MMCS 2022

3rd Molecules Medicinal Chemistry Symposium

Shaping Medicinal Chemistry for the New Decade

27–29 JULY 2022, ROME, ITALY

Program and Abstract Book

Organizers

MDPI Academic Open Access Publishing since 1996

molecules

SAPIENZA UNIVERSITÀ DI ROMA

Sponsors

CDD VAULT Complexity Simplified

LIFE CHEMICALS

MCE MedChemExpress

o2h discovery

Media Partners

antibiotics

biomolecules

EFMC European Federation for Medicinal Chemistry

pharmaceuticals

symmetry

3rd Molecules Medicinal Chemistry Symposium: Shaping Medicinal Chemistry for the New Decade

Sapienza Università di Roma

Roma, Italy

27 – 29 July 2022

Conference Chair

Prof. Dr. Rino Ragno

Prof. Dr. Diego Muñoz-Torrero

Organised by

Conference Secretariat

Ana Sanchis

Pablo Velázquez

Lucy Chai

Email: mmcs@mdpi.com

CONTENTS

Conference Committee

Dr. Jóhannes Reynisson FRSC

Prof. Dr. Florenci V. González

Prof. Dr. Maria Emília Sousa

Prof. Dr. Anne Roivainen

Prof. Dr. Mariarosaria Miloso

Prof. Dr. Giuseppe Manfroni

Prof. Dr. Mariana Spetea

Prof. Dr. Jorge Salvador

Prof. Dr. Bernard Maigret

Prof. Dr. Massimo Bertinaria

Prof. Dr. Helen Osborn

Prof. Dr. Josef Jampilek

Prof. Dr. Dante Rotili

Prof. Dr. Sergio Valente

MMCS2022 – Shaping Medicinal Chemistry for the New Decade
27 – 29 July 2022, Rome, Italy

	Wednesday 27 July 2022	Thursday 28 July 2022	Friday 29 July 2022
Morning	Check-in **Opening Ceremony** *Session 1*	*Session 4. Part 2*	*Session 6. Part 3*
	Coffee Break	**Coffee Break**	**Coffee Break**
	Session 3	*Session 6. Part 1*	*Session 5. Part 1*
	Lunch & Free Workshop	**Lunch & Poster Session A**	**Lunch & Poster Session B**
Afternoon	*Session 4. Part 1*	*Session 6. Part 2*	*Session 5. Part 2*
	Coffee Break	**Social Events**	
	Session 2		
	Best Posters Presentations		**Closing Remarks & Awards Ceremony**

Wednesday 27 July 2022: 08:15 - 13:00 / 15:00 - 18:45

Thursday 28 July 2022: 09:00 - 12:45/ 14:45 – 16:30 / **Conference Dinner: 20:00**

Friday 29 July 2022: 09:00 - 12:45 / 14:30 - 16:00

Conference Programme

Day 1 - Wednesday, 27 July 2022, 08:15 - 18:45 (CEST)

08:15 - 08:45	Registration Desk Open (Check-in)
08:45 - 09:00	Welcome Chairs Prof. Rino Ragno and Prof. Diego Muñoz-Torrero Open ceremony and greetings from the Director of the Department and the deputy-rector at the International Area of Sapienza: Prof. Claudio Villani and Prof. Bruno Botta

S1. Molecules Against Drugs Resistant Microorganisms and SARS-CoV-2

Chaired by Prof. Maria Emília Sousa

09:00 - 09:30	**Keynote Talk : Svetlana Tsogoeva** *"Multi-Step Domino Reactions: Facile Access to Antiviral, Antimalarial and Anticancer Agents"*
09:30 - 10:00	**Keynote Talk : Kelly Chibale** *"Combating Multi-Drug Resistance in Tuberculosis: strategies underpinned by Medicinal Chemistry*
10:00 - 10:15	**Thanigaimalai Pillaiyar** *"Small molecule inhibitors of SARS-CoV-2 Mpro: Enzyme inhibition and mechanism, antiviral activity, structure-activity relationship, and X-ray structure determination"*
10:15 - 10:30	**Angelo Oneto** *"Potent, non-peptidic irreversible inhibitors of SARS-CoV-2 main protease"*
10:30 - 10:45	**Elisabetta di Bello** *"Effects of Structurally Different HDAC Inhibitors against Trypanosoma Cruzi, Leishmania and Schistosoma mansoni"*
10:45 - 11:00	**Eleonora Proia** *"Ligand-Based and Structure-Based Predictive Models for SARS-CoV-2 Main Protease Ligands"*

11:00 – 11:30 Coffee Break

S3. Machine Learning in Drugs Design

Chaired by Prof. Rino Ragno

11:30 - 12:00	**Keynote Talk : Gabriele Cruciani** *"Exploiting PROTAC technology as an innovative for the treatment of lethal prostate cancer"*

12:00 - 12:15	**Maxime Langevin** *"What kind of applicability domain for molecular generative artificial intelligence?"*
12:15 - 12:30	**Barak Akabayov** *"Expanding the chemical space of a hit molecule obtained by NMR fragment screening using machine-learning"*
12:30 - 12:45	**Franco Lombardo** *"Human Dose Prediction in Drug Discovery: (when) is it possible?"*
12:45 - 13:00	**Vesna Rastija** *"Antitumor activity of rhodanine derivatives: Quantitative structure-activity relationship and molecular docking study"*
13:00 - 15:00 Lunch Break	
14:15 - 15:00 Workshop: **Live Demonstration of CDD Vault for Accelerating Drug Discovery**	
S4. Natural Compounds in Drug Discovery – Part I Chaired by Prof. Mariana Spetea	
15:00 - 15:30	**Keynote Talk : Janine Cossy** *"Natural products: A good starting point for accessing bioactive compounds"*
15:30 - 15:45	**Göklem Üner** *"A new perspective to natural product chemistry: non-apoptotic cell death induction via small molecule based supramolecular particles originating from sapogenins"*
15:45 - 16:00	**Mariana Spetea** *"The plant-derived alkaloid, sewarine, as a novel kappa-opioid receptor ligand: An experimental assessment and molecular docking"*
16:00 - 16:15	**Laura Treiber** *"Synthesis of the upper binding arm of kibdelomycin, a novel broad-spectrum, Gram-positive focussed antibiotic without cross-resistance to known gyrase inhibitors"*
16:15 - 16:30	**Filippo Umberto Sapienza** *"New potential applications of essential oils; an insight on Py-OE (3dQsar)"*
16:30 -17:00 Coffee Break	

S2. Targeting Proteins for Degradation: PROTACS, PHOTACS, LYTACs, and molecular glues

Chaired by Prof. Massimo Bertinaria

17:00 - 17:30	**Keynote Talk : Carles Galdeano** *"Expanding the toolbox of E3 ligases for PROTACs: drugging the Fbxw7 E3 ligase"*
17:30 - 18:00	**Keynote Talk : Manfred Jung** *"Dual inhibitors of acetylation and fatty acid-deacylation by the NAD dependent histone deacetylase Sirtuin2 (Sirt2)"*
18:00 - 18:15	**Maurizio Pellecchia** *"NMR- and structure-guided design of Lysine covalent ligands for PPI antagonists and degraders"*
18:15 - 18:45	**Selected Posters 3-min Flash Presentations:** **Dariia Samofalova** *"Identification Of Novel Potential Inhibitors Of Dpre1 From Mycobacterium Tuberculosis And Mycobacterium Bovis"* **Olga Bobileva** *"3-(Adenosylthio)methyl benzoic acid with modified adenosine as SARS-CoV-2 methyltransferases inhibitors"* **Bahne Stechmann** *" Identifying molecules against SARS-CoV-2 and future pandemics collaboratively within an open-access research infrastructure initiative "* **Federica Blua** *"Design and synthesis of encorafenib-based BRAF-V600E degraders"* **Salvatore Galati** *"VenomPred: A Machine Learning Based Platform for Molecular Toxicity Predictions"* **Arianna Colcerasa** *"Synthesis and biological evaluation of Schistosoma mansoni Sirtuin2 (SmSirt2) inhibitors"* **Noemí Villella** *"Targeting Plasmodium falciparum dihydroorotate dehydrogenase: design, synthesis, co-crystallization and biological evaluation of new 3-hydroxypyrazole scaffold-based inhibitors"* **Manuel Schriefer** *"Synthesis of the lower binding arm of kibdelomycin, a novel gyr B- and topo IV-inhibitory antibiotic"* **Alessandra Salerno** *"A fragment-based approach for the development of trypanothione reductase inhibitors as antileishmanial agents"* **Marc Panosetti** *"Design, synthesis and biological evaluation of new RNA ligands targeting miR-210: modulation of the circadian clock for cancer chemotherapy"*

Day 2 - Thursday 28 July 2022

S4. Natural Compounds in Drug Discovery – Part II

Chaired by Prof. Mariana Spetea

09:00 - 09:30	**Keynote Talk : Markus Kalesse** *"Stereoselective Sparteine-free 1,2-Metallate Rearrangements in the Syntheses of Chondrochlorene and Meridamycin"*
09:30 - 09:45	**Maria Carpena** *"How to solve the quantification issues inherited from the antioxidant assays based on single-electron transfer? A mathematical approach"*
09:45 - 10:00	**Sandra Kovachka** *"Panicein A Hydroquinone (PAH) and analogs overcome chemotherapy resistance in cancer cells"*
10:00 - 10:15	**Abdellah Ezzanad** *"Effect of neurogenesis promoters, phorbol esters and derivatives of 12-deoxyphorbol esters, on the inhibition of cytochrome P450"*

10:15 - 11:00 – Coffee Break

S6. Medicinal Chemistry Tales – Part I

Chaired by Dr. Jóhannes Reynisson FRSC

11:00 - 11:30	**Keynote Talk : Mauro Maccarrone** *"Plant-Derived & Endogenous Cannabinoids: Different in Nature"*
11:30 - 11:45	**Flavio Emery** *"Synthesis and structural activity relationships of novel isoindolone series against Trypanosoma brucei rhodesiense"*
11:45 - 12:00	**Elizabeth Lopes** *"Identification of a Novel Scaffold for Dual MDM2/X Inhibition"*
12:00 - 12:15	**Carmen Cerchia** *"Discovery of novel naphthylphenylketone and naphthylphenylamine derivatives as Cell Division Cycle 25B (CDC25B) Phosphatase Inhibitors via in silico design"*
12:15 - 12:30	**Rosana Ribić** *"Design, synthesis and evaluation of immunostimulating activities of mannosylated desmuramyl peptides containing lipophilic substituents"*

12:30 - 12:45	**Eleonora Diamanti** *"Targeting the Energy-Coupling Factor Transporters: a novel antibacterial target"*
12:45 - 14:45 *13:45 - 14:45*	**Lunch** ***Poster Session A***

S6. Medicinal Chemistry Tales – Part II

Chaired by Dr. Jóhannes Reynisson FRSC

14:45 - 15:15	**Keynote talk: Rebecca Wade** *"Mapping dynamic protein binding sites for the design of selective anti-infective agents"*
15:15 - 15:30	**Chiara Borsari** *"Volume Scanning, a Rational Approach to Covalent PI3Ka Inhibitors"*
15:30 - 15:45	**Ouldouz Ghashghaei** *"Facilitated Access to Novel Aryl Hydrocarbon Receptor Ligands through Extended Multicomponent Reactions"*
15:45 - 16:00	**Johannes Reynisson** *"The cytotoxic potential of cationic triangulenes against tumour cells"*
16:00 - 16:15	**Cristina Maccallini** *"The inhibition of iNOS as a promising strategy against cancer development: good news from novel phenyl-amidine based compounds"*
16:15 - 16:30	**Ariadna Gil-Martinez** *" Targeting multimeric G-quadruplex structures using reinforced ligands"*
20 :00	**Conference Dinner**

Day 3 - Friday 29 July 2022

S6. Medicinal Chemistry Tales – Part III

Chaired by Dr. Jóhannes Reynisson FRSC

09:00 - 09:30	**Keynote Talk : *Olalla* Vázquez** *"Our Journey To Achieve Photoinhibition of Haematopoiesis in Vivo"*
09:30 - 09:45	**Marta Giorgis** *"Targeting Acute Myelogenous Leukemia using potent human dihydroorotate dehydrogenase inhibitors based on the 2-hydroxypyrazolo[1,5-a]pyridine scaffold: from academy to clinic"*
09:45 - 10:00	**Jorge González García** *" Delivery of a potent G-quadruplex DNA binder to cancer cells by aptamer functionalized liposomes"*
10:00 - 10:15	**Sundus Erbas Cakmak** *"Regulation Of Gene Expression With Smart Activatable Therapeutics"*
10:15 - 10:30	**Stefan Laufer** *"Discovery And Development Of A Mkk-4 Inhibitors To Increase Liver Regeneration"*

10:30 – 11:15 – Coffee break

S5. Multitarget Drug Discovery – Part I

Chaired by Prof. Dante Rotili

11:15 - 11:45	**Keynote Talk : Maria Laura Bolognesi** *"Expanding the polypharmacology toolbox for neurodegenerative diseases"*
11:45 - 12:00	**Elisabetta Marini** *"Multitarget antioxidant NO-donor organic nitrates: a novel approach to overcome nitrates tolerance"*
12:00 - 12:15	**Jussara Amato** *"Anticancer activity of G-quadruplex DNA-targeting monohydrazone based compounds"*
12:15 - 12:30	**Laura Fumagalli** *"Novel series of multitarget ligands for the treatment of Type 2 Diabetes."*
12:30 - 12:45	**Diogo Rodrigo Moreira** *"Studies of potency and efficacy of an optimized dihydroartemisinin-quinoline hybrid against multiple stages of the Plasmodium life cycle"*

12:45 – 14:30	**Lunch**
13:30 – 14:30	***Poster Session B***
	S5. Multitarget Drug Discovery – Part II Chaired by Prof. Dante Rotili
14:30 - 15:00	**Keynote Talk : Antonello Mai** *"Epi-Polypharmacology: A Medicinal Chemistry Perspective"*
15:00 - 15:15	**Chiara Disraeli** *"Synthesis, biological activity and physicochemical properties evaluation of antiplasmodial pyrimido[1,2-a]benzimidazoles"*
15:15 - 15:30	**Raquel Gil-Edo** *"Multitarget inhibitors as anticancer agents with immunomodulatory and antiangiogenic properties"*
15:30 - 15:45	**Maurinne Bonnet** *"Design, synthesis and biological evaluation of novel RNA binders"*
15:45 - 16:00	**Giorgia Canini** *"In silico characterization of glucosylceramide synthase (GCS) binding site by Induced Fit Docking, Molecular Dynamics and Metadynamics"*
	Awards Ceremony & Closing Remarks

Certificate of Attendance

Participants of the event will be able to download an electronic Certificate of Attendance by accessing their dashboards on Sciforum.net once the event is concluded. The certificates will be found under "My Certificates" category.

Welcome from the chairs

Dear Colleagues,

It is with great pleasure that we announce the 3rd Molecules Medicinal Chemistry Symposium—Shaping Medicinal Chemistry for the New Decade (MMCS2022), to be held in Rome, Italy, from 27 to 29 July 2022.

The conference is co-organized by the Sapienza University of Rome, Department of Drug Chemistry and Technology, and MDPI, the publisher of the open-access journal Molecules. The conference is a follow-up to the successful 2nd Molecules Medicinal Chemistry Symposium—Facing Novel Challenges in Drug Discovery (MMCS2019), held in May 2019 in Barcelona, Spain. This turned out to be a very fruitful forum that delved into numerous topics. The excellent atmosphere was created by the presence of more than 190 attendees from 30 countries.

Encouraged by the great success of the MMCS2019, the three-day MMCS2022 will continue to address a large audience and a wide variety of current medicinal chemistry topics. The MMCS2022 will be organized into a number of thematic sessions on the medicinal chemistry of particularly challenging diseases, novel and revisited drug discovery approaches, and medicinal chemistry stories about recently implemented projects in areas not covered in the other sessions—target and hit identification, hit-to-lead optimization, the tuning of physicochemical and pharmacokinetic properties, preclinical and clinical development, etc.
In each session, two prominent and inspiring keynote speakers will share the program with a number of oral communications selected from among the contributions submitted by young researchers. The program will be complemented by poster presentations and social events.

We hope that you all have a great experience and enjoy your stay in Rome!

Prof. Rino Ragno
Conference Chair

Prof. Diego Muñoz-Torrero
Conference Chair

molecules
an Open Access Journal by MDPI

Molecules is the leading international, peer-reviewed, open access journal of chemistry. Molecules is published semimonthly online by MDPI. The International Society of Nucleosides, Nucleotides & Nucleic Acids (IS3NA), the Spanish Society of Medicinal Chemistry (SEQT) and the International Society of Heterocyclic Chemistry (ISHC) are affiliated with *Molecules* and their members receive a discount on the article processing charges.

Journal Webpage: https://www.mdpi.com/journal/molecules
Open Access — free for readers, with article processing charges (APC) paid by authors or their institutions.
Rapid Publication: manuscripts are peer-reviewed, and a first decision provided to authors approximately 14.2 days after submission; acceptance to publication is undertaken in 2.8 days (median values for papers published in this journal in the first half of 2022).
Recognition of Reviewers: reviewers who provide timely, thorough peer-review reports receive vouchers entitling them to a discount on the APC of their next publication in any MDPI journal, in appreciation of the work done.

General Information

What is Sciforum?

Sciforum is an event planning platform that supports open science by offering the opportunity to host and participate in academic conferences. It provides an environment for scholarly exchange, discussion of topics of current interest, building of networks and establishing collaborations.

The Benefits of Sciforum

Sciforum helps conference organizers to run online and physical conferences efficiently. The organizers reduce their administrative efforts thanks to an online tool that supports all aspects of conference organization, including setting up and maintaining the conference website, managing the peer-review process, publishing the conference proceedings, handling and coordinating the conference schedule, registration, billing, sponsors, etc.

We are here to help you to

- Set up your conference website
- Handle abstract & full paper submission and peer review
- Handle online registrations and billing
- Build your conference program & schedule, record the conference income & expenses
- Build customized mailing lists
- Have access to useful data from your conference (participants list, registration details, book of abstracts, etc.)
- Copy-edit, manage & publish your conference proceedings
- Manage Awards & post event surveys

For more information, please visit: **www.sciforum.net**

For any queries, please contact: **info@sciforum.net**

3rd Molecules Medicinal Chemistry Symposium will be held at Sapienza Università di Roma, Roma, Italy, on 27 – 29 July 2022.

This conference seeks to gather together experts in the field of medicinal chemistry, and aims to provide a forum for discussion regarding recent innovative medicinal chemistry projects, focused particularly (but not only) on challenging diseases, novel and revisited drug discovery approaches, and medicinal chemistry stories about recently implemented projects in areas not covered in the other sessions—target and hit identification, hit-to-lead optimization, the tuning of physicochemical and pharmacokinetic properties, preclinical and clinical development, etc.

Conference Venue

Sapienza University of Rome
Piazzale Aldo Moro, 5, 00185 Roma RM, Italy

Registration Desk

The desk for registration, information and distribution of documents will be open from 08:15 on 27 July 2022.

Use of Masks in Sapienza University

Masks should be worn in the venue facilities during the talks and when moving across common areas. You may take them off during the coffee or lunch breaks, and when going outside the building. Thank you for your collaboration!

Disclaimer

Delegates will receive a name-badge at the Information Desk, upon registration. The badge must be worn prominently in order to gain access to the congress area during all scientific and social events. Admission will be refused to anyone not in possession of an appropriate badge.

Insurance

The organizers do not accept liability for personal accident, loss, or damage to private property incurred as a result of participation in the *3rd Molecules Medicinal Chemistry Symposium*. Delegates are advised to arrange appropriate insurance to cover travel, cancellation costs, medical, and theft or damage of belongings.

Rome and Italy

Rome today is one of the most idyllic tourist destinations in the world, due to the incalculable immensity of its archaeological and art treasures, as well as for the charm of its unique traditions, the beauty of its panoramic views, and the majesty of its magnificent "villas" (parks).

Rome has been one of the world's most visited cities for the past two millennia. In the Roman times, Rome was the centre and the most powerful city of Western Civilization, ruling all the Mediterranean, Northern Africa, England and parts of the Middle East. Afterwards, it became one of the most important cities in Christianity, since the pope, the head of the Roman Catholic Church, resides in Rome. It became a worldwide centre of pilgrimage, and later in the Renaissance, as the city became a major European capital of the arts, education, philosophy and trade; becoming an important crossroads for bankers, artists and other people in general.

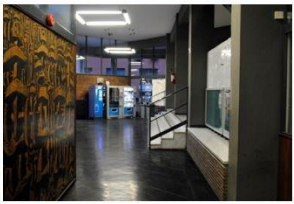
Most popular tourist attractions

Rome's two most popular tourist destinations are the Vatican Museums and Colosseum (the world's 37th and 39th most popular tourist destinations respectively). Other popular sites include St Peter's Basilica, the Forum Romanum, the Pantheon, the Trevi Fountain, the Spanish Steps, Via Condotti, Via Veneto, the Capitoline Museums, the Villa Borghese gardens, the Villa Giulia, Piazza Navona, the Basilica di Santa Maria Maggiore, the Archbasilica of Saint John Lateran, the Piazza del Popolo, the Castel Sant'Angelo, the Campo de' Fiori, the Quirinal Palace, the Lateran Palace and the Palazzo Barberini, to name a few.

For a complete overview, visit https://www.turismoroma.it/en.

Sapienza University of Rome

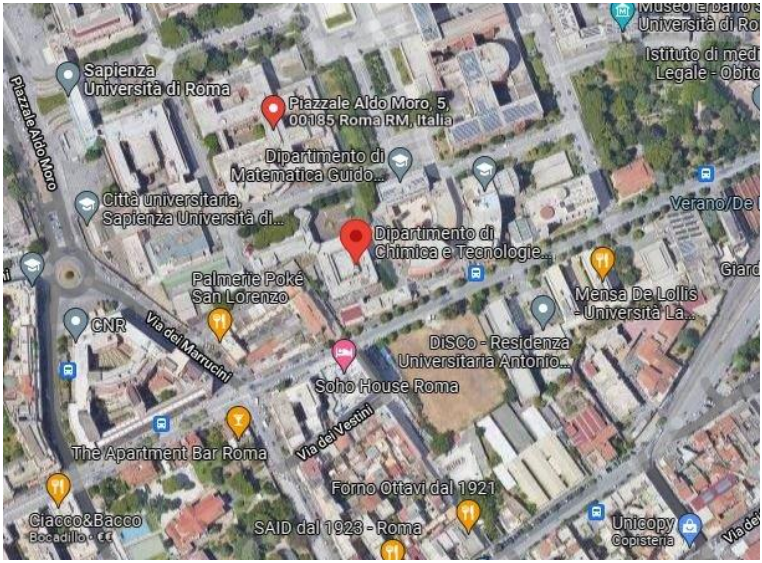
The Sapienza University of Rome is a research university that is located in Rome, Italy. Formally known as Università degli Studi di Roma "La Sapienza", it is one of the largest European universities by enrolment and one of the oldest in history, founded in 1303. The University is one of the country's most prestigious universities and commonly ranks first in both national and continental rankings. In 2018, 2019 and 2021 it ranked first in the world for classics and ancient history.



How to Reach the Venue

Venue: Department of Chemistry and Pharmaceutical Technologies - Sapienza University of Rome

Address: Piazzale Aldo Moro, 5, 00185 Roma RM, Italy



Keynote speakers

Prof. Dr. Kelly Chibale
Drug Discovery and Development (H3D) Centre, University of Cape Town (UCT), South Africa

Prof. Dr. Janine Cossy
Molecular Chemistry and Catalysis, ESPCI Paris, France

Prof. Dr. Svetlana B. Tsogoeva
Friedrich-Alexander-Universität Erlangen-Nürnberg, Germany

Prof. Dr. Maria Laura Bolognesi
Dipartimento di Farmacia e Biotecnologie, Università di Bologna, Italy

Prof. Dr. Olalla Vázquez
Philipps-Universität Marburg, Germany

Prof. Dr. Mauro Maccarrone
Department of Biotechnological and Applied Clinical Sciences, University of L'Aquila, L'Aquila (Italy)

Prof. Dr. Antonello Mai
Sapienza University of Rome, Roma, Italy

Prof. Dr. Gabriele Cruciani
Chemistry Department, University of Perugia, Italy

Prof. Dr. Manfred Jung
University of Freiburg, Germany

Prof. Dr. Markus Kalesse
Institute of Organic Chemistry, Leibniz University Hannover, Germany

Prof. Dr. Rebecca Wade
Heidelberg Institute for Theoretical Studies (HITS) and Heidelberg University, Germany

Dr. Carles Galdeano
Serra Hunter Lecturer Professor, University of Barcelona, Spain

Social Events

Conference Dinner

Thursday 28 July, 20:00
Price: 60€ *Tickets must be purchased in advance.*

Depending on the final number of attendees and the actual COVID restictions, the gala dinner of MMCS2022 will take place at one of the two following places, both near the Colosseum:

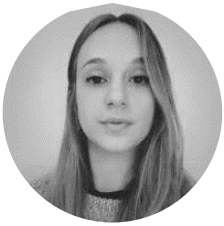
1. Ristorante Pizza Forum Roma - Forno a Legna located at via San Giovanni in Laterano 34/38

2. Caffè Rossi Martini located at piazza del Colosseo n. 3

Contact persons during the event

Pablo Velázquez

Email: pablo.velazquez@mdpi.com



Carmen Ruiz

Email: carmen.ruiz@mdpi.com

Ana Sanchis

Email: ana.sanchis@mdpi.com

Abstracts

Session 1. Molecules against Drug Resistant Microorganisms and SARS-CoV-2

<u>Keynote talk</u> - Multi-Step Domino Reactions: Facile Access to Antiviral, Antimalarial and Anticancer Agents

Svetlana B. Tsogoeva

1 Organic Chemistry Chair I, Friedrich-Alexander-University of Erlangen-Nürnberg, Nikolaus-Fiebiger-Straße 10, 91058 Erlangen, Germany

The domino process is a powerful tool to economically and sustainably build up complex molecular architectures which drastically reduces the number of work-up and purification steps.[1] Recently we developed new metal-free multi-step domino reactions and one-pot processes for the waste-reducing and cost-effective preparation of versatile frameworks, which otherwise are difficult to access via traditional methods. The developed new methods engage malononitrile and other simple and readily available reagents (including visible light and oxygen) in a wide range of domino reactions to construct new antiviarl, antimalarial, anticancer and antischistosomal agents. [2-7]
The in vitro tests against multidrug-resistant P. falciparum strains (Dd2 and K1), human cytomegalovirus (HCMV) and multidrug-resistant P glycoprotein-overexpressing CEM/ADR5000 leukemia cells revealed the selected products and some corresponding artemisinin-containing hybrid compounds as highly active agents, outperforming the clinical reference drugs. These recent results will be discussed in the talk.

[1] B. Grau et al. Angew. Chem. Int. Ed. 2021, 60, 22307.
[2] C. M. Bock, et al. Chem. Eur. J., 2016, 22, 5189.
[3] F. E. Held, A. et al. Nature Commun., 2017, 8: 15071.
[4] A. Çapcı, et al. Angew. Chemie. Int. Ed., 2019, 58, 13066.
[5] A. Çapcı, et al. Chem. Eur. J. 2020, 26, 12019.
[6] L. Herrmann, et al. ChemMedChem, 2022, e202200005.
[7] L. Kersting, et al. ChemPhotoChem, 2022, https://doi.org/10.1002/cptc.202200109.

NOTES

Keynote talk - Combating multi-drug resistance in tuberculosis: strategies underpinned by medicinal chemistry

Kelly Chibale

1 University of Cape Town

The increase in drug resistant forms of Mycobacterium tuberculosis (Mtb), the causative agent of tuberculosis (TB), has led to the inclusion of Mtb amongst the World Health Organization's priority list of antimicrobial resistance (AMR) pathogens. This is justified on the basis of the grim statistic of drug-resistant TB (DR-TB) accounting for ~ 29% of deaths attributable to AMR. The current TB drug regimens for both drug-sensitive TB (DS-TB) and DR-TB have an unsatisfactory side effect profile and are plagued by long treatment durations leading to poor patient adherence and subsequently further acquisition of drug resistance and treatment failure. For these reasons novel TB drugs with novel mechanisms of action (MoAs) for use in novel regimens are urgently needed.

This talk will describe our phenotypic- and target-based approaches towards addressing DR-TB. Identification of drug discovery small molecule starting points (aka hits) from either of these approaches is followed by medicinal chemistry optimization, with attendant deconvolution of the mechanism of action (MoA) in the case of phenotypic drug discovery. The identification of small molecule hits active against Mtb and subsequent structure-activity-relationship and MoA deconvolution studies will be presented.

NOTES

Small molecule inhibitors of SARS-CoV-2 M^{pro}: Enzyme inhibition and mechanism, antiviral activity, structure-activity relationship, and X-ray structure determination

Thanigaimalai Pillaiyar[1], Philipp Flury[1], Olga Eppler[1], Laura Schäkel[2], Elany Barbosa Da Silva[3], Haixia Sud[4], Tianqing Nied[4], Stephanie Luedtke[3], Cheila Rocha[5], Marvin R.I. Petry[2], Nadine Krüger[5], Anthony J. O'Donoghue[3], Yechun Xu[4], Michael Gütschow[2], Christa E. Müller[2], Stefan Laufer[1]

[1] Institute of Pharmacy, Pharmaceutical/Medicinal Chemistry and Tübingen Center for Academic Drug Discovery, Eberhard Karls University Tübingen, Auf der Morgenstelle 8, 72076 Tübingen, Germany
[2] PharmaCenter Bonn, Pharmaceutical Institute, Pharmaceutical & Medicinal Chemistry, University of Bonn, An der Immenburg 4, D-53121 Bonn
[3] Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California San Diego, La Jolla, California, United States of America
[4] CAS Key Laboratory of Receptor Research, and Drug Discovery and Design Center, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, China
[5] Infection Biology Unit, German Primate Center, Leibniz Institute for Primate Research Göttingen, Kellnerweg 4, 37077 Göttingen, Germany

The development of antiviral drugs that are effective against SARS-CoV-2 is a major priority in the battle against COVID-19, especially with the emergence of variants that may elude vaccines [1]. The main protease (Mpro, 3CLpro) is an attractive target in coronaviruses because of its essential role in viral replication and transcription [1,2]. We designed, synthesized, and evaluated a series of small molecules for inhibition of SARS-CoV-2 Mpro based on previous findings [3], which resulted in potent inhibitors with IC50 values of around 10 nM. Preliminary structure–activity relationship studies provide a basis for further optimization to obtain drugs against COVID-19. These inhibitors also exhibited promising antiviral activity in cell-based assays without showing cytotoxicity. To obtain insights into the molecular interactions, co-crystallization of selected compounds with SARS-CoV-2 Mpro was achieved and crystal structures were determined confirming the covalent bond formation of inhibitors with the protease. The potent SARS-CoV-2 Mpro inhibitors were also found to inhibit the Mpro of other β-coronaviruses, including SARS-CoV-1 and MERS-CoV, with similar potency, suggesting potential application for the treatment of a broader spectrum of coronaviral infections.

[1] T Pillaiyar, Med. Res. Rev. 2021, 41, 72-135. [2] Y. Chen, J. Med. Virol. 2020, 92, 418–423. [3] Breidenbach, J.; Lemke, C.; Pillaiyar, T. et al. Angew. Chem. 2021, 133, 10515-10521

NOTES

Potent, non-peptidic irreversible inhibitors of SARS-CoV-2 main protease

Angelo Oneto, Christa Elizabeth Müller, Michael Gütschow, Laura Schäkel, Ghazl Al Hamwi, Marvin Petry, Thanigaimalai Pillaiyar, Vigneshwaran Namasivayam

University of Bonn

Various effective vaccines against SARS-CoV-2, which causes COVID-19, have recently been developed and are broadly applied. Nevertheless, many severe infections are still observed due to the emergence of resistant mutations, vaccine non-responders, and a large number of non-vaccinated people [1,2]. Therefore, effective therapeutics against the current and future variants of this virus are urgently needed. The main protease (Mpro) of SARS-CoV-2, a virus-encoded cysteine protease, plays an essential role in viral protein processing and pathogenesis [3,4]. The enzyme is highly conserved among coronaviruses (including SARS-CoV-1, SARS-CoV-2, and MERS-CoV), and mutations are rare. To date, several peptide-like Mpro inhibitors are in development (MW 488 – 720 g/mol), but only few non-peptidic small molecule inhibitors have been reported [5]. We synthesized a series of novel chloropyridyl ester derivatives (MW 233 – 376 g/mol) and studied their structure-activity relationships employing a robust enzyme assay previously established by ourselves [6]. In addition to the compounds' inhibitory activity, enzyme inhibition kinetics, inhibition mechanism, and further properties were evaluated to optimize the compounds' potency and drug-like properties. Highly potent SARS-CoV-2 small molecule Mpro inhibitors were developed which may be superior to peptidomimetic drugs and provide excellent starting points for further drug development.

NOTES

Effects of Structurally Different HDAC Inhibitors against *Trypanosoma Cruzi, Leishmania* and *Schistosoma mansoni*

Elisabetta Di Bello[1], Beatrice Noce[1], Rossella Fioravanti[1], Clemens Zwergel[1], Sergio Valente[1], Dante Rotili[1], Daniela Trisciuoglio[2], Marina M. Mourão[3], Policarpo Sales-Jr[3], Suzanne Lamotte[4], Eric Prina[4], Gerald F. Späth[4], Cécile Häberli[5, 6], Jennifer Keiser[5, 6], Antonello Mai[1, 7]

[1] Department of Drug Chemistry and Technologies, Sapienza University of Rome, P. le A. Moro 5, 00185 Rome, Italy
[2] Institute of Molecular Biology and Pathology, National Research Council (CNR), Via degli Apuli 4, 00185 Rome, Italy
[3] Instituto René Rachou, Fundação Oswaldo Cruz, Avenida Augusto de Lima, 1715, 30190-002 Belo Horizonte, Brazil
[4] Institut Pasteur, Université Paris Cité, INSERM U1201, Unité de Parasitologie Moléculaire et Signalisation, Paris, France
[5] Swiss Tropical and Public Health Institute, 4002 Basel, Switzerland
[6] University of Basel, Basel, Switzerland
[7] Pasteur Institute, Cenci-Bolognetti Foundation, Sapienza University of Rome, Italy

Neglected tropical diseases (NTDs), including trypanosomiasis, leishmaniasis, and schistosomiasis, represent a severe problem in under-developed regions of the world even today regarding morbidity and mortality worldwide every year. (1) Current anti-parasitic drugs carry several limitations such as toxicity, no efficacy towards all the forms of the parasites' life cycle, and/or induction of resistance. (2) Epigenetic mechanisms and changes in chromatin structure play an essential role in parasite growth and survival, and the impact of histone proteins is expected to be strong in parasites with complex life cycles and multiple developmental stages. (3) The use of epigenetic drugs has been suggested as strategic for treating NTDs. We tested structural different histone deacetylases inhibitors (HDACi) **1**-**9**, chosen from our in-house library or newly synthesized, against *T. cruzi, Leishmania,* and *S. mansoni*. Among them, **4** emerged as the most potent against all the tested parasites, but it was toxic towards host cells hampering further studies. The retinoic 2'-aminoanilide **8** was less potent than **4** in all parasitic assays, but as its toxicity is considerably lower, it could be the starting structure for further development. In *T. cruzi*, compound **3** exhibited a single-digit micromolar inhibition of the parasite growth, joined to moderate toxicity. In *S. mansoni*, the **4**'s close analogs **17**-**20** were tested in NTS and adult worms displaying high death induction against both the parasite forms. Among them, **17** and **19** exhibited very low toxicity in human RPE cells, being promising compounds for further optimization.

NOTES

Ligand-Based and Structure-Based Predictive Models for SARS-CoV-2 Main Protease Ligands

Eleonora Proia, Manuela Sabatino, Lorenzo Antonini, Filippo Sapienza, Rino Ragno

Rome Center for Molecular Design, Department of Drug Chemistry and Technology, Sapienza University of Rome, P.le Aldo Moro 5, 00185 Rome, Italy

The main protease (M^{pro}) of SARS-Cov-2 is an essential enzyme for the maturation of functional proteins implicated in viral replication and transcription. The peculiarity of its specific cleavage site joint with its high degree of conservation among all coronaviruses promotes it as an attractive target to develop broad-spectrum inhibitors, with high selectivity and a tolerable safety profile. The present study provides the combined use of three-dimensional quantitative structure–activity relationships (3-D QSAR) and comparative molecular binding energy (COMBINE) analysis to build robust and predictive statistical models through the well-established web portal *3d-qsar.com*. Models were trained on experimental binding poses of co-crystallized M^{pro} inhibitors and validated on available literature data. After an exhaustive search of optimal parameters to enhance both the robustness and predictiveness of the models, we obtained final statistical values of r^2 , q^2 and SDEP with reliable performances. Despite the different nature (ligand-based and structure-based) of the employed methods, their outcome converged. The obtained results will guide future rational design and/or virtual screening campaigns with the aim of discovering new potential anti-coronavirus candidates, minimizing both time and financial resources.

NOTES

Abstracts

Session 2. Targeting Proteins for Degradation: PROTACS, PHOTACS, LYTACs, and Molecular Glues

Keynote talk - Expanding the toolbox of E3 ligases for PROTACs: drugging the Fbxw7 E3 ligase

Carles Galdeano

Department de Farmàcia i Tecnologia Farmacèutica i Fisicoquímica, Facultat de Farmàcia i Ciències de l'Alimentació, Institut de Biomedicina (IBUB), Universitat de Barcelona

E3 ligases have been largely described as a relevant target family in disease. Besides, the irruption of the targeted protein degradation strategy has situated this target family at the forefront. The recent development and identification of a handful number of specific drug-like molecules targeting E3 ligases have exceptionally improved the perspectives of the PROTAC strategy. Here, I will first present a structure-based computational approach to study E3 ligases ligandability. We identified ligandable allosteric pockets in the major part of the 24 E3 ligases studied. Remarkably, the Fbxw7 E3 ligase presented an extremely interesting scenario of allosteric pockets for drug discovery purposes. Given also its biological relevance in cancer recruiting crucial oncogenes such as c-Myc, cyclin-E, and Notch, we selected the "undruggable" Fbxw7 as a benchmark E3 ligase to develop a structure-based approach that combines computational and biophysical techniques intending to discover small molecules. In summary, I will also present how we have been able to identify small molecules that target allosterically Fbxw7 in the low digit micromolar range. Preliminary results have shown that these molecules can enhance the activity of Fbxw7 (positive allosteric modulators) by degrading c-Myc in a dose-dependent and proteasome-dependent manner. In parallel, a fragment-based program has been performed and fragments in the low micromolar range have been identified. These fragments could be employed as head-ligands of future Fbw7-based PROTACs.

NOTES

Keynote talk - Dual inhibitors of acetylation and fatty acid-deacylation by the NAD dependent histone deacetylase Sirtuin2 (Sirt2)

Anja Vogelmann, Manfred Jung

University of Freiburg, Freiburg, Germany

Sirtuins are NAD-dependent histone deacetylases (HDACs) that also remove long-chain fatty acids from the epsilon-amino group of lysines in histones but also other proteins. We present a structure guided approach which resulted in potent dual inhibitors of deacetylation and the removal of long-chain fatty acids in-vitro and in cells for Sirtuin2 (Sirt2). These inhibitors exhibit improved antiproliferative activities in cultured cancer cells. To decipher the contributions of enzymatic inhibition versus cellular updake we successfully developed the first Nan-BRET-assay for hSirt2 using a fluorescent Sirt2 inhibitor and a Nano-Luc fusion of Sirtuin2. This assay showed that the net cellular effect is not a result from different cellular uptake but rather the enzyme inhibition profile. This is a powerful tool to show cellular target engagement for Sirt2 inhibitors and the new inhibitors are valuable tools to dissect the molecular consequences of deacetylation versus removal of long chain fatty acids in cell biology.

NOTES

NMR- and structure-guided design of Lysine covalent ligands for PPI antagonists and degraders

Maurizio Pellechia

University of California, Riverside, School of Medicine, USA

NMR-guided screening strategies to design potent and effective ligands targeting protein-protein interactions (PPIs) include our recently developed fHTS by NMR, that can guide the design of nanomolar agents even for challenging drug targets. Moreover, we also found that the design of potent ligands can be aided by introduction of properly juxtaposed electrophiles targeting surface Lys residues, that are more often found at protein interfaces compared to Cys residues. We found that incorporation of aryl-fluorosulfates can lead to pharmacologically viable irreversible agents that are cell permeable and orally bioavailable. Perhaps most importantly, the approach is also useful in devising novel E3-directed covalent degraders.

NOTES

Abstracts

Session 3. Machine Learning in Drug Design

<u>Keynote talk</u> - Exploiting PROTAC technology as an innovative for the treatment of lethal prostate cancer

Gabriele Cruciani

Chemistry Department, University of Perugia, Italy

Over the last two decades, the field of proteolysis targeting chimeras (PROTACs) has gained tremendous attention and has emerged as a powerful new approach in drug design, being focused on the degradation of disease-causing proteins. PROTACs are hetero-bifunctional compounds which combine a ligand for a protein of interest (POI) and an E3 ubiquitin ligase binder connected via a proper chemical linker. The forced close proximity between POI and E3 ligase, resulting in POI–PROTAC–E3 ternary complexes, triggers POI polyubiquitylation and its subsequent proteasome-dependent degradation. To date, PROTAC technology has been applied to a wide range of target proteins involved in different cancer types or other diseases, and several PROTACs are currently under clinical investigation.

Despite increasing efforts devoted to PROTACs synthesis and investigation of their biological mechanism of action, numerous challenges still exist in PROTAC development, mainly concerning their rational design. In particular, *in silico* tools for the prediction of efficient ternary complexes formation as well as for the modulation of their physicochemical and pharmacokinetics properties are still limited. Indeed, the high molecular weight (600-1900 Da) and high TPSA (100-400) make PROTACs solubility, bioavailability, and delivery the most significant hurdles to overcome for clinical progression.

Here, we will describe the discovery and optimization of compound **MTX-23**, a PROTAC able to induce the degradation of androgen receptor splice variant 7 (AR-V7) as 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, resistance inevitably occurs, and one of the major mechanisms of resistance to SAT involves the emergence of androgen-receptor splice variants, such as AR-V7.

In addition, a review of the key points to be addressed to approach a rational design of PROTACs endowed with suitable preliminary pharmacokinetic properties will be presented.

NOTES

What kind of applicability domain for molecular generative artificial intelligence?

Maxime Langevin[1, 2], Christoph Grebner[1], Stefan Guessregen[1], Susanne Sauer[1], Yi Li[1], Hans Matter[1], Marc Bianciotto[1]

[1] *Sanofi R&D*
[2] *Ecole Normale Supérieure*

Molecular generative artificial intelligence is drawing significant attention in the drug design community [1], with different experimentally validated proof of concepts already published [2, 3]. Nevertheless, generative AI algorithms have been criticized for sometimes generating unrealistic, unsynthesizable, or unstable structures [4]. This calls for methods to keep those algorithms to generate structures in reasonable portions of the chemical space. Although the concept of applicability domains (AD) for QSAR models has been well studied [5], its counterpart for generative models is not yet defined. In this work, we use state-of-the-art generative methods based on Recurrent Neural Networks on both public and internal datasets to generate novel structures that are predicted actives by a QSAR model, while constraining the generative model to stay within a given AD. Our work investigates 16 AD definitions, combining different criteria, such as structural similarity, similarity of physico-chemical properties, PAINS filters, and Quantitative Estimate of Drug-Likeness. We assess the structures generated using the different AD both from a qualitative and quantitative point of view, and we identify the AD definitions that are the best suited for generating drug-like molecules with generative AI algorithms.

*1. Schneider, P. et al. Rethinking drug design in the artificial intelligence era. Nat Rev Drug Discov **19**, 353–364 (2020).*
*2. Merk, D., Friedrich, L., Grisoni, F. & Schneider, G. De Novo Design of Bioactive Small Molecules by Artificial Intelligence. Mol Inform **37**, 1700153 (2018).*
*3. Zhavoronkov, A. et al. Deep learning enables rapid identification of potent DDR1 kinase inhibitors. Nat Biotechnol **37**, 1038–1040 (2019).*
*4. Walters, W. P. & Barzilay, R. Critical assessment of AI in drug discovery. Expert Opin Drug Dis **16**, 1–11 (2021).*
*5 Tropsha, A. & Golbraikh, A. Predictive QSAR Modeling Workflow, Model Applicability Domains, and Virtual Screening. Curr Pharm Design **13**, 3494–3504 (2007).*

NOTES

Expanding the chemical space of a hit molecule obtained by NMR fragment screening using machine learning

Barak Akabayov

[1]*Ben-Gurion University of the Negev*

Data-driven algorithms have emerged as powerful platforms that can consolidate bioisosteric rules for preferential modifications on small molecules with a common molecular scaffold.

This paper will present complementary machine learning models to grow the size and optimize the binding properties of molecules targeting an RNA hairpin within the bacterial ribosome. The data used for training the learning models were based on NMR fragment screening and virtual screening.[1] Visual, geometrical, and chemical features were extracted that enhance the binding to the targeted RNA.[2] Functional validation was conducted after synthesizing new small molecules pinpointed computationally and revealed specific inhibitors that target bacterial translation and, as a result, kill bacteria.[2]

[1] B. Tam, D. Sherf, S. Cohen, S. Adi Eisdorfer, M. Peretz, A. Soffer, D. Vilenchik, S.R. Akabayov, G. Wagner, B. Akabayov. Discovery of small-molecule inhibitors targeting the ribosomal peptidyl transferase center (PTC) of M. tuberculosis. Chemical Science., 2019, 10, 8764-8767.
[2] B. Grimberg, V.S. Tiwari, B. Tam, L. Gur-Arie, D. Gingold, L. Polachek, B. Akabayov. Machine learning approaches to optimize small-molecule inhibitors for RNA targeting. Journal of Cheminformatics., 2022, 14, 4.

NOTES

Human Dose Prediction in Drug Discovery: (when) is it possible?

Franco Lombardo

Founder/Principal, CMAXDMPK, LLC, USA

The presentation will cover the aspects of whether useful early dose prediction is possible, and at what stage it could be reasonably performed. The data available at each stage are a key aspect and research groups in industry and academia may have to become a bit more daring in investing earlier rather than later in a more extensive profiles, especially to determine clearance, or compounds. There is, clearly, a conundrum where more accurate data can only be obtained once some determination and "at risk" studies in vivo are performed. But the rewards of advancing compounds with high probability of success in Phase I and beyond are also high.

NOTES

Antitumor activity of rhodanine derivatives: Quantitative structure-activity relationship and molecular docking study

Vesna Rastija[1], Maja Molnar[2]

[1] Department of Agroecology and Environmental Protection Faculty of Agrobiotechnical Sciences Osijek, Josip Juraj Strossmayer University of Osijek,
[2] Department of Applied Chemistry and Ecology, Faculty of Food Technology Osijek, Josip Juraj Stossmayer University of Osijek

Rhodanines have been reported to possess antibacterial, antiviral, antimalarial, antifungal, and antitumor activity. A series of novel rhodanine derivatives were assayed for their cytotoxic activity against a panel of human cancer cell lines. Quantitative structure-activity relationship (QSAR) analysis was performed on the anticancer activity against cutaneous T lymphocyte (cell line Hut-78) since 98 % of compounds have achieved the concentration achieving 50% cell growth inhibition (GI_{50}) 100 µM. QSAR study of the anticancer activity against HUT-78 achieved the model that satisfies the fitting and internal cross-validation criteria (N = 28; R^2 = 0.75; Q^2_{LOO} = 0.64):

$\log GI_{50} = 2.51 - 3.46\ MATS2e - 1.05\ MATs7e - 0.15\ RDF060p$

Descriptors included in the model revealed the importance of the presence of atoms with higher polarizability in the outer region of molecules. Molecules that possess 3-methoxy group (**ALR9**) and 3-bromine (**ALR16**) at the benzene ring proved to be potential drug candidates against human T cell lymphoma since inhibited 50% growth of cells Hut-78 at the very low concentration (3 and 7.5 µmol dm^{-3}, respectively). Also, the presence of electronegative atoms at the topological distances 2 and 7, such as hydroxyl and ethoxy groups as substituents of the benzylidene group decreases the activity.
A molecular docking study confirmed the findings of the QSAR study regarding the structural features related to the inhibition of nonreceptor protein-tyrosine kinase (c-Src) (PDB ID: G6H) and indicated importance of the oxygen atoms from phenoxy and rhodanine groups, and rhodanine sulphur atoms as hydrogen bond acceptors in key interactions with binding site residues, as well as benzene rings in generations of van der Waals interactions.
Our study suggests that rhodanine derivatives could be developed as novel tyrosine kinase inhibitors in the treatment of leukemia.

NOTES

Abstracts

Session 4: Natural Compounds in Drug Discovery

<u>Keynote talk</u> - Natural products: A good starting point for accessing bioactive compounds

Janine Cossy

[1] *Molecular, Macromolecular Chemistry and Materials ESPCI Paris, CNRS, PSL University, 10 rue Vauquelin, 75231 Paris Cedex 05 France*

Natural products are a good source of inspiration to access bioloigically active compounds. If we are considering the drugs on the market, 6% are natural products, such as taxol, 32% are derivatives of natural products, for example taxotere, 23% are mimes of natural products and 39% are purely synthetic compounds.

In general, natural products are produced in small quantities and/or to obtain them, the natural environment is destroyed. One alternative to access natural products is chemical synthesis. One major challenge to produce complex natural products, by synthesis, is the design and execution of concise and efficient approaches to these molecules by generating the minimum of wastes. Strategies and methods that rapidly lead to the skeleton framework of natural biologically active compounds are attractive.

For our part, we are involved in the synthesis of hemicalide, an antitumor compound, extracted from a marine sponge which has an original mode of action and has an IC50 of 0.1 nm. We will describe the strategy and the method that we have developed to construct the carbon skeleton of this compound as well as the methods to built up the substituted six-membered ring lactones present in hemicalide. In addition, we will show that by replacing aromatic rings by strained rings or by introducing macrocycles, the biological activity of molecules can be increased.

NOTES

Keynote talk - Stereoselective Sparteine-free 1,2-Metallate Rearrangements in the Syntheses of Chondrochlorene and Meridamycin

Markus Kalesse

Leibniz University Hannover, Institute of Organic Chemistry, Schneiderberg 1B, 30167 Hannover, Germany

The synthesis of polyketides relies to a large extent on a distinct set of C–C bond-forming transformation of which aldol reactions are certainly the most prominent and useful ones as they are able to control up to two new chiral centers in the C–C bond-forming event. Here we report the stereoselective synthesis of two natural products which rely at pivotal C-C disconnections on a 1,2-metallate rearrangement as an advancement of the work originally developed by Hoppe and Matteson. This 1,2-metallate rearrangement of vinyl boronates yields stereoselectively allylic alcohols which are usually constructed through a Nozaki-Hiyama-Takai-Kishi (NHTK) reaction, of which stereo as well as enantioselective variants are available. However, in the context of complex natural product syntheses it often provides unsatisfactory yields and/or selectivities. The two projects for which we had to overcome these obstacles were the syntheses of chondrochloren and meridamycin. We will not only describe our endeavors towards these two natural product but discuss a set of polyketidal substrates and their diastereoselective transformation in 1,2-metallate rearrangements in order to provide a general concept for its use in total syntheses.

NOTES

A new perspective to natural product chemistry: non-apoptotic cell death induction via small molecule based supramolecular particles originating from sapogenins

Göklem Üner[1], Erdal Bedir[1], Onur Serçinoğlu[2], Petek Ballar Kırmızıbayrak[3]

[1] Department of Bioengineering, Faculty of Engineering, İzmir Institute of Technology, 35430 Urla, İzmir, Turkey
[2] Department of Bioengineering, Faculty of Engineering, Gebze Technical University, 41400 Gebze, Kocaeli, Turkey
[3] Department of Biochemistry, Faculty of Pharmacy, Ege University, 35100 Bornova, İzmir, Turkey

Induction of distinct cell death pathways is critical to deal with tumor heterogeneity and therapeutic resistance. In our previous study, we prepared a semi-synthetic saponin analog (AG-08) from a non-cytotoxic parent compound and reported initiation of non-canonical necrotic cell death as well as inhibition of autophagy, lysosomal impairment/tubulation and general proteolysis (1). Since AG-08 was found to lose its activity after freeze thaw without any change in its chemical structure, here, we aimed to investigate the possibility of particles formation leading to necrotic effects. Both Nile Red encapsulation assay and molecular dynamics simulations indicated that the parent compound (Astragenol) was not capable to form ordinary supramolecular structures while AG-08 formed circular supramolecular structures. AG-08 particles were internalized via a clathrin, dynamin and actin-independent but cholesterol dependent non-canonical endocytosis pathway. Additionally, microarray assay showed that these structures were affecting several cell signaling pathways including unfolded protein response, immune response, oxidative and heat stress. Moreover, we prepared 18 analogs to reveal the role of residues on the formation of supramolecular structures and biological activities. The results demonstrated that unique structural features were required for the formation of particulate structures and exceptional cell death mechanism. Collectively, AG-08 and four of its analogs form particles that trigger necrotic cell death with an unprecedented mechanism. Although small molecule based supramolecular assemblies have widely been accepted as nuisance in drug discovery studies, our results signify that these colloidal particles are of significant for the field of anti-cancer drug development studies, especially as immunotherapy agents.

[1] Uner G, Tag O, Erzurumlu Y, Kirmizibayrak PB, Bedir E. Identification of a Noncanonical Necrotic Cell Death Triggered via Enhanced Proteolysis by a Novel Sapogenol Derivative. Chemical Research in Toxicology 2020; 33: 2880-91.

NOTES

The plant-derived alkaloid sewarine as a novel kappa-opioid receptor ligand: An experimental assessment and molecular docking

Aquilino Lantero[1], Stefan Salcher[2], Muhammad F Asim[1], Kristina Puls[3], Helmut Schmidhammer[1], Gerhard Wolber[3], Petra Obexer[4], Mariana Spetea[1]

[1] Department of Pharmaceutical Chemistry, Institute of Pharmacy and Center for Molecular Biosciences (CMBI), University of Innsbruck, Innsbruck, Austria
[2] Tyrolean Cancer Research Institute, Department of Internal Medicine V, Medical University Innsbruck, Innsbruck, Austria
[3] Institute of Pharmacy, Freie Universität Berlin, Berlin, Germany
[4] Tyrolean Cancer Research Institute, Department of Internal Medicine II, Medical University Innsbruck, Innsbruck, Austria

Natural products are an excellent source of promising lead compounds for the generation of new therapeutic drugs. *Rhazya stricta,* an alkaloid-rich herb, is used in traditional oriental medicine to treat several human diseases, including tumors. Applying a pharmacophore-based virtual screening strategy, we discovered a new κappa-opioid receptor (KOR) ligand, sewarine, a natural alkaloid from *R. stricta.* We present a pharmacological and molecular modeling study on sewarine investigating (i) binding and signaling at the rodent and human KOR, and (ii) antiproliferative and anticancer effects in vitro. Sewarine shows high KOR selectivity and similar binding affinity to the guineapig and human KOR. While sewarine shows antagonism in the rodent KOR, it is a partial agonist in the human KOR. It effectively inhibits proliferation and induces apoptosis in lymphoblastic leukemia, neuroblastoma, and breast cancer cells. The apoptotic effect of sewarine is mediated via the activation of caspase pathways and by modulating pro-apoptotic and anti-apoptotic proteins, and it involves the KOR. Molecular docking of sewarine to the crystal structure of the human KOR provides important insights into the binding mode to the receptor. Our results established the significant anticancer activity of sewarine in vitro and thus provided valuable information on the role of the KOR in apoptosis, as well as the first evidence and rationale for the anticancer effects of alkaloid extracts of *R. stricta*.

NOTES

Synthesis of the upper binding arm of kibdelomycin, a novel broad-spectrum, Gram-positive focussed antibiotic without cross-resistance to known gyrase inhibitors

Laura Treiber, Manuel Georg Schriefer, Rainer Schobert

Organic Chemistry I, Universität Bayreuth, Germany

A rapidly growing number of bacteria are developing resistance to commonly used antibiotics. This renders entire groups of antibiotic agents ineffective. An opportunity to solve this problem is the discovery of new antibiotic compound classes by screening natural products. 2011 this was achieved by discovery of kibdelomycin, an antibiotic agent isolated from *kibdelosporangium sp.* (MA7385). It was found identical to amycolamicin, which was isolated from the culture broth of the soil actinomycete *Amycolatopsis sp.* MK575-fF5.[1][2] The unique structure of kibdelomycin is characterized by the novel sugars amycolose, attached to a pyrrole moiety by an amide bond, and amykitanose tethered via an *N*-glycosidic linkage to a 3-acyl tetramic acid, which are connected by a decalin linker. The structural peculiarities result in a pronounced activity especially against gram-positive bacteria. [1][2] Like other antibiotics kibdelomycin inhibits the ATPase activity of topoisomerase IV and of DNA gyrase, but by a novel, dual binding mode, accessing a binding pocket which is unavailable to other topoisomerase IV- and gyrase B-inhibitors. The total synthesis of kibdelomycin allows to elucidate the role of the upper binding arm (3-acyl tetramic acid and amykitanose) in its mechanism of action and its structure dependency on its antibiotic activity. Our retrosynthetic approach towards this fragment starts from naturally occurring L-rhamnose and L-valine. At first, the stereocenter at C-4 must be inverted by oxidation and diastereoselective reduction. Next, the hydroxyl groups at C-2, C-3 and C-4 are regioselectively functionalised. The stability of the carbamate ester at C-4 is crucial and a suitable time for introduction needs to be found. A key step is the stereoselective coupling of the sugar amykitanose to the amino acid by an *N*-glycosylation. The resulting *N*-aminoglycoside readily undergoes hydrolysis and so must be converted to a β-ketoamide. Lacey-Dieckmann-cyclisation leads to formation of the 3-acyl tetramic acid.

NOTES

New potential applications of essential oils. An insight on Py-OE (eo.3d-qsar.com)

Filippo Umberto Sapienza[1], Manuela Sabatino[2], Lorenzo Antonini[2], Rino Ragno[2]

[1] Sapienza University of Rome - Dept of Drug Chemistry and Technology
[2] Sapienza University of Rome, Dept of Drug Chemistry and Technology

Nowadays, use of natural compounds with mainstream pharmaceutical has gained the momentum and scientific investigation on essential oils (EOs) composition and the related biological profile are continuously growing.
EOs are concentrated oils derived from plants usually obtained by steam or hydro-distillation.
They are a complex mixtures containing from a few to hundreds of compounds and can be derived from nearly any plant such as Apiaceae, Asteraceae, Lamiaceae, Myrataceae.
The chemical components belong to a wide variety of chemical classes: aliphatic and aromatic compounds, hydrocarbons, alcohols, aldehydes, ketones, esters, phenols, acids.
Despite the large amount of information, the data available are often unstructured, not organized, partial and chaotic, simply waiting to be stocked and managed.
Contrary to the field of Pharmaceutical/Medicinal chemistry which takes advantage from complex chemical databases like PubChem and ChEMBL, in the case of EOs is not yet avialable such a structured database and for this reason, we decided to develop one called Py Oe 3d qsar; a database that collects all the information about plants, biological activity, targets and chemical compositions of these Eos.
The data were either retriaved from scientific literature or generated within our group.
The database compilation allowed us to gain information about the most studied plants, their distillation timing and the characterization of extracts obtained from these plants. Furthermore, it gave us the possibility to understand which, among those extracts, were the ones mostly present within a certain essential oil. Finally, owing to the database compilation we defined the appropriate targets used to test the essential oils.
A further analysis of this database is reported below in order to show a potential avenues for future applications of essential oils in related fields.

NOTES

How to solve the quantification issues inherited from the antioxidant assays based on single-electron transfer? A mathematical approach

M. Carpena[1], Hui Cao[1], Jianbo Xiao[1, 2], J. Simal-Gandara[1], M. A. Prieto[3, 4]

[1] *Nutrition and Bromatology Group, Faculty of Food Science and Technology, University of Vigo, Ourense Campus, E32004 Ourense, Spain*
[2] *International Research Center for Food Nutrition and Safety, Jiangsu University, Zhenjiang 212013, China*
[3] *Nutrition and Bromatology Group, Faculty of Food Science and Technology, University of Vigo, Ourense Campus, E32004 Ourense, Spain.*
[4] *Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal*

Developing a suitable mathematical application to test the antioxidant potential of standard and new therapeutic agents is essential for the research community to conduct more accurate evaluations. Despite the abundance of procedures for describing antioxidant effects in Single Electron Transfer assays (SET), based on theoretical interpretations, they are somewhat inadequate, mainly because of the lack of (1) unequivocal experimental design, because of the widespread use of simplistic procedures to quantify the effects of joint responses, based on a single dose value and (2) detailed mathematical hypotheses to quantify the dose-response values, making difficult to evaluate the statistical consistency of the results. This communication aims to develop a mathematical procedure for SET antioxidant assays. The model was developed by studying the dose-response behavior of butyl-hydroxytoluene (BHT) (0.0-600.0 µM) as the reference antioxidant and evaluating the results of the DPPH bleaching reaction from a kinetic point of view by two approaches: *(A)*: kinetic dose-response of BHT; *(B):* non-linear dose-response of BHT at the fixed points selected in the previous study (t = 5, 30 min, 90 min). Then, the inhibitory concentration, IC_{50} was computed for each respective fixed time and compared with the computed kinetic IC_{50} value for all asymptotic values found by the proposed model. The approach was experimentally demonstrated in one classical SET assay (DPPH), but can be directly expanded to other types of SET assays. The methodology here propose is more complex than other approaches, however, it allows us to move forward from the classical measurements of the antioxidant activity and their controversial aspects. This model helps to describe the response as a time-dose function and establish comparisons between compounds. Furthermore, the application may facilitate the ranking process and the selection of appropriate concentrations of natural antioxidants to replace synthetics, which is currently a growing topic for disease prevention.

NOTES

Panicein A Hydroquinone (PAH) and analogs overcome chemotherapy resistance in cancer cells

Sandra Kovachka[1, 2], Giuliano Malloci[3], Paolo Ruggerone[3], Isabelle Mus-Veteau[2], Stéphane Azoulay[1]

[1] Université Côte d'Azur, CNRS, ICN, 28 Avenue Valrose, 06108, Nice, CEDEX 2, France
[2] Université Côte d'Azur, CNRS, IPMC, 660 Route des Lucioles, 06560, Valbonne, France
[3] Dipartimento di Fisica, Università di Cagliari, Cittadella Universitaria, I-09042, Monserrato, CA, Italy

Chemotherapy resistance is one of the major challenges in cancer treatment. Thus, the development of inhibitors of biological mechanisms involved in multidrug resistance (MDR) meets an important medical need but still represents a challenging task. Major MDR targets are multidrug efflux systems, such as the ATP Binding Cassette (ABC) transporters. However, their inhibition leads to severe side effects mainly related to the ubiquitous localization of these transporters[1]. Therefore, alternative therapeutic targets are necessary.

The Hedgehog receptor Patched1 (Ptch1), part of the Hedgehog signaling pathway involved in tissue development in embryogenesis and tissue homeostasis in adults, was recently shown to transport different chemotherapeutics out of cancer cells therefore contributing significantly to MDR phenomena in cancer treatment. Ptch1 is known to be over-expressed in many types of cancers and due to the peculiarity of its pH dependent efflux mechanism, its inhibition was shown as a successful strategy in improving chemotherapy efficacy without toxicity for healthy cells or potential side-effects. To date, only few compounds have been identified as efficient Ptch1 inhibitors, among which panicein A hydroquinone (PAH), a meroterpenoid natural compound[2].

We describe here the first stereoselective synthesis for the E and Z isomers of PAH and we apply the methodology to several analogs with the aim of building a structure-activity relationship. The biological activity of the derivatives, in combination with chemotherapy, was evaluated in melanoma cells. Molecular insights into the mechanism by which the compounds bind to Ptch1 and inhibit chemotherapeutics transport are also addressed by using *in silico* methodologies[3]. Altogether, these data pave the way for the design and development of the next generation of Ptch1 inhibitors.

[1]. Kathawala et al., Drug Resist. Updat., 2015, 18, 1.
[2]. Kovachka et al., Eur. J. Med Chem., 2022, 114306.
[3]. Kovachka et al., Phys. Chem. Chem. Phys., 2021, 23, 8013.

NOTES

Effect of neurogenesis promoters, phorbol esters and derivatives of 12-deoxyphorbol esters, on the inhibition of cytochrome P450

Abdellah Ezzanad[1], Glen J.P. McCann[2], Neill J. Horley[2], Carmen Castro[3], Rosario Hernández-Galán[1], A. Christy Hunter[4], Antonio J. Macías-Sánchez[1]

[1] Departamento de Química Orgánica and Instituto de Investigación en Biomoléculas (INBIO), Facultad de Ciencias, Universidad de Cádiz, 11510 Puerto Real, Cádiz (Spain).
[2] Leicester School of Pharmacy, De Montfort University, Leicester LE1 9BH, United Kingdom.
[3] Área de Fisiología, Facultad de Medicina, Universidad de Cádiz and Instituto de Investigación e Innovación Biomédica de Cádiz (INiBICA), Cádiz (Spain).
[4] School of Pharmacy, College of Science, University of Lincoln, Lincoln, United Kingdom.

In a previous work, phorbol esters and 12-deoxyphorbol esters with the ability to bind to specific PKCs, induced neural progenitor cell (NPC) proliferation **[1].** Development of these compounds as therapeutic agents requires exploration of its chemistry, in order to find structural modifications with improved activity and the right pharmacokinetics, which includes an evaluation of their metabolism and possible interferences with key human metabolic pathways.
Cytochrome P450 (CYP) enzymes are a group of hemoproteins, with particular relevance for the production of endogenous metabolites such as sterols, fatty acids eicosanoids among others **[2].** CYP enzymes also play an important role in the metabolism of clinical drugs and other xenobiotics. CYP are also expressed in the brain,**[3]** where they play a key role in the genesis of neurosteroids and neuroprotection (CYP19A1) [4].
In this communication, we describe, based on a high-throughput fluorescence assay of CYP inhibition, the in vitro inhibition of isoforms CYP3A4 and CYP19A1 by phorbol esters and 12-deoxyphorbol esters derivatives. Structure–activity relationships and molecular interaction of inhibitors with CYP are explored by molecular docking with CYP19A1.

[1] Ezzanad A, Gómez-Oliva R, Escobar-Montaño F, Díez-Salguero M, Geribaldi-Doldan N, Dominguez-Garcia S et al. ***2021****. "Phorbol Diesters and 12-Deoxy-16-hydroxyphorbol 13,16-Diesters Induce TGFα Release and Adult Mouse Neurogenesis. " J Med Chem;* ***64****: 6070–6084.*
[2] Zanger, Ulrich M., and Matthias Schwab. ***2013****. "Cytochrome P450 Enzymes in Drug Metabolism: Regulation of Gene Expression, Enzyme Activities, and Impact of Genetic Variation." Pharmacology and Therapeutics* ***138****(1): 103–41.*
[3] Toselli, Francesca, Peter R. Dodd, and Elizabeth M.J. Gillam. ***2016****. "Emerging Roles for Brain Drug-Metabolizing Cytochrome P450 Enzymes in Neuropsychiatric Conditions and Responses to Drugs." Drug Metabolism Reviews* ***48****(3): 379–404.*
[4] Ponti, G.; Farinetti, A.; Marraudino, M.; Panzica, G.; Gotti, S. ***2018****. "Sex Steroids and Adult Neurogenesis in the Ventricular-Subventricular Zone." Front. Endocrinol. (Lausanne). 9, 156.*

NOTES

Abstracts

Session 5: Multitarget Drug Discovery

Keynote talk - Expanding the polypharmacology toolbox for neurodegenerative diseases

Maria Laura Bolognesi

Department of Pharmacy and Biotechnology, Alma Mater Studiorum - University of Bologna

Since at least the early 2000s, the development of polypharmacological tools against neurodegeneration has emerged as a dynamic and fertile field of drug discovery.[1] They can respond to the limitations of conventional single-target drugs by better confronting the multifactorial nature of neurodegenerative diseases. Various medicinal chemistry strategies have been developed over the years, greatly expanding the available toolbox. In this talk, we will outline these useful molecular tools, spanning from hybrids, codrugs and conjugates. Selected successful examples[2] of each approach will be presented to document that the development of these tools remains significant, notwithstanding the enormous attrition rates of the neurodegenerative disease therapeutic area and the indisputable problem with translatability. We are positive that medicinal chemists, through ingenious effort driven by hypotheses, hard work and an open science approach, will be able to make the most out of the opportunity currently offered by multi target drug discovery in the battle against neurodegeneration.

Bibliography

(1) Cavalli, A.; Bolognesi, M. L.; Minarini, A.; Rosini, M.; Tumiatti, V.; Recanatini, M.; Melchiorre, C. Multi-target-directed ligands to combat neurodegenerative diseases. J Med Chem ***2008****, 51 (3), 347-372. DOI: 10.1021/jm7009364.*

(2) Rossi, M.; Freschi, M.; de Camargo Nascente, L.; Salerno, A.; de Melo Viana Teixeira, S.; Nachon, F.; Chantegreil, F.; Soukup, O.; Prchal, L.; Malaguti, M.; et al. Sustainable Drug Discovery of Multi-Target-Directed Ligands for Alzheimer's Disease. J Med Chem ***2021****, 64 (8), 4972-4990. DOI: 10.1021/acs.jmedchem.1c00048. Albertini, C.; Naldi, M.; Petralla, S.; Strocchi, S.; Grifoni, D.; Monti, B.; Bartolini, M.; Bolognesi, M. L. From Combinations to Single-Molecule Polypharmacology-Cromolyn-Ibuprofen Conjugates for Alzheimer's Disease. Molecules* ***2021****, 26 (4). DOI: 10.3390/molecules26041112. Rossi, M.; Petralla, S.; Protti, M.; Baiula, M.; Kobrlova, T.; Soukup, O.; Spampinato, S. M.; Mercolini, L.; Monti, B.; Bolognesi, M. L. α-Linolenic Acid-Valproic Acid Conjugates: Toward Single-Molecule Polypharmacology for Multiple Sclerosis. ACS Med Chem Lett* ***2020****, 11 (12), 2406-2413. DOI: 10.1021/acsmedchemlett.0c00375.*

NOTES

Keynote talk - Epi-Polypharmacology: A Medicinal Chemistry Perspective

Antonello Mai

"Sapienza" University of Rome, Italy

The multi-target approach (polypharmacology) can overcome some main limitations of the single target therapy leading to a superior therapeutic effect, a decrease of adverse reactions, and a reduction of potential mechanism(s) of drug resistance. Multitargeting drugs are the result of the conjugation of two or more single target inhibitors which can be connected by a linker, after cleaved in physiological conditions (mutual prodrugs), or by a stable linker, allowing each active portion to interact with its specific target without interfering with the interactions established by the others, or can be obtained by merging molecules together, preserving and connecting in the final multitarget ligand only functional and reactive groups (warheads). In detail in epigenetics, the goal of the multi-epi-target approach consists in the development of small molecules able to simultaneously and (often) reversibly bind different specific epi-targets, or one epi-target and another target not related to epigenetics.[1]

To date, two dual histone deacetylase/kinase inhibitors (CUDC-101 and CUDC-907) are in clinical trials for treatment of cancer.[2,3] In the lecture, we will discuss our med chem experience in epi-polypharmacology, from the first hybrids ATRA/HDAC inhibitors[4] to pan-KDM inhibitors,[5-7] dual HAT/EZH2[8] and HDAC/EZH2 inhibitors,[9] dual HDAC/LSD1[10] and G9a/LSD1 inhibitors,[11,12] all useful to fight cancer diseases.

References:

1. *Tomaselli, D. et al. Med. Res. Rev. 2020, 40, 190-244.*
2. *Shimizu, T. et al. Clin Cancer Res. 2014, 20, 5032-5040.*
3. *Oki, Y. et al. Haematologica 2017, 102, 1923-1930.*
4. *De Bellis, F. et al. Cancer Res. 2014, 74, 2328-2339.*
5. *Rotili, D. et al. J. Med. Chem. 2014, 57, 42-55.*
6. *Benedetti, R. et al. Cancers (Basel) 2019, 11, 2027.*
7. *Benedetti, R. et al. Cancers (Basel) 2021, 13, 543.*
8. *Petraglia, F. et al. Oncotarget 2018, 9, 25630-25646.*
9. *Romanelli, A. et al. ACS Med. Chem. Lett. 2020, 11, 977-983.*
10. *Kalin, J.H. et al. Nat. Commun. 2018, 9, 53.*
11. *Speranzini V. et al. Sci Adv. 2016, 2, e1601017.*
12. *Menna M. et al. Eur J Med Chem. 2022 Jul 5;237:114410.*

NOTES

Multitarget antioxidant NO-donor organic nitrates: a novel approach to overcome nitrates tolerance

Elisabetta Marini[1], Marta Giorgis[2], Barbara Rolando[2], Konstantin Chegaev[2]

[1] *University of Turin, Department of Drug Science and Technology*
[2] *University of Turin, Departmente of Drug Science and Technology*

Chronic use of glyceryl trinitrate (GTN) is limited by serious side effects, such as tolerance and endothelial dysfunction of coronary and resistance arteries. Although GTN is used as a drug since more than 130 years, the mechanisms of the vasodilatory effects and of tolerance development to organic nitrates are still incompletely elucidated. New synthesized organic nitrates with and without antioxidant properties were characterized for their ex vivo tolerance profile, in order to investigate the oxidative stress hypothesis of nitrate tolerance. The organic nitrates studied showed different vasodilation and tolerance profiles, probably due to the ability or inability of the compounds to interact with the aldehyde dehydrogenase-2 enzyme (ALDH-2) involved in bioactivation. Furthermore, nitrooxy derivatives endowed with antioxidant properties did not determined the onset of tolerance, even if bioactivated by ALDH-2. The results of this study allows to deepen one of the complex mechanisms underlying the phenomenon of nitrate tolerance. Indeed, the characterization of the ex-vivo tolerance profile of organic nitrates with antioxidant properties allows to support the hypothesis of the ROS involvement in inactivating ALDH-2. This study provides fresh insight into the mechanisms responsible for nitrate tolerance, suggesting a potential role for multitarget drugs, namely antioxidant NO-donor organic nitrates, as a therapeutic tool in the prevention or control of the tolerance that accompanies the chronic use of GTN in patients.

NOTES

Anticancer activity of G-quadruplex DNA-targeting monohydrazone based compounds

Jussara Amato[1], Giulia Miglietta[2], Rita Morigi[2], Nunzia Iaccarino[1], Alessandra Locatelli[2], Alberto Leoni[2], Bruno Pagano[1], Giovanni Capranico[2], Antonio Randazzo[1]

[1] University of Naples Federico II, IT
[2] University of Bologna, IT

G-quadruplexes (G4s) are noncanonical DNA secondary structures formed by G-rich sequences with important roles in the regulation of basic nuclear processes, including promoter activity, chromatin remodeling and replication, genome instability, and epigenetic alterations. Several specific G4 ligands have been shown to selectively stabilize G4 structures in living cells and trigger genome instability and cell killing, therefore supporting G4s as targets for anticancer drug developments. Searching for potent and selective G4 binders, here we describe a small series of new monohydrazone derivatives designed as analogues of a lead which was proved to stabilize G4 structures and increase R loop levels in human cancer cells. To investigate the G4 binding properties of the new molecules, in vitro biophysical studies were performed employing both telomeric and oncogene promoter G4-forming sequences. The obtained results allowed the identification of a highly selective G4 ligand that, when studied in human cancer cells, proved to be able to stabilize both G4s and R loops and showed a potent cell killing activity associated with the formation of micronuclei, a clear sign of genome instability.

Reference: Amato, J.; Miglietta, G.; Morigi, R.; Iaccarino, N.; Locatelli, A.; Leoni, A.; Novellino, E.; Pagano, B.; Capranico, G.; Randazzo A. J. Med. Chem. 2020, 63, 3090−3103.

NOTES

Novel series of multitarget ligands for the treatment of Type 2 Diabetes.

Laura Fumagalli[1], Angelica Artasensi[1], Andrea Angeli[2], Carmen Lammi[1], Claudiu Supuran[2], Giulio Vistoli[1]

[1] Department of Pharmaceutical Sciences "Pietro Pratesi", University of Milan, Via Mangiagalli 25, I-20133 Milan, Italy
[2] Department of Pharmaceutical Sciences "NEUROFARBA", University of Florence, Via Ugo Schiff 6, 50019 Sesto Fiorentino, Florence, Italy.

Recently multitarget drugs have raised considerable interest owing to their advantages in the treatment of complex disease, like Diabetes Mellitus (DM). In fact, the DM refers to a group of metabolic disorders characterized and identified by hyperglycemia caused by defects in insulin secretion, insulin action, or both, and disturbances of carbohydrate, lipid and protein metabolism [1].
Considering the multifactorial nature, DM could be approached not as a single disorder but rather as an interconnected combination of risk factors and complications. Thus, multitarget drugs which could reduce hyperglycemia and concomitantly inhibit the progression of complications may be a valuable therapeutic option for the management of this chronic condition [2]. With the aim of finding new multitarget antidiabetic compounds, repurposing and morphing approaches were applied on WB-4101, a well-known adrenergic ligand since computational molecular docking demonstrated that WB-4101 can fit into the pockets of two enzymes that can be exploited in the antidiabetic therapy, namely Dipeptidyl Peptidase IV (DPP IV) and Carbonic Anhydrase II (CA II), even if it lacks some required interactions.
Thus, we designed different WB-4101 derivatives corroborated by computational investigations. In such new derivatives the amine moiety has been morphed as well as the methoxy groups, and a sulfonamide function has been inserted to fulfill the lacking interactions.
Furthermore, computational and pharmacological investigations were performed also on CA V, a mitochondrial Carbonic Anhydrase isoform involved in glucose metabolism [3].
This work allow to extend the knowledge about structural requirements needed to bind DPP IV and CA II/V. Moreover, two newly synthesized derivatives exhibit a satisfactory nanomolar potency towards the targeted enzymes.

*[1] Bawa P, Pradeep P, Kumar P, Choonara YE, Modi G, Pillay V. Drug Discov. Today **21** (2016), 1886.*
*[2] Artasensi, A.; Pedretti, A.; Vistoli, G.; Fumagalli, L. Molecules **25** (2020), 1987.*
*[3] Supuran CT, Di Fiore A, De Simone G. Expert Opin Emerg Drugs. **13(2)** (2008), 383.*

NOTES

Studies of potency and efficacy of an optimized dihydroartemisinin-quinoline hybrid against multiple stages of the Plasmodium life cycle

Diogo Rodrigo Moreira[1], Helenita Costa Quadros[2], Aysun Çapcı[3], Lars Herrmann[3], Sarah D'Alessandro[4], Diana Fontinha[5], Raquel Azevedo[5], Wilmer Villarreal[6], Nicoletta Basilico[4], Miguel Prudêncio[5], Svetlana B. Tsogoeva[3]

[1] Fiocruz Instituto Gonçalo Moniz
[2] Fundação Oswaldo Cruz (Fiocruz), Instituto Gonçalo Moniz, Salvador
[3] Friedrich-Alexander University of Erlangen-Nürnberg
[4] Dipartimento di Scienze Farmacologiche e Biomolecolari, Università degli Studi di Milano
[5] Faculdade de Medicina, Universidade de Lisboa
[6] Universidade Federal do Rio Grande do Sul

A recently developed dihydroartemisinin-quinoline hybrid, named 163A, has been shown to display potent activity against the asexual blood stage of Plasmodium, the malaria parasite. Here, we determined its in vitro cytotoxicity to mammalian cells, its potency to suppress P. berghei hepatic infection and to decrease the viability of P. falciparum gametocytes, in addition to determining whether the drug exhibits efficacy of a P. berghei infection in mice. This hybrid compound has a low level of cytotoxicity to mammalian cells and conversely a high level of selectivity. It is potent in the prevention of hepatic stage development as well as in killing gametocytes, denoting a potential blockage of the malaria transmission. The hybrid presents a potent inhibitory activity for beta-hematin crystal formation, in which subsequent assays revealed that its endoperoxide component undergoes bioactivation by reductive reaction with ferrous heme towards the formation of heme-drug adducts, in parallel the 7-chloroquinoline component has binding affinity for ferric hemin. Both structural components of hybrid co-operate to enhance the inhibition of beta-hematin and this bitopic ligand property is essential for arresting the growth of asexual blood parasites. We demonstrated the in vivo efficacy of the hybrid as an erythrocytic schizonticide agent in comparison to a chloroquine/artemisinin combination therapy. Collectively, the findings suggest that the bitopic property of hybrid is highly operative on the heme detoxification suppression and this provides compelling evidence for explaining the action of hybrid for asexual blood stage. For sporozoite and gametocyte stages, hybrid conserves the potency typically observed for endoperoxide drugs and this is possibly achieved due to the redox chemistry of endoperoxide component with ferrous heme.

NOTES

Synthesis, biological activity and physicochemical properties evaluation of antiplasmodial pyrimido[1,2-a]benzimidazoles

Chiara Disraeli[1, 2], Godwin Akpeko Dziwornu[2], Laura Baldini[1], Kelly Chibale[3]

[1] *University of Parma, Italy*
[2] *University of Cape Town, South Africa*

Malaria still causes high rates of morbidity and mortality, especially within tropical and sub-tropical regions of the world. Pyrido[1,2-*a*]benzimidazole (PBI) derivatives have demonstrated promising antimalarial activity, among other biological properties. In an attempt to improve antiplasmodium potency and physicochemical properties, especially solubility, further structure−activity relationship (SAR) studies have led to the identification of the pyrimido[1,2-*a*]benzimidazole congener.
Herein, we present a new series of pyrimido[1,2-*a*]benzimidazoles with good *in vitro* antiplasmodium activity against the malaria parasite *Plasmodium falciparum*. SAR studies involved the incorporation of aliphatic amine side chains containing various water solubilizing H-bonding donors or acceptors (SAR1), the introduction of small hydrophobic substituents on the pyrimido[1,2-*a*]benzimidazole core scaffold with the aim to disrupt molecular planarity and thereby potentially enhance aqueous solubility (SAR2), and the exploration of substituted phenyl rings incorporating representative Craig plot groups with diverse lipophilic and electronic effects or amide groups (SAR3).
Some compounds from SAR1 and SAR3 showed great *in vitro* antiplasmodium activity against the asexual blood stage (ABS) parasites, whereas activity against gametocytes was broadly negligible. The most soluble analogues resulted from SAR3 studies. SAR2 derivatives demonstrated lowered activity and solubility, suggesting the importance of a residual molecular planarity. Compounds with the most potent *in vitro* ABS activity (IC_{50}1µM) were profiled for cytotoxicity against the Chinese Hamster Ovarian cell line (CHO). Generally, good selectivity indices were obtained (SI>10). Selected compounds were evaluated for metabolic stability using human, mouse, and rat liver microsomes and for permeability (LogD). A number of synthesized derivatives was able to inhibit β-hematin formation in the NP-40 assay, showing IC_{50} values comparable to chloroquine (IC_{50} = 17µM).
The pyrimido[1,2-*a*]benzimidazole reported here are a good starting point for identification of potentially new antimalarial agents.

NOTES

Multitarget inhibitors as anticancer agents with immunomodulatory and antiangiogenic properties.

Raquel Gil-Edo, Eva Falomir, Miguel Carda

Departamento de Química Inorgánica y Orgánica, Universidad Jaume I, E-12071 Castellón, Spain

In last few years, Programmed Death Ligand-1 (PD-L1) has become an interesting target in the study of new anticancer treatments as their implication as immune checkpoint. Besides, it is been demonstrated that PD-L1 also promotes the angiogenesis in some cancers by binding with Vascular endothelial growth factor receptor 2 (VEGFR-2), known as one of the most implicated receptors in the angiogenesis and consequent progression of the tumour[1]. This makes especially interesting to find a multitarget agent capable to inhibit this both proteins. Previous studies developed in our group[2],[3], revealed Urea and Triazole as good scaffolds for designing multitarget inhibitors. Here we are presenting the results we obtained in preliminary biological studies on some compounds, that have been designed and synthetised, containing urea or triazole unit.

*[1] Yang, Y.; Xia, L.; Wu, Y.; Zhou, H.; Chen, X.; Li, H.; Xu, M.; Qi, Z.; Wang, Z.; Sun, H.; Cheng, X. Programmed death ligand-1 regulates angiogenesis and metastasis by participating in the c-JUN/VEGFR2 signaling axis in ovarian cancer. Cancer Communications **2021**, 41, 511-527*

*[2] Martín Beltrán, C.; Gil-Edo, R.; Hernandez-Ribelles, G.; Agut, R.; Marí-Mezquita, P.; Carda, M.; Falomir, E. Aryl urea based scaffolds for multitarget drug discovery in anticancer immunotherapies Pharmaceuticals; **2021**, 4 pp. 1-11*

*[3] Conesa-Milián, L; Falomir, E.; Murga, J.; Carda, M.; Marco, J.A.; Novel multitarget inhibitors with antiangiogénicos and immunomodulator properties. European Journal of Medicinal Chemistry **2019,** 170, pp. 87-98*

NOTES

Design, synthesis, and biological evaluation of novel RNA binders

Maurinne Bonnet, Audrey Di Giorgio, Maria Duca

[1] *Institut of Chemistry of Nice, France*

The design and synthesis of nucleic acid ligands recently became a major issue in medicinal chemistry. RNA is one of the most promising biological targets for the discovery of innovative drugs in a large number of pathologies [1]. Various biologically relevant RNAs that could serve as drug targets have been identified, such as microRNAs, bacterial RNAs, and viral RNAs[2]. Given that some of the reported RNA ligands still lack selectivity, large efforts to develop specific binders recently succeded with the FDA approval of Risdiplam (Evrysdi™, Roche) as an mRNA splicing modifier.[3] This, together with a large number of marketed antibiotics able to bind prokaryotic ribosomal RNA inhibiting protein synthesis in bacteria, proves that RNA binders could represent promising therapeutic tools.
The aim of the present work is to take advantage of the covalent binding mode of a DNA-alkylating agent, the prodrug Temozolomide[4], for the design of novel RNA ligands able to covalently bind the target. This strategy led to the design of more selective and efficient ligands composed of (a) a well-known RNA ligand to interact with the target and (b) a covalent DNA ligand to strongly bind to the target. We first evaluated the stability and biological activity of the synthesized compounds against HIV-1 TAR RNA, which allowed us to select one compound for electrophoretic mobility shift assays in order to highlight the site of interaction. Finally, mass analysis confirmed the concept of covalent RNA binders, opening the way for the development of new series of efficient RNA ligands.

[1] Disney, M. D. J. Am. Chem. Soc. 2019, 141, 6776–6790.
[2] (a) Di Giorgio, A.; Duca, M. MedChemComm. 2019, 10, 1242–1255. (b) Warner, K. D.; Hajdin, C. E.; Weeks, K. M. Nat. Rev. Drug Discovery 2018, 17, 547–558.
[3] Ratni, H. et al. J. Med. Chem. 2018, 61, 6501–6517.
[4] Babu, N. et al. CrystEngComm 2012, 15, 666–671.

NOTES

In silico characterization of glucosylceramide synthase (GCS) binding site by Induced Fit Docking, Molecular Dynamics and Metadynamics.

Giorgia Canini[1], Ettore Lo Cascio[1], Stefano Della Longa[2], Giuseppina Nocca[1, 3], Alessandro Arcovito[1, 3]

[1] Dipartimento di Scienze Biotecnologiche di Base, Cliniche Intensivologiche e Perioperatorie, Università Cattolica del Sacro Cuore, Largo Francesco Vito 1, 00168 Roma, Italy.
[2] Department of Life, Health and Environmental Sciences, University of L'Aquila, 67100 L'Aquila, Italy
[3] Fondazione Policlinico Universitario "A. Gemelli", IRCCS, Largo A. Gemelli 8, 00168 Roma, Italy.

Glucosylceramide synthase (GCS) is an enzyme that catalyzes the first reaction of ceramide glycosylation in sphingolipid metabolism. Niemann-Pick type C (NPC) disease is characterized by an accumulation of cholesterol, sphingomyelin and glycosphingolipids, this last derived from glucosylceramide. Accumulation occurs in visceral organs, such as liver and spleen, but also in the brain, where there is a change of distribution of unesterified cholesterol and a contemporary accumulation of glycosphingolipids.
In this study we analyzed the protein structure of GCS derived from AlphaFold Protein Structure Database in the presence and in the absence of the supposed metal cofactor Mn2+. Following these results, Induced Fit Docking and MD simulations of endogenous substrates UDP-glucose and ceramide, were performed to characterize the binding site of the enzyme in order to trap the best pose of the activation state.
These results lay the foundation for a future study of drug repurposing for NPC disease, by means of a screening of a database of clinically used compounds.

NOTES

Abstracts

Session 6. Medicinal Chemistry Tales

<u>Keynote talk</u> - Plant-Derived & Endogenous Cannabinoids: Different in Nature

Mauro Maccarrone

[1] *University of L'Aquila*

During the last 60 years the relevance for human health and disease of cannabis (*Cannabis sativa* or *Cannabis indica*) ingredients, like the psychoactive compound Δ^9-tetrahydrocannabinol (THC), cannabidiol, additional 120+ phytocannabinoids (pCBs) and 440+ non-cannabinoid compounds, has become apparent. THC was identified in 1964, and approximately 30 years later (in 1992), the molecular reasons for the biological activity of cannabis extracts were made clearer by the discovery of anandamide (*N*-arachidonoylethanolamine). The latter is the first member of a new family of bioactive lipids collectively termed "endocannabinoids (eCBs)", that are able to bind to the same receptors activated by THC. In addition to eCBs, that include several *N*-acylethanolamines and acylesters, also a complex array of receptors, metabolic enzymes, transporters (transmembrane, intracellular and extracellular carriers) were discovered, and altogether they form the so-called "eCB system" that finely tunes the manifold biological activities of eCBs. Here, we describe the major pCBs and the main components of the eCB system to appreciate their differences and mutual interactions, as well as the potential of using pCB/eCB-based drugs as novel therapeutics to treat human diseases, both in the central nervous system and at the periphery.

NOTES

Keynote talk - Mapping dynamic protein binding sites for the design of selective anti-infective agents

Rebecca Wade

[1] Molecular and Cellular Modeling Group, Heidelberg Institute for Theoretical Studies (HITS), Schloss-Wolfsbrunnenweg 35, 69118 Heidelberg, Germany
[2] Zentrum für Molekulare Biologie (ZMBH), DKFZ-ZMBH Alliance and Interdisciplinary Center for Scientific Computing (IWR), Heidelberg University, Germany

Proteins are constantly in motion, changing their shape and wiggling and jiggling around. The dynamic nature of protein structures provides challenges and opportunities for drug design. Beyond this, a key requirement of the design of anti-infectives is achieving selectivity for the parasite target proteins over human proteins. I will describe our recent experience in mapping protein binding sites for the design of anti-parasitic agents against trypanosomatids and anti-viral agents against SARS-CoV-2.
We apply molecular dynamics simulations techniques and the TRAPP (Transient Pockets in Proteins, trapp.h-its.org) toolbox to explore protein binding pocket flexibility and assess pocket druggability [1]. We combine these approaches with systematic comparative mapping of on- and off-targets and virtual screening to identify potent enzyme inhibitors [2,3]. We also employ simulations to reveal both direct and dynamic allosteric mechanisms underlying the SARS-CoV-2 antiviral activity of the polysaccharide heparin; these provide a basis for the design of heparin derivatives with improved antiviral activity [4].

[1] Yuan J, Han SB, Richter S, Wade RC, Kokh DB (2020). Druggability Assessment in TRAPP Using Machine Learning Approaches, J. Chem. Inf. Model. 60(3):1685-1699.
[2] Gossen, J et al. (2021) A blueprint for high affinity SARS-CoV-2 Mpro inhibitors from activity-based compound library screening guided by analysis of protein dynamics ACS Pharmacol. Transl. Sci. 4(3):1079-1095.
[3] Pöhner I et al. (2022) Multitarget, Selective Compound Design Yields Potent Inhibitors of a Kinetoplastid Pteridine Reductase 1 J Med. Chem. DOI: 10.1021/acs.jmedchem.2c00232
[4] Paiardi G, Richter S, Oreste P, Urbinati C, Rusnati M, Wade RC (2022). The binding of heparin to spike glycoprotein inhibits SARS-CoV-2 infection by three mechanisms, J. Biol. Chem. 298(2):101507

NOTES

Keynote talk - Our Journey To Achieve Photoinhibition of Haematopoiesis *in Vivo*

Olalla Vázquez

[1] *Department of Chemical Biology, Faculty of Chemistry, Philipps-Universität Marburg, Hans-Meerwein-Straße 4, 35043, Marburg, Germany*

All cells in an organism contain the same DNA sequence but vary greatly in gene expression. Epigenetics deals with these phenotype changes that retains the same DNA sequence. Importantly, misregulation of these epigenetic processes is implicated in the pathophysiology of numerous human diseases,[1] including: cancer, autoimmune disorders and neurodegenerative disease. Therefore, epigenetic regulation is at the core of both natural and pathological states. The current available methods do not have sufficient spatiotemporal resolution to deal with the challenges of targeting the dynamic epigenome. We and others[2] envision that light could offer new possibilities and achieve molecular functionality. Reversible photoswitches, which have demonstrated their potential in diverse areas such as material science, have hardly implemented as genome regulators. Modulating the epigenome to tune transcription profiles, and cellular phenotypes in a programmable manner is of wide interest, and may ultimately lead to novel epigenetics-based therapeutics. Here, we will present our journey to achieve photoinhibition of the histone methyltransferase MLL1, which is a crucial player in the formation of blood cells.[3-5]

REFERENCES

*[1]. Portela, A. & Esteller, M. Epigenetic modifications and human disease. Nat Biotechnol **28**, 1057-1068, doi:10.1038/nbt.1685 (2010).*

*[2]. Hartmann, D., Smith, J. M., Mazzotti, G., Chowdhry, R. & Booth, M. J. Controlling gene expression with light: a multidisciplinary endeavour. Biochem Soc Trans **48**, 1645-1659, doi:10.1042/BST20200014 (2020).*

*[3]. Albert, L. et al. Controlled inhibition of methyltransferases using photoswitchable peptidomimetics: towards an epigenetic regulation of leukemia. Chem Sci **8**, 4612-4618, doi:10.1039/c7sc00137a (2017).*

*[4]. Albert, L. et al. Bistable Photoswitch Allows in Vivo Control of Hematopoiesis. ACS Cent Sci **8**, 57-66, doi:10.1021/acscentsci.1c00434 (2022).*

[5]. Albert, L. et al. Modulating Protein-Protein Interactions with Visible-Light-Responsive Peptide Backbone Photoswitches. Chembiochem 20, 1417-1429, doi:10.1002/cbic.201800737 (2019).

NOTES

Synthesis and structural activity relationships of novel isoindolone series against *Trypanosoma brucei rhodesiense*

Flavio da Silva Emery[1], Daniel Gedder Silva[1], Pedro Ivo Palácio Leite[1], Louis Maes[2]

[1] *School of Pharmaceutical Sciences at Ribeirao Preto of the University of Sao Paulo*
[2] *University of Antwerp*

Sleeping sickness (Human African Trypanosomiasis - HAT) is caused by two subspecies of trypanosoma brucei: gambiense and rhodesiense. While T. b. gambiense causes a chronic illness with symptoms after a longer incubation period; T. b. rhodesiense causes a more acute disease, with more aggressive symptoms after a few days or weeks of the bite of infected tsetse flies. Existing medicines are not effective, although without treatment the disease is invariably fatal. We tackled the need of new anti-HAT drugs by varying the heterocyclic scaffolds of hits found in a screening campaign against different trypanosoma subtypes [1,2]. Among the most promising scaffold was the dihydroisoindol core ring, which was optimized toward a series of selective hits to T. b. rhodesiense in vitro. Several compounds of this series exhibited an in vitro EC50 ≤ 1µM against T. b. rhodesiense and no detectable activity against other parasites, as T. b. gambiense, T. cruzi amastigotes or L. infantum. All potent compounds were furthermore tested for toxicity against MRC-5 and PMM cell lines. The compounds exhibited no significant toxicity to both cell lines. With high potency, low toxicity, and good water solubility we have found a promising series of compounds for advancing HAT drug discovery.

NOTES

Identification Of A Novel Scaffold For Dual Mdm2/X Inhibition

Elizabeth A. Lopes[1], Mi Wang[2], Shaomeng Wang[2], Daniel J. V. A. dos Santos[3], Maria M. M. Santos[4]

[1] Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Av. Prof Gama Pinto 1649-003, Lisbon, Portugal.
[2] Rogel Cancer Center, College of Pharmacy, University of Michigan, Ann Arbor, MI 48109 USA.
[3] CBIOS – Research Center for Biosciences & Health technologies, Universidade Lusófona de Humanidades e Tecnologias, Campo Grande 376, 1749–024 Lisboa, Portugal
[4] Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Av. Prof Gama Pinto 1649-003, Lisbon, Portugal

The discovery of p53 has disclosed a wide field of novel p53-based therapies, not only for cancer but for other diseases such as neurodegenerative or malaria. This protein is a highly attractive target due to its relevance in the maintenance of the integrity of human genome as it is involved in cell cycle arrest and programmed cell death. In almost 50% of cancer malignancies, the tumor suppressor function of p53 function is inactivated by overexpression of negative regulators (e.g., MDM2 and MDMX). So, inhibition of p53-MDM2/X interactions, with consequent activation of 53, is expected to be a valuable approach in fighting cancer. Several p53-MDM2 interaction inhibitors have been developed and some are currently in clinical trials, however most MDM2 inhibitors lack significant potency against MDMX. For this reason, it is imperative to develop new chemical families able to inhibit both MDM2 and MDMX [1].

In this communication, we report on the identification and structure-based optimization of novel dual MDM2/X inhibitors. By screening three virtual libraries (Drugbank, MOE, and NCI) in crystallographic structures of MDM2 and MDMX we identified 4 ligands that mimic the main interactions established by the p53 amino acids Phe19, Leu22, Trp23, and Leu26 involved in the interaction of p53 with both MDMs. Binding assays with the proteins, revealed that one ligand is a dual inhibitor of MDM2 and MDMX. Hit-to-lead optimization to improve activity was developed by constructing a virtual library of new derivatives to better explore the binding pockets of MDM2 and MDMX. Details of these studies will be disclosed. Taken together, the identified molecules represent a promising starting scaffold for the development of novel anti-cancer agents through activation of p53 by dual inhibition of MDM2/X.

NOTES

Discovery of novel naphthylphenylketone and naphthylphenylamine derivatives as Cell Division Cycle 25B (CDC25B) Phosphatase Inhibitors via *in silico* design

Carmen Cerchia[1], Rosarita Nasso[2], Matteo Mori[3], Stefania Villa[3], Arianna Gelain[3], Alessandra Capasso[4], Federica Aliotta[4], Martina Simonetti[4], Rosario Rullo[5], Mariorosario Masullo[6], Emmanuele De Vendittis[4], Maria Rosaria Ruocco[4], Antonio Lavecchia[7]

[1] Department of Pharmacy, " Drug Discovery" Laboratory, University of Naples Federico II, Via D. Montesano, 49, 80131 Naples, Italy
[2] Department of Molecular Medicine and Medical Biotechnology, University of Naples Federico II, Via S. Pansini 5, 80131 Naples, Italy; Department of Movement Sciences and Wellness, University of Naples " Parthenope" , 80133 Naples, Italy
[3] Department of Pharmaceutical Sciences, University of Milan, Via Mangiagalli, 25, 20133 Milan, Italy
[4] Department of Molecular Medicine and Medical Biotechnology, University of Naples Federico II, Via S. Pansini 5, 80131 Naples, Italy
[5] Department of Molecular Medicine and Medical Biotechnology, University of Naples Federico II, Via S. Pansini 5, 80131 Naples, Italy; Institute for the Animal Production Systems in the Mediterranean Environment, Via Argine 1085, 80147 Naples, Italy
[6] Department of Movement Sciences and Wellness, University of Naples " Parthenope" , 80133 Naples, Italy
[7] Department of Pharmacy, " Drug Discovery" Laboratory, University of Naples Federico II, Via D. Montesano, 49, 80131 Naples, Italy

CDC25 phosphatases are members of the family of dual-specificity phosphatases (DSPs) and play a critical role in the regulation of the cell cycle. The overexpression of CDC25s in many human cancers supports their clinical significance and has encouraged the pursuit of specific small-molecule inhibitors. A structure-based optimization of NSC28620, previously reported by us as a new CDC25 inhibitor endowed with promising anticancer activity, led to the identification of a series of novel naphthylphenylketone and naphthylphenylamine derivatives. Five compounds showed higher inhibitory activity than the initial lead, with Ki values in the low micromolar range. Kinetic analysis, intrinsic fluorescence studies, and induced fit docking simulations provided a mechanistic understanding of the activity of these derivatives. All compounds were tested in the highly aggressive human melanoma cell lines A2058 and A375; among them, one compound potently inhibited cell proliferation and colony formation, causing an increase of the G2/M phase and a reduction of the G0/G1 phase of the cell cycle in both cell lines.

NOTES

Design, synthesis and evaluation of immunostimulating activities of mannosylated desmuramyl peptides containing lipophilic substituents

Danijela Mihelec[1], Željka Car[1], Vesna Petrović Peroković[1], Ranko Stojković[2], Rosana Ribić[3]

[1] Department of Chemistry, Faculty of Science, University of Zagreb, Horvatovac 102a, HR-10000 Zagreb, Croatia
[2] Ruđer Bošković Institute, Bijenička cesta 54, HR-10000 Zagreb, Croatia
[3] University Center Varaždin, University North, Jurja Križanića 31b, HR-42000 Varaždin, Croatia

Muramyl dipeptide (MDP, *N*-acetylmuramyl-L-alanyl-D-*iso*glutamine) is the smallest peptidoglycan fragment capable to trigger the immune response. MDP acts as a pathogen-associated molecular pattern and activates the NOD2 (Nucleotide Binding Oligomerization Domain Containing 2) receptor. The main parameter for the improvement of its pharmacological properties is lipophilicity. Up to now, our research was directed towards desmuramyl peptides containing adamantane and their mannose derivatives. Namely, mannose receptors presented on immunocompetent cells are considered to be pattern-recognition receptors, as well as NOD2, and therefore they can affect the immune reactions. Here we present the design and synthesis of mannosylated desmuramyl peptides containing lipophilic substituents attached at D-*iso*glutamine/D-glutamic acid of dipeptide pharmacophore through 1,2,3-triazole moiety. Their immunostimulating activities are evaluated *in vivo* in the mouse model using ovalbumin as an antigen. Lipophilic substituents attached over α-COOH of D-Glu contribute to the immunostimulation while the presence of free γ-COOH at D-Glu/D-*iso*Gln is important for establishing interactions with native receptor.

NOTES

Targeting the Energy-Coupling Factor Transporters: a novel antibacterial target

Eleonora Diamanti

[1] *HIPS - Helmholtz-Institut für Pharmazeutische Forschung Saarland*

Energy-Coupling Factor (ECF) transporters are transmembrane proteins involved in the uptake of several vitamins into a range of bacteria. They are mainly present in Gram-positive bacteria (*e.g.*, *Streptococcus pneumoniae*, *Enterococcus faecium*) and importantly absent in humans.
A tri-component ECF module that is conserved among different species and a vitamin-specific type S-component make their architecture a unique and unprecedented structural feature. Importantly, experiments with a CRISPRi library confirmed the ECF module as an essential part for the survival of the pathogen *S. pneumoniae,* making these novel transporters an attractive drug target.
Here, we have established an *in vitro*, whole-cell and SPR assay to screen for and validate inhibitors of the ECF transporter, leading to three chemically different families of compounds.
A focused Structure-Activity Relationship (SAR) study led to identify compounds in the low-micromolar range both *in vitro* and *in cellulo* environment and, the molecular dynamic studies indicated the potential binding site of our ECF inhibitors that is at the interface between the ECF module and the S-component, making them potentially protein-protein inhibitors. [1—3]
As no red-flags has been found on the ADME-T studies, we also tested our best compound in preliminary PK studies that showed an oral bioavailability of around 80%.

Literature:
[1] Diamanti E.. et al, Hirsch A.K. H. (2021): Targeting the energy-coupling factor (ECF) transporters: identification of new tool compounds, 10.26434/chemrxiv-2021-xq08b-v2
[2] Bousis S., Winkler S., Haupenthal J., Fulco F., Diamanti E., Hirsch A. K. H. An Efficient Way to Screen Inhibitors of Energy-Coupling Factor (ECF) Transporters in a Bacterial Uptake Assay Int. J. Mol. Sci. ***2022****, 23, 2637.*
[3] Kiefer A. F., Bousis S., Hamed M.M., Diamanti E., Haupenthal J. Hirsch A. K. H. Structure-Guided Optimization of Small-Molecule Folate Uptake Inhibitors Targeting the Energy-Coupling Factor Transporters, J. Med. Chem. ***2022****, Accepted.*

NOTES

Volume Scanning—A Rational Approach to Covalent PI3Ka Inhibitors

Chiara Borsari[1], Erhan Keles[1], Jacob McPhail[2], Alexander Schäfer[3], Thorsten Schäfer[1], Matthias Gstaiger[3], John Burke[2], Matthias Wymann[1]

[1] Department of Biomedicine, University of Basel, Mattenstrasse 28, 4058 Basel, Switzerland
[2] Department of Biochemistry and Microbiology, University of Victoria, Victoria, British Columbia V8W 2Y2, Canada
[3] Department of Biology, Institute of Molecular Systems Biology, ETH Zurich, Otto-Stern-Weg 3, 8093 Zürich, Switzerland

Inhibitors of the phosphatidylinositol 3-kinase (PI3K) pathway are considered valuable assets in cancer therapy. A considerable effort has been dedicated to the development of drugs targeting PI3Ks, which are evaluated in preclinical/clinical studies. Here, we present a strategy to convert a phase II clinical candidate, a pan-PI3K inhibitor (PQR309/bimiralisib), into a highly selective covalent PI3Ka inhibitor aiming to minimize on-target metabolic side effects of PI3K inhibitor cancer therapy. We exploited a rational approach to increase the target selectivity by covalently targeting PI3Ka at the non-conserved Cys862.

A combination of warhead activity design, proximity screening, and an optimized orientation allowed tight control of reversible inhibitor binding in combination with isoform-specific covalent reaction. To avoid off-target reactions, the warhead reactivity was determined and optimized for selectivity and Cys862 modification. An extensive structure–activity relationship (SAR) study was performed, and a wide range of linear and restricted rotation linkers was introduced. A comprehensive understanding of the kinetics of irreversible inhibition acquired by kinetic TR-FRET assays and subsequent determination of k_{chem}, k_{inact} and calculated K_i allowed compound selection with minimal off-target reactivity and high PI3Ka selectivity. X-ray crystallography and MS-based proteomics validated the covalent modification of Cys862. Our pilot compounds exceed specificity and potency over an experimental dimethyl-substituted enone, CNX-1351. Moreover, our compounds display increased stability in rat liver microsomes and outperform the rapidly metabolized CNX-1351.

Our strategy to investigate and tune the reactivity of warheads represents a major step forward in the rational design of covalent chemical tools. Moreover, we provide highly selective probes 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.

NOTES

Facilitated Access to Novel Aryl Hydrocarbon Receptor Ligands through Extended Multicomponent Reactions

Ouldouz Ghashghaei[1], Marina Pedrola[1], Francesca Seghetti[1], Frederick Hartung[2], Katharina Rolfes[2], Thomas Haarmann-Stemmann[2], Rodolfo Lavilla[1]

[1] *Universitat de Barcelona*
[2] *IUF Leibniz Research Institute for Environmental Medicine*

Facilitated synthetic methodologies play a key role in the success of Medicinal Chemistry projects, by providing convenient access to screening libraries. In this context, Multicomponent Reactions (MCRs) have been among the strategies of choice due to the unique advantages they offer. Being single-step and modular operations, a large number of relavant derivatives around a scaffold could be readily prepared. Extended MCRs are processes in which the initial MCR adduct continues to evolve into a more sophisticated molecule in a one-pot manner. These operations can further elevate the structural diversity and complexity of classic MCR adducts.
Aryl hydrocarbon Receptor (AhR) signalling pathway has been a highly attractive research topic in recent years due its regulatory role in a variety of physiological processes, including immune responses, carcinogenesis, and xenobiotic metabolism. Moreover, due to its dual nature, both activating and inhibiting agents could be beneficial for eventual therapeutic purposes. Nevertheless, as the receptor's binding site is not fully described, the discovery and tuning of the new ligands heavily relies on screening libraries.
6-Formylindolo(3,2-b)carbazole (FICZ) and its derivatives are among the most common activators of AhR. However, their preparation often requires multi-step synthesis. Here we present a novel MCR-based methodology to access a variety of indolocarbazoles in a single-step from commercially available building blocks. The representative compounds prepared by the described approach have confirmed to be potent activators of AhR pathway. Moreover, novel *linkable* AhR ligands with -COOH residues have been designed to conveniently assemble chemical probes and studies are ongoing to unravel their therapeutic potential. Latest results around the unpublished derivatives and their properties will be presented.

NOTES

The cytotoxic potential of cationic triangulenes against tumour cells

Euphemia Leung[1], Lisa I Pilkington[2], Mohinder M. Naiya[2], David Barker[2], Ayesha Zafar[2], Chatchakorn Eurtivong[3], Jóhannes Reynisson[4]

[1] Auckland Cancer Society Research Centre, University of Auckland, Grafton, Auckland 1023, New Zealand
[2] School of Chemical Sciences, University of Auckland, City Centre, Auckland 1010, New Zealand
[3] Program of Chemical Biology, Chulabhorn Graduate Institute, Chulabhorn Royal Academy of Sceince, Bangkok 10210, Thailand
[4] School of Pharmacy and Bioengineering, Keele University, Hornbeam building, Staffordshire ST5 5BG, United Kingdom

TOTA (Trioxatriangulenium ion) is a close-shelled cabocation known to intercalate strongly with the DNA double helix (*J. Am. Chem. Soc.* 2003, **125**, 2072). The cytotoxicity of **TOTA** and its four close structural analogues, **ADOTA**, **Pr-ADOTA**, **Pr-DAOTA** and ***n*-Butyl-TATA** were tested against the breast cancer cell line MDA-MB-231 and colon cancer cell line HCT116. The most potent derivatives **Pr-ADOTA** and **Pr-DAOTA** had IC_{50} values of ~80 nM for MDA-MB-231 but slightly higher for HCT116 in the low hundreds nM range. A 3D model assay of HCT116 spheroids was also used, mimicking a tumour environment, again both **Pr-ADOTA** and **Pr-DAOTA** were very active with IC50 values of 38 nM and 21 nM, respectively. DNA damage assay and molecular docking to the DNA double helix strongly suggest that the mechanism of action is due to the intercalation between the base pairs of the carbenium ions. Finally, a robust density functional theory (DFT) model was built to predict the pK_{R+} stability values, *i.e.*, to design derivatives, which predominantly have a non-intercalating buckled form in healthy tissues followed by a nucleophilic attach of water on the central carbon, but a planar form at relatively low pH values rendering them *only* cytotoxic in the interior of tumours.

NOTES

The inhibition of iNOS as a promising strategy against cancer development: good news from novel phenyl-amidine based compounds.

Encarnación Camacho[1], Pasquale Amoia[2], Belén Rubio[3, 4], Francisco Franco-Montalbán[1], Dora Carrión[4], Rosa Amoroso[2], Cristina Maccallini[2]

[1] Department of Medicinal and Organic Chemistry, Faculty of Pharmacy, University of Granada, Granada, Spain.
[2] Department of Pharmacy, University "G. d'Annunzio", Chieti-Pescara, via dei Vestini, 31, 66100 Chieti, Italy
[3] GENYO, Centre for Genomics and Oncological Research, Pfizer/University of Granada/Andalusian Regional Government, PTS, Avda. de la Ilustración 114, 18016 Granada, Spain.
[4] Department of Medicinal and Organic Chemistry, Faculty of Pharmacy, University of Granada, Granada, Spain.

The dual role of the inducible Nitric Oxide Synthase (iNOS) in tumor progression is of considerable interest in cancer biology [1]. Increased iNOS activity has been positively correlated with the degree of malignancy in glioma, gynecological tumors, and in the colon, prostate and breast cancers. Therefore, the inhibition of iNOS was proposed as a valuable strategy to counteract tumor development and chemoresistance. In this context, we have disclosed CM544 and FAB1020, two acetamidine-based compounds which demonstrated in vitro anti-glioma activity, counteracting C6 rat glioma cells proliferation [2,3]. Moreover, we have recently obtained interesting results from some imidazolyl-derivatives which, acting as dual iNOS and aromatase inhibitors, showed antiproliferative effects against the MCF-7 breast cancer cell line [4].
As part of an ongoing project about the discovery of new iNOS inhibitors, we have synthesized a new set of phenyl-amidine based compounds containing some aryl-sulphonamide moieties with the aim to study their effect against the progression of triple negative breast cancer (TNBC). Interestingly, the most potent human iNOS inhibitor (IC_{50} = 0.065μM) was able to counteract TNBC MDA-MB-231 cells proliferation in a dose-dependent manner, reducing also cells migration. A docking study was also performed to shed light on the binding mode of this compound into the iNOS.

[1] Vannini, F. Et al. Redox Biology 6 (2015) 334-343
[2] Maccallini, C. Et al. Eur. J. Med. Chem. 152 (2018) 53-64
[3] Maccallini, C. Et al. ACS Med. Chem. Lett. 11 (2020) 1470-1475
[4] Maccallini, C. et al. J. Enzyme Inhib. Med. Chem. 36 (2021) 1632-1645

NOTES

Delivery of a potent G-quadruplex DNA binder to cancer cells by aptamer functionalized liposomes

Pont Isabel, Cristina Galiana-Rosello, Gil-Martinez Ariadna, Mulet Laura, M. Teresa Albelda, Enrique García-España, Jorge Gonzalez-Garcia

[1] *University of Valencia, Institute of Molecular Science (ICMol)*

Drug delivery systems have become an integral tool in research and clinical applications to administrate drug(s) and enhance their therapeutic effect with improved biodistribution. The translation from the bench to the clinics was successfully achieved with the drug-loaded liposomal system Doxil®, which allows, with less cardiotoxicity, a more efficient accumulation of the drug doxorubicin in ovarian and breast tumours than when using a non-liposomal approach. Since then, several liposomal drug formulations have been approved by the FDA or are currently in late-stages of the clinical trials.
Besides drug delivery, the essential characteristic for efficient cancer therapies is the drug target. The novel targets involve epigenetic alterations, including histone modification, nucleosome remodelling and other non-coding mediated structures. One of the most attractive non-coding structures in anticancer drug development are G-quadruplex (G4) DNA.
Herein, we present a family of G4 DNA binders constituted by a triphenylamine scaffold linked to one, two or three appended either open-chain or macrocyclic polyamine substituents and showing capacity as G4 binders. Particularly, the trisubstituted ligands have along with a strong stabilization effect, a good selectivity for G4 *vs*. duplex DNA structures. However, in viability studies with cancer cells, both trisubstituted ligands show lower cytotoxicity than the less-charged mono- and disubstituted derivatives. This limitation is overcome by their encapsulation in liposomes and aptamer-liposome systems. The IC_{50} values decrease one order of magnitude for untargeted liposomes whereas aptamer-targeted liposomes show further increases in cytotoxicity of several orders of magnitude of IC_{50} values. Confocal microscopy shows that while non-targeted liposomes are in the cytoplasmic region stuck to the nuclear membrane, the targeted ones reach the interior of the nucleus. These data suggest that the aptamer AS1411 helps to efficiently transport and deliver the ligand TPA3P into the nucleus.

NOTES

Targeting Acute Myelogenous Leukemia using potent *human* dihydroorotate dehydrogenase inhibitors based on the 2-hydroxypyrazolo[1,5-*a*]pyridine scaffold: from academy to clinic

Marta Giorgis[1], Stefano Sainas[1], Paola Circosta[2], Alice Passoni[3], Renzo Bagnati[3], Marilena Marraudino[4], Brigitta Bonaldo[4], Stefano Gotti[4], Riccardo Miggiano[5], Davide Ferraris[5], Marta Alberti[5], Agnese Chiara Pippione[1], Chiara Vigato[1], Donatella Boschi[1], Giuseppe Nicola Saglio[2], Marco Lucio Lolli[1]

[1] *Department of Drug Science and Technology of Turin University*
[2] *Department of Clinical and Biological Science of Turin University*
[3] *Department of environmental health sciences Istituto di ricerche farmacologiche Mario Negri IRCCS*
[4] *Neuroscience Institute Cavalieri Ottolenghi NICO*
[5] *Department of Drug Sciences UPO University*

The connection in the late 2016 beetwen Acute Myelogenous Leukemia (AML) and dihydroorotate dehydrogenase (hDHODH), a key enzyme in pyrimidine biosynthesis, has attracted significant interest from pharmaceutical companies who have recognized a possible new therapeutic target for AML. In 2018, using a bioisosteric approach supported by structure-based techniques, we discovered MEDS433, a potent hDHODH inhibitor (IC_{50} = 1.2 nM), able to induce myeloid differentiation in AML cell lines (THP1 and U937) in the low nM range (EC_{50} = 40 and 26 nM), which shows better qualities than brequinar (EC_{50} = 249 nM on THP1 and 189 nM on U937), currently in phase I/II clinical trial for AML. These incredible *in vitro* results encouraged us to deeper the MEDS433 properties in order to optimize the design of the future certified preclinical study necessary to open the doors of the Phase I clinical trial. In this occasion, the pharmacokinetics, metabolism, toxicity as well as the *in vivo* efficacy in leukemic xenograft mouse model (IP, PO) of MEDS433 are presented. The strategy that allowed the discovery of MEDS700, a MEDS433 backup compound, superior to MEDS433 itself in terms of in vitro efficacy and minor toxicity is also presented.

NOTES

Targeting multimeric G-quadruplex structures using reinforced ligands

Ariadna Gil Martínez, Andrea Lázaro Gómez, Isabel Pont Niclòs, Cristina Galiana Rosellò, Enrique García-España Monsonís, Jorge González García

[1] *University of Valencia, Institute of Molecular Science, C./ Catedrático José Beltrán 2, Paterna 46980, Spain*

Non-canonical nucleic acid structures have attracted considerable attention of researchers from many science fields, including chemistry, biology, physics, materials and nanotechnology. They include triplexes, *i*-motifs or G-quadruplexes (G4). The later one is a supramolecular assembly of two or more tetrads, which arise from the hydrogen bonding network of four coplanar guanines. The stability and topology of G4 structures are mainly controlled by the alkali metal cation employed, the base sequence and the nature of the nucleic acid (DNA or RNA). Strikingly, a large number of putative G-quadruplex forming sequences have been identified in the genomes of human, microorganisms and viruses, and evidence suggest their pivotal role in key biological processes.[1] In particular, telomeres are regions enriched with putative G4-forming DNA sequences and have been associated to ageing and cancer. Therefore, G4 structures are currently tested as a therapeutic target to block telomere elongation in cancer cells.[2] Telomere sequences comprise hundreds of TTAGGG repeats which forms a superstructure constituted by multiple G4s, termed as multimeric G4s (multG4s).

Herein, we present our synthetic efforts to develop new organic molecules [3] able to interact with multG4s. We have prepared a family of linear, macrocyclic and cryptand ligands taking into account the first generation of G4 binders with triphenylamine moieties.[3] A range of biophysical assays (FRET melting, fluorescence spectroscopy and gel electrophoresis) has been used to characterise the interaction towards multG4s, monomeric G4s and duplexes. Our results point out the importance of the organic core scaffold, the number and class of the substituents, the molecule net charge and the reinforced structure of the ligands to bind strongly multimeric G4s.[4] Among the series, cryptand-like ligands arise as potent and selective G-quadruplex binders of multimeric structures, which can be applied to target the telomeric regions of cancer cells.

NOTES

Regulation Of Gene Expression With Smart Activatable Therapeutics

Sundus Erbas Cakmak

[1] *Konya Food and Agriculture University, Department of Molecular Biology and Genetics*

Guanine rich regions of DNA and RNA hydrogen bond with one another to form three stacked planes called G quadruplex. These structures are shown to be located at DNA telomeres and promoter regions of certain genes. G quadruplex structures are shown to fold and unfold dynamically and their association with certain proteins are important for the regulation of transcription and translation. In the project, novel cationic G quadruplex binding ligand is synthesized and its activity towards G quadruplex stabilization is analyzed. Ligand is designed to bear bulky substituents which prevents intercalation. The bulky groups are enzymatically removed by reductive enzymes upon exposure to cancer microenvironment, under hypoxic conditions. Cells are incubated with the G-quadruplex stabilizer and after 24h incubation, gene expression of MCF7 cancer cells under normal and hypoxic conditions is analyzed. Results indicate a significant decrease in the expression of oncogenes in hypoxic conditions. These results demonstrate the activation of the agent under oxygen deficient hypoxic conditions, a characteristic solid tumor environment. Among the genes which are silenced, there are transcription factor HIFa. Considering the role of this transcription factor in the formation of hypoxic conditions, decrease in gene expression is expected to result in reversal of hypoxic phenotype. Hence, novel G-quadruplex stabilizer presented in the research would have the potential to be used in therapeutic applications.

Funding Agent: TÜBİTAK, Project No: 120C125

NOTES

Discovery and development of a mkk-4 inhibitors to increase liver regeneration

Stefan Laufer

[1] *Department of Pharmaceutical/Medicinal Chemistry, Eberhard Karls Universität, Auf der Morgenstelle 8, Tübingen Center for Academic Drug Discovery, 72076 Tübingen, DE, Germany*

Currently, the therapeutic options for treatment of liver failure are very limited. In an in vivo RNAi screen, mitogen-activated protein kinase kinase 4 (MKK4) has recently been identified as a major regulator in hepatocyte regeneration. By functional genetic silencing of MKK4. the target was validated in various experimental disease models. These data strongly supported the concept, that selective MKK4-inhibition with a small molecule-represents a promising and attractive approach for treatment of a complex and multi-factorial disease. Further to the observation that the approved BRAFV600E inhibitor vemurafenib shows a high affinity to and moderate functional inhibition potency against MKK4, our hit optimization concept included classical iterative SAR-optimization but also a scaffold-hopping approach by changing the core heterocycle from 1H-pyrrolo[2,3-b]pyridine to 1H-pyrazolo[2,3-b]pyridine. Both approaches followed a mandatory multiparameter optimization. In vivo RNAi experiments also revealed, that MKK7 and JNK1 are anti-targets and thereby defined the specification regarding the kinase selectivity. In both series, highly selective MKK4-inhibitors down to low nanomolar range with excellent selectivity profile against MKK7/JNK1 could be achieved.
LN3118 was identified as a tool compound with an IC50-value against MKK4 of 0,1 µM and an attractive selectivity profile and was used to validate MKK4 as a druggable target for treatment of liver disease. In experimental 2/3-hepatectomy in the mouse, orally administered LN3118 achieved a two-fold increase of hepatocyte proliferation. In acute CCl4-induced liver injury, cell death was prevented by 70%. Furthermore, LN3118 demonstrated therapeutic activity in two subchronic animal models. LN3118 reduced alcohol-induced steatosis and significantly reversed CCl4-induced liver fibrosis. The latest generation of compounds included highly potent MKK4-inhibitors with a pharmacological, toxicological and pharmacokinetic profile, which meet the specification of a clinical development candidate. Latest profiling and selection strategy for candidate selection will be presented.

NOTES

Abstracts

Poster Exhibition

A1. *In vivo* toxicity, redox-modulating capacity and intestinal permeability of novel aroylhydrazone derivatives with high *in vitro* antimycobacterial activity

Simeon Dimitrov[1], Rumyana Simeonova[2], Milka Mileva[1], Stanislav Philipov[3], Iva Valkova[2], Almira Georgieva[1], Elina Tzvetanova[1], Reneta Petrova[4], Violina Angelova[2], Violeta Valcheva[1]

[1] *The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, Sofia, Bulgaria*
[2] *Faculty of Pharmacy, Medical University of Sofia, Sofia, Bulgaria*
[3] *Department of Human Anatomy, Histology, General and Clinical Pathology and Forensic Medicine, Faculty of Medicine, Sofia University St. Kliment Ohridski, Sofia, Bulgaria;*
[4] *National Diagnostic and Research Veterinary Medical Institute "Prof. Dr. G. Pavlov", Sofia, Bulgaria*

Despite significant progress in the development of new drugs against tuberculosis, many therapies and preventive measures do not lead to the expected favorable health results for various reasons. This opens the way to identify novel, structurally diverse compounds, whose structure–activity–toxicity relationships must be thoroughly elucidated prior to further development. We present the aroylhydrazone compounds (3a,b,c) about their: (i) acute and subacute toxicity in mice; (ii) redox-modulating capacity by *in vivo* and *in vitro* investigations; (iii) pathomorphological observation in differentiated tissue specimens; (iv) intestinal permeability; and (v) *in vitro* antimycobacterial activity. They were *characterized* by ^{1}H-NMR, ^{13}C NMR and HRMS spectroscopic data. The minimum inhibitory concentration (MIC) was determined using the broth microdilution assay against *M. tuberculosis H37Rv.* The screening identified 3a (MIC=0.0730 µM, cytotoxicity - HEK-293T IC_{50} = 256.7 µM, SI=3516), 3b (MIC=0.3969 µM, cytotoxicity - HEK-293T IC_{50} = 785 µM, SI=1978.83) and 3c (MIC=0.4412 µM, cytotoxicity - HEK-293T IC_{50} = 279.5 µM, SI=633.49) as a new promising hit compounds against *M. tuberculosis* H37Rv. According to the Hodge and Sterner toxicity scale, 3a,b,c are classified as slightly toxic with an LD_{50} > 2000 mg/kg for both oral and intraperitoneal administration. Changes in behavior, and amounts of food and water intake were not observed during 14 days oral administration at two doses of 1/10 and 1/20 of the LD_{50}. The histological examination proved that the tissue findings do not show toxic changes. Liver findings showed isolated changes without a pathological organ profile. The *in vitro* antioxidant assays confirmed the results found *ex vivo*. High GIT permeability at all tested pH values was possess for 3a and 3b. These compounds display promising antitubercular drug-like properties and can be used for further investigation. This work was supported by the Bulgarian National Science Fund (Grant KP-06-PN- 41/3, 2020).

A2. Novel pyrazole- and pyrrole- based compounds active against multiple stages of P.falciparum

Federico Appetecchia[1], Douglas G Paton[2], Alexandra Probst[2], Laura De Vries[2], Tasneem Rinvee[2], Chiara Tammaro[1], Dyann F. Wirth[2], Flaminia Catteruccia[2], Giovanna Poce[1]

[1] La Sapienza -Università di Roma
[2] Department of Immunology and Infectious Diseases, Harvard T.H. Chan School of Public Health, 665 Huntington Avenue, Boston, MA 02115, U.S.A.

Spreading of antimalarial resistance represents a major threat to the effective treatment of malaria, an infectious disease caused by Plasmodium parasites. From a screening of 125 compounds from an in-house library, we have identified 12 of them having pyrazole- and pyrrole- based structures that are active against early and late stages (preferentially ring and trophozoite stages) of *P. falciparum* with **CL241**, **CL191** and **CL203** to be the most potent (IC_{50}: 0.7-1.2 μM) *in vitro*. Interestingly, after a short time of exposure *in vitro* culture they lead to an unusual amorphous phenotype suggesting that they may act through a novel mechanism of action. From transmission-blocking activity assays, we also found that the pyrazole derivative **CL239** is active against late-stage gametocytes (SMFA assay) and compounds **CL191** and **CL89**hamper parasite development in mosquito stages (topical exposure assay). All these findings together, not only lays the foundation for a new lead identification and optimization to the development of new clinical candidates, but also the development for further vector-targeted malaria control strategies that could be used in combination anti-malarial drugs to treat multi-drug resistant malaria. Further studies on the activity against drug-resistant strains and for target identification are ongoing.

A3. A high-throughput-chemistry, direct-to-biology approach discovers novel reactive fragments targeting SARS-CoV-2

Harry Wilders[1, 2], David Fallon[1], Nicholas Tomkinson[2], Jacob Bush[1]

[1] *GlaxoSmithKline*
[2] *University of Strathclyde*

Advances in drug target identification strategies have resulted in the discovery of increasing numbers of proteins linked to human disease. Whilst a major advancement for medicine, the novel nature of these targets often necessitates the subsequent discovery and development of ligands at an equal pace. Reactive fragments have been extensively applied to the identification of protein binders. Hit reactive fragments typically have low potency, and extensive optimisation is required to develop them towards lead-like compounds. This would previously have been laborious and time-consuming. We report a technology that is capable of rapidly synthesising and screening cysteine-targeting reactive fragments and their elaborated analogues. Synthesis is performed within 384-well plates, with follow up screening performed against recombinant protein without prior purification in a direct-to-biology (D2B) manner. The resultant screening platform can be used to synthesise and screen a full 384-well plate of compounds in 48 hours, with two iterative rounds of hit optimisation possible within 30 days, a remarkable improvement on the time required for current hit optimisation strategies. Crucially, appropriate biochemical optimisation and controls enable the identification and elimination of false positives which have often complicated similar approaches. This technology has been applied to the discovery and ongoing optimisation of novel ligands for SARS-CoV-2-3CL - a protein target of great importance for potential treatments against COVID-19 for which there is an unmet clinical need.

A4. Chiral xanthones reversing antimicrobial resistance: enantioselectivity in efflux pump inhibition

Fernando Durães[1, 2], Sara Cravo[1, 2], Joana Freitas-Silva[2], Nikoletta Szemerédi[3], Maria Tiritan[1, 4], Paulo Martins-da-Costa[2, 5], Eugénia Pinto[6, 7], Gabriella Spengler[3], Carla Fernandes[1, 2], Madalena Pinto[1, 2], Emília Sousa[1, 2]

[1] a Laboratory of Organic and Pharmaceutical Chemistry, Department of Chemical Sciences, Faculty of Pharmacy, University of Porto, Portugal
[2] b Interdisciplinary Centre of Marine and Environmental Research (CIIMAR), University of Porto, Portugal
[3] d Department of Medical Microbiology, Albert Szent-Györgyi Health Center and Albert Szent-Györgyi Medical School, University of Szeged, Hungary
[4] CESPU, Institute of Research and Advanced Training in Health Sciences and Technologies (IINFACTS), Gandra, Portugal
[5] c ICBAS – Institute of Biomedical Sciences Abel Salazar, University of Porto, Portugal
[6] b Interdisciplinary Centre of Marine and Environmental Research (CIIMAR), Terminal de Cruzeiros do Porto de Leixões, Av. General Norton de Matos s/n, 4450-208 Matosinhos, Portugal
[7] g Laboratory of Microbiology, Department of Biological Sciences, Faculty of Pharmacy, University of Porto, Rua de Jorge Viterbo Ferreira, 228, 4050-313 Porto Portugal

Antimicrobial resistance is a public health issue that has been dangerously rising over the past years and has relevance in the case of bacteria. These microorganisms can change their target or permeabilize their membrane but can also develop enzymes which inactivate the drug or overexpress efflux pumps [1]. Efflux pumps are transport proteins responsible for expelling substances toxic to the bacteria, in which antibiotics are included [2].

The quest for the identification of bacterial efflux pump inhibitors (EPIs) is underway, and several inhibitors have been discovered [3]. Our recent disclosure of xanthones as a bacterial efflux pump inhibitor [4] led us to investigate a series of chiral derivatives of xanthones and study their potential as bacterial EPIs, while analyzing enantioselectivity in this scope. The library was characterized for antimicrobial effects against clinically relevant bacterial and fungal strains and for synergistic effects with antibiotics in resistant strains of *Escherichia coli* and *Enterococcus faecalis*. Although no compound showed antimicrobial activity, one enantiomer and one xanthone precursor showed synergy with cefotaxime against *E. coli*. After studying their EPI potential, we found that three different enantiomers presented inhibitory activity in *Salmonella enterica* serovar *Typhimurium* SL1344, while only one enantiomer was active against a methicillin- and oxacillin-resistant strain of *Staphylococcus aureus*. Compounds shown to inhibit efflux pumps were tested for their effects against related virulence and resistance mechanisms, such as quorum-sensing and biofilm formation. Overall, different effects for enantiomeric

pairs were noted, suggesting that enantioselectivity plays an important role in microbial resistance mechanisms.

FCT UIDB/04423/2020, UIDP/04423/2020, PTDC/SAU-PUB/28736/2017 (POCI-01–0145-FEDER-028736), COMPETE 2020, Portugal 2020 ERDF, ATLANTIDA (NORTE-01-0145-FEDER-000040), NORTE2020, CHIRALBIO ACTIVE-PI-3RL-IINFACTS-2019. F.D. acknowledges FCT for his PhD grant (SFRH/BD/144681/2019).

[1]. J Nat Rev Micro 2010, 8, 251-259.
[2]. J Antimicrob Chemother 2003, 51, 9-11.
[3]. Curr Med Chem 2018, 25, 6030-6069.
[4]- Antibiotics 2021, 10, 600.

A5. Fucoidan as a protective agent against SARS-CoV-2 in Vero cells

David Alam-Escamilla

[1] *Creamos Mas SA de CV*

The recent periodic influx of massive quantities of pelagic Sargassum spp. (sargasso) into the Caribbean has posed ecological, social, and economic challenges to the region. Sustainable use of the biomass is crucial to mitigate the negative impacts of beached algae. Sargassum is a source of fucoidan, a marine-algae-derived compound. Fucoidan is a compound widely consumed in several countries as a medicinal, herbal, nutraceutical, or dietary supplement; its consumption in humans is considered safe. Several biological activities have been attributed to this marine compound, including immunomodulatory, antioxidant, and anti-inflammatory activity. Recent studies have strongly suggested that fucoidan could inhibit the entry of the SARS-CoV-2 virus into human cells, and previously, we found that fucoidan induces the increment of intracellular calcium flux and promotes the recovery of ΔΨm in PBMCs from COVID-19 patients. The aim of this study was to determine antioxidant activity and the in vitro effects of fucoidan with a cytopathic effect induced by SARS-CoV-2 infection on VERO cells, strengthening the possibility that fucoidan could ameliorate the immune response in COVID-19 patients.

A6. High content Screening identification of new molecular tools against SARS-CoV-2

Simona Sestito[1], Roberta Ibba[1, 2], Erika Plicanti[3], Federico Riu[1], Sandra Piras[1], Michele Lai[3], Giula Freer[3], Antonio Carta[1]

[1] Department of Chemistry and Pharmacy, University of Sassari, Sassari, Italy
[2] Department of Biotechnology, Chemistry and Pharmacy, University of Siena, Siena, Italy
[3] Retrovirus Center and Virology Section, Department of Translational Research and New Technologies in Medicine and Surgery, University of Pisa, Pisa, Italy

In 2019, a virus later known as SARS-CoV-2 started to infect some regions in China; the infection came up very contagious and shortly thereafter it became pandemic, infecting more than 240 million people all over the globe (WHO - October 2021). Both academia and industry directed most of their efforts through the identification of vaccines. While the vaccines drastically stemmed the pandemic, new drugs active against RNA viruses are still an urgent need. In fact, the access to vaccination and the emerging of novel SARS CoV-2 variants poses a serious threat to how the massive immunization might last.
In this context, we planned screening of a selected set of compounds from our in-house library to evaluate the SARS-CoV-2 entry inhibition. The selected molecules were previously proved as antiviral compounds by inhibiting the entry of other RNA viruses. Starting from this point, we analyzed the effect of our compounds on SARS-CoV-2 entry mechanism.
For this purpose, we generated a spike-pseudotyped lentiviral vector (LV) and an ACE2-expressing HEK 293T cell line. LVs were obtained using GFP or Luciferase as reporter genes. Then, cells were transduced using LVs in the presence of compounds at two different concentrations (5 and 0.5 µM). The percentage of transduction was evaluated by High Content Confocal screening (GFP+cells/total) and by luminescence analysis (luciferase expression).
Our screening selected the compounds RI26, RI94, RI95 4SMA, 10MG and 15MG as moderate inhibitors of SARS CoV-2 entry. Although this effect alone would not efficiently prevent SARS CoV-2 infection, the dissection of how these compounds affect the cell metabolism and the subsequent MedChem optimization might lead to the generation of novel antiviral drugs against Coronavirus infections.

A7. Identifying molecules against SARS-CoV-2 and future pandemics collaboratively within an open-access research infrastructure initiative

Bahne Stechmann

[1] *EU-OPENSCREEN*

Poster selected for a flash presentation

The SARS-CoV2 demonstrated that a concerted, collaborative effort is needed to address emerging pandemics quickly. Open access to state-of-the-art technology platforms, research teams with expertise in multiple scientific fields and to research data are crucial to progress drug discovery projects in academia.
As the European research infrastructure for early drug discovery, EU-OPENSCREEN [1-2] supports international scientists in identifying novel chemical probes. Through its 20 academic partner institutes across Europe, it offers complementary expertise and instrumentation for the development of novel chemical probes to study SARS-CoV-2 and emerging pathogens. EU-OPENSCREEN partner sites jointly use a unique diversity compound collection. The primary screening data will be made available to the scientific community through its open-access European Chemical Biology Database. In response to the Covid-19 pandemic, EU-OPENSCREEN is actively involved in a range of activities to study SARS-CoV-2, with a focus on drug repurposing activities and the development of HTS assays for the rapid identification of small molecule modulators of viral proteins [3-4]
A unique per-competitive compound sharing model allows medicinal chemists to make their compounds accessible to a broader scientific community and thereby expose them to a range of biological targets with the aim to uncover novel bioactivities of their compounds. The submitted compounds are screened in suitable bioassays by a wider community of biologists, which would otherwise not be feasible in individual one-to-one-collaborations.
Here we will present an overview of the SARS-CoV-2 projects of EU-OPENSCREEN and explain the opportunities and benefits of the unique per-competitive compound sharing model.

References: [1] Silvestri A, et al., Drug Discov Today. 2021;26(6):1369-1381 [2] Brennecke P, et al., SLAS Discov. 2019;24(3):398-413 [3] Kuzikov M, et al., ACS Pharmacol. Transl. Sci. 2021, 4, 3, 1096–1110 [4] Schuller M, et al., Sci Adv. 2021 Apr 14;7(16):eabf8711

A8. Molecular Dynamics simulation of SARS-CoV-2 main protease reveals role of Q189 loop flexibility in recognition of novel inhibitors

Amgad Albohy[1], Mohamed A. Said[2], Mohamed A. Abdelrahman[2], Hany S. Ibrahim[2]

[1] Department of Pharmaceutical Chemistry, Faculty of Pharmacy, The British University in Egypt (BUE), El-Sherouk City, Suez Desert Road, Cairo 11837, Egypt
[2] Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Egyptian Russian University, Badr City, Cairo, P.O. Box 11829, Egypt

Since the recognition of COVID-19 as pandemic almost 2 years ago, several trials have been taken to find a small molecule antiviral against its causative organism, SARS-CoV-2. This was done through the study of several viral targets such as viral main protease, Papain-like protease, RNA dependent RNA polymerase, methyl transferase, spike protein as well as other targets. SARS-CoV-2 main protease was among the first targets that was crystalized and investigated. In this study, we screened ChEMBL database using in-house generated pharmacophore to find new hits for this important target. Top hits were docked into the protein and investigated using molecular dynamics. The results were compared to dynamics study of the apoprotein and main protease complexed with peptidomimetic inhibitor N3 derived from PDB 6LU7. The flexibility of Q189 loop was found to play a key role in recognizing new inhibitors. This key finding have been seen before with other viral proteases and will play a key role in the design of novel inhibitors against SARS-CoV-2 main protease.

A9. The PADAM oxidation route for the synthesis of SARS-CoV-2 Main Protease inhibitors

Sveva Pelliccia[1], Paola Storici[2], Elisa Costanzi[2], Enzo Tramontano[3], Angela Corona[3], Carmen Cerchia[1], Vincenzo Summa[1]

[1] Department of Pharmacy, University of Naples, Federico II via D. Montesano 49, Napoli, 80131, Italy
[2] Protein Facility, Elettra Sincrotrone Trieste S.C.p.A., SS 14 - Km 163, 5 in AREA Science Park, Trieste, 34149, Italy
[3] Department of Life and Environmental Sciences, University of Cagliari, Monserrato, 09042, Italy

In 2019, SARS-CoV-2 caused worldwide the current outbreak named COVID-19. Despite multiple countermeasures implemented and approved DNA, RNA and protein subunits vaccines an additional step forward has been made thanks to approved drugs targeting the CoV RdRp (e.g. Remdesivir i.v., Molnupiravir p.o.) and the CoV 3CL Protease (Nirmatrelvir) although they suffer of modest efficacy or suboptimal PK properties. It is an urgent global need to identify new direct-acting antiviral drugs (DDAs) against this pathogen and new emerging viruses, in order to prevent the progression to severe disease or new pandemic. In particular, the main protease (M^{pro}) of SARS-CoV-2 is a cysteine protease playing the essential role in viral replication, thus being identified as a solid target for the development of effective antiviral drugs. [1] The knowledge of both catalytic mechanism and substrate specificity of the M^{pro} triggered the idea to exploit multicomponent reactions (MCRs) as a fast and versatile synthetic tool toward novel M^{pro} inhibitors. Accordingly, the Passerini reaction-amine deprotection-acyl migration (PADAM) oxidation route, was employed for the development of novel small peptidomimetic compounds with a ketoamide warhead behaving as covalent reversible inhibitors.[2] The peptidomimetics prepared were evaluated in SARS-CoV-2 M^{pro} biochemical assay and in antiviral cell-based assays, showing IC_{50} and EC_{50} in nanomolar / low micromolar range. Furthermore, X-ray co-crystal structures of protease–inhibitor complexes were determined as a part of this study, revealing the molecular determinants of the interaction with the M^{pro} and providing key hints for further optimization.

References

*[1] a)Cannalire, R.; Tramontano, E.; Summa, V. A Focus on Severe Acute Respiratory Syndrome (SARS)Coronavirus (SARS-CoVs) 1 and 2. Book Series: Methods and Principles in Medicinal Chemistry Chapter 18,Wiley **2022**. [2] a)Banfi,L. , Guanti, G.; Riva, R. Passerini multicomponent reaction of protected a-aminoaldehydes as a tool for combinatorial synthesis of enzyme inhibitors. Chem. Commun., **2000**, 985–986.*

A10. Identification Of Novel Potential Inhibitors Of Dpre1 From *Mycobacterium Tuberculosis* And *Mycobacterium Bovis*

Dariia Samofalova

[1] Life Chemicals Europe GmbH, Leonhardsweg 2, 82008 Unterhaching, Germany
[2] Institute of Food Biotechnology and Genomics Natl. Academy of Sci. of Ukraine

Poster selected for a flash presentation

Tuberculosis is a well-known bacterial infectious disease that has gradually acquired all the hallmarks of social status, both in Ukraine and around the world. From a pharmacological point of view, one of the best approaches is the identification and development of the novel inhibitors targeting unique proteins of the genus Mycobacterium. Such molecular targets, being essential and conservative for mycobacteria genius, lack obvious homologues in humans and animals, therefore limited side toxic effects in patients are expected.

Our project "Creation of new effective inhibitors of Z-ring formation in order to obtain new anti-tuberculosis drugs with antimitotic action" (National Research Foundation of Ukraine: 0120U104882, https://nrfu.org.ua/) is focused on a large-scale examination of different microorganism functions and possibilities of their inhibition in search of novel inhibitors of Mycobacterium tuberculosis and Mycobacterium bovis.

Enzymes, involved in the biosynthesis of fatty acids and peptidoglycans that compose the Mycobacterium cell wall, were considered as highly attractive targets in the frame of this project. In silico structural and biological studies of essential molecular targets related to the mitotic apparatus and cell wall biosynthesis have been performed. Based on the bioinformatics analysis of the known sites of ligand–protein interaction, the reconstruction of target proteins in complex with reference compounds was performed and models for pharmacophore search and docking were built.

Herein, we present docking results of the Life Chemicals proprietary HTS Compound Collection against Mtb DprE1. Inhibition of DprE1 interrupts the cell wall biosynthesis in mycobacteria, leading to cell death. In total, 649 potential DprE1 inhibitors and modifiers were identified. They have become a starting point for the synthesis of new derivatives and analogues and their biochemical evaluation. Moreover, on our previous research some representatives belong to the compound families already reported in the literature as potent DprE1 inhibitors (Balabon O. et al., 2020).

A11. 3-(Adenosylthio)methyl benzoic acid with modified adenosine as SARS-CoV-2 methyltransferases inhibitors

Olga Bobileva, Raitis Bobrovs, Evelina Elva Sirma, Iveta Kanepe

Latvian Institute of Organic Synthesis

Poster selected for a flash presentation

Coronavirus RNAs are protected from degradation by a cap moiety at the 5`-end. Two self-encoded nonstructural proteins nsp14 and nsp16 in complex with nsp10 are responsible for viral RNAs methylation and formation of 5`-cap. In the absence of functional nsp14 or nsp16 methyltransferase, the replication of the virus is diminished as an uncapped viral RNAs are recognized by host cell immune system.[1,2] Since nsp14 and nsp16 are highly conserved proteins among human coronaviruses, SARS-CoV-2 nsp14 and nsp16 inhibitors should have activity also against other existing and possibly future coronaviruses.
Methyltransferases employ S-adenosyl methionine (SAM) as a source of methyl group. We have targeted SAM-binding site and recently reported nanomolar SARS-CoV-2 methyltransferases inhibitor 3-(adenosylthio)methyl benzoic acid (**1**) based on SAM methionine modifications.[3] In the present work, further modifications of compound **1** are described. Based on docking studies, we have designed compounds with additional groups at the adenine moiety to explore chemical space around it. Compound **1** analogues with sulfur atom modifications were prepared to mimic SAM methyl group and explore mRNA binding cavity. Synthesis, structure-activity relationships and cell membrane permeability of a small library of compound **1** analogues will be discussed.

Acknowledgement
This work is supported by European Regional Development Fund project No. 1.1.1.2/VIAA/4/20/747

References

[1] J. Virol. **2013**, 87 (11), 6296–6305.
[2] J. Virol. **2014**, 88 (8), 4251–4264.
[3] ACS Med. Chem. Lett. **2021**, 12 (7), 1102–1107.

A12. Targeted Degradation of Trypanothione Reductase as potential new approach to treat leishmaniasis

Alessandra Salerno[1], Francesca Seghetti[1], Jessica Caciolla[1], Elisa Uliassi[1], Riccardo Ocello[1], Eleonora Testi[1], Ignacio Enrique Tobal Salcedo[2], Melissa Guardigni[3], Eleonora Fiorentino[4], Stefania Orsini[4], Annarita Fiorillo[5], Cécile Exertier[6], Marinella Roberti[1], Matteo Masetti[1], Andrea Milelli[3], Gianni Colotti[6], Veronica Morea[6], Trentina Di Muccio[4], Andrea Ilari[6], Maria Laura Bolognesi[1]

[1] Department of Pharmacy and Biotechnology, Alma Mater Studiorum - University of Bologna, Via Belmeloro 6, 40126 Bologna, Italy
[2] Departamento de Química Orgánica, Facultad de Ciencias Químicas, Universidad de Salamanca. Plaza de los Caídos 1-5, 37008 Salamanca, Spain
[3] Department for Life Quality Studies, Alma Mater Studiorum-University of Bologna, Corso d' Augusto 237, 47921 Rimini, Italy
[4] Dipartimento di Malattie Infettive, Istituto Superiore di Sanità Viale Regina Elena, 299, 00161 Rome, Italy
[5] Department of Biochemical Sciences, Sapienza University, P.le A.Moro 5, 00185 Rome, Italy
[6] Institute of Molecular Biology and Pathology, Italian National Research Council, IBPM-CNR, c/o Department of Biochemical Sciences, Sapienza University, P.le A.Moro 5, 00185 Rome, Italy

Leishmaniasis is a vector-borne neglected disease affecting 12 million people in tropical, subtropical, and Mediterranean areas, which represents a global health concern. Most of the available treatments are inadequate, due to poor efficacy, high toxicity, and emerging resistance. Therefore, new drugs are needed. Trypanothione Reductase (TR) is an attractive therapeutic target, since it is Leishmania-specific and essential. However, most TR inhibitors have low potency and efficacy, likely because of the high TR expression and presence of a large and featureless binding site. (10.3390/molecules25081924). We envisaged that these two drawbacks could be overcome by exploiting the novel therapeutic strategy based on Proteolysis Targeting Chimeras (PROTACs) (10.1016/j.cell.2019.11.031).PROTACs are heterobifunctional small molecules that couple a binder of a protein of interest (POI) to an E3 ubiquitin ligase-recruiting moiety via a suitable linker. The induced proximity between the POI and the E3 ubiquitin ligase elicits ectopic ubiquitination and subsequent degradation of the specific disease-related protein. For this reason, PROTAC-induced TR degradation may be advantageous, although, little is known about structure and role of *Leishmania* ubiquitination machinery. With these concepts in mind, we developed a series of putative TR-directed PROTACs with the dual aim of obtaining innovative therapeutic tools for leishmaniasis and chemical probes for deciphering biochemical components of *Leishmania* ubiquitination machinery. As first step, in silico docking studies have been

performed to rationalize structural derivatization of TR known inhibitors into PROTACs. In parallel, putative Leishmania ubiquitination genes have been identified by bioinformatic analyses. Then, a series of degraders have been synthesized and tested to confirm TR binding and residual inhibitory activity. Mechanistic studies on TR degradation and anti-leishmanial phenotypic assays are ongoing. This project, spanning from basic science to drug discovery, might contribute to solving a big- and still open-question: whether targeted protein degradation can be extended to the anti-parasitic field.

A13. Design and synthesis of encorafenib-based BRAF-V600E degraders

Federica Blua[1], Marco Marino[1], Simone Gastaldi[1], Federica Maione[2], Eleonora Gianquinto[1], Federica Di Nicolantonio[2], Massimo Bertinaria[1]

[1] Department of Drug Science and Technology, University of Turin, Via Pietro Giuria 9, 10125-Torino, Italy
[2] Department of Oncological Sciences, University of Torino School of Medicine, 10060-Candiolo, Italy

Poster selected for a flash presentation

BRAF is a member of the RAF family kinases that play an important role in the RAS-ERK pathway, transducing signals downstream of RAS to MERK and ERK kinases. Dysregulation of this signaling pathway, which normally regulates cell proliferation and survival, is closely related to cancer development and progression, particularly for melanoma, colorectal cancer, thyroid cancer, non-small-cell lung cancer, and hairy-cell leukemia. BRAF mutations, such as BRAF-V600E, which is the most common cancer-causing mutant, are directly associated with aberrant activation of the signaling pathway, thus promoting oncogenesis. Inhibitors of BRAF, such as vemurafenib, dabrafenib, and encorafenib, have shown good results in the clinic for the treatment of melanoma, although their use is short-lived due to the rapid onset of a resistance mechanism that can reactivate the signaling pathway.

A novel BRAF-inhibition technique consists in the use of proteolysis-targeting chimeras (PROTACS), which are bifunctional molecules capable of inducing the degradation of a protein of interest (POI) through the activation of the ubiquitination system. In this work, we designed and synthesized new PROTACS, connecting encorafenib to pomalidomide or lenalidomide through different linkers. In this series of compounds, the linker between the BRAF targeting moiety and the ligase binder has been modulated in length and flexibility to allow for the optimal spatial arrangement of the two protein partners. The ternary complex between one selected compound, BRAF, and cereblon has been modeled to investigate the atomistic details of the interaction. The synthesized compounds, tested on isolated BRAF-V600E, showed IC50 values of 40–88 nM, comparable to that shown by encorafenib (21 nM). Four selected compounds were able to inhibit the proliferation of colon cancer cells (Colo205) and the melanoma cell line (A375) in a concentration-dependent manner with IC50 values in the submicromolar range; tests on the targeted degradation of BRAF-V600E in the same cell lines are being performed.

A14. DeLA-Drug: A Deep Learning Algorithm for Automated Design of Drug-like Analogues

Giuseppe Lamanna[1], Teresa Maria Creanza[2], Pietro Delre[3], Marialessandra contino[4], Nicola Corriero[1], Michele Saviano[5], Giuseppe Felice Mangiatordi[5], Nicola Ancona[6]

[1] CNR - Institute of Crystallography, 70126 Bari, Italy
[2] CNR - Institute of Intelligent Industrial Technologies and Systems for Advanced Manufacturing, 70126 Bari, Italy
[3] Italy; CNR - Institute of Crystallography, 70126 Bari, Italy
[4] Department of Pharmacy - Pharmaceutical Sciences, University of Bari "Aldo Moro", via E. Orabona, 4, I-70125 Bari, Italy
[5] CNR – Institute of Crystallography, Via Amendola 122/o, 70126 Bari, Italy
[6] CNR - Institute of Intelligent Industrial Technologies and Systems for Advanced Manufacturing, Via Amendola 122/o, 70126 Bari, Italy

We present *DeLA-Drug*,[1] a recurrent neural network (RNN) model composed of two Long Short-Term Memory (LSTM) layers and conceived for data-driven generation of drug-like compounds. *DeLA-Drug* captures the syntax of SMILES strings of more than 1 million molecules belonging to the ChEMBL28 database and generates analogues starting from a single user-defined query compound by employing a new strategy called Sampling With Substitutions (SWS). The generative model preserves drug-likeness and synthetic accessibility of the known bioactive compounds belonging to the ChEMBL28 repository. The absence of any time-demanding fine-tuning procedure enables *DeLA-Drug* to perform a fast generation of focused libraries for further high-throughput screening and makes it a suitable tool for performing *de-novo* design even in low-data regimes. *DeLA-Drug*, available as a free web platform (http://www.ba.ic.cnr.it/softwareic/deladrugportal/), can help medicinal chemists interested in generating analogues of compounds already available in their laboratories and, for this reason, good candidates for an easy and low-cost synthesis.

[1] Creanza, T. M.; Lamanna, G.; Delre, P.; Contino, M.; Corriero, N.; Saviano, M.; Mangiatordi, G. F.; Ancona, N. DeLA-Drug: A Deep Learning Algorithm for Automated Design of Druglike Analogues. J. Chem. Inf. Model. ***2022****, 62 (6), 1411–1424. https://doi.org/10.1021/acs.jcim.2c00205.*

A15. Leveraging information from essential oils biological assays through machine learning: PDIA3 as a case study

Lorenzo Antonini[1], Manuela Sabatino[1], Eleonora Proia[1], Filippo Sapienza[1], Giuliano Paglia[2], Margherita Eufemi[2], Fabio Altieri[2], Rino Ragno[1]

[1] Rome Center for Molecular Design, Department of Drug Chemistry and Technology, Sapienza University of Rome, Piazzale Aldo Moro 5, 00185 Rome, Italy
[2] Department of Biochemical Sciences "Alessandro Rossi Fanelli", Sapienza University of Rome, Piazzale Aldo Moro 5, 00185 Rome, Italy

In the last ten years, essential oils (EOs) have been widely investigated as antibacterial agents and, more recently, also evaluated for their potential antiviral and anticancer activity. Although EOs chemical composition is almost always known, a rigorous analysis on the relationship between composition and assay response is often missing. Machine learning (ML) techniques represent a powerful tool to elucidate the assay outcome, highlight patterns in the data, and assign "importance" to EOs chemical components in relation to the observed biological activity. In this work 30 EOs extracted through steam distillation from *Calamintha Glandulosa*, *Foeniculum Vulgare*, *Melissa Altissima*, and *Ridolfia Segetum* were tested to assess their inhibitory effect on protein disulfide isomerase A3 (PDIA3) using a FITC-insulin assay. PDIA3 is an enzyme that catalyzes disulfide bond formation through its thiol-oxidoreductase and protein disulfide isomerase activities. The IC_{50} returned by the assays and EOs chemical compositions were used to train ML binary classification models. Robust statistical models were obtained through extensive Bayesian hyperparameter optimization and using dimensionality reduction methods. Algorithms used spanned from classical logistic regression, support vector machines to decision trees, and gradient boosting. Model's performances were assessed using the Matthews correlation coefficient metric in fitting and cross-validation (20% out). The best model was used to perform a feature importance study by means of a model agnostic method (Permutation Feature Importance) joint with a partial dependence analysis. This approach allowed to identify the components having the highest impact on the experimentally observed PDIA3 inhibition. Most promising compounds were selected and sent to biochemical assay to confirm the predicted importance. This approach is a first attempt to exploit information from EOs biochemical assays making them available for drug design purposes. It is also a first attempt to democratize this fast-growing research field, proposing a workflow for results analysis.

A16. Quantized computational QSAR framework for molecular toxicity virtual screening

Mauro Nascimben[1, 2], Lia Rimondini[1]

[1] *Department of Health Sciences, Università del Piemonte Orientale UPO, Novara, Italy*
[2] *Enginsoft SpA, Padua, Italy*

Spiking neural networks (SNN) are computational learning systems that shape neuron activity from the point of view of membrane potential and synaptic conductance, simulating the behavior of biological cells in the mammal nervous system. They work on spike trains and encode information through the timing of the spikes; thus, weights in SNN are modified according to the timing of information exchange between presynaptic and postsynaptic neurons (Hebbian learning rule). In present investigation, a QSAR-SNN framework has been developed as a virtual screening tool analyzing structural information encoded in molecular fingerprints (MF). MFs are binary sequences that can be passed directly as inputs to SNNs without requiring transformations because their structure resembles the binary encoding of spike trains. Numerical experiments focused on testing SNN architectures and neuron models for classification purposes of molecules on the following benchmarks: toxicity assessment, adverse drug reactions in marketed drugs, and blood-brain barrier permeability. Currently developed architecture employs the leaky-integrate-and-fire neuronal model and a fast sigmoid surrogate derivative to overcome the discrete nature of binary data and accomplish backpropagation. In addition, this trick favors the integration of NN traditional optimizers and loss functions. Input MFs were the short MAACS, architecture was shallow with one hidden layer, and the output of the last SNN layer was converted into a label by assigning class membership to the neuron that fired more spikes. Balanced accuracy was measured on ten rounds with random data splits (train/validation/test). Test outcomes for two-classes toxicity prediction of compounds (nuclear receptor of anti-androgenic effects 98.17±0.24%, ligand-binding domain of estrogen receptor 97.85±0.41%, genotoxicity 98.68±0.25%), permeability to molecules of the blood-brain barrier (93.65±1.0%) and drugs that exhibited toxicity or not (96.02±1.74%) show good precision for harmful activity detection. Future developments of the SNN design will evaluate more advanced neuron models with adaptive and learnable parameters.

A17. Self-explainable Graph Neural Network for Molecular Property Prediction using Concept Whitening

Michela Proietti, Alessio Ragno, Roberto Capobianco

[1] *Dept. of Computer, Control and Management Engineering "Antonio Ruberti", Sapienza University of Rome*
[2] *Sony AI*

Molecular property prediction is a key task in the field of drug discovery. Even if many deep learning architectures have successfully managed to obtain accurate predictions, there is a need to make such models interpretable by humans. In this work, we adapt an explainability method originally developed for convolutional neural networks, called concept whitening, to graph neural networks. This module constrains the latent space to represent some concepts of interest—in our case, several molecular properties—that are then used to make the prediction. In this way, we have an inherently interpretable model which simply takes as input the molecular graph representation. The network is trained and tested using several benchmark datasets from MoleculeNet.

A18. VenomPred: A Machine Learning Based Platform for Molecular Toxicity Predictions

Salvatore Galati[1], Miriana Di Stefano[1, 2], Elisa Martinelli[1], Marco Macchia[1], Adriano Martinelli[1], Giulio Poli[1], Tiziano Tuccinardi[1, 3]

[1] Department of Pharmacy, University of Pisa, 56126 Pisa, Italy
[2] Department of Life Sciences, University of Siena, 53100 Siena, Italy
[3] Center for Biotechnology, Sbarro Institute for Cancer Research and Molecular Medicine, College of Science and Technology, Temple University, Philadelphia, PA 19122, USA

Poster selected for a flash presentation

A rapid and efficient in-silico assessment of the potential toxicity methods plays an important role in the selection of lead compounds and in ADMET studies since in vitro and in vivo methods are often limited by ethics, time, budget and other resources. In this context, we present our new web tool VenomPred, a user-friendly platform for evaluating the potential mutagenic, hepatotoxic, carcinogenic, and estrogenic effects of small molecules. VenomPred platform employs several in-house Machine Learning (ML) models developed with datasets derived from VEGA QSAR, a software that includes a comprehensive collection of different toxicity models and has been used as a reference for building and evaluating our ML models. The results showed that our models achieved equal or better performance than those obtained with the reference models included in VEGA QSAR. In order to improve the predictive performance of our platform, we adopted a consensus approach combining the results of different ML models, which was able to predict chemical toxicity better than the single models. This improved method was thus implemented in the VenomPred platform, a freely accessible webserver that takes the SMILES (Simplified Molecular-Input Line-Entry System) strings of the compounds as input and sends the prediction results providing a probability score about their potential toxicity.

A19. Evaluation of antimicrobial and antitumor activities from *Chamaemelum nobile* (L) All

Marta Barral-Martínez[1], Paula Garcia-Oliveira[1, 2], Lucia Cassani[1, 2], Antia G Pereira[1, 2], Javier Echave[1], Jovana Petrović[3], Marina Soković[3], Tiane C. Finimundy[4], Ricardo Calhelha[4], Jesus Simal-Gandara[1], Lillian Barros[4], Miguel A. Prieto[1, 2]

[1] Nutrition and Bromatology Group, Department of Analytical and Food Chemistry, Faculty of Food Science and Technology, University of Vigo, Ourense Campus, E32004 Ourense, Spain.
[2] Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolo-nia, 5300-253 Bragança, Portugal
[3] Institute for Biological Research "Siniša Stanković"- National Institute of Republic of Serbia, University of Belgrade.
[4] Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolonia, 5300-253 Bragança, Portugal.

Since ancient times plants have been used in natural therapies as a remedy against diseases. However, the use of medicinal plants is growing because they have many compounds of great interest with antioxidant, anti-inflammatory, and antimicrobial properties [1]. In particular, *Chamaemelum nobile* (L) All. belonging to the Asteraceae family is known for treating insomnia, pain, inflammation and also, it has been used in foods, dyes, cosmetics and traditional remedies [2]. In this context, the objective of this work was to evaluate the antimicrobial and antitumor activities of *C. nobile*. For this purpose, plant extracts were obtained through maceration (1 h, 45°C) using methanol (60%) as solvent. Antimicrobial activity was analyzed in terms of the minimum inhibitory concentration (MIC) against different Gram-positive (*Staphylococcus aureus* (ATCC 11632), *Bacillus cereus* (clinical isolate), *Listeria monocytogenes* (NCTC 7973)) and Gram-negative bacteria (*Escherichia coli* (ATCC 25922), *Salmonella* Typhimurium (ATCC 13311) and *Enterobacter cloacae* (ATCC 35030)). Regarding antitumor activity, four human tumor cell lines were used (gastric adenocarcinoma (AGS), colorectal adenocarcinoma (Caco-2), breast adenocarcinoma (MCF-7), and non-small cell lung cancer lines (NCI-H460)). Results suggested that *C. nobile* showed great antibacterial activity with MIC values ranged between 0.25 and 0.5 mg/mL. Regarding antitumor activity, this plant exerted significant cytotoxic effects against all tumor cell lines studied. Considering these results, using *C. nobile* to obtain extracts with biological properties could be interesting to be further used during the development of new formulations in the pharmaceutical, nutraceutical, or cosmetic industries.

***References**:*

*[1]. Garcia-Oliveira, M. Fraga-Corral, A. G. Pereira, C. Lourenço-Lopes, C. Jimenez-Lopez, M. A. Prieto, J. Simal-Gandara J. Food Chem (**2020**), 127197.*
*[2]. Garcia-Oliveira, M. Barral-Martinez, M. Carpena, P. Gullón, M. Fraga-Corral, P. Otero, M. A. Prieto, J. Simal-Gandara. Food & Function (**2021**), 12(7), 2850-2873.*

A20. Antimalarial Activity of Novel Substituted *N*-Arylcinnamamides

Dominika Pindjakova[1], Jiri Kos[1, 2], Gilles Degotte[3, 4], Tomas Strharsky[5], Pierre Francotte[3], Michel Frédérich[4], Josef Jampilek[1]

[1] *Department of Analytical Chemistry, Faculty of Natural Sciences, Comenius University, Ilkovicova 6, 84215 Bratislava, Slovakia*
[2] *Department of Biochemistry, Faculty of Medicine, Masaryk University, Kamenice 5, Brno 62500, Czech Republic*
[3] *Laboratory of Medicinal Chemistry, CIRM, Department of Pharmacy, University of Liege, Avenue Hippocrate 15, 4000 Liege, Belgium*
[4] *Laboratory of Pharmacognosy, CIRM, Department of Pharmacy, University of Liege, Avenue Hippocrate 15, 4000 Liege, Belgium*
[5] *Regional Centre of Advanced Technologies and Materials, Czech Advanced Technology and Research Institute, Palacky University, Slechtitelu 27, 783 71 Olomouc, Czech Republic*

Malaria is one of the most important infectious diseases. In 2019, there were an estimated 229 million cases of malaria worldwide and the estimated number of malaria deaths stood at 409,000 in this year. To face the development of resistances in *Plasmodium falciparum*, the causative agent of malaria, to all the current antimalarials, there is an urgent need of innovations in the therapeutic arsenal [1]. One of the possibilities is the search for derivatives of natural substances [2]. Derivatives based on cinnamic acid scaffold demonstrate a wide range of significant anti-infective effects [3–5]. However, their antiplasmodial activities are described poorly. Therefore, selected ring-substituted cinnamanilides were investigated as noteworthy small multi-target compounds [6] with the presumed ability to inhibit some of the protozoan's life processes. In vitro evaluation of a library of almost two hundred compounds inspired by mildly active 3,4-dihydroxycinnamic acid (IC_{50} ca. 80 µM) on chloroquine-sensitive strains of *P. falciparum* showed that more than 90 compounds showed IC_{50} 10 µg/mL, and can be considered as highly active antimalarial agents promising for further investigation. In addition, the wide range of tested concentrations (from 100 µg/mL to 1 µg/mL) should allow to define strong and comprehensive structure-activity relationships and subsequently to optimize the structure of the final antimalarial agent.

Acknowledgement: This study was supported by the Slovak Research and Development Agency (APVV-17-0373), grant of Comenius University (UK/320/2022), and by FRS-FNRS (FC23283) & Fondation Léon Frédéricq.

References

1. *WHO – Malaria, https://www.who.int/news-room/fact-sheets/detail/malaria (accessed on 11 October 2021).*

2. *Jampilek, J. Design of antimalarial agents based on natural products. Org. Chem.* ***2017****, 21, 1824–1846.*

3. *Pospisilova, S.; Kos, J.; Michnova, H.; Kapustikova, I.; Strharsky, T.; Oravec, M.; Moricz, A.M.; Bakonyi, J.; Kauerova, T.; Kollar, P.; Cizek, A.; Jampilek, J. Synthesis and spectrum of biological activities of novel N-arylcinnamamides. J. Mol. Sci.* ***2018****, 19, 2318.*

4. *Gaikwad, N.; Nanduri, S.; Madhavi, Y.V. Cinnamamide: An insight into the pharmacological advances and structure-activity relationships. J. Med. Chem.* ***2019****, 181, 111561.*

5. *Strharsky, T.; Pindjakova, D.; Kos, J.; Vrablova, L.; Michnova, H.; Hosek, J.; Strakova, N.; Lelakova, V.; Leva, L.; Kavanova, L.; Oravec, M.; Cizek, A.; Jampilek, J. Study of biological activities and ADMET-related properties of novel chlorinated N-arylcinnamamides. J. Mol. Sci.* ***2022****, 23, 3159.*

6. *Yang, T.; Sui, X.; Yu, B.; Shen, Y.; Cong, H. Recent advances in the rational drug design based on multi-target ligands. Med. Chem.* ***2020****, 27, 4720–4740.*

A21. Antimicrobial activity screening for drug development of *Camellia japonica* flowers (*var.* Conde de la Torre)

Antia G. Pereira[1, 2], Catarina Lourenço-Lopes[1], Jovana Petrović[3], Marina Soković[3], Franklin Chamorro[4], Aurora Silva[5, 6], Rosa Perez-Gregorio[6, 7], Paz Otero[1], Jesús Simal-Gandara[1], Lucia Cassani[6, 8], Lillian Barros[2], Miguel A. Prieto[1, 8]

[1] *Nutrition and Bromatology Group, Department of Analytical and Food Chemistry, Faculty of Food Science and Technology, University of Vigo - Ourense Campus, Ourense, Spain*
[2] *Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal*
[3] *Institute for Biological Research "Siniša Stanković"- National Institute of Republic of Serbia University of Bel-grade, Belgrade, Serbia*
[4] *Nutrition and Bromatology Group, Department of Analytical and Food Chemistry, Faculty of Food Science and Technology, University of Vigo, Ourense Campus, E32004 Ourense, Spain;*
[5] *REQUIMTE/LAQV, Instituto Superior de Engenharia do Porto, Instituto Politécnico do Porto, Rua Dr António Bernardino de Almeida 431, 4200-072 Porto, Portugal;*
[6] *Nutrition and Bromatology Group, Department of Analytical and Food Chemistry, Faculty of Food Science and Technology, University of Vigo, Ourense Campus, E32004 Ourense, Spain*
[7] *LAQV-REQUIMTE Department of Chemistry and Biochemistry. Faculty of Sciences. University of Porto. Rua do Campo Alegre s/n 4169-007 Porto, Portugal*
[8] *Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolonia, 5300-253 Bragança, Portugal*

The continuous increased resistance of pathogenic microorganisms to antibiotics is a serious public health problem worldwide. In the last decades, numerous studies have been focused on exploring and developing effective natural additives with antimicrobial potential based on natural matrices such as plants and new strategies to prevent this multi-resistance. (1). This new research line is in accordance with the increasing consumer demands for more organic and natural products. In this sense, using bioactive molecules from *Camellia japonica* flowers as bio-preservatives appears as a possible alternative since they have shown interesting biological properties (2). Among the bioactive compounds of camellias include, phenolic compounds, anthocyanins, pigments, polysaccharides, and polyunsaturated fatty acids (3). In this work, the antimicrobial activity of *C. japonica* flowers (*var.* Conde de la Torre) was evaluated through determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) against food-related microorganisms. Extracts were obtained by an easy, conventional and profitable extraction method such as maceration (50 °C, 1h) using methanol (60%) as solvent. Results indicated that *C. japonica* flowers (*var.* Conde de la Torre) showed a significant antimicrobial activity against *Escherichia coli* since MIC and MBC values were 0.48 and 0.95 mg/mL, respectively. In addition, for the rest of tested microorganisms, MIC and MBC values were in the range of 0.95-1.90 mg/mL and 1.90-3.80 mg/mL. In conclusion, *C. japonica* flowers (*var.* Conde de la Torre) showed potential to be used as antimicrobial agent with promising applications in the food and pharmaceutical industries.

A22. Ingol derivatives as PKC-modulating compounds to promote neurogenesis.

Fátima Vela[1], Ricardo Gómez Oliva[2], Abdellah Ezzanad[1], Sergio Martínez Ortega[2], Antonio J. Macías-Sánchez[1], Carmen Castro[2], Rosario Hernández-Galán[1]

[1] Departamento de Química Orgánica and Instituto de Investigación en Biomoléculas (INBIO), Facultad de Ciencias, Universidad de Cádiz, 11510 Puerto Real, Cádiz (Spain)
[2] Área de Fisiología, Facultad de Medicina, Universidad de Cádiz and Instituto de Investigación e Innovación Biomédica de Cádiz (INiBICA), Cádiz (Spain)

Neurodegenerative, cerebrovascular, or traumatic injuries in the central nervous system produce irreversible neuronal loss [1] for which there is no effective treatment so far. It is known that the brain has the capacity to generate new neurons from neural stem cells, but only in an adequate neurogenic environment [2]. Consequently, the discovery of new drugs that generate such an environment is of great interest.
In previous works, we reported the capacity of lathyrane-type diterpenes to stimulate the proliferation or differentiation of neural precursor cells (NPC) through the activation of classical or novel PKC isozymes [3]. Small differences in the substitution pattern of this macrocyclic scaffold produce significant changes in its effect on NPCs.
This communication describes the development of a library of ingol-type lathyranes through molecular derivatization, directed toward the establishment of structure–activity relationships regarding their neurogenesis-promoting activity.

*[1] Blennow, K., Hardy, J. & Zetterberg, H. The neuropathology and neurobiology of traumatic brain injury. Neuron **76**, 886–899 (2012).*
*[2] a) Jin, K. et al. Neurogenesis in dentate subgranular zone and rostral sub-ventricular zone after focal cerebral ischemia in the rat. Proc. Natl Acad. Sci. USA **98**, 4710–4715 (2001); b) Fallon, J. et al. In vivo induction of massive proliferation, directed migration, and differentiation of neural cells in the adult mammalian brain. Proc. Natl Acad. Sci. USA **97**, 14686–14691 (2000).*
*[3] a) Murillo-Carretero, M. et al. ELAC (3,12-di-O-acetyl-8-O-tigloilingol), a plant derived lathyrane diterpene, induces subventricular zone neural progenitor cell proliferation through PKCb activation. Br. J. Pharm. **174**, 2373–2392 (2017); b) Flores-Giubi, E. et al. Lathyrane, premyrsinane, and related diterpenes from Euphorbia boetica: effect on in vitro neural progenitor cell proliferation. J. Nat.Prod. **82**, 2517–2528 (2019); c) Domínguez-García, S. et. al. A novel PKC activating molecule promotes neuroblast differentiation and delivery of newborn neurons in brain injuries Cell Death and Disease **11**, 262-281 (2020).*

A23. Prenylated derivatives of *trans*-cinnamic acids effective against clinical Fusarium spp. responsible of onychomycosis infection

Paola Carta[1], Maria Antonietta Dettori[1], Davide Gaetano Fabbri[1], Safa Oufensou[2], Stefano Casalini[2], Wiem Chtioui[2], Virgilio Balmas[2], Quirico Migheli[2], Giovanna Maria Delogu[1]

[1] Institute of Biomolecular Chemistry – National Research Council, IT-07100 Sassari, ITALY
[2] Department of Agricultural Science, Sect. of Plant Pathology - University of Sassari, IT-07100 Sassari, ITALY

Trans-cinnamic acids are widely distributed in fruits (e.g., apple and orange), vegetables (e.g., bean, potato, and onion), and cereals (e.g., maize and wheat bran) [1]. They occupy a key place as intermediates in the synthesis of pharmaceuticals, dyes, flavorings, cosmetics, thermoplastics, and materials. Among cinnamic acids, *p*-coumaric acid, caffeic acid and ferulic acid consist of a *trans*-α,β-unsaturated carboxylic chain bonded to a phenol, catechol, and guaiacyl unit, respectively. They belong to the class of phenolic compounds considered the most desirable food components because of their excellent antioxidant activity and nutraceutical properties and therefore they find wide-ranging application in medicine and agriculture in virtue of their antimicrobial, anti-inflammatory and antitumoral activities [2]. Onychomycosis, a chronic nail fungal infection caused by *Fusarium* spp., is an important public health concern being responsible for disseminated infections, particularly in patients undergoing cancer therapy or those affected by immunological deficiency. With the aim to identify novel antimicrobial agents and to overcome resistance phenomena due to the massive use of conventional antifungal agents in onychomycosis therapy [3], we have synthetized a series of prenylated derivatives of *p*-coumaric, caffeic and ferulic acids. It is generally acknowledged the efficiency of prenylated phenols in crossing bacterial and fungal membranes, as well as their role in exerting antimicrobial activity [4]. All cinnamic acids derivatives were tested in vitro on six *Fusarium* spp. isolates particularly those belonging to three species complexes: *F. oxysporum, F. solani,* and *F. fujikuroi*; significant fungal growth inhibition on all *Fusarium* strains was observed evidencing a compound, namely *p*-coumaric acid 3,3'-dimethyl allyl ester, as the most active one [5].

Bibliography

[1] J. Agric. Food Chem. ***2012****, 60, 44; [2] J. Sci. Food Agric.* ***2020****, 100, 483; [3] J. Fungi* ***2017****, 3, 18; [4] Trop. J. Pharm. Res.* ***2014****, 13(2), 307; [5] Molecules* ***2021****, 26, 658.*

A24. Therapeutic potential of natural products and thiols in maternal and child diseases

Marília O. F. Goulart[1], Tauane Santos Rocha[1, 2], Jadriane Almeida Xavier[1, 2], Monika Oliveira[1, 2], Antonio Euzebio Goulart Santana[1, 3], Rayane Martins Botelho[1, 4], Keyla Nobre Pires[1, 4], Jaqueline C. Santos[1, 4], Monique A. Vila Nova[1, 4], Karen Borbely[1, 5], Camilla Mendes Gonçalves[1, 4], Alexandre URBAN Borbely[1, 4], Marilene Tenório Fragoso[1, 2], Raphaela Costa Ferreira[1, 6], Fabiana Andrea Moura[1, 5], Alane Menezes de Oliveira[1, 5]

[1] UNIVERSIDADE FEDERAL DE ALAGOAS
[2] Instituto de Química e Biotecnologia, Maceio, Alagoas, Brasil, 57072-970
[3] Centro de Ciências Agrárias, Maceió, Alagoas, Brasil
[4] Instituto de Ciências Biológicas e da Saúde, Laboratório de Biologia Celular, CEP 57072-970 Maceió, AL, Brazil
[5] Faculdade de Nutrição, UFAL, Maceió, Alagoas, BRazil
[6] Instituto de Ciências Biológicas e da Saúde, CEP 57072-970 Maceió, AL, Brazil

Essential aspects concerning maternal-child health during pregnancy are related to pre-eclampsia and ZIKA Virus (ZIKV). The last one infects the developing central nervous system cells and causes severe anatomical, physiological and cognitive alterations. In this context, preventing placental infection by ZIKV is vital to protect the fetus. As such, the present work aims are divided into two parts. The first one compares the redox imbalance and inflammation biomarkers in the placenta and umbilical cords of pregnancies with and without pre-eclampsia (PE). The second one addresses the investigation of natural products, which may inhibit ZIKV infection of trophoblast cells, an excellent model to test ZIKV molecular interactions and potential interveners. The higher levels of GSH and GPx and the lower levels of IL-6 and TNF-α found in the PE placenta, and umbilical cord may result from adaptive mechanisms to maintain the oxidative and inflammatory balance, corroborating the positive influence of low-molecular-weight thiols. The potential of some natural extracts against ZIKV infection was evaluated using an *in vitro* method. Polyphenol-rich ethanolic extracts obtained from peels and whole fruits of pink pepper (*Schinus terebinthifolius*) and *Passiflora edulis* seed extract could reduce ZIKV infection and modulate placental responses to protect them. The supernatant viral load and cellular viral RNA were detected by qRT-PCR, while cellular protein NS1 amount was detected by flow cytometry and immunofluorescence. Proliferation was accessed by Ki67 immunofluorescence, and cell cycle phases were quantified. Our results also indicated that the extracts in several concentrations were non-toxic and remarkably decreased the viral load of both MR766 and PE243 strains in cellular supernatants, besides other effects on specific targets. In conclusion, the results unveil the role of antioxidants in protecting maternal-child health, adding value to familiar farm products.

A25. Total oligomeric flavonoids (TOF) of the herb tubers Cyperus rotundus induce growth inhibition, antioxidant activity and apoptosis in different cancer cell lines.

Zaynab Abdulghany, Noah Mahmood, Amer Tawfeeq

[1] *iraqi center for cancer and medical genetics research / mustansiriyah university*

Total oligomeric flavonoids (TOF) were extracted from the dry rhizomes of locally harvested Cyprus rotundus. The free radical scavenging activity for the extraction was assessed using DPPH at different concentrations. The treated cells were cultured in the presence of TOF extracts at different concentrations extended from 50µg/ml to 1000µg/ml with 50µg/ml increment each time for 24, 48, and 72 h. Cells viability was determined by MTT assay and calculated as a percentage of control untreated cells. extract inhibited all cancer cells proliferation by the range of 67.09% to 52.41% at a concentration of 350 µg/ml (IC50) during an incubation time of 24 h. Cell membrane integrity was destructed in AMGM, AMN3, MCF7, AMJ13, SKOV3, and RD cell lines by 54.50%, 53.67%, 65.55%, 54.43%, and 45.77%. While the REF and Vero cells were distracted by 3.5%, and 11.5% respectively after 24 h of TOF treatment at IC50. REF cells did not show any fragments of their DNA with MW lower than 10,000. All AMGM, AMN3, MCF7, AMJ13, SKOV3, and RD cells possessed a smear of DNA fragmentation extended from 3000 to 400. Cells and nucleus morphological changes for apoptosis were evident in AMGM, MCF7, AMJ13, SKOV3, and RD cancer cells when treated with IC50 for 24 have assessed using acridine orange and propidium iodide staining. The distraction of mitochondrial membrane potential for in AMGM, MCF7, AMJ13, SKOV3, and RD cells treated with IC50 of TOF for 24 h indicated of the cells with apoptotic cells phenotype. The treatment with 1000 µg/ml increased the apoptotic cells up to a range of 79.89% to 89.5%. The total antioxidant activity in AMGM, AMJ13, AMN3, and HeLa cells was increased during exposure to TOF at different concentrations. results indicate that TOF of Cyperus rotundus has apparent anti-proliferative activity against cancer cells tested due to the induction of apoptosis and can be claimed to have therapeutic potential for cancer.

A26. Synthesis of the lower binding arm of kibdelomycin, a novel gyr B- and topo IV-inhibitory antibiotic

Manuel Georg Schriefer, Laura Treiber, Rainer Schobert

University of Bayreuth

Poster selected for a flash presentation

Tetramic acids, and their 3-acyl derivatives in particular, are known as highly active compounds with inhibitory effects against cancer, fungi, bacteria and specific kinases and phosphatases. A probable rationale is their structural similarity to inorganic phosphate and their property to sequester biologically essential metal ions via chelate complex formation. Kibdelomycin, a.k.a. amycolamicin, shows a novel and unique mode of antibacterial action. It sits like a horseshoe in the bacterial gyrase B or topoisomerase IV and inhibits the binding of ATP. The sites and pockets kibdelomycin binds to are not addressed by any other commercial gyrase or topoisomerase inhibitory antibiotics, which accounts for its efficacy against resistant germs such as MRSA or other Gram-positive bacteria. Our synthesis of kibdelomycin will shed some light on the contributions of the individual fragments (amykitanose, tetramic acid, decalin, amycolose) to its mode of action and activity as an antibiotic. As the amycolose part shows cytotoxicity, it is interesting to elucidate how its connection to the other moieties will affect the antibiotic potency. Our synthetic approach starts from fragments, which are then coupled in a convergent way, finally resulting in the first total synthesis of kibdelomycin. Our synthesis of the amycolose part follows the biosynthesis starting from glucose, adding a C_2-synthon, and establishing the amine which builds the amide with the chlorinated pyrrole. Another crucial step is the beta-selective glycosylation of the 2-deoxy-sugar amycolose with the decalin fragment, which itself is to be built up via a stereoselective Diels-Alder cycloaddition. We will test the resulting advanced coupling product for antibacterial activity, as it already features the amycolose part, which occupies the ATP-binding pocket of bacterial gyrase and topoisomerase IV, and the nonpolar decalin part which shows van-der-Waals-interactions with the enzyme in this region.

A27. Design, synthesis and biological evaluation of new RNA ligands targeting miR-210: modulation of the circadian clock for cancer chemotherapy

Marc Panosetti[1], Audrey Di Giorgio[2], Benedetto Grimaldi[3], Maria Duca[2]

[1] Institute of Chemistry of Nice (ICN) - Université Côte d'azur and Istituto Italiano di Tecnologia (IIT)
[2] Institute of Chemistry of Nice (ICN) - Université Côte d'azur
[3] Istituto Italiano di Tecnologia (IIT)

Poster selected for a flash presentation

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. Numerous observations support 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. Notably, the expression of MNT under diverse conditions, such as hypoxia and cancer, appears to be regulated by miR-210[2]. The laboratory of Dr. Duca (ICN) has experience in the design of multimodal small molecules targeting miRNAs. 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 circadian activity through the targeting of the miR-210/MNT axis. The molecules generated represent a valuable pharmacological tool for studying the role of miR-210 in circadian clock regulation. Moreover, these molecules provide suitable chemical scaffolds for the development of innovative clock modulators for treating circadian-related pathologies. The anticancer activity of the miR-210 inhibitors will be also assessed.

*[1] L. Ercolani, et al. Pharmacological Research **2015**, 100, 288–295.*
*[2] O. Blaževitš, et al. IJMS **2020**, 21, 2294.*
*[3] D. D. Vo, et al. Chem. Eur. J. **2016**, 22, 5350–5362.*
*[4] D. D. Vo, et al. ACS Chem. Biol. **2014**, 9, 711–721.*

B1. New bioorganometallic-heterocylic compounds based on ferrocene as potential antitumor agents

Miguel Gallardo[1], Rodrigo Arancibia[1], Yosselin Huentupil[1], Rodrigo López[2], Pascal Roussel[3], Natacha Henry[3]

[1] Universidad de Concepción, Concepción, Chile.
[2] Universidad Austral de Chile, Valdivia, Chile.
[3] Université de Lille, Unité de Catalyse et Chimie du Solide, Lille, France

Organic compounds containing 2-pyridinyl or 2-pyrrolyl moieties have been studied as potential antitumor agents [1,2]. Recently, our research group has been involved in the development of organometallic derivatives bound covalently to these bioactive entities through imine bridges as a new class of biological agents.
With the aim to get more insights in this type of compounds, we would like to report the synthesis and characterization of new ferrocenyl sulfonyl hydrazones and diamine with 2-pyrrolyl and 2-pyridinyl entities.
All compounds were isolated as pure samples and characterized by spectroscopic techniques and the structure for one of the compounds was confirmed by X-ray crystallography.
The biological evaluation of the compounds has been carried out against two lung cancer cell lines (H1299 and A549). In the case of the A549 cell line, all compounds exhibited an IC50 greater than 290 µM. On the other hand, the H1299 cell line is more sensitive to this type of compounds, with IC50 values greater than 176 µM.
At present the biological studies as trypanocidal agents are under investigation.

[1] M. Sadia, et. al., Journal of King Saud University - Science, 33 (2021) 101331.
[2] B. Wang, et. al., Bioorganic & Medicinal Chemistry Letters, 27 (2017) 1428.

Acknowledgments: *This work was funded by the FONDECYT-Chile (Project 1190327) and National Agency for Research and Development (ANID), National Doctorate scholarships (Chile/2018 – 21181138)*

B2. [60]Fullerene to Alleviate the Consequences of Cosmic Radiation

Yuri Mackeyev[1], Joseph BK Kim[1, 2], Elena Vishnyakova[3], Sunil Krishnan[1]

[1] *Department of Radiation Oncology, Mayo Clinic; 4500 San Pablo Rd., Jacksonville, FL 32224*
[2] *Division of Radiation Oncology, The University of Texas MD Anderson Cancer Center; 1515 Holcombe St., Houston, TX 77030*
[3] *AGNI Medical LLC (Florida L20000071772)*

Our work addresses the C_{60}-ser, a [60]fullerene derivatized with serinol, which we found to be an outstanding radioprotector and mitigator of space radiation-induced health risk. Having an exceptional water solubility, contrary to its parent C_{60} compound, it readily traverses physical barriers within tissues and cells to achieve excellent concentrations in all tissues within the body, and protects cells against proton radiation-induced damage. We evaluated the magnitude of, the mechanisms of, and the range of cellular phenotypes, protection by C_{60}-ser from proton irradiation injury in epithelial, endothelial, neuronal and mesenchymal cells (HepG2, HUVEC, N2A, and NIH3T3 cells used as prototypes) with a clinical proton irradiator. Our results confirmed that (i) even 100 µM doses C_{60}-ser is non-toxic, (ii) C_{60}-ser protects cells from radiation injury as shown by clonogenic survival assays, (iii) protects via scavenging radiation-induced free radicals by reduced reactive oxygen species, (iv) the oxidative stress inhibition also results in mitochondrial metabolic reprogramming and decreased levels of unrepaired DNA damage. Our result presents a compelling story that C_{60}-ser is a strong radioprotector and even more powerfully demonstrates that there may be convergent benefits to using this agent during space travel because it counteracts the common mechanistic pathway of unchecked oxidative stress that underlies carcinogenesis, early and late central nervous system effects, cardiovascular diseases, and accelerated aging seen with long duration space travel.

B3. Design, synthesis, and bioactivity evaluation of novel cinnoline derivatives as antitumor agents

Raid Abdel-Jalil[1], Eman Mahmoud[2], Raphael Stoll[3], Ebrahim Saeedian Moghadam[1]

[1] *Sultan Qaboos University*
[2] *Hashemite University*
[3] *Ruhr-Universität Bochum*

Cancer is a globally health problem because of the severity and drug resistance in many cases and therefore there is an urgent need to design and develop new anticancer agents.
Herein, we report the synthesis of novel 3-(4-substituted-1-piperazinyl)-4-methylbenzo[*h*]cinnoline derivatives following a multi-step synthesis protocol. The reaction of naphthylamine with α-chloroacetylacetone affords a new hydrazonyl chloride. The reaction of latter with some piperazine derivatives yields 1-(4-substituted-1-piperazinyl)-1-(naphthylhydrazono)-2-propanone which upon cyclization, using poly phosphoric acid , furnishes 3-(4-substituted-1-piperazinyl)-4-methylbenzo[*h*]cinnolines in good yields. The structures of the prepared compounds were confirmed by different spectroscopic techniques and and four new compounds were further confirmed by X-ray single crystal diffraction technique. The primary cytotoxicity assay of selected compounds were investigated against NCI-60 Human Tumor Cell Line at a single high dose concentration. Among the tested compounds , one intermediate was further evaluated for five dose criteria at five different minimal concentrations against the full panel of the 60 human tumor cell lines which exhibited activity against leukemia, Melanoma and Renal cancer and some selectively for lung cancer.

B4. Novel bis-dipyridothiazines - synthesis and *in silico* analysis

Emilia Martula, Beata Morak-Młodawska, Małgorzata Jeleń

[1] *Department of Organic Chemistry, Faculty of Pharmaceutical Sciences, The Medical University of Silesia in Katowice, Jagiellońska 4, 41-200 Sosnowiec, Poland*

Phenothiazines are an important class of heterocyclic compounds with wide spectrum of biological properties. Recent reports have shown promising anticancer, antiplasmid, antibacterial, anti-inflammatory and immunosuppressive activities for classical and new phenothiazines [1]. Previously synthesized dipyridothiazine derivatives (1,6-, 1,8-, 2,7- and 3,6-diazaphenothiazines) were shown to possess interesting antiproliferative, anticancer, antioxidant and immunosuppressive activities [2-7]. In continuation of our search we obtained new derivatives of dipyridothiazines – bis-dipyridothiazines in the reactions of selected dipyridothiazines with a,a`-dichloro-p-xylene, a,a`-dichloro-o-xylene, 2,6-bis(chloromethyl)-pyridine, in the presence of sodium hydride.
Using 1H and 13C NMR, two-dimensional spectroscopy (1H-1H COSY, ROESY, HSQC, HMBC), mass spectrometry (HR MS) the right structure of the products was determined. For all new compounds, preliminary pharmacokinetic and lipophilicity studies were performed using available Internet servers: SwissADME, SwissTargetPrediction, and PASS; showing promising properties [8,9]. Drug-likeness properties of novel compounds were evaluated using a predictive bioavailability radar model from the SwissADME web tool. The descriptors of physicochemical properties for selected compounds were projected next on the optimal range for each property to be considered drug-like. Further studies of biological activity have been planned.

References

[1] K. Pluta, B. Morak-Młodawska, M. Jeleń, Eur. J. Med. Chem., 138, 774 (2017).
[2] B. Morak-Młodawska, K. Pluta, A. N. Matralis, A. P. Kourounakis, Archiv Pharm., 343, 268 (2010).
[4] B. Morak-Młodawska, K. Pluta, M. Zimecki, M. Jeleń, J. Artym, M. Kocięba, Med. Chem. Res. 24, 1408 (2015).
[5] B. Morak-Młodawska, K. Pluta, M. Latocha, K. Suwińska, M. Jeleń, D. Kuśmierz. J. Enzyme Inhib. Med. Chem. 31 (2016) 1512-1519.
[6] J. Zhang, M. Chen, Z. Wenzhi, P. N.P. Okechukwu, B. Morak-Młodawska, K. Pluta, M. Jeleń, A. Md Akim, K-P. Ang, K.K Ooi. Drug Des. Develop. Ther., 11, 3045–3063 (2017).
[7] B. Morak-Młodawska, K. Pluta, M. Jeleń, M. Latocha, D. Kuśmierz, Molecules, 24, 267 (2019).
[8] http://www.swissadme.ch/
[9] http://www.way2drug.com/passonline/

B5. Novel, potent and selective drug-like butyrylcholinesterase inhibitors with antiagregating properties

Anna Pasieka[1], Dawid Panek[1], Paula Zaręba[1], Gniewomir Latacz[1], Alba Espargaró[2, 3], Raimon Sabaté[2, 3], Anna Więckowska[1], Barbara Malawska[1]

[1] Faculty of Pharmacy, Jagiellonian University Medical College, Cracow, Poland
[2] Department of Pharmacy and Pharmaceutical Technology and Physical-Chemistry, School of Pharmacy and Food Sciences, University of Barcelona, Barcelona, Spain
[3] Institute of Nanoscience and Nanotechnology (IN2UB), Barcelona, Spain

Forgetting is the main symptom of Alzheimer's disease (AD) – a progessive neurodegenerative disorder affecting millions people around the world. This memory loss is mainly caused by disturbances observed in cholinergic neurotransmission. Butyrylcholinesterase (BuChE) is one of the crucial enzymes responsible for maintaining of this homeostasis. BuChE is a serine hydrolase catalyzing the hydrolysis of choline and non-choline esters. For many years, the role of BuChE in the cholinergic neurotransmission was considered as irrelevant. Nowodays, more and more often scientists empathize its role in the hydrolysis of acetylcholine (ACh) - a neurotransmitter linked to cognitive functions when acetylcholinesterase (AChE) is absent or insufficient. It is observed that in the progression of AD the level of BuChE significantly increases (120% of normal values) and thus, the activity of AChE is reduced [1]. Besides this, the aggregation of protein tau and amyloid-β are considered as primary causes of AD development. Thus, we decided to obtain new selective BuChE inhibitors with antiaggregating properties as promising anti-AD agents.

Based on our previous results [2,3], we designed and synthetized a novel series of hydroxyetylenamine derivatives containg fluorene scaffold. All of them fulfilled the druglikeness criteria. Among them, we identified cycloheptyl derivative **15** (*N*-(3-((cycloheptylmethyl)amino)-2-hydroxypropyl)-9*H*-fluorene-9-carboxamide) with a high inhibitory potency against *eq*BuChE (IC_{50} = 38 nM). We performed a resolution of racemic mixture to pure enantiomers and determined the influence of chirality on the biological properties. Moreover, we evaluated antiaggregating properties against protein tau and amyloid-β using recombinant *Escherichia coli* cells and conducted preliminary ADME-tox *in vitro* assessment.

[1] Giacobini E., Neurochem. Res. 28 (2003) 515–522.
[2] Panek D et al. ACS Chem Neurosci 9(5) (2018) 1074-1094.
[3] Pasieka A et. al. Eur. J. Med. Chem. 218 (2021) 113397.
Acknowledgments: This work was supported by NCN grant 2020/37/N/NZ7/01081.

B6. Selective inhibition of cancer cell proliferation by the action of styrylcarbamates

Amelia Bou Puerto, Miguel Carda, Eva Falomir

[1] *Departamento de Química Inorgánica y Orgánica, Universitat Jaume I, E-12071 Castellón, Spain*

In previous studies, we established that styryl carbamates are good scaffolds for the development of new anticancer multitarget drugs. Based on this scaffold, we designed small molecules to target PD-L1, a transmembrane protein that is able to control the body's immune responses, including those towards cancer cells. The blockage of interactions between PD-L1 and its receptor, PD-1, reactivates the T-cell anti-tumor response, so this is a good anticancer target for the design of new drugs.

Here, we present a series of 18 styrylcarbamates that have been synthesised and characterized. The IC_{50} values of all the compounds were determined in four human cell lines: three cancer cell lines (HT-29, A-549, and MCF-7) and a non-cancer cell line (HEK-293). In general, these compounds showed IC_{50} values above 90 µM in HEK-293, and all of them were found to be cytotoxic in at least one cancer cell line. Next, we selected five derivatives based on their good selectivity rates as inhibitors of cancer proliferation, to study their effect on PD-L1. We performed a preliminary assay in A-549 cells co-cultured with defensive Jurkat T cells, which showed promising results.

B7. Structure-based molecular modeling approach in search for multi-target-directed ligands blocking phosphodiesterases 4B, 8A and TRPA1 ion channel with a potential application in the treatment of asthma and COPD

Alicja Gawalska, Adam Bucki, Marcin Kołaczkowski

[1] *Department of Medicinal Chemistry, Jagiellonian University Medical College, ul. Medyczna 9, 30-688 Kraków*

Asthma and COPD are characterized by complicated pathophysiology associated with chronic inflammation, bronchoconstriction, and bronchial hyperresponsiveness resulting in airway remodeling. The currently available therapies do not address all of the most important pathological processes in the course of both diseases. Therefore, it is an urgent need to work out the comprehensive solutions affecting pathological processes of both diseases. The development of multi-target-directed-ligands (MTDLs) gives the potential to broaden theraputic spectrum and cumulatively stronger effect.

The aim of the study is to develop a comprehensive strategy to search for MTDL blocking PDE4B/PDE8A/TRPA1, using structural models of these proteins, allowing the selection of novel MTDL chemotypes.

To this end, the properly prepared and validated structural models of biological targets—PDE8A, PDE4B, TRPA1—were used. All structural models were optimized with the known inhibitors and antagonists. In the case of TRPA1 and PDE8A, the optimization of their models was followed by the prediction of the binding mode of the inhibitors, HC-030031 and PF-04957325, respectively. These models were evaluated in retrospective virtual screening (RVS) and are characterized by following parameters: $EF1\%_{PDE8A/PDE4B/TRPA1}$=31/10/33,$BEDROC(\alpha=20)_{PDE8A/PDE4B/TRPA1}$=0.901/0.243/0.785. The dynophores were prepared for each biological target and validated in RVS ($EF1\%_{PDE8A/PDE4B/TRPA1}$=54.3/15.8/76.8, $AUC_{PDE8A/PDE4B/TRPA1}$= 1/0.89/0.98). On their basis, virtual screenings, composed of two stages: fitting to the dynophore-hypotheses and docking to the structural models, were performed. A common group of compounds placed within top results, were selected as potential novel chemotypes of MTDLs.

Further studies focused on purchase of selected compounds and in vitro activity tests, will be performed to provide reliable results to aid the discovery of PDE4B/PDE8A/TRPA1 MTDLs.

Financially support: National Science Centre, Poland (grant no. 2020/37/N/NZ7/02365).

B8. Synthesis and biological evaluation of triazole derivatives as inhibitory agents of cancer multitargets

Alberto Pla-López, Eva Falomir, Miguel Carda

[1] *Departamento de Química Inorgánica y Orgánica, Universidad Jaume I, E-12071 Castellón, Spain*

In order to develop new anticancer treatments, PD-L1[1] (Programmed Death Ligand-1) and VEGFR-2[2] (Vascular Endothelial Growth Factor Receptor-2) have become two relevant targets due to their importance in disturbing the tumor microenvironment by promoting the action of immune cells and avoiding the formation of new vascular vessels, respectively. According to preliminary docking studies, small molecules containing a triazole moiety in their structures could establish interactions in the active center of PD-L1. Besides, previous studies have shown that the oncogenic transcription factor c-Myc is the responsible for the upregulation of VEGFR-2 and PD-L1 in cancer cells. As a consequence, a group of small triazole derivatives have been designed and synthetized by using Click Chemistry[3] as strategy to obtain them starting from an alkyne and an azide. After this, it has been tested these derivatives in different cancer cell lines (HT-29, A-549 and MCF-7). Specifically, it has been determined its cellular viability in all three cell lines and the interaction with the biological targets PD-L1, VEGFR-2 and c-Myc. Results have shown promising inhibitory capacity for some derivatives in the three targets.

[1] Yu, X.; Gao, R.; Li, Y.; Zeng, C. Regulation of PD-1 in T cells for cancer immunotherapy. Eur. J. Pharmacol. ***2020****, 15, 881:173240.*
[2] Lian, L.; Li, X. L.; Xu, M. D.; Li, X. M.; Wu, M. Y.; Zhang, Y.; Tao, M.; Li, W.; Shen, X. M.; Zhou, C.; Jiang, M. VEGFR2 Promotes Tumorigenesis and Metastasis in a Pro-Angiogenic-Independent Way in Gastric Cancer. BMC Cancer ***2019****, 19 (1), 479–489*
[3] Hou, J.; Liu, X.; Shen, J.; Zhao, G.; Wang, P.G.(2012) The impact of click chemistry in medicinal chemistry. Expert Opinion on Drug Discovery ***2012****, 7:6, 489-501.*

B9. Triorganotin derivatives act as metabolic inhibitors towards oral squamous cell carcinoma (OSCC) cells through suppression of glucose uptake.

Beatrice Macchi[1], Elena Valletta[2], Antonella Minutolo[2], Claudai Matteucci[2], Franca Cordero[3], Oscar Daniel Cicero[4], Greta Petrella[5], Francesca Marino-Merlo[6], Antonio Mastino[7]

[1] Dept of Chemical Science and Technology University of Rome Tor Vergata
[2] Department of Experimental Medicine University of Rome Tor Vergata Rome Italy
[3] Department of Organic Chemistry Ugo Schiff University of Florence Italy
[4] Department of Chemical Science and Technology, University "Tor Vergata", Rome, Italy
[5] 1Department of Chemical Science and Technology, University "Tor Vergata", Rome, Italy
[6] Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina
[7] Institute of Translational Pharmacology, C.N.R., Rome Italy

Chemotherapy has uncovered the vantage to use metal-derived platinum complex for the treatment of a number of solid tumors. However, the systemic toxicity and the drug resistance of the developed drugs have encouraged the research for new similar compounds. Recently organotin derivatives have been investigated as promising anti- cancer drug candidates. The proliferation/growth inhibition of adherent cell lines in vitro by organotin derivatives was mostly ascribed to induction of cell death or autophagy. Nevertheless, the impact of organotin ligand structure and the influence of dose on organotin toxicity has not been fully clarified. In the present study the biological activity of a newly synthetized tributyltin trifluoroacetate (TBT-OCOCF3), in comparison with commercial Bis(trbutyltin) Oxide (TBT-O), Tributyltin Chloride (TBT-Cl) and cis-Diaminedichloroplatinum (Cisplatin), was assayed in oral squamous cell carcinoma (OSCC) CAL-27 tumor cells, and in the non tumorigenic cell lines MCF10A. The results showed that CAL-27 cell line was more susceptible to TBT-OCOF3 toxicity and metabolic inhibition with respect to Cisplatin. This was not owed to an interaction with DNA, as revealed through NMR investigations, but rather to inhibition of glucose uptake. Only a small percentage of the cells underwent apoptotic cell death, rather mostly underwent a not well-identified form cell death resembling necrosis, at 24 hours after treatment with organotin derivatives, in a dose-dependent effect fashion. The evaluation of cell death in the presence of specific inhibitors suggests that organotin compounds induced cell death takes place at an early time post treatment and in response to low concentrations of the compounds, and that an autophagic and/or necroptotic responses are involved in the occurring phenomenon. Further investigation is in progress to clarify these aspects. Results of this study suggest that a combination of organotin derivatives with specific inhibitors of cell signaling might be a promising treatment versus tumor cells.

B10. *In silico* and *in vitro* evaluation of ASP9521 – a compound supporting anticancer activity of daunorubicin through the inhibition of aldo-keto reductase 1C3 and carbonyl reductase 1.

Marek Jamrozik[1], Kamil Piska[2], Adam Bucki[1], Paulina Koczurkiewicz-Adamczyk[2], Benedykt Władyka[3], Elżbieta Pękala[2], Marcin Kołaczkowski[1]

[1] Department of Medicinal Chemistry, Jagiellonian University Medical College, Medyczna 9 st, Kraków, Poland
[2] Department of Pharmaceutical Biochemistry, Jagiellonian University Medical College, Medyczna 9 st, Kraków, Poland
[3] Department of Analytical Biochemistry, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Gronostajowa 7 st, Kraków, Poland

The search for effective anticancer drugs is one of the biggest challenges of modern pharmacotherapy. Anthracycline antibiotics (ANT) are among the most widely used group of anticancer drugs. Unfortunately, ANT metabolism (two-electron reduction of a carbonyl moiety) performed mainly by carbonyl reductase 1 (CBR1) and aldo-keto reductase 1C3 (AKR1C3), leads to the formation of metabolites with no activity. Inhibition of the metabolism can lead to improvement of the pharmacological action of ANT [1].
We observed a structural similarity of ASP9521 (potent AKR1C3 inhibitor tested in monotherapy of prostate cancer [2]) to CBR1 inhibitors and assessed its potential to interact with this enzyme in series of *in silico* simulations. The compound was docked to the CBR1 model (PDB: 1WMA), previously optimized using structures of reference inhibitors and validated in retrospective virtual screening ($BEDROC_{\alpha=20}$: 0.690; $EF_{1\%}$: 18). The binding mode was in line with the one for known CBR1 inhibitors (H-bonds with Ser139, Tyr193, Met234; π-π interaction with Trp229). The stability of ASP9521 within enzyme's active site was observed in 20 ns molecular dynamics simulation. Finally, inhibitory activity was confirmed in enzymatic assay with purified CBR1 (IC_{50} = 44 µM). Dual AKR1C3-CBR1 activity of ASP9521 was applied in series of *in vitro* experiments, where the compound administered together with various concentrations of daunorubicin (0,05-1 µM) significantly improved cytotoxic activity of the drug on A549 and HepG2 cancer cells.
This study presents a new concept of overcoming drug resistance to ANT using inhibitors of two crucial metabolic enzymes – AKR1C3 and CBR1. Further studies on structure-activity relationship are necessary to obtain inhibitors with more balanced and potent activity.

Acknowledgements:
This study was funded by National Science Centre's grant 2020/37/N/NZ7/01097.

[1] Koczurkiewicz-Adamczyk et al.; Eur J Pharm Sci. 2020 Nov 1;154:105511,
[2] Loriot et al.; Invest New Drugs. 2014 Oct;32(5):995-1004.

B11. Design and synthesis of a novel series of imidazo[1,2-b]pyridazines as antifungals against *Madurella mycetomatis*, the prime causative agent of Eumycetoma

Lamis Yahia Mohamed Elkheir[1, 2, 3], Pierre-Olivier Delaye[3], Wendy van de Sande[4], Pierre Besson[5], Magdi Awadalla Mohamed Elhussein[2], Sébastien Roger[6], Ahmed Hassan Fahal[1], Cécile Enguehard-Gueiffier[3]

[1] *The Mycetoma Research Centre, University of Khartoum, Khartoum, Sudan*
[2] *Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Khartoum, Sudan*
[3] *SIMBA, Faculty of pharmacy , University of Tours, France*
[4] *Erasmus Medical Center, Rotterdam, the Netherlands*
[5] *EA 4245 -Transplantation, Immunologie et Inflammation (T2I), Faculty of Medicine, University of Tours, France.*
[6] *EA 4245 -Transplantation, Immunologie et Inflammation (T2I), Faculty of Medicine, University of Tours, France*

Mycetoma is a neglected invasive infection endemic to tropical and subtropical regions. Presenting as subcutaneous inflammation, mycetoma spreads to involve deep structures of the skin, muscles, and bones, leading to deformities, disabilities, and even mortality. Mycetoma can be caused by either fungi (eumycetoma) or bacteria (actinomycetoma), and in the host, these causative agents are sequestered in grains, a unique feature of mycetoma. Treatment is dependent on the causative agent. Actinomycetoma is treated with antimicrobial agents with high success rates, but eumycetoma is treated with conventional antifungal agents, mainly itraconazole, and surgery with low cure rates and a high incidence of recurrence. Therefore, there is a need to identify novel chemical entities for the treatment of eumycetoma. In this project, we started by in vitro screening a library of 45 compounds with diverse chemical structures against the prime causative agent of eumycetoma *Madurella mycetomatis*. Seven compounds were able to inhibit *M. mycetomatis* growth. Four of them had a common imidazo[1,2-*b*]pyridazine backbone that was used as a core for the hits' library expansion. Using a simple three-step synthetic pathway consisting of classic heterocyclization, palladium catalysed Suzuki cross-coupling, and nucleophilic aromatic substitution, we designed and synthesised 60 diversely pharmacomodulated products with high yields and purity. Nine of these products showed promising in vitro activity against *M. mycetomatis* and good predicted pharmacokinetic profiles for oral administration. Fifteen more products are currently being tested for their in vitro activity. Cytotoxicity evaluation was performed using NIH-3T3 fibroblasts, and all products were less toxic than itraconazole. In vivo testing using *Galleria mellonella* larvae as an experimental model is currently underway. Ultimately, we are hoping to design a product that could move further in the drug development pipeline and potentially be advanced into an effective and safe drug for the treatment of eumycetoma.

B12. Development of activatable fluorophore for use in Super-resolution Fluorescence Microscopy

Aminesena Başer, Sündüs Erbaş Çakmak, Betül Altunkaynak

[1] *Konya Food and Agriculture University*

Activatable fluorophore having control over emission through photocleavage will be developed for Super-resolution Fluorescence Microscopy (SRFM). SRFM enables high-resolution live bioimaging that cannot be obtained by a light microscope. The technique requires specially designed fluorophores. Requirements change depending on sub-technique and reversible/irreversible photo conversion, change in emission (spontaneously or controlled) are some characteristic properties. Up-to-date, fluorophores used for SRFM are proteins, derivatives of rhodamine/cyanine. Increasing variety, developing fluorophores having new photophysical conversions facilitate bioimaging, enable proper fluorophore selection. In the project, a fluorophore activated by photocleavage is proposed to visualize HaloTag-fused proteins. The proposed compound bears modules among which energy transfer occurs. Molecule releases acceptor upon photocleavage by 500-530 nm light. Since energy transfer does not occur to the released acceptor, donor emission increases. Fluorophore activated by photocleavage would be suitable for Photoactivated Localization Microscopy (PALM). Energy-transfer-dependent high Stoke's shift enables the use of molecule in Stimulated Emission Depletion Microscope (STED). In contrast to literature using cytotoxic UV light with low tissue penetration, low energy, biocompatible visible light activates molecules. There are just certain fluorophores for SRFM and there can be innovation for alternative photophysical conversion. In the project, photocleavage-dependent energy transfer as a means of activation mode is proposed. Fluorophore was synthesized by chemical synthesis. The characterizations of the materials were done by nuclear magnetic resonance and mass spectrometry. Preliminary photocleavage, photophysical, and cell culture analysis were performed. The fluorophore will be given to the cell at 10 µM concentration and by MTT assay, the viability of the cell is determined to be above 80%. Protein binding analysis and SRFM cell imaging will be performed.

B13. The Py-ComBinE Web App as a Tool to build Structure-Based Quantitative Models. Application to the BCL-2 Protein family inhibitors.

Manuela Sabatino[1], Lorenzo Antonini[2], Eleonora Proia[2], Rino Ragno[2]

[1] *Sapienza, University of Rome*
[2] *Sapienza, University of Rome.*

Apoptosis is a highly conserved process suicide of cells. The main regulators of this physiological process belong to BCL-2 family, responsible for the mitochondria-mediated pathway and their abnormal regulations have been observed in many cancers [1]. Targeting these proteins with small inhibitors has been a goal in the oncology community, and several efforts have been undertaken to identify new potential anti-cancer compounds. In this study, a structure-based approach was applied on BCL-2/ BCL-X_L Through the revisited Python implemented Wade's comparative binding energy (COMBINE) technique. [2] Using electrostatic (ELE), steric (STE), desolvation (DRY), and hydrogen bonding (HB) ligand–protein per residue interaction energies preliminary models showed encouraging r^2 and q^2 values, indicating a relevant role of the steric interaction. Optimized models were developed by simulating annealing feature selection and external prediction leading to q^2 values ranging from 0.492 (STE) to 0.778 (STE.ELE.DRY.HB). Inspection of the final models led to the rationalizing of the inhibitors selectivity, indicating those residues mainly responsible of the ligand–protein interaction. In particular, PHE39A and TYR43A for the steric ligand–protein interaction and GLU38A for the hydrophobic interactions. Interesting is the residue GLU71A. This, being associated with a negative coefficient in all COMBINE field models, if contacted by a small molecule seems to be able to decrease compound inhibitory capability.

B14. Antidiabetic and antioxidative synergistic potential between zn(ii) and caffeic acid: improving therapeutic potential through complexation

Limpho Martha Ramorobi[1, 2], Godfrey R Matowane[1, 2], Samson S. Mashele[1, 2], Susanna L Bonnet[3], Tshepiso J Makhafola[2], Chika Ifeanyi Chukwuma[2]

[1] Department of Health Sciences, Faculty of Health and Environmental Sciences, Central University of Technology, Bloemfontein, 9301, Free State, South Africa.
[2] Centre for Quality of Health and Living (CQHL), Faculty of Health and Environmental Sciences, Central University of Technology, Bloemfontein, 9301, Free State, South Africa.
[3] Department of Chemistry, Faculty of Natural and Agricultural Sciences, University of the Free State, Bloemfontein, South Africa.

Objectives: In this study the innate bioactivities of zinc(II) and caffeic acid were considered in synthesizing a novel complex with promising antioxidant and anti-hyperglycaemic attributes. **Methods:** The complex synthesis was done using a 2:1 mole ratio between caffeic acid and zinc acetate and structurally characterized using NMR, FT-IR, mass-spectroscopy and HPLC techniques. Its cellular toxicity was assessed in Chang liver cells and L-6 myotubes. In vitro, cellular, and isolated tissue models were used to evaluate the antioxidant and anti-hyperglycaemic properties of the complex, relative to its precursor. **Results:** Zinc(II) and caffeic acid interacted via $Zn:O_4$ coordination, with Zn(II) coordinating with two moieties of caffeic acid. The complex showed in vitro radical scavenging, α-glucosidase and α-amylase inhibitory activity that was up to 2.6 folds stronger than that of caffeic acid. Its ability to inhibit lipid peroxidation (IC_{50} = 26.4 µM) and GSH depletion (IC_{50} = 16.8 µM) in hepatocytes was comparable to that of ascorbic acid (IC_{50} = 24.5 and 29.2 µM) and about 2 folds stronger than caffeic acid. Complexation improved the glucose uptake activity of caffeic acid in L-6 myotubes (EC_{50} = 23.4 versus 169 µM) and isolated rat muscle tissues (EC_{50} = 339 versus 603 µM). The complex was not hepatotoxic and myotoxic. **Conclusions:** Data suggest a synergistic antioxidant and anti-hyperglycaemic interactive potential between zinc and caffeic acid, which could be attributed to the $Zn:O_4$ coordination.

B15. Design and preliminary biological evaluation of dihydro-benzazepine tricyclic derivatives as new NEDD4 covalent inhibitors

Maurizio Pasi[1], Raffaella Amici[1], Adrian Andronache[1], Agostino Bruno[1], Anna Cappa[1], Marco Ciro'[1], Daniele Fancelli[1], Giovanni Faga'[1], Raimondo Fattori[1], Elena Maspero[1], Giuseppe Meroni[1], Sebastiano Pasqualato[2], Michela Robusto[1], Alessia Romussi[1], Luca Sartori[1], Paolo Trifiro'[1], Manuela Villa[1], Stefania Vultaggio[1], Janine Weber[1], Simona Polo[1], Mario Varasi[1], Ciro Mercurio[1]

[1] IFOM – Istituto Fondazione di Oncologia Molecolare, Experimental Therapeutics Program, Via Adamello 16, 20139 Milano, Italy
[2] Biochemistry and Structural Biology Unit, Department of Experimental Oncology, European Institute of Oncology, Via Adamello 16, 20139 Milano, Italy

Protein ubiquitination is a post-translational modification that can direct proteins for degradation. Three enzymes were involved: ubiquitin-activation enzyme (E1), -conjugation enzymes (E2s), and -ligases (E3s). Ubiquitination resulted to be dysregulated in various types of tumours . [1]. NEDD4 is an HECT type E3 ligase and appears to be a promising target for drug discovery. To date, the data suggest that several NEDD4-like E3s could function as oncogenic proteins. NEDD4 in particular was found to be highly expressed on several cancer types. [2]
Inhibitors of NEDD4 with a suboptimal potency and absence of data on cancer cells have been identified [3]. In addition, some indole-3-carbinol (I3C) analogues were found to inhibit NEDD4 with IC50 in the high micromolar range [4]. In this context, there is still the need to identify NEDD4 inhibitors, which would represent an interesting approach for cancer therapy and for other diseases.
Starting from Norclomipramine, a high micromolar NEDD4 inhibitor, and using a multidisciplinary approach, new NEDD4 covalent inhibitors have been identified and preliminary SAR of dihydro-benzazepine tricyclic derivatives was developed. Design, synthesis , biochemical activity and selectivity on NEDD4 family members, as well as antiproliferative activity on human Ewing's Sarcoma TC71 cancer cells and preliminary ADME characterizations of the synthetized compounds will be reported.

Acknowledgments:
This work was supported by AIRC under IG 2018 ID 21328, project PI Mercurio Ciro and by a generous donation to IFOM from Ravelli Family.

References

*[1] Senft D. et al. Nature Reviews Cancer, **2018,** 18, 69–88; [2]; Eide P.W. et al. Cell Signal., **2013**, 25(1), 12-18; Amodio N. et al. Am. J. Pathol., **2010**, 177, 2622-2634; Huang Z.I. et al. Oncol. Lett., **2017**, 14(3), 2649-2656; Weng M. et al. Oncotarget, **2017**, 8(12), 20288-20296; Zhang Y. et al. Gynecol. Oncol., **2015**, 139(1), 127-33; [3] Kathman S.J. et al. J. Am. Chem. Soc., **2015**, 137(39), 12442-5; [4] Quirit J.G. et al. Biochem. Pharmacol., **2017**, 127, 13-27.*

B16. Dipeptidyl-Peptidase III Cancer Mutations

Sara Matić[1], Sanja Tomić[1], Mihaela Matovina[1], Sandra Sobočanec[1], Goran Pipalović[1], Monika Martinčić[1], Marija Pinterić[2]

[1] *Ruđer Bošković Institute, 10000 Zagreb, Croatia*
[2] *Ruđer Bošković Institute, Bijenička 54, 10000 Zagreb, Croatia*

Kelch-like ECH-associated protein 1 (KEAP1) – NRF2 (Nuclear factor [erythroid-derived 2]-like 2 protein) pathway is the major regulator of cytoprotective responses to oxidative stress. We investigated the influence of mutations of dipeptidyl peptidase III (DPP III) listed in cBioPortal for Cancer Genomics on its affinity for the Kelch domain of KEAP1 and on the KEAP1 – NRF2 pathway.
DPP III is a metallopeptidase with a proposed role in the final stages of protein turnover in cell. It is also involved in blood pressure regulation, pain modulation and, through its interaction with KEAP1, in the modulation of the response to oxidative stress. Several studies have reported increased levels and activity of DPP III in malignant tissues and an association with the disease progression.
The ETGE motif (which is also present in NRF2) is essential for the DPP III – KEAP1 interaction. Binding of DPP III to KEAP1 results with release of NRF2 from complex with KEAP1 and activation of genes involved in the oxidative stress response controlled by NRF2. In human DPP III the ^{480}ETGE^{483} motif is at the peak of the flexible loop, and it is bound to the structured part of the catalytic domain by strong hydrogen bonds with Arg623 and Arg624. The R623W variant found in human cancer increases the affinity of DPP III for the Kelch domain for two orders of magnitude. In addition, the R623W variant significantly upregulated the expression of NQO1 mRNA compared to HEK293T cells transfected with an empty vector and the WT protein. Moreover, we identified P479S mutation as beneficial for the DPP III – KEAP1 interaction. It should be noted that the KEAP1-interacting loop of DPP III becomes more similar to the KEAP1-interacting loop of Nrf2 (EETGE) by mutating P479 to Ser.

B17. Discovery of Human Constitutive Androstane Receptor (CAR) Agonists with Imidazo[1,2-a]pyridine Structure

Ivana Mejdrova[1], Jan Dusek[2], Krystof Skach[1], Alzbeta Stefela[2], Josef Skoda[2], Karel Chalupsky[1, 3], Klara Dohnalova[3, 4], Ivona Pavkova[5], Thales Kronenberger[6, 7, 8], Azam Rashidian[6], Vojtech Duchoslav[1], Tomas Smutny[1], Petr Pavek[2], Radim Nencka[1]

[1] Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, Flemingovo nám. 2, 166 10 Prague 6, Czech Republic
[2] Department of Pharmacology and Toxicology, Faculty of Pharmacy in Hradec Kralove, Charles University, Akademika Heyrovskeho 1203, 500 05, Hradec Kralove, Czech Republic
[3] Czech Centre for Phenogenomics, Institute of Molecular Genetics of the Czech Academy of Sciences, Vídeňská 1083, 142 20, Prague; Czech Republic
[4] 1st Medical Faculty, Charles University, Katerinska 32, 112 08, Prague, Czech Republic
[5] University of Defense, Trebeska 1575, Hradec Kralove, 500 02, Czech Republic
[6] Department of Internal Medicine VIII, University Hospital of Tübingen, 72076 Tübingen, Germany
[7] School of Pharmacy, Faculty of Health Sciences, University of Eastern Finland, 70211 Kuopio, Finland
[8] Department of Pharmaceutical and Medicinal Chemistry, Institute of Pharmaceutical Sciences, Eberhard Karls Universität, 72076 Tübingen, Germany

Constitutive androstane receptor (CAR, NR1I3) is the nuclear receptor with significant roles in many hepatic functions, such as gluconeogenesis, fatty acid oxidation, biotransformation, liver regeneration, and clearance of steroid hormones, bile acid, cholesterol, and bilirubin. Thus, CAR has been proposed as a hypothetical target receptor for the therapy of metabolic or liver diseases. Currently known prototype high-affinity human CAR agonists such as CITCO (6-(4-chlorophenyl)imidazo[2,1-*b*][1,3]thiazole-5-carbaldehyde-*O*-(3,4-dichlorobenzyl)oxime) have limited selectivity activating also the pregnane X receptor (PXR) receptor, a related receptor of the NR1I subfamily.
Through the synthesis of 2-substitued imidazo[1,2-*a*]pyridine, its iodination in 3 position with subsequent introduction of TMS-acetylene group mediated by Sonogashira cross-coupling followed by a “click” reaction with variety of aromatic azides, we discovered several derivatives of 3-(1*H*-1,2,3-triazol-4-yl)imidazo[1,2-*a*]pyridine that directly activate human CAR in nanomolar concentrations, but do not activate PXR.
Compound MI-676 regulates CAR target genes in humanized CAR mice (humanized PXR-CAR-CYP3A4/3A7 mice, model 11858), does not activate other nuclear receptors after treatment with primary human hepatocytes, does not significantly interact with cytochrome P450 enzymes, and is nontoxic in cellular or genotoxic assays (HepG2, COS-1), and in rodent toxicity studies (rodent liver and its subsequent RT-qPCR analysis).
Our findings of potent and selective human CAR agonists with *in vivo* activity reinforce the role of CAR as a possible therapeutic target in metabolic diseases.

B18. Dynamics matter: Deciphering Opioid Receptor Subtype Specificity of the Peripheral Analgesic HS-731

Kristina Puls[1], Gerhard Wolber[1], Mariana Spetea[2], Helmut Schmidhammer[2]

[1] Department of Pharmaceutical Chemistry, Institute of Pharmacy, Freie Universität Berlin, Königin-Luise-Str. 2+4, 14195 Berlin, Germany
[2] Department of Pharmaceutical Chemistry, Institute of Pharmacy and Center for Molecular Biosciences Innsbruck (CMBI), University of Innsbruck, Innrain 80-82, 6020 Innsbruck, Austria

Peripherally restricted opioids, like HS-731, are analgesics with improved safety properties. HS-731 is a full agonist at the μ- and δ-opioid receptors (MOR; DOR) and a partial agonist at the κ-opioid receptor (KOR). Nonetheless, its binding mode and opioid receptor (OR) subtype selectivity determinants remain elusive. Thus, we performed an in-silico evaluation including molecular dynamics simulations, and developed dynamic 3D pharmacophore models (dynophores). Our study revealed that K6.39 and the non-conserved epitope 6.58 are responsible for the experimentally determined affinity differences of HS-731 at the classical ORs (MOR; DOR, KOR). At the MOR, HS-731 takes part in more frequent and stronger charge interactions than in DOR and KOR, in correlation with the highest affinity of HS-731 measured at the MOR. At the DOR and KOR, ionic interactions were detected with comparable frequency but interaction distance measurement over the simulation time revealed stronger interactions at the DOR, for which HS-731 shows higher affinity. We rationalized the partial agonism of HS-731 at the KOR. A salt bridge between $K227^{5.39}$ and $E297^{6.58}$ in the transmembrane helices (TM) 5 and 6 occurring during MD simulations is assumed to force the KOR to adopt an intermediate state conformation with decreased TM6 outward movement hindering receptor activation. Even though HS-731 binds with low nM affinity to the classical ORs, we experimentally revealed the absence of specific binding to the nociceptin/orphanin FQ peptide receptor in concentrations up to 10 μM and discovered that $Y130^{3.33}$ that points deep into the orthosteric binding pocket is responsible for this lack of binding.

B19. Endothelium-targeted delivery of vitamin K_1 using hyaluronan-based capsules

Anna Kieronska-Rudek[1, 2], Agnieszka Kij[1], Małgorzata Janik-Hazuka[3], Justyna Bednorz[3], Szczepan Zapotoczny[3], Stefan Chlopicki[1, 2]

[1] Jagiellonian Centre for Experimental Therapeutics (JCET), Jagiellonian University, Cracow, Poland,
[2] Faculty of Medicine, Chair of Pharmacology, Jagiellonian University Medical College, Cracow, Poland,
[3] Department of Physical Chemistry and Electrochemistry, Faculty of Chemistry, Jagiellonian University, Cracow, Poland

The regulatory role of vitamin K (VK) goes beyond coagulation and calcification, and includes also anti-inflammatory as well as senolitic activity. Considering numerous functions of VK, it might well be used for the prevention and treatment of age-dependent endothelial dysfunction. However, the delivery of VK to the endothelium and vascular tissue is difficult due to hydrophobic character of VK and its interconversion in intestine. Therefore, the aim of our research was to obtain stable, hyaluronan-based capsules templated on oil cores, enabling hydrophobic VK transport and ensuring high affinity to vascular tissue.

Hydrophobic derivative of the hyaluronic acid (HyC12) was prepared as described previously [1,2]. Attached alkyl chains enable self-organization of the modified polysaccharide at the oil-water interface stabilizing the emulsion. Capsules were obtained directly in ultrasound-assisted emulsification process. The solution of a given polysaccharide derivative was mixed with an oil phase with dissolved vitamin K_1 (VK_1). The mixture was firstly homogenized using vortex shaker and sonicated. Then, the uptake of capsules in isolated aorta and perivascular adipose tissue (PVAT) surrounding the aorta were studied *ex vivo* after 24h incubation with encapsulated or non-encapsulated VK_1 (5mM). The measurements of VK_1 and endogenous K_2MK-4 concentration in tissues, were performed using UHPLC-MS/MS method.

The obtained emulsion containing capsules of hydrodynamic diameters mostly below 500 nm. Importantly, no symptoms of capsules degradation during storage (4°C) as well as after incubation (37°C) were observed.

Our results indicated that capsules containing VK_1 were effectively stabilized by hydrophobically modified hyaluronic. Concluding, hyaluronan-based capsules appears to be promising pharmacological tool for the delivery of VK and other hydrophobic vitamins directly to vessel wall and endothelium.

J. Szafraniec, et al. (2017). Nanoscale 9, 18867 – 18880.
2. Czyzynska-Cichon et al. (2021). Int. J. Nanomedicine, 16, 1377-1390

B20. New compositions of electrochemical DNA biosensors receptor layers for fast and sensitive detection of SARS-CoV-2 biomarkers

Anna Szymczyk[1], Robert Ziółkowski[1], Anita Joniak[1], Aleksandra Kramarska[2], Joanna Kawalec[1], Elżbieta Malinowska[1, 2]

[1] *Chair of Medical Biotechnology, Faculty of Chemistry, Warsaw University of Technology, Stanisława Noakowskiego 3, 00-664 Warsaw, Poland*
[2] *Centre for Advanced Materials and Technologies CEZAMAT, Warsaw University of Technology, Poleczki 19, 02-822 Warsaw, Poland*

To meet the requirements of novel therapies, effective treatments should be supported by diagnostic tools characterized by appropriate analytical and working parameters. The example of recent COVID-19 pandemic has shown the importance of early diagnosis and rapid prevention of the virus spread. It would be advisable to develop fast, reliable and cost-efficient tests that could be fully performed outside of the laboratory and which will allow for early stage virus infection. Assays based on nucleic acids turned out to be an effective approach. In this point of view, electrochemical biosensors, because of their miniaturization and integration possibility as detectors in more sophisticated devices dedicated to complete and automated molecular analysis, seem to be of particular importance. Because of limitations in amplification techniques, real-life applications of such biosensors rarely employ specific sequence detection and selectivity is secured only by starters used in polymerase chain reaction. An interesting approach to make diagnostic tool more specific and to eliminate possible false positive results, is the detection of amplified nucleic acid strand by electrochemical biosensor. Moreover, the construction of this biosensor and detection mechanism used, should allow for simple and effective integration in microfluidic device. The presented research is focused on the development of fast and versatile “on-off” signalling electrochemical genosensor based on electrochemical stem-loop probe for the detection of SARS-CoV-2. Specific nucleotide sequences (RdRp, E and N) were chosen as genetic biomarkers and human β-actin gene was used as an intrinsic control of correct swab taking. During research several hairpin-like probes, labelled with methylene blue as a redox molecule, were investigated. Also biosensor’s interfacial region was optimized in the point of view of its conductivity and obtained current intensity. We confirmed the possibility of fast and selective detection of SARS-CoV-2 sequences amplified in asymmetric PCR process.

B21. Photostability and phototoxicity of ocular drugs, azelastine, ketotifen and timolol

Karolina Lejwoda[1, 2], Anna Gumieniczek[2], Agata Filip[3]

[1] *Doctoral School of Medical University of Lublin, Poland*
[2] *Chair and Department of Medicinal Chemistry, Medical University of Lublin, Poland*
[3] *Department of Cancer Genetics with Cytogenetics Laboratory, Medical University of Lublin, Poland*

Medicinal substances are constantly influenced by various external factors, including radiation in the UV-Vis range. Photostability of drugs may be defined as the response of the drug or drug's product to the exposure to solar light that can lead to physical or chemical changes. Many drugs can absorb radiation in the UV-Vis range, which may induce photodegradation and generation of toxic photoproducts.
In the present experiment, several ocular drugs which are applied in solutions (i.e., azelastine, ketotifen, timolol) were exposed to different doses of radiation in the UV-Vis range under different pH values. Next, they were analyzed by validated high performance liquid chromatography (HPLC) methods, to estimate the percentage of degradation and calculate kinetic parameters, i.e., the degradation rate constant (k), the degradation time of the 10% substance (t0.1), and degradation time of the 50% substance (t0.5).
In the case of azelastine, its photostability was low at pH 7.0, 10.0, and 13.0. Ketotifen was shown to be photolabile at pH 7.0 and 10.0. In turn, timolol was shown to be photolabile in the whole range of pH values. For ketotifen, several photodegradation products were separated and identified using the UPLC-MS/MS method.
Moreover, the generation of singlet oxygen and superoxide anion were examined, together with the molar extinction coefficient (MEC) evaluation, to estimate their phototoxic risk. According to the current recommendations, all of these substances may present phototoxic activity

B22. Structure-based design and modular synthesis of novel PI4K class II inhibitors with 4-aminoquinazoline scaffold

Mbilo Misehe[1, 2], Radim Nencka[3], Marika Matoušová[1], Dominika Chalupská[1], Helena Mertlíková-Kaiserová[1], Evžen Bouřa[1]

[1] a Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Flemingovo nam. 2, 166 10 Prague 6, Czech Republic
[2] b Department of Organic Chemistry, Faculty of Science, Charles University in Prague, Hlavova 2030/8, 128 43 Prague 2, Czech Republic
[3] a, Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Flemingovo nam. 2, 166 10 Prague 6, Czech Republic*

Novel 4-aminoquinazoline-6-carboxamide derivatives bearing differently substituted aryl or heteroaryl groups at the position 7 of the core were rationally designed, synthesized and evaluated for their biological activity *in vitro* as phosphatidylinositol 4-kinase IIα (PI4KIIα) inhibitors. The straightforward approach and procedures devised and employed allowed the sequential, modular synthesis and broad functionalization of the scaffold in six steps solely. Our previous knowledge of a crystal structure of PI4KIIα, combined with extensive docking studies focused on its ATP-binding site were employed in this work to perform the hereby reported SAR investigation aiming at the highest possible affinity, while retaining appropriate interactions with the hinge region of the isoenzyme. The analysis of this data enabled us to design and prepare a novel series of compounds. Several derivatives exhibited significant activity against PI4KIIα *in vitro*, suggesting that this rational approach to the design of novel lipid kinase inhibitors can be further developed and employed to synthesize new compounds with higher affinity and potential selectivity for this molecular target.

B23. Study of the cytotoxic activity of ethanolic extracts from four native plants of Northern Chile

Dioni Arrieche[1], Hector Carrasco[2], Javier Pardo[3], Carlos Jara[4], Joan Villena[4], Lautaro Taborga[1], Jaime Carmi[5]

[1] Laboratorio de Productos Naturales, Departamento de Quimica, Universidad Tecnica Federico Santa Maria
[2] Instituto de Ciencias Químicas Aplicadas, Facultad de Ingeniería, Universidad Autónoma de Chile, Llano Subercaseaux, 2801, San Miguel, Santiago, 8900000, Chile
[3] Programa de conservación de Flora Nativa del Norte de Chile, Biorestauración consultores, Copiapó, 1530000, Chile
[4] Laboratorio de Bioensayos (Biología Celular del cáncer), Centro de Investigaciones Biomédicas, Facultad de Medicina, Universidad de Valparaíso, Angamos 680, Reñaca, 2520000, Chile
[5] Laboratorio de Analisis Instrumental, Departamento de Química, Universidad Tecnica Federico Santa María, Sede José Miguel Carrera

The plant species of this study were collected in one of the most extreme environments on the planet: Atacama Desert, Chile. The organisms that habit this environment must cope with high temperatures during the day, low temperatures at night, strong ultraviolet radiation, and low availability of nutrients, so they are forced to develop different strategies to survive, hence the importance of their phytochemical studies and its biological activities. The cytotoxic activity of ethanolic extracts from the plant's *Krameria cistoidea* (AP and S), *Pintoa chilensis* (AP and S), *Nolana albescens* (AP), and *Skytanthus acutus* (AP) was analyzed. The ethanolic extracts obtained by maceration were subjected to exhaustive sonication (x3) at a temperature of 50ºC for 1 h., filtered, and the solvent was removed under reduced pressure, obtaining the ethanolic extracts. Cell viability was estimated using the cell staining method (Sulforhodamine B, SRB) on the HT29 and MCF-7 cell lines, using the MCF-10A as a control. Only two extracts showed interesting activity with IC_{50} values of 99.5 (HT29) and 100.3 (MCF-7) µg/mL for *S. acutus* (AP) and 111.25 (MCF-7) µg/mL for *P. chilensis* (S). The selectivity index (SI) showed a selective effect of the *P. chilensis* (S) extract on the MCF-7 cell line. These results correlate with flow cytometry, studying the production of reactive oxygen species (ROS), as well as oxidative damage (lipid peroxidation). In conclusion, the results showed that the extract studied produces a greater ROS production as well as a major effect oxidative damage on the MCF-7 cell line compared with the control cell line, where a lower production of ROS and less oxidative damage are observed, so it could be acting as an antioxidant in this cell line.

B24. Virtual screening as a tool for the discovery of new BGT-1 transporter inhibitors

Kamil Łątka[1], Kristine Sloth Wilhelmsen[2], Stefanie Kickinger[2], Petrine Wellendorph[2], Marek Bajda[1]

[1] Department of Physicochemical Drug Analysis, Faculty of Pharmacy, Jagiellonian University Medical College, 30-688 Cracow, Medyczna 9, Poland
[2] Department of Drug Design and Pharmacology, Faculty of Health and Medical Sciences, University of Copenhagen, Universitetsparken 2, 2100, Copenhagen, Denmark

Gamma-aminobutyric acid (GABA) is one of the main inhibitory neurotransmitters in the central nervous system. The level of GABA within synapses is regulated by transporters GAT-1, BGT-1, GAT-2, and GAT-3. Studies indicate that blocking particular types of these transporters may be beneficial in the treatment of diseases such as epilepsy, depression, anxiety, and neuropathic pain. Although the potential of GABA transporters as therapeutic targets appears to be very high, the only drug that has been approved for medical use so far is tiagabine, a selective inhibitor of GAT-1. The pool of compounds active toward other transporter types, especially BGT-1, is still limited.
Therefore, we carried out a virtual screening of the ZINC 15 database to find new inhibitors of BGT-1 transporters. From over 230 million commercially available compounds, approximately 748,000 were retrieved that met the preferred criteria for BGT-1 inhibitors in terms of molecular weight and lipophilicity (MW 200, LogP 1). Then, compounds containing fragments crucial for activity were selected (5113 amino acid derivatives) using simplified pharmacophore models built based on known ligands. This pool of molecules was docked into BGT-1 homology models and scored according to a method pre-validated by retrospective virtual screening (BEDROCα=20: 0.776). The highest-rated compounds were visually analysed with respect to the interactions created at the main binding site of the transporter. Finally, we chose 12 molecules, which were purchased and tested in vitro in recombinant BGT-1 and GAT-3 [^{3}H]GABA uptake assays. We identified a new hit with moderate activity toward BGT-1 (IC_{50} = 48.9 µM) and GAT-3 (IC_{50} = 46.8 µM). This compound is a good starting point to design derivatives with increased activity and selectivity targeting a specific type of GABA transporters.

Acknowledgements:
This study was funded by Jagiellonian University Medical College (N42/DBS/000115, N42/DBS/000213).

B25. Synthesis and biological evaluation of *Schistosoma mansoni* Sirtuin2 (*Sm*Sirt2) inhibitors

Arianna Colcerasa[1], Dante Rotili[2], Antonello Mai[2], Elizabeth Ramos-Morales[3], Christophe Romier[3], Manfred Jung[1]

[1] *University of Freiburg*
[2] *Sapienza Università di Roma*
[3] *Institut de génétique et de biologie moléculaire et cellulaire (IGBMC)*

Poster selected for a flash presentation

The modulation of epigenetic regulators, expressed in the genome of the *Schistosoma mansoni* parasite, represents a promising therapeutic strategy to develop a new treatment against the neglected tropical disease schistosomiasis [1]. In fact, up to date Praziquantel is the only available drug and alternatives are urgently required [2]. Five isotypes of the class III Histone deacetylases called Sirtuins were identified in the parasite as orthologs of the human ones. Among them, *Schistosoma mansoni* Sirtuin2 (*Sm*Sirt2) displayed a high level of transcription in a constant manner during all life-cycle stages of the parasite and, thus, it was postulated as a feasible epigenetic antischistosomal target [3]. Further characterizations reported *sm*Sirt2 as both deacetylase and demyristoylase [4]. Additionally, the screening of the Kinetobox library [5] led to the identification of three drug-like hits and further optimization concerning TCMDC-143295 has followed [5]. Here, we report extended structure–activity–relationship studies of TCMDC-143159, which enabled the optimization of the scaffold with improved potency (IC_{50} values in the low micromolar range) and conserved selectivity over the human ortholog, as a required feature for antiparasitic drugs. These compounds did not show cytotoxicity when tested via MTS assay in HL-60 leukemia cells. Further *in vitro* investigations about *sm*Sirt2 catalytic activities and potential inhibition by small molecules will follow as well as phenotypic experiments to evaluate the potential antiparasitic activity of this new promising scaffold.

[1]. Hailu, G. S. et al.: J. Med. Chem. ***2017****, (60): 4780–4804.*
[2]. Cioli, D. et al.: Mol. Biochem. Parasitol. ***2014****, (195), 23– 29.*
[3]. Lancelot, J. et al.: PLoS Negl. Trop. Dis. ***2013****, (7): e2428.*
[4]. Monaldi, D. et al.: J. Med. Chem. ***2019****, (62): 8733–8759.*
[5]. Peña, I. et al.: Sci. Rep. ***2015****, (5): 8771.*

B26. Targeting *Plasmodium falciparum* dihydroorotate dehydrogenase: design, synthesis, co-crystallization and biological evaluation of new 3-hydroxypyrazole scaffold-based inhibitors.

Noemi Villella[1], Agnese Chiara Pippione[1], Stefano Sainas[1], Marta Giorgis[1], Giulio Poli[2], Tiziano Tuccinardi[2], Marta Alberti[3], Riccardo Miggiano[3], Davide Maria Ferraris[3], Luca Marchisio[1], Sandrine Cojean[4], Efrain Salamanca[5], Alberto Jose Gimenez[5], Barbara Buccinnà[6], Cristina Ramondetti[6], Marco Piccinini[6], Marco Lucio Lolli[1], Donatella Boschi[1]

[1] Department of Science and Drug Technology, University of Turin, via P. Giuria 9, 10125, Turin (Italy)
[2] Department of Pharmacy, University of Pisa, via Bonanno 6, 56126, Pisa (Italy)
[3] Department of Pharmaceutical Sciences, University of Piemonte Orientale, via Bovio 6, 28100, Novara (Italy)
[4] UFR de Pharmacie, Universitè Paris-Saclay, 5 rue Jean-Baptiste Clement, 92296, Chatenay-Malabry (France)
[5] Institute for Pharmacological and Biochemical Sciences, San Andrés University, 3299, La Paz (Bolivia)
[6] Department of Oncology, University of Turin, Via Michelangelo 27/B, 10125, Turin (Italy)

Poster selected for a flash presentation

Malaria is a global parasitic infectious disease caused by *Plasmodium* parasites. New antimalarial agents are required due to the developing of drug resistance to commercialized therapies. Many efforts were taken to discover new target and *Dihydroorotate Dehydrogenase* (DHODH) was defined as a promising target for novel anti-malarial therapy. DHODH is an FMN-dependent, mitochondrial enzyme, involved in the *de novo* biosynthesis of pyrimidines. Generally, cells can acquire pyrimidines using two different pathways: the *de novo* biosynthesis and the *salvage* pathway. *Plasmodium* species can gain pyrimidines only from the *de novo* pathway so blocking this way offers a therapeutic opportunity to kill the parasite. *P. falciparum* DHODH (*Pf*DHODH) has been validated as a drug target and different inhibitors were tested in clinical trials.
Assaying several hydroxylated compounds present in our library, we identified a 3-hydroxypyrazole scaffold-based compound as a *Pf*DHODH inhibitor with an activity in the µM range and selective over the *human* isoform. With the aim of increasing its potency, we performed a SAR study, modulating several positions of its structure. The most potent analogue, able to inhibit *Pf*DHODH with an IC_{50} at 10 µM and selective against mammalian cells (IC_{50}>200 µM) has been co-crystallized with the isolated protein, clarifying its experimental binding mode and explaining the key interactions within the binding site. Moreover, this compound

showed low cytotoxicity and activity against synchronized cultures of *P.falciparum* infected erythrocytes (IC_{50}: 61.72 µM). Starting from this knowledge and applying a bioisosteric replacement, a third generation of analogues has been designed and synthesized, leading to a new pool of compounds. Among them, compound **M591** showed an increased potency compared to the lead (IC_{50}: 1.6 µM).
In this occasion, the design, synthesis, co-crystallization and preliminary data about the biological evaluation of these new potential inhibitors in the *Pf*DHODH landscape are presented.

B27. Study of the interaction of far-red fluorescence ligands with G-quadruplex structures

Cristina Galiana-Rosello, Gil-Martinez Ariadna, Mulet Laura, Lazaro Andrea, Sonia López Molina, Enrique García-España, Jorge Gonzalez-Garcia

[1] University of Valencia, Institute of Molecular Science (ICMol)

Non-canonical nucleic acid structures have emerged as molecular controlling gates of biological processes acting as epigenetic markers. Unusual nucleic acid structures include triplexes, i-motifs, three-way junctions, holiday junctions or G-quadruplexes (G4s). The later one is formed from stacks of two or more planar guanine tetrads that arise from hydrogen bonding network of four guanines whereas these structures are assembled and stabilized by alkali metal cations. A large number of putative G-quadruplex forming sequences have been identified in the human genome and evidences suggest their pivotal role in key biological processes. These G4 structures have been proposed as potential targets by small molecules for therapeutic intervention.

Even G4s have been fully proved to exist *in vitro*; its existence *in vivo* still remains an active debate. Some of the most direct evidence has been obtained by using antibodies to visualize G4 structures in fixed cells. Because of the limitation of the antibody technology, a large number of optical probes has been reported to date to visualize these structures in live cells. Mostly, small-molecule optical probes are based on changes in the emission intensity in the visible range. However, this approach in microscopy has important drawbacks such as photon scattering, high absorption and autofluorescence of cells.To overcome this issue, we developed small far-red fluorescent probes, which emission intensity is tightly regulated by the interaction with G4s.

Herein, we present the interaction studies of a series of cyanine ligands, termed as C1-C5, with G4s and duplex DNAs by a range of biophysical assays. The ligands are characterised by a polymethine moiety with different length, which influences strongly the binding to G4s. Among all the ligands, C3, shows a strong stabilization effect and high binding affinity towards G4s as well as selectivity for G4s over duplexes.

B28. A fragment-based approach for the development of trypanothione reductase inhibitors as antileishmanial agents

Francesca Seghetti[1], Alessandra Salerno[1], Jessica Caciolla[1], Riccardo Ocello[1], Eleonora Testi[1], Eleonora Fiorentino[2], Marinella Roberti[1], Matteo Masetti[1], Gianni Colotti[3], Veronica Morea[4], Trentina Di Muccio[2], Cécile Exertier[3], Andrea Ilari[3], Annarita Fiorillo[5], Maria Laura Bolognesi[1]

[1] Department of Pharmacy and Biotechnology, Alma Mater Studiorum - University of Bologna, Via Belmeloro 6, 40126 Bologna, Italy
[2] Dipartimento di Malattie Infettive, Istituto Superiore di Sanità Viale Regina Elena, 299, 00161 Rome, Italy
[3] Institute of Molecular Biology and Pathology, Italian National Research Council, IBPM-CNR, c/o Department of Biochemical Sciences, Sapienza University, P.le A.Moro 5, 00185 Rome, Italy
[4] Institute of Molecular Biology and Pathology, Italian National Research Council, IBPM-CNR, c/o Department of Biochemical Sciences, Sapienza University, P.le A.Moro 5, 00185 Rome, Italy;
[5] Department of Biochemical Sciences, Sapienza University, P.le A.Moro 5, 00185 Rome, Italy

Poster selected for a flash presentation

Leishmaniasis is a vector-borne neglected disease with high morbidity and mortality rates, considered an emergent worldwide threat. The disease is endemic in tropical and subtropical areas but also in southern Europe, comprising up to 1.2 million cases each year. Currently used drugs possess several drawbacks, such as limited efficacy, toxic side-effects, and resistance development. All in all, this alarming scenario advocates for the development of new drugs. Trypanothione reductase (TR), a validated antileishmanial target, is a parasite-specific enzyme critical for antioxidant defence. The absence of TR in the host, its vital role for the parasite and the development of numerous TR inhibitors make this enzyme an attractive target for antileishmanial drugs (10.3390/molecules25081924). However, among the TR-inhibiting compounds reported so far, only few molecules possess adequate antiparasitic activity, mainly due to two aspects: i) survival of the parasites is affected when TR activity is reduced by more than 90% (10.1046/j.1365-2958.1998.00968.x); ii) a large and featureless TR active site hampers the developability of effective inhibitors (doi.org/10.1371/journal.pntd.0003773). However, as TR features different sub-pockets, it provides an interesting scope for screening fragments for these sub-pockets and linking them to design larger molecules with optimal binding profile. On this basis, an initial crystallographic fragment screening allowed us to identify twelve fragments able to bind TR sub-pockets. Among the screened ligands, the five compounds interacting with trypanothione-binding site were selected to design TR inhibitors. Supported by docking studies, a small library of

compounds was obtained by merging, linking and/or growing the selected fragments. The molecules have been synthetized and are being tested to evaluate TR binding and inhibitory activity, followed by anti-leishmanial phenotypic assays. In conclusion, this approach will potentially contribute to discovering lead candidates with the aim to optimize the leishmanicidal profile and to perform a preliminary structure-activity relationship study.

MDPI