTIVITY AND ADME PROPERTIES

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Macrocycles in drug discovery have been defined as a ring system consisting of 12 or more atoms. These compounds with properties outside Lipinski's rule of 5 have raised particular interest in the field of medicinal chemistry[1]. In fact, they can modulate novel targets that have difficult, large, and featureless binding sites. The macrocycle allows a molecule to achieve a degree of structural pre-organization, such that key functional groups can interact across extended binding sites in proteins without a major entropic loss upon binding. This can result in high affinity and selectivity for protein targets, while preserving sufficient bioavailability to reach intracellular locations[2]. Despite the molecular weight of the macrocycles which doesn't respect the "lipinski's rule of 5", the macrocycles have shown favorable physicochemical and pharmacokinetic properties such as good solubility, lipophilicity, metabolic stability and bioavailability[3]. A recent survey lists 70 marketed macrocycle drugs and 35 macrocycles in clinical development, and these drugs belong to different classes including peptidic and nonpeptidic natural products[2] such as cyclosporine A and Tacrolimus respectively, non-natural (synthetic) peptides and non-natural (synthetic) macrocycles such as the dual JAK2/FLT3 inhibitor pacritinib[1], now in advanced Phase III trials. Given the drug-like physicochemical and pharmacokinetic properties of macrocycles, the macrocyclization has found a lot of application in the field of kinase, protease and polymerase inhibitors and other biological targets[4]. In this poster, we describe the impact of macrocyclization on ADME properties as well as its role in improving target selectivity, based on the various scientific research carried out in latest years in this field.

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