CSSMC

1ST CROATIAN SUMMER SCHOOL ON MEDICINAL CHEMISTRY

Book of Abstracts

1ST CROATIAN SUMMER SCHOOL ON MEDICINAL CHEMISTRY

September 14–17, 2025, Rijeka, Croatia

Book of Abstracts

IMPRESSUM

Publisher: Croatian Chemical Society

Editors:

Višnja Stepanić

Nela Malatesti

Vesna Gabelica Marković

Robert Vianello

Nikola Basarić

Sanja Koštrun

Ivana Perković

Vesna Petrović Peroković

Silvana Raić Malić

Ines Vujasinović

Cover design: Marta Perković

ISBN: 978-953-8334-17-7

Zagreb, 2025.

ORGANISED BY

Croatian Chemical Society

Section for Medicinal and Pharmaceutical Chemistry

University of Rijeka, Faculty of Biotechnology and Drug Development

SPONSORS


Altium


AnAs


ThermoFisher
SCIENTIFIC


KEFO


Labtim


Selvita


Ru Ve
Ru-Ve
tvoj lab partner


Enamine

SCIENTIFIC AND ORGANISING COMITTEE:

Višnja Stepanić, chair
Ruđer Bošković Institute, Zagreb

Nela Malatesti, co-chair
University of Rijeka, Faculty of Biotechnology and Drug Development

Vesna Gabelica Marković, co-chair
University of Zagreb, Centre for research, development and transfer technology

Robert Vianello, co-chair
Ruđer Bošković Institute, Zagreb

Nikola Basarić
Ruđer Bošković Institute, Zagreb

Sanja Koštrun
Selvita Ltd.

Ivana Perković
University of Zagreb Faculty of Pharmacy and Biochemistry

Vesna Petrović Peroković
University of Zagreb Faculty of Science

Silvana Raić Malić
University of Zagreb Faculty of Chemical Engineering and Technology

Ines Vujasinović
Selvita Ltd.

WELCOME NOTE

Dear colleagues,

It is our great pleasure to welcome you to the First Croatian Summer School on Medicinal Chemistry (CSSMC)! The 1st CSSMC is held at the Faculty of Biotechnology and Drug Development of University of Rijeka, from September 14 to 17, 2025. The school has been organized by medicinal chemists from six Croatian institutions with the support of the Croatian Chemical Society, the Faculty of Biotechnology and Drug Development of University of Rijeka and our valued sponsors.

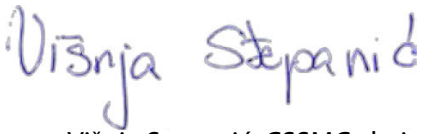
The school brings together experienced medicinal chemists and experts in drug discovery from both academia and industry to deliver an intensive four-day training program covering key concepts, approaches, and techniques used in the discovery and design of novel drugs and biologically active molecules.

A group of 49 participants form Bosnia and Herzegovina, Croatia, Italy, Serbia, Slovenia, and Türkiye will engage in topics such as drug targets, lead optimization, chemical libraries, characterization of mechanisms of action, ADME optimization as well as the application of AI/ML and molecular modelling. These themes will be explored through a dynamic mixture of lectures, interactive sessions, and workshops. In addition, participants will have the opportunity to present their own ideas and research on posters and in pitch presentation session, and to gain new perspectives on bridging science, industry, and innovations.

Last but not least, taking place in the beautiful coastal city of Rijeka, Croatia, the school also offers the chance to network and collaborate in a vibrant, supportive environment, enriched by social events.

We truly believe that the knowledge, skills, and connections you gain here will boost your research potential, open up career opportunities, inspire new discoveries and, most of all, that you will enjoy your time with us in Rijeka.

On behalf of the Scientific and Organizing Committee,



Višnja Stepanić, CSSMC chair

PROGRAMME

Sunday, 14 September 2025

Hall 030 • Faculty of Biotechnology and Drug Development, University of Rijeka

08:00 – 09:00	**REGISTRATION AND POSTER SETUP**
09:00 – 09:10	**OPENING CEREMONY** **Višnja Stepanić**, CSSMC Chair, Ruđer Bošković Institute, Croatia
09:10 – 09:20	**WELCOME ADDRESS** **Vladislav Tomišić**, Croatian Chemical Society, President **Ivana Ratkaj**, University of Rijeka, Faculty of Biotechnology and Drug Development, Croatia, Vice-Dean for Science
	Chair: Sanja Koštrun, Selvita Ltd. Croatia
09:25 – 10:45	**Principles of Early Drug Discovery: Finding the Right Ligand for the Right Target – I** **Marko Anderluh**, University of Ljubljana, Faculty of Pharmacy, Slovenia
10:45 – 11:15	**COFFEE BREAK sponsored by KEFO Ltd.**
11:15 – 12:45	**Principles of Early Drug Discovery: Finding the Right Ligand for the Right Target – II** **Marko Anderluh**, University of Ljubljana, Faculty of Pharmacy, Slovenia
12:45 – 14:00	**LUNCH BREAK**
	Chair: Silvana Raić-Malić, University of Zagreb Faculty of Chemical Engineering and Technology, Croatia
14:00 – 15:30	**Never Ending Battle Against a Perfect Enemy: Can We Finally Decipher Tumour Communication** **Ivana Ratkaj**, University of Rijeka, Faculty of Biotechnology and Drug Development, Croatia
15:30 – 15:45	**SHORT BREAK**
15:45 – 17:15	**Drug Targeting: Where We Are, How We Got Here, and Where We're Headed?** **Ivana Perković**, University of Zagreb Faculty of Pharmacy and Biochemistry, Croatia
17:30 – 19:30	**TRSAT SIGHTSEEING – A guided tour**
20:00	**WELCOME RECEPTION**

Monday, 15 September 2025

Hall 030 • Faculty of Biotechnology and Drug Development, University of Rijeka

	Chair: Višnja Stepanić, Ruđer Bošković Institute, Croatia
09:00 – 10:00	**Compound Libraries in Modern Drug Discovery** **Ivan Kondratov**, Enamine Ltd. Germany
10:00 – 10:45	**Workshop: Design of Compound Libraries – I** **Ivan Kondratov**, Enamine Ltd. Germany
10:45 – 11:15	**COFFEE BREAK sponsored by Enamine Ltd.**
11:15 – 12:45	**Workshop: Design of Compound Libraries – II** **Ivan Kondratov**, Enamine Ltd. Germany
12:45 – 14:00	**LUNCH BREAK**
	Chair: Ivana Perković, University of Zagreb Faculty of Pharmacy and Biochemistry, Croatia
14:00 – 15:30	**Implementing AI in Drug Discovery** **Jörg Wichard**, Selvita S.A., Krakow, Poland
15:30 – 15:45	**SHORT BREAK**
15:45 – 16:00	**The Journey of Innovation – introductory lecture** **Vesna Gabelica Marković**, University of Zagreb, Technology Transfer Office, EIT Health Croatia Representative, Croatia
16:00 – 17:00	**Bridging gaps: A Roundtable on Science, Industry & Innovation** Host: **Vesna Gabelica Marković**, University of Zagreb, Technology Transfer Office, EIT Health Croatia Representative, Croatia Guests: **Marko Anderluh** (University of Ljubljana, Slovenia), **Nikola Basarić** (Ruđer Bošković Institute, Croatia), **Silvana Raić Malić** (University of Zagreb, Croatia), **Maša Safundžić Kučuk** (JGL, Croatia), **Jőrg Wichard** (Selvita S.A., Krakow, Poland)
17:00	**GROUP PHOTO**
18:30	**SCHOOL DINNER**

Tuesday, 16 September 2025

Hall 030 • Faculty of Biotechnology and Drug Development, University of Rijeka

	Chair: Robert Vianello, Ruđer Bošković Institute, Croatia
09:00 – 10:00	**Synthesis of BODIPY Compounds as Potential Drug Delivery Systems and Cancer Phototherapeutics** **Nikola Basarić**, Ruđer Bošković Institute, Zagreb, Croatia — *last minute replacement!*
10:00 – 10:30	**COFFEE BREAK sponsored by AnAs Ltd.**
10:30 – 12:45	**Hands-On Session: Predicting ADMET and Physicochemical Properties Using Online Resources** **Višnja Stepanić**, Ruđer Bošković Institute, Zagreb, Croatia
12:45 – 14:00	**LUNCH BREAK**
	Chair: Vesna Petrović Peroković, University of Zagreb Faculty of Science, Croatia
	Workshops with two groups exchange
14:00 – 15:30	**Integrating LC and LC-MS in Drug Discovery and Development: Principles, Practice, and Applications** **Ivan Grgičević**, Labtim Ltd. Croatia..... **Lab O-275**
15:45 – 17:15	**Technological Infrastructure for the Future of Therapeutics** **Iva Kušec**, Altium International Ltd. Croatia..... **Lab O-274**
18:00 – 19:30	**PITCH PRESENTATIONS**
20:00	**PIZZA & BEER PARTY**

Wednesday, 17 September 2025

Hall 030 • Faculty of Biotechnology and Drug Development, University of Rijeka

	Chair: Nikola Basarić, Ruđer Bošković Institute, Croatia
09:00 – 10:30	**Optimizing Drug Behavior: A DMPK Perspective – I** **Jasna Padovan**, Selvita Ltd. Croatia
10:30 – 11:00	**COFFEE BREAK sponsored by Altium Ltd.**
11:00 – 12:30	**Optimizing Drug Behavior: A DMPK Perspective – II** **Jasna Padovan**, Selvita Ltd. Croatia
12:30 – 13:30	**LUNCH BREAK**
	Chair: Nela Malatesti, Faculty of Biotechnology and Drug Development, University of Rijeka, Croatia
13:30 – 14:15	**Molecule Makeover: Engineering the Perfect Preclinical Drug** **Milan Mesić**, University of Rijeka, Faculty of Biotechnology and Drug Development, Croatia
14:15 – 15:00	**Incretins: The Hormones That Sparked a Therapeutic Revolution** **Milan Mesić**, University of Rijeka, Faculty of Biotechnology and Drug Development, Croatia
15:00 – 16:30	**Lead Optimization for Inhaled Drugs: Tactics, Differences from Oral Therapies and Case Studies** **Alessandro Acetta**, CHIESI Italia S.p.A., Italy
16:30 – 17:00	**CLOSING REMARKS** **Višnja Stepanić**, CSSMC Chair, Ruđer Bošković Institute, Croatia

POSTER PRESENTATIONS

P01	**Jovana Ajduković**, An Matheeussen, Natascha Van Pelt, Maja Marinović, Svetlana Fa Nedeljković, Marina Savić, Guy Caljon *Androstane-based heterocycles: Evaluation of antiparasitic activity, in silico protein affinity and (eco)toxicological insight*
P02	**Anja Beč**, Lucija Vrban, Leentje Persoons, Dirk Daelemans, Kristina Starčević, Robert Vianello, Marijana Hranjec *Biological activity and computational analysis of novel acrylonitrile and imino-coumarin derived benzimidazoles*
P03	**Emina Bečić** *Assessment of the anticancer potential of transition metal complexes with metformin via molecular docking: Insights from a literature review*
P04	**Sofija Bekić**, Ivana Kuzminac, Milica Stevanović, Edward Petri, Anđelka Ćelić *10-Alkoxy estrane derivatives as potential anticancer agents: interactions with steroid receptors and inhibition of aldo-keto reductases 1C3 and 1C4*
P05	**Lucija Bilandžija**, Bárbara Teixeira, Miguel Prudencio, Zrinka Rajić *Antiplasmodial activity of novel β-carboline-artemisinin hybrids*
P06	**Ida Boček Pavlinac**, Corina Šljubura, Katarina Zlatić, Marijeta Kralj, Ivana Fabijanić, Marijana Radić Stojković, Marijana Hranjec *Synthesis, biological evaluation and interaction with ct-DNA of mono and diamidino-substituted imidazo[4,5-b]pyridines*
P07	**İlayda Boyraz**, Bilge Bıçak *The nifedipine molecule used in the treatment of Raynaud syndrome: An in silico study*
P08	**Dea Demeter**, Nikola Basarić, and Višnja Stepanić *Predictive profiling of BODIPY dyes: Insights into their drug-likeness*
P09	**Dorian Dulčić**, Višnja Stepanić *Harnessing transcriptomic signatures for drug discovery: Repurposing potential, off-target detection, and safety forecasting*
P10	**Marina Galić**, Tamara Rinkovec, Leentje Persons, Dirk Daelemans, Marijana Radić Stojković, Rosana Ribić, Robert Vianello and Marijana Hranjec *Synthesis of biologically active benzoxazoles*
P11	**Petra Katalinić**, Ivo Crnolatac *DNA nanopores functionalized with antimicrobial peptides*
P12	**Natan Koraj**, Ema Faganeli, Sushmaa Dangudubiyyam, Anne Harduin-Lepers, Marko Anderluh *Structure-activity relationship study of ST6Gal1 inhibitors aimed at improving cell permeability*
P13	**Petra Kovačec**, Leentje Persoons, Dirk Daelemans, Tatjana Gazivoda Kraljević *Synthesis and biological activity of new benzoxazolinone–coumarin hybrids*

P14	**Antonia Krsnik**, Jelena Osmanović Barilar, Leonarda Vlahov, Ana Babić Perhoč, Jan Homolak, Davor Virag, Melita Šalković-Petrišić, Ana Knezović *Region-specific insulin signaling dynamics in the rat brain following intranasal insulin administration*
P15	**Ivana Kuzminac**, Edward Petri, Marija Sakač, Dimitar Jakimov, Maja Marinović *Synthesis, in silico ADMET properties, target prediction, docking studies, and in vitro antiproliferative activity of C19-oxime and -nitrile androstane aromatise inhibitors*
P16	**Antonija Mamić**, Kristina Butković, Leentje Persoons, Dirk Daelemans, Robert Vianello, Mihailo Banjanac, Marijana Hranjec *Synthesis, antiproliferative and antibacterial activity in vitro of new pyridine benzamides and acrylonitriles*
P17	**Ana Petrović**, Marija Hefer, Sanja Đokić, Jelena Kesić, Robert Smolić, Vesna Kojić, Jovana Francuz, Srđan Bjedov, Martina Smolić *Lactone-based derivatives induce apoptosis via Caspase-3 activation in drug-resistant K562 cells*
P18	**Marina Punčec**, Tomislav Šmuc, Višnja Stepanić *How reliable are in silico predictions? A comparative study of ML models*
P19	**Milena Rašeta**, Sofija Bekić, Sanja Berežni, Gordana Gojgić-Cvijović, Anđelka Ćelić, Edward Petri *Phenolic profile, AKR1C inhibition, and steroid receptor binding of Cyclocybe aegerita extracts: Insights into anticancer and metabolic therapeutic potential*
P20	Danijela Beneš, Vesna Petrović Peroković, Željka Car, Ranko Stojković, **Rosana Ribić** *Synthesis and immunological propeties of cholesterol derivatives of mannosylated desmuramyl peptide*
P21	**Leon Sačer**, Marina Ter, Doris Babić, Martin C. Taylor, John M. Kelly, Silvana Raić-Malić *Synthesis and structural characterization of new nitro(triazole/imidazole)-appended quinolines with potential antitrypanosomal activity*
P22	**Marina Savić**, Tijana Kovačević, Sofija Bekić, Anđelka Ćelić *New steroid hydrazones as potential ligands for estrogen receptors*
P23	**Gjino Šutić** *Yellow K2 – potential next generation NSAID*
P24	**Luca Tongiorgi**, Simone Albani, Francesco Musiani *Selection of promising hit candidates through an automated filtering workflow for structure-based virtual screening: a case study on TMPRSS2*
P25	**Lucija Vrban**, Robert Vianello *SARS-CoV-2 spike protein interference with monoamine oxidase B: Potential links to neurodegenerative diseases*
P26	**Dejana Vujnović**, Martina Mušković Lukić, Nela Malatesti *Preparation of A_3B meso-(N,N-diethylaminophenyl)porphyrin with a long alkyl chain and N-quaternisation*

LECTURES AND WORKSHOPS

Alessandro Accetta | CHIESI Italia S.p.A., Italy
Title: *Differences from Oral Therapies, and Case Studies* (**L01**)

Alessandro Accetta is head of Medicinal Chemistry Unit-1 in the Department of Medicinal Chemistry & Drug Desing Technologies at Chiesi Farmaceutici (Parma, Italy). He completed his PhD studies in organic chemistry at the University of Parma (Parma, Italy) in March 2010. His research activity is focused on medicinal chemistry for pulmonary diseases, with emphasis on inhalation design. He is co-author of 12 publications and 21 patent applications.

Marko Anderluh | University of Ljubljana, Faculty of Pharmacy, Slovenia
Title: *Principles of Early Drug Discovery: Finding the Right Ligand for the Right Target* (**L02**)

Prof. Marko Anderluh is a professor at the University of Ljubljana where he teaches Medicinal Chemistry and Pharmacology. He is president of the Slovenian Pharmaceutical society and intensively involved in the work of the European Federation for Medicinal Chemistry. He authored 100+ SCI-indexed papers (h-index 25). His research focuses on glycodrugs, bacterial resistance and radioligands.

Vesna Gabelica Marković | University of Zagreb Centre for research, development and transfer technology, Croatia
Title: *The Journey of Innovation; Bridging gaps: A Roundtable on Science, Industry & Innovation* (**L03**)

Vesna Gabelica Marković is the Innovation Community Manager at the University of Zagreb and a representative of EIT Health. She leads initiatives that foster innovation in local community healthcare. With over 30 years of experience across academia and industry, she brings deep expertise and a broad perspective to her work.

At the CSSMC, Vesna will introduce participants to innovation from a business perspective, a topic often underrepresented in academic education. She will also moderate a Roundtable on Science, Industry & Innovation featuring our distinguished lecturers from both academia and industry, designed to be engaging, insightful, and educational!

Tatjana Gazivoda Kraljević | University of Zagreb Faculty of Chemical Engineering and Technology, Croatia

Title: *Sustainable Synthesis of Biologically Active Compounds Supported by AI Tools* (**L04**)

Tatjana Gazivoda Kraljević is a Full Professor and Head of the Department of Organic Chemistry at the University of Zagreb Faculty of Chemical Engineering and Technology. She has over 20 years of experience in synthetic organic and medicinal chemistry, with a particular focus on the development and sustainable synthesis of novel biologically active heterocyclic compounds.

Nikola Basarić | Ruđer Bošković Institute, Zagreb, Croatia

Title: *Synthesis of BODIPY Compounds as Potential Drug Delivery Systems and Cancer Phototherapeutics* (**L05**)

Nikola Basarić obtained his education at the University of Zagreb, where he conducted his graduate studies of chemical engineering and received Ph.D. in chemistry in 2002 with M. Šindler. He did postdoctoral work at the KU Leuven, Belgium in the F. C. De Schryver's laboratory for photochemistry and spectroscopy, working with N. Boens, and at the University of Victoria, Canada, working with P. Wan. From 2006 he is employed at the Ruđer Bošković Institute in Zagreb in Croatia where he conducts research in organic photochemistry.

Ivan Grgičević | Labtim d. o. o., Croatia

Title: *Integrating LC and LC-MS in Drug Discovery and Development: Principles, Practice, and Applications* (**L06**)

Ivan Grgičević is specialized in analytical chemistry, business development and lab management. He is currently Business Development Manager and Genotoxic Impurities Lab Manager at Labtim Adria Ltd. He holds an MSC in Medicinal Chemistry, an EXEC MBA and is pursuing a PhD in Chemistry. Ivan is proficient in a wide range of chromatography and mass spectrometry techniques, with an expertise in method development, validation and GMP compliance.

At the CSSMC, Ivan will be delivering a hands-on workshop on integration of LC and LC-MS in drug discovery and development, combining theory with practice and real-world application.

Ivan Kondratov | Enamine Germany

Title: *Compound Libraries in Modern Drug Discovery; Design of Compound Libraries* (**L07**)

Ivan Kontradov defended his PhD in the Institute of Biorganic Chemistry and Petrochemistry, NAS of Ukraine in 2008. At Enamine Ltd. (Kyiv, Ukraine) he held different positions from the Team Lead to the Head of Medicinal Chemistry. In 2023, Ivan and his colleagues launched the German site of Enamine – Enamine Germany GmbH. His research is in the field of heterocyclic chemistry, synthesis of Building Blocks for Drug Discovery, unnatural amino acids, organofluorine chemistry, medicinal chemistry and compound library design.

At the CSSMC, he will introduce participants to the state-of-the-art in chemical library design and application. In addition to his lecture, he will lead a hands-on workshop to demonstrate how these tools can help accelerate research projects.

Iva Kušec | Altium International d.o.o., Croatia

Title: *Technological Infrastructure for the Future of Therapeutics* (**L08**)

Iva Kušec earned her MSc in chemistry at the Faculty of Science, University of Zagreb. She was awarded a PLIVA scholarship and worked for 3 years at PLIVA in the quality control laboratory. Then she joined Altium International as an application specialist in the Life Science sector, supporting users in analyses and the implementation of new technologies and soutions.

At the CSSMC, Iva will lead an inspiring workshop on state-of-the-art technological infrastructure shaping the future of therapeutics, including a live demonstration of the Seahorse instrument, a powerful tool for analyzing cellular metabolism and mitochondrial function.

Milan Mesić | University of Rijeka, Faculty of Biotechnology and Drug Development

Title: *Molecule Makeover: Engineering the Perfect Preclinical Drug; Incretins: The Hormones That Sparked a Therapeutic Revolution* (**L09**)

Milan Mesić is a Full Professor of Medicinal Chemistry at the University of Rijeka's Faculty of Biotechnology and Drug Development. Prof. Mesić has extensive experience in pharmaceutical R&D, having held leadership positions at GSK, Galapagos, Fidelta and PLIVA. His research includes small-molecule synthesis for CNS, cancer, inflammatory and antimalarial targets.

Jasna Padovan | Selvita Ltd., Croatia
Title: *Optimizing Drug Behavior: A DMPK Perspective* (**L10**)

Dr. Jasna Padovan is Senior Director of DMPK at Selvita, with over 25 years of experience in biotech and pharma. She leads global DMPK strategies with expertise in ADME, PK/PD, and bioanalysis, supporting projects from discovery to early development. Dr. Padovan has served as Test Facility Manager for the GLP bioanalytical lab in Zagreb, built high-throughput ADME screening platforms, and led multidisciplinary teams. She is also a dedicated mentor, fostering a collaborative, innovation-driven culture, and author or co-author of over 20 scientific publications.

Ivana Perković | University of Zagreb Faculty of Pharmacy and Biochemistry, Croatia
Title: *Drug Targeting: Where We Are, How We Got Here, and Where We're Headed* (**L11**)

Ivana Perković is an Associate Professor at the University of Zagreb Faculty of Pharmacy and Biochemistry. Her research focuses on the synthesis of small molecules with potential antimalarial activity. She investigates the binding of various molecular scaffolds to natural product harmine-based compounds. She is also interested in the synthesis of quorum sensing inhibitors as potential novel therapeutic agents.

Ivana Ratkaj | University of Rijeka, Faculty of Biotechnology and Drug Development, Croatia
Title: *Never Ending Battle Against a Perfect Enemy: Can We Finally Decipher Tumor Communication* (**L12**)

Ivana Ratkaj is an Associate Professor at the Faculty of Biotehnology and Drug Development in Rijeka. Her research interests are in cancer biology, focusing on elucidating the mechanisms of anti-tumor agents through transcriptomic and proteomic analyses. Her current scientific work is centered on *in vitro* studies investigating the biological effects of compounds for application in photodynamic therapy, as well as the mechanisms of intercellular communication.

Višnja Stepanić | Ruđer Bošković Institute, Zagreb
Title: *Hands-On Session: Predicting ADMET and Physicochemical Properties Using Online Resources* (**L13**)

Dr. Višnja Stepanić is a senior scientist at the Ruđer Bošković Institute in Zagreb with a PhD in theoretical and computational chemistry. She has over 30 years of experience in academia and industry and has contributed to numerous interdisciplinary and international projects. Her expertise spans molecular modeling, chemoinformatics, machine learning, medicinal chemistry, biochemistry, and ADMET profiling. She is active in EU-funded research, COST networks, and national projects. She also serves as a reviewer, mentor, and organizer of scientific events, and has authored over 50 publications.

Jörg Wichard | Selvita S.A., Krakow, Poland
Title: *Implementing AI in Drug Discovery* (**L14**)

Dr. Jörg Wichard is head of the AI team at Selvita. He holds a PhD in Physics from the University of Göttingen. His previous experience includes serving as a Computational Toxicologist at Bayer AG (2010-2021), where he developed and applied *in silico* tools for predictive toxicology. Earlier postdoctoral and research work centered on ensemble learning, machine learning in GPCR drug discovery, and computational peptide design. Dr. Wichard has a wide scientific experience from different places such as AGH Kraków, Schering AG, Bayer Crop Science in France, the Molecular Modeling Group at FMP Berlin and from Charité - Berlin University of Medicine. He is working in the field of Artificial Intelligence and Machine Learning related to Life Science more than two decades.

POSTERS

Androstane-based heterocycles: Evaluation of antiparasitic activity, *in silico* protein affinity and (eco)toxicological insight

Jovana Ajduković[1,*], An Matheeussen[2], Natascha Van Pelt[2], Maja Marinović[3], Svetlana Fa Nedeljković[3], Marina Savić[1], Guy Caljon[2]

[1]*Department of Chemistry, Biochemistry and Environmental Protection, Faculty of Sciences, University of Novi Sad, Trg Dositeja Obradovića 3, Novi Sad, Serbia*

[2]*Laboratory of Microbiology, Parasitology and Hygiene, Faculty of Pharmaceutical, Biomedical and Veterinary Sciences, Antwerp University, Antwerp, Belgium*

[3]*Department of Biology and Ecology, Faculty of Sciences, University of Novi Sad, Trg Dositeja Obradovića 2, Novi Sad, Serbia*

*Corresponding Author's E-mail: jovana.ajdukovic@dh.uns.ac.rs

Infectious diseases are an ongoing threat to human and animal health and the cause of significant economic losses. According to the WHO, human trypanosomiasis is one of the 13 most neglected tropical diseases. While treating bacterial infections continually progresses through the discovery of new antibiotics, parasitic infections remain more challenging to treat due to their high diversity. *Trypanosoma cruzi* relies heavily on glucose metabolism for its survival and proliferation in the host, so glucose-6-phosphate dehydrogenase (G6PD) could be an important target for antiparasitic drug discovery. This motivated the scientific community to focus on the discovery of safe, effective, and affordable antiparasitic drugs.

Steroids, particularly those with heterocycles, are promising drug candidates as they can easily penetrate cell membranes and bind to nuclear receptors. It is known that they possess anticancer, anti-inflammatory, antimicrobial, and antiviral activity, but they are also recognized as antiparasitic agents [1]. Therefore, our main objective was the development of steroid-based compounds with different heterocyclic rings in their skeleton, as efficient antitrypanosomal agents. The A-, B- or D-ring modified androstane derivatives were combined with lactone, lactam or pyridine moieties in multistep synthetic procedures, which were then tested against several parasitic species. Interestingly, only derivatives with 17-picolinylidene function showed strong and selective activity against *T. cruzi*, consistent with the results obtained for some non-steroidal pyridinyl-substituted pyrazoles [2]. The most promising compounds were evaluated *in silico* for their binding affinity to G6PD. Molecular docking analyses suggest strong binding of our derivatives to the enzyme, so they may act as inhibitors through steric hindrance, physically preventing the substrate from entering the active site. Several compounds were also subjected to evaluation of their toxicity, which is an important part of the drug design process.

Keywords: Steroid; Heterocyclic ring; Parasites; Antitrypanosomal activity; Molecular docking.

Acknowledgement: The Authors acknowledge the Provincial Secretariat for Higher Education and Scientific Research of the Autonomous Province of Vojvodina (Project No. 003056514 2024 09418 003 000 000 001/01) for the financial support and the COST Action CA21111: One Health Drugs against Parasitic Vector-Borne Diseases in Europe and Beyond (OneHealthDrugs).

References:

[1] Kuzminac I. *et al*. Curr Topics Med Chem. 2023;23:791-815.
[2] Winge T. *et al*. ChemMedChem. 2024;19:e202400220.

Biological activity and computational analysis of novel acrylonitrile and imino-coumarin derived benzimidazoles

Anja Beč[1], Lucija Vrban[2], Leentje Persoons[3], Dirk Daelemans[3], Kristina Starčević[4], Robert Vianello[2], Marijana Hranjec[1,*]

[1]*Department of Organic Chemistry, University of Zagreb Faculty of Chemical Engineering and Technology, Marulićev trg 19, Zagreb, Croatia*

[2]*Laboratory for the Computational Design and Synthesis of Functional Materials, Division of Organic Chemistry and Biochemistry, Ruđer Bošković Institute, Bijenička cesta 54, Zagreb, Croatia*

[3]*KU Leuven, Department of Microbiology, Immunology and Transplantation, Laboratory of Virology and Chemotherapy, Rega Institute, Leuven, Belgium*

[4]*Department of Chemistry and Biochemistry, University of Zagreb Faculty of Veterinary Medicine, Heinzelova 55, Zagreb, Croatia*

*Corresponding Author's E-mail: mhranjec@fkit.hr

Benzimidazole, a small nitrogen-containing heterocyclic scaffold, is a key structural motif found in numerous natural and synthetic compounds with diverse biological activities, including notable antitumor effects [1-3]. This work presents the biological evaluation and computational analysis of three novel benzimidazole derivatives. All compounds were synthesized using established and optimized organic synthesis methods previously developed within our research group. The synthesized compounds were evaluated for their *in vitro* antiproliferative activity against various cancer cell lines and antioxidative activity using *several spectroscopic methods*. Computational docking and molecular dynamics simulations confirmed the high affinity of potent derivatives for the tubulin colchicine site and justified the suitability of the employed skeleton by identifying crucial protein–ligand interactions promoting binding. Furthermore, computational Density Functional Theory (DFT) analysis was employed to elucidate the chemical processes involved and identify the structural and electronic characteristics responsible for the observed antioxidant activity.

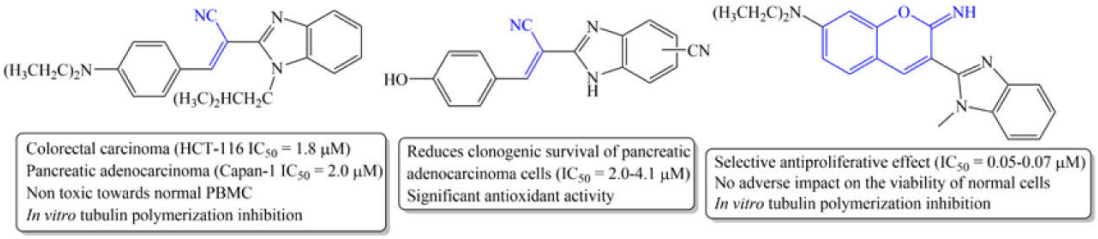


Figure 1. Acrylonitrile and imino-coumarin derived benzimidazoles

Keywords: Antiproliferative activity; Antioxidative activity; Benzimidazole; Computational analysis.

Acknowledgement: This work was funded by the Croatian Science Foundation (IP-2024-05-7208).

References:

[1] Perin N, Hok L, Beč A, Persoons L, Vanstreels E, Daelemans D, Vianello R, Hranjec M. Eur J Med Chem. 2021;211:113003.

[2] Beč A, Persoons L, Daelemans D, Starčević K, Vianello R, Hranjec M. Bioorg Chem. 2024;147:107326.

[3] Boček Pavlinac I, Persoons L, Beč A, Vrban L, Daelemans D, Vianello R, Hranjec M. Bioorg Chem. 2025;154:107991.

Assessment of the anticancer potential of transition metal complexes with metformin via molecular docking: Insights from a literature review

Emina Bečić*

University of Sarajevo – Faculty of Pharmacy, Zmaja od Bosne 8, Sarajevo, Bosnia & Herzegovina

*Corresponding Author's E-mail: emina.becic@ffsa.unsa.ba

Metformin is a widely used drug for the treatment and management of type 2 diabetes mellitus [1]. Beyond its glucose-lowering effect, metformin exhibits a range of pleiotropic health benefits, including a reduced risk of cancer and inhibition of tumorigenesis [2]. Numerous studies have demonstrated that metformin use is associated with a lower incidence of cancer among diabetic patients, and its potential role in cancer therapy is increasingly being explored in non-diabetic individuals as well [3, 4]. Moreover, it has been confirmed that metformin complexes with transition metals may exhibit improved pharmacokinetic properties and enhanced biological activity compared to metformin alone, thereby increasing its efficacy and therapeutic potential [5]. In this paper, the anticancer activity of metformin complexes with Ni(II) and Cu(II) is compared, based on a review of the available literature. The potential anticancer activity of metformin complexes with Ni and Cu was evaluated via molecular docking by assessing their interaction with the B-DNA sequence. The results suggest that all complexes bind to DNA via groove binding. Metformin complexes with Ni(II) demonstrate stronger interactions with DNA compared to Cu(II) complexes. The highest interaction energy has $[Ni(met)(opda)_2]Cl_2$, highlighting it as a promising candidate for further investigations in the development of anticancer agents.

Complex	Interaction Energy	Reference
$[Cu(Cl)_2(met)(o\text{-phen})]$	**24.88**	[6]
$[Cu(Cl)_2(met)(en)]$	**14.86**	[6]
$[Cu(Cl)_2(met)(opda)]$	**13.75**	[6]
$[Ni(met)(o\text{-phen})_2]Cl_2$	**38.169**	[7]
$[Ni(met)(opda)_2]Cl_2$	**34.849**	[7]
$[Ni(met)(2–2'bipy)_2]Cl_2$	**30.989**	[7]

met = metformin, *o*-phen = *ortho*-phenanthroline, en = ethylenediamine, opda = *ortho*-phenylenediamine

Keywords: Metformin; Anticancer activity; Molecular docking.

References:

[1] Sam S, Ehrmann DA. Diabetologia. 2017;60(9):1656-61.
[2] Vancura A, Bu P, Bhagwat M, Zeng J, Vancurova I. Trends Pharmacol Sci. 2018;39(10):867-78.
[3] Lv Z, Guo Y. Front Endocrinol. 2020;11:191.
[4] Pernicova I. Nat Rev Endocrinol. 2014;10(3):143-56.
[5] Rusanov DA, Zou J, Babak MV. Pharm. 2022;15(4):453.
[6] Vasantha P, Sathish Kumar B, Shekhar B, Anantha Lakshmi PV. Mater Sci Eng C. 2018;90:621-33.
[7] Rajeshwari K, Vasantha P, Kumar BS, Lakshmi PVA. Biol Trace Elem Res. 2022;200(12):5351-64.

10-Alkoxy estrane derivatives as potential anticancer agents: interactions with steroid receptors and inhibition of aldo-keto reductases 1C3 and 1C4

Sofija Bekić[1,*], Ivana Kuzminac[1], Milica Stevanović[1], Edward Petri[2], Anđelka Ćelić[2]

[1]*Department of Chemistry, Biochemistry and Environmental Protection, Faculty of Sciences, University of Novi Sad, Trg Dositeja Obradovića 3, Novi Sad, Serbia*

[2]*Department of Biology and Ecology, Faculty of Sciences, University of Novi Sad, Trg Dositeja Obradovića 2, Novi Sad, Serbia*

*Corresponding Author's E-mail: sofija.bekic@dh.uns.ac.rs

Steroid hormones are key regulators of both normal reproductive development and malignant growth in hormone-dependent cancers. Modulation of levels or activity of steroid hormones has emerged as promising treatment for such cancers. Therefore, intensive research has been focused on the synthesis of modified steroid molecules in order to obtain compounds with improved anticancer activity. In this study, the biological activity of newly synthesized 10-alkoxy estrane derivatives was evaluated against steroid receptors and steroid-converting aldo-keto reductases (AKRs), biological targets in the treatment of hormone-dependent cancers. Estrane derivatives were evaluated *in vitro* for their relative binding affinities for the ligand-binding domains (LBDs) of estrogen receptor α (ERα) and β (ERβ), androgen (AR) or glucocorticoid receptor (GR) using a fluorescent assay in yeast [1]. LBD of each steroid receptor was expressed in-frame with yellow fluorescent protein in yeast and fluorescence intensity changes upon addition of ligand were measured using fluorimetry. In addition, evaluation of inhibition potential against human recombinant AKR1C3 and AKR1C4 was performed by fluorescence spectroscopy. None of the tested compounds exhibited binding to GR and AR, indicating no agonistic activity. This is important for the design of steroidogenic enzyme inhibitors to be used in anti-hormone treatment, because compounds that could interact with AR may promote tumor growth in hormone-dependent cancers. As expected, these estrane derivatives showed affinity towards ER isoforms, and were found to be more selective for α than β isoform. Our results suggest potential role of 10-alkoxy estrane derivatives in modulating ER-mediated pathways, but additional studies are needed to determine whether these compounds act as agonists or antagonists. Furthermore, these compounds were shown to be potent inhibitors of AKR1C3 and AKR1C4, suggesting their potential as therapeutic agents for conditions such as hormone-related cancers and metabolic disorders. Although there is significant interest in finding AKR1C3 inhibitors due to its overexpression in various cancers and acute myeloid leukemia, as well as its contribution to the development of drug resistance through the metabolism of chemotherapeutic agents, none have yet been approved for clinical use. Findings of this study highlight the potential of 10-alkoxy estrane derivatives as promising anticancer agents or adjuvants due to their ability to modulate both ER signaling and AKR1C activity. Their ability to be structurally modified also makes them ideal for the design of novel steroidal agents with enhanced anticancer properties.

Keywords: Steroid receptor; Aldo-keto reductase; Hormone-dependent cancer; Estrane derivative.

Acknowledgement: The authors gratefully acknowledge the financial support of the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Grants No. 451-03-137/2025-03/ 200125 & 451-03-136/2025-03/ 200125) and the financial support of the Provincial Secretariat for Higher Education and Scientific Research of the Autonomous Province of Vojvodina (Project No. 003056514 2024 09418 003 000 000 001/1).

References:

[1] Bekić S, Marinović M, Petri E, Sakač M, Nikolić A, Kojić V, Ćelić A. Steroids 2018;130:22-30.

Antiplasmodial activity of novel β-carboline-artemisinin hybrids

Lucija Bilandžija[1], Bárbara Teixeira[2], Miguel Prudencio[2], Zrinka Rajić[1,*]

[1]*Department of Medicinal Chemistry, Faculty of Pharmacy and Biochemistry University of Zagreb, Ante Kovačića 1, Zagreb, Hrvatska*

[2]*Instituto de Medicina Molecular João Lobo Antunes, Faculdade de Medicina Universidade de Lisboa, Av. Prof. Egas Moniz, Lisboa, Portugal*

*Corresponding Author's E-mail: zrinka.rajic@pharma.unizg.hr

Malaria is a life-threatening infectious disease caused by *Plasmodium* parasites. In 2023, the disease affected approximately 263 million people and resulted in an estimated 597,000 deaths worldwide. The high mortality rate, coupled with the emergence of resistance to existing antimalarial therapies, highlights the urgent need for the development of novel and effective therapeutic agents [1]. One promising approach in antimalarial drug discovery is molecular hybridization which has the potential to enhance therapeutic efficacy and overcome some limitations associated with combination therapies. To this end, we have designed harmisinins, hybrids comprising β-carboline and artemisinin moieties, linked *via* triazole ring or amide bond. *β*-Carbolines represent a structurally diverse class of compounds, which have been shown to interact with multiple biological targets. In the context of malaria, they exhibit inhibitory activity against *P. falciparum* heat shock protein 90 (*Pf*Hsp90) [2]. Artemisinin is a sesquiterpene lactone with a characteristic endoperoxide bridge, which represents a cornerstone of current antimalarial treatment regimens, exhibiting rapid action and well-characterized mechanisms of activity against *P. falciparum* [3]. The synthesis of harmisinins required the preparation of their constituent building blocks, each of which was obtained through a multistep synthetic route. The triazole-type (TT) harmisinins were synthesized *via* copper(I)-catalyzed azide–alkyne cycloaddition (CuAAC) between artesunate-derived azide and *β*-carboline-based terminal alkynes, or alternatively, between dihydroartemisinin-derived alkyne and *β*-carboline-based azides. The amide-type (AT) harmisinins were obtained through amide bond formation, involving the coupling of artesunate or different dihydroartemisinin-based carboxylic acids with *β*-carboline-based amine counterparts. Screening of *in vitro* activity of harmisinins against the hepatic stages of *Plasmodium* parasite was performed employing a previously described method [4, 5]. Both AT and TT harmisinins were initially tested at two concentrations, 1 and 10 μM, followed by the determination of the concentration that causes 50% growth inhibition (IC_{50}) for the most active compounds. Most of the harmisinins exerted remarkably higher activity than the parent compound harmine. The experiments revealed that 7 harmisinins display activities in single digit micromolar or sub-micromolar range. Overall, the hybridization of artemisinin with β-carboline derivatives presents a promising approach for addressing the challenges of malaria. In our future investigations, we will focus on the elucidation of harmisinins' mechanisms of action.

Keywords: *β*-Carboline; Artemisinin; Hybridization; Malaria.

References:

[1] World malaria report 2024: addressing inequity in the global malaria response.

[2] Kamboj A, Sihag B, Singh Brar D, Kaur A, Salunke DB. Eur J Med Chem. 2021;221:113536.

[3] Zeng Z, Chen D, Chen L, He B, Li Y. Eur J Med Chem. 2023;247:115000.

[4] Machado M, Sanches-Vaz M, Cruz JP, Mendes AM, Prudêncio M. Front Cell Infect Microbiol. 2017;7:329.

[5] Ploemen IHJ, Prudêncio M, Douradinha BG, Ramesar J, Fonager J, van Gemert, GJ, Luty AJF, Hermsen CC, Sauerwein RW, Baptista FG *et al*. PLoS ONE 2009;4:7881.

Synthesis, biological evaluation and interaction with *ct*-DNA of mono and diamidino-substituted imidazo[4,5-*b*]pyridines

Ida Boček Pavlinac[1,*], Corina Šljubura[1], Katarina Zlatić[2], Marijeta Kralj[2], Ivana Fabijanić[3], Marijana Radić Stojković[3], Marijana Hranjec[1]

[1]*Department of Organic Chemistry, Faculty of Chemical Engineering and Technology, University of Zagreb, Marulićev trg 19, Zagreb, Croatia*

[2]*Division of Molecular Medicine, Institute Ruđer Bošković, Bijenička cesta 54, Zagreb, Croatia*

[3]*Ruđer Bošković Institute, Division of Organic Chemistry and Biochemistry, Bijenička cesta 54, Zagreb, Croatia*

*Corresponding Author's E-mail: ibocek@fkit.unizg.hr

Amidine groups are important in drug discovery due to their strong basicity and ability to form stable hydrogen bonds and electrostatic interactions, which enhance binding affinity to biological targets, including DNA [1]. Nitrogen heterocycles are crucial because they mimic natural purines and offer structural diversity and metabolic stability, making them valuable scaffolds in the design of therapeutics [2]. Together, these features enable selective DNA binding and modulation of biological activity.

This work presents the synthesis and biological evaluation of 2,6-disubstituted imidazo[4,5-*b*]pyridines. Precursors were prepared via Suzuki coupling reactions followed by nitro group reduction, while the key cyclic intermediates were obtained through a DMSO-mediated cyclization in the presence of $Na_2S_2O_5$ [3]. Unsubstituted amidines were synthesized using lithium hexamethyldisilazane, while substituted derivatives were prepared via the Pinner reaction. The structures of the newly synthesized compounds were confirmed using ^1H and ^{13}C NMR spectroscopy as well as mass spectrometry. Biological evaluation included *in vitro* assessment of antiproliferative activity across a diverse panel of human cancer cell lines. Additionally, spectroscopic investigations of the interactions between the most active derivatives and calf thymus DNA (*ct*-DNA) were conducted using UV/Vis absorption, fluorescence emission and circular dichroism (CD) spectroscopy.

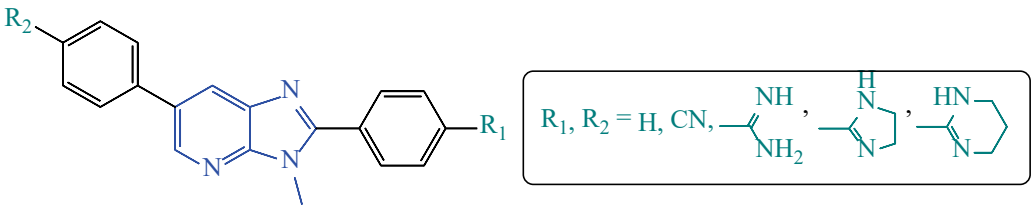


Figure 1. Structures of newly prepared amidino-substituted imidazo[4,5-*b*]pyridines

Keywords: Amidines; Imidazo[4,5-*b*]pyridines; Antiproliferative activity; ct-DNA.

This work was funded by Croatian Science Foundation under the projects IP-2024-05-7208 (Synthesis of new benzazole heterocycles as potential antitumor and antibacterial agents) and IP-2018-01-4694 (Molecular recognition of DNA:RNA hybrid and multistranded structures in bioanalytical and in vitro systems).

References:

[1] Qian K *et al*. RSC Med Chem. 2021;12:95-102.
[2] Boček Pavlinac I *et al*. Molecules 2023;28:34.
[3] Abou-Elkhair RAI *et al*. Org Lett. 2016;18:4714-17.

The nifedipine molecule used in the treatment of Raynaud syndrome: An *in silico* study

İlayda Boyraz[1], Bilge Bıçak [1,*]

[1] *Department of Physics, Faculty of Science, Istanbul University, Istanbul, Türkiye*

*Corresponding Author's E-mail: boyrazilayda484@gmail.com

Raynaud's Syndrome is a vascular disease characterised by narrowing of the blood vessels, usually caused by triggers such as cold or stress. Nifedipine, a dihydropyridine derivative calcium channel blocker, is used in the treatment of this syndrome due to its vasodilator effects [1]. In this study, a comprehensive molecular modeling analysis was performed to better understand the structural, electronic and pharmacokinetic properties, and to determine the interaction profile of the nifedipin with voltage-dependent L-type calcium channel subunit alpha-1S [2]. Density Functional Theory (DFT) method was used to perform molecular geometry optimization, The Highest Occupied of Molecular Orbitals- The Lowest Unoccupied Molecular Orbitals (HOMO-LUMO) and molecular electrostatic potential (MEP) analyses [3]. Additionally, molecular docking and Absorption, Distribution, Metabolism, Excretion and Toxicity (ADMET) analyses were performed to determine the binding profile of nifedipine with voltage-dependent L-type calcium channel subunit alpha-1S and pharmacokinetic properties [4]. In this study, the molecular structure and interaction profile of the calcium channel blocker nifedipine were elucidated and a reference drug profile was presented from the literature.

Keywords: Calcium-channel blocker; Molecular modeling; Density Functional Theory; Molecular docking; ADMET.

References:

[1] Birnstingl M. The Raynaud syndrome. Postgrad Med J. 1971;47(547):297-310.

[2] Elliott WJ, Ram CV. Calcium channel blockers. J Clin Hypertens (Greenwich). 2011;13(9):687-9.

[3] Marinho ES, Márcia MM. A DFT study of synthetic drug topiroxostat: MEP, HOMO, LUMO. Int J Sci Eng Res. 2016;7(8):1264.

[4] Norinder U, Bergström CA. Prediction of ADMET Properties. ChemMedChem. 2006;1(9):920-37.

Predictive profiling of BODIPY dyes: Insights into their drug-likeness

Dea Demeter[1], Nikola Basarić[2], and Višnja Stepanić[2,*]

[1]*University of Zagreb Faculty of Science, Horvatovac 102a, Zagreb, Croatia*

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

*Corresponding Author's E-mail: Visnja.Stepanic@irb.hr

BODIPY dyes (Boron-Dipyrromethene compounds, Figure 1) are versatile fluorescent molecules used across many fields due to their exceptional photophysical properties, such as high fluorescence quantum yield, photostability, and chemical tunability [1,2]. They have been used for fluorescent imaging and cell labeling, for tumor targeting through conjugaton with peptides or nanoparticles, in biosensor development as well as in photodynamic therapy (PDT) [2,4]. BODIPYs modified with heavy atoms (e.g., iodine, bromine) act as efficient photosensitizers to generate singlet oxygen for killing cancer cells [4].

BODIPY derivatives exhibit unique physicochemical characteristics that set them apart from other organoboron molecules and from drugs. In order to guess their drug-likeness profile, we have compared them with drugs as well as other classes of organoboron compounds. The comparison has been made in terms of calculated structural, physicochemical and ADMET properties. All compounds were gathered from the public chemical databases ChEMBL and PubChem, or from recently published article [3] describing BODIPY dyes that exhibit enhanced antiproliferative activity on exposure to visible light irradiation. Molecular representations were based on structural fingerprints computed using RDKit, as well as physicochemical features calculated by using quantitative structure-activity relationship (QSAR) models in-built in Simulations Plus and DataWarrior software. Differentiating features were identified using statistical analyses, including unsupervised machine learning techniques such as Principal Component Analysis (PCA), t-distributed Stochastic Neighbor Embedding (t-SNE), and Uniform Manifold Approximation and Projection (UMAP). Molecules were also compared using criteria from Lipinski's Rule of Five and Veber's rule of drug-likeness.

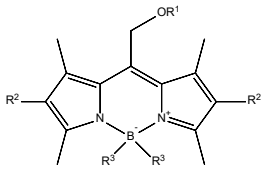


Figure 1. BODIPY core with potential substitution sites

Keywords: BODIPY; Drug-likeness; Machine Learning; QSAR.

Acknowledgement: NPOO-KP3-24 BODIPY dyes as precursors of borenium cations in photochemical reactions: investigation of the reaction mechanism and application in cancer phototherapy

References:

[1] Grams RJ, Santos WL, Scorei IR, Abad-García A, Rosenblum CA, Bita A, Cerecetto H, Viñas C, Soriano-Ursúa MA. Chem Rev. 2024;124:2441-511.

[2] Kamkaew A *et al*. Chem Soc Rev 2013;42:77-88.

[3] Zlatić K, Popović M, Uzelac L, Kralj M, Basarić N. Eur J Med Chem. 2023;259:115705-20.

[4] Das BC, Nandwana NK, Das S, Nandwana V, Shareef MA, Das Y, Saito M, Weiss LM, Almaguel F, Hosmane NS, Evans T. Molecules 2022;27:2615-51.

Harnessing transcriptomic signatures for drug discovery: Repurposing potential, off-target detection, and safety forecasting

Dorian Dulčić, Višnja Stepanić*

Division of Electronics, Ruđer Bošković Institute, Bijenička cesta 54, Zagreb, Croatia

*Corresponding Author's E-mail: Visnja.Stepanic@irb.hr

Developing safe and effective therapeutics remains a significant economic and research challenge. Traditional approaches in drug discovery, commonly centered around single biological targets, often fail to elicit the intended biological response and may overlook off-target effects, which could result in toxicity [1]. Transcriptomic profiling - the comprehensive quantitative analysis of mRNA expression upon exposure to various pharmacological agents of interest, could contribute to the elucidation of the mechanism of action of a compound of interest, detection of its off-targets, and predicting its side effects, thereby enabling its therapeutic repurposing.

In drug repurposing, transcriptomic profiling of differential gene expression (DGE) enables the identification of new therapeutic uses for already approved or shelved compounds. By comparing the DGE of a disease state with the DGE of drug-treated/perturbed cells, compounds that reverse disease-specific transcriptional profiles can be computationally matched to new indications, which is the concept known as signature reversion. Signature reversion is a hallmark feature of the LINCS L1000 platform, which facilitates large-scale, *in silico* drug-disease matching [2]. A successful example is the repurposing of Vorinostat, a drug initially developed for cancer, to reverse HIV latency [3].

Transcriptomic profiling also helps to uncover off-target effects and predict drug-induced toxicities, offering insights often overlooked by traditional protein binding or phenotypic assays. By capturing DGE across the entire genome, this approach reveals unintended modulation of molecular pathways that may result in systemic adverse outcomes. Enrichment of transcriptomic alterations occurring across diverse cellular networks helps delineate a drug's pharmacological footprint. A successful example is the transcriptomic discovery of the mechanism underlying Doxorubicin-induced cardiotoxicity through gene enrichment validation from experimentally acquired RNAseq data, which was later verified in vitro using immunoblotting [4]. In toxicogenomics, drug-induced gene expression signatures can be screened against known adverse outcome pathways (AOPs), such as those associated with oxidative stress, apoptosis, or fibrosis. Open-access resources such as DrugMatrix [5] systematically correlate transcriptomic signatures with pathohistological findings in rat target tissues, providing a comprehensive reference database for mechanistic interpretation of toxicogenomic data.

Altogether, transcriptomic profiling offers an unbiased and integrative method for linking drug-induced phenotypic changes to events at the molecular level. Combined with other omics (proteomics and metabolomics) and computational tools, it is the basis of systems pharmacology. It holds great promise for precision medicine, enabling individualized drug efficacy and induced toxicity prediction.

Keywords: Transcriptomics; Drug Repurposing; Toxicogenomics; Signature Reversal.

References:

[1] Sadri A. OSF 2022: 2022-04-06 (preprint)
[2] Subramanian A *et al*. Cell 2017;171(6):1437-1452.e17.
[3] Archin N *et al*. Nature 2012;487:482-485.
[4] McSweeney KM *et al*. Cell Death Discov. 2019;5:102.
[5] Cong G *et al*. bioRxiv 2024: 2024-03 (preprint)

Synthesis of biologically active benzoxazoles

Marina Galić[1,*], Tamara Rinkovec[2], Leentje Persons[3], Dirk Daelemans[3], Marijana Radić Stojković[4], Rosana Ribić[5], Robert Vianello[2] and Marijana Hranjec[1]

[1]*Department of Organic Chemistry, University of Zagreb Faculty of Chemical Engineering and Technology Organisation, Marulićev trg 19, Zagreb, Croatia*

[2]*Laboratory for the Computational Design and Synthesis of Functional Materials, Ruđer Bošković Institute, Zagreb, Croatia*

[3]*KU Leuven Department of Microbiology, Immunology and Transplantation, Rega Institute, Leuven, Belgium*

[4]*Division of Organic Chemistry and Biochemistry, Ruđer Bošković Institute, Bijenička cesta 54, Zagreb, Croatia*

[5]*Department of Nursing, University North, Koprivnica, Croatia*

*Corresponding Author's E-mail: mgalic@fkit.unizg.hr

Heterocyclic compounds represent one of the most significant classes of organic compounds in organic and medicinal chemistry since many of them are used as key structural building blocks for the synthesis of various biologically active molecules [1,2]. As a structural analog of the nucleobases adenine and guanine, benzoxazole stands out as an important nitrogen-containing heterocycle. Namely, benzoxazole interactions with macromolecules such as DNA, RNA or proteins are commonly attributed to the bioisosterism with the naturally occurring nucleotides. The present heteroatoms enable the realization of non-covalent interactions important in rational drug design [1,3]. Studies on their antitumor activity have shown that different benzoxazole derivatives can bind to various biological targets, among the most important ones are different protein kinases [3]. In this work, we present the results of biological evaluation of acrylonitrile (**1** and **2**), benzamide (**3**) and iminocoumarine (**4**) derived benzoxazoles. Antiproliferative activity *in vitro* of prepared compounds was tested against several human cancer cells as well as antiviral activity *in vitro* against several virus strains. For most active acrylonitrile compounds interaction with *ct*-DNA was studied by several spectroscopic methods including UV/Vis, fluorescence and CD spectroscopy as well as thermal melting experiments. Besides, the inhibitory potential of acrylonitrile derivatives was evaluated using a RIPK2 inhibition assay.

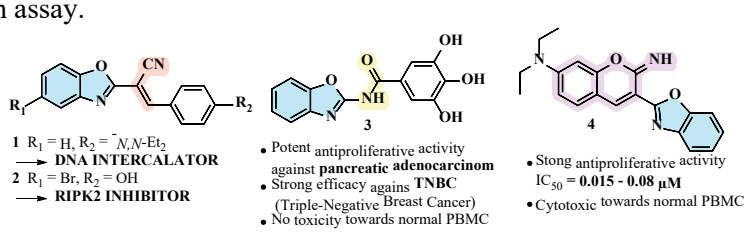


Figure 1. Structures of investigated benzoxazole derivatives

Keywords: Benzoxazole; Acrylonitrile; Benzamide; Iminocoumarine; Biological activity.

Acknowledgement: The Croatian Science Foundation funded this work within project BenzHetPot IP-2024-05-7208.

References:

[1] Kumar A, Singh AK, Singh H, Vijayan V, Kumar D, Naik J, Thareja S, Yadav JP, Pathak P, Grishina M. Pharma. 2023;16:299.

[2] Beč A, Persoons L, Daelemans D, Starčević K, Vianello R, Hranjec M. Bioorg Chem. 2024; 147(16):107326.

[3] Di Martino S, De Rosa M. Top Curr Chem. 2024;382:33.; Wong XK, Yeong KY, ChemMedChem 2021;16: 3237-62.

DNA nanopores functionalized with antimicrobial peptides

Petra Katalinić*, Ivo Crnolatac

Department of Organic Chemistry and Biochemistry, Ruđer Bošković Institute, Bijenička Cesta 54, Zagreb, Croatia

*Corresponding Author's E-mail: pkatalin@irb.hr

Bacterial resistance poses a significant challenge in modern medicine. It allows bacteria to prosper, despite the presence of antibiotics, leading to increased healthcare costs and, ultimately, mortality [1]. As one of the potential solutions, antimicrobial peptides are being explored for their activity against various microorganisms [2]. These peptides can destabilize bacterial membranes due to their amphipathic character [3]. To enhance the therapeutic efficacy and stability of the antimicrobial peptides, we focused our research on conjugating peptides with DNA nanopores. Such conjugates provide more structural stability than peptides, while allowing precise targeting of the bacterial membrane. This study is based on the click chemistry synthesis and analysis of peptide-oligonucleotide conjugates [4]. The compounds were characterized by circular dichroism spectrometry, HPLC and gel electrophoresis. The final products were subjected to microbiological testing.

Keywords: Antimicrobial peptides; Bacteria; Oligonucleotides; PMAP-23.

Acknowledgement: This work has been funded by the European Union – NextGenerationEU.

References:

[1] Odonkor ST, Addo KK. Int J Biol Med Res. 2011;2(4):1204-10.
[2] Bahar AA, Ren D. Pharm. 2013;6(12):1543-75.
[3] Kim J, Park S, Yoon M, Hahm K, Park Y. Amino Acids 2011;40(1):183-95
[4] Jewett JC, Sletten EM, Bertozzi CR. J Am Chem Soc. 2010;132(11):3688-90.

Structure-activity relationship study of ST6Gal1 inhibitors aimed at improving cell permeability

Natan Koraj[1,*], Ema Faganeli[1], Sushmaa Dangudubiyyam[2], Anne Harduin-Lepers[2], Marko Anderluh[1]

[1]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Ljubljana, Aškerčeva cesta 7, Ljubljana, Slovenia*

[2]*Institute for Structural and Functional Glycobiology, University of Lille, RdC 59655 Villeneuve d'Ascq Cedex, Lille, France*

*Corresponding Author's E-mail: natan.koraj@ffa.uni-lj.si

Sialyltransferases perform sialic acid transfer to glycoproteins and glycolipids in the Golgi apparatus. α-2,6-sialyltransferase (ST6Gal1) does so via an α-2,6-linkage and plays an important role in cancer progression. Rillahan *et al*. discovered a series of glycosyltransferase inhibitors using high-throughput screening, of which the compound JFD00458 (Figure 1) showed the strongest inhibitory activity against ST6Gal1 with an IC_{50} of 10.8 μM. However, this inhibitor is not expected to cross the cell membranes and reach the Golgi apparatus *in vitro* or *in vivo* due to its high polarity [1]. In this work, we designed and synthesized JFD00458 derivatives with the goal of increasing cell permeability. Additionally, we synthetised a series of compounds to further explore structure-activity relationship in order to improve potency. We also used a recently developed biochemical assay to evaluate ST6Gal1 inhibitors [2]. Our structure-activity study demonstrates that potent inhibition can be maintained with simultaneous cellular permeation.

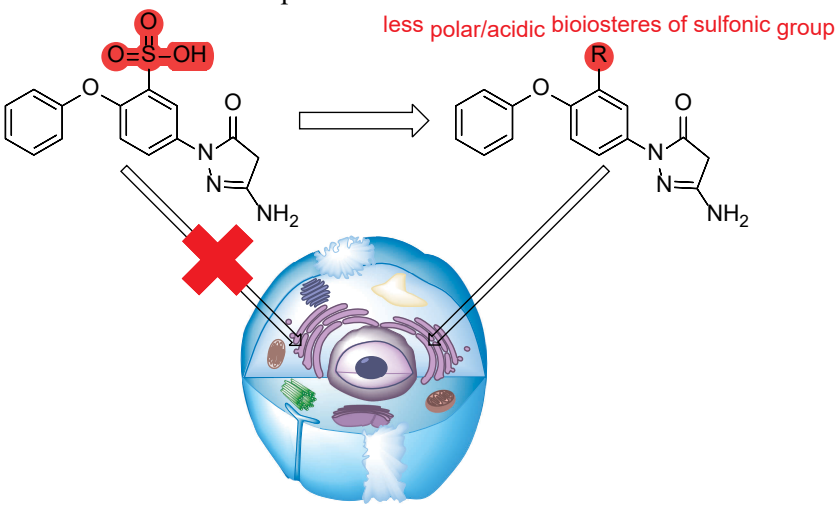


Figure 1. JFD00458 and derivatives – expected cell permeability.

Keywords: ST6Gal1; Sialyltransferase inhibitor; Structure–activity relationship (SAR); Cell permeability; Glycosyltransferase inhibition.

Acknowledgement: This project has received funding from the European Union's Horizon Europe research and innovation programme under the Marie Skłodowska-Curie grant agreement 101119601 — GlyCanDrug.

References:

[1] Rillahan CD *et al*. Angew Chem Int Ed Engl. 2011;50(52):12534-7.

[2] Clairene Filipe K *et al*. Chembiochem. 2025;26(5):e202400539.

Synthesis and biological activity of new benzoxazolinone–coumarin hybrids

Petra Kovačec[1,*], Leentje Persoons[2], Dirk Daelemans[2], Tatjana Gazivoda Kraljević[1]

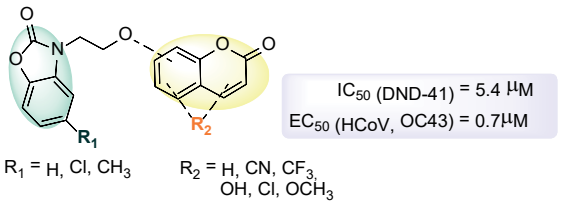
[1]*University of Zagreb Faculty of Chemical Engineering and Technology, Zagreb, Croatia*

[2]*KU Leuven, Department of Microbiology, Immunology and Transplantation, Laboratory of Virology and Chemotherapy, Rega Institute, Leuven, Belgium*

*Corresponding Author's E-mail: pkovacec@fkit.unizg.hr

Coumarin hybrids have emerged as a promising class in medicinal chemistry due to their diverse biological activities, including anticancer, antibacterial, antiviral, and antifungal properties. In addition to established coumarin hybrids featuring cores such as quinoline, imidazole, thiazole, indole, and triazole, novel derivatives incorporating coumarin and 2-benzoxazolinone scaffolds are being developed [1]. The 2-benzoxazolinone core, in particular, represents an attractive scaffold for drug development due to its weakly acidic nature and balanced lipophilic and hydrophilic properties, which facilitate structural modification and support a wide range of biological activities [2].

In this study, we prepared benzoxazolinone–coumarine hybrids linked via an ethyl spacer in order to evaluate their antitumor and antiviral potential. The synthetic route involved the cyclization of 2-aminophenol to 2-benzoxazolinone using 1,1'-carbonyldiimidazole, followed by nitrogen alkylation with dibromoethane under basic conditions to yield *N*-bromoethyl-2-benzoxazolinone. This intermediate was subsequently reacted with 4-hydroxycoumarin and 7-hydroxycoumarin to afford the target hybrids. The structures were confirmed by ^{1}H- and ^{13}C-NMR spectroscopy, and compounds were subjected both antitumor and antiviral evaluations. The most potent antitumor activity was observed for the compound bearing a 5-chloro substitution on the benzoxazolinone ring and a 4-trifluoromethyl group on the coumarin moiety. Notably, the highest antiviral activity was exhibited by the hybrid with the same benzoxazolinone substitution and an unsubstituted coumarin ring.



Keywords: Coumarin; Benzoxazolinone; Biological activity.

Acknowledgement: This work was supported by the Croatian Science Foundation under the project HRZZ-IP-2022-10-9420.

References:

[1] Zhang L, Xu Z. Eur J Med Chem. 2019;181:111587-606

[2] Prasher P, Mall T, Sharma M. Arch Pharm. 2023;356(9):1-31

Region-specific insulin signaling dynamics in the rat brain following intranasal insulin administration

Antonia Krsnik[1,2], Jelena Osmanović Barilar[1,2], Leonarda Vlahov[1,2], Ana Babić Perhoč[1,2], Jan Homolak[1,2], Davor Virag[1,2], Melita Šalković-Petrišić[1,2], Ana Knezović[1,2,*]

[1]*Department of Pharmacology, School of Medicine University of Zagreb, Zagreb, Croatia*

[2]*Croatian Institute for Brain Research, School of Medicine University of Zagreb, Zagreb, Croatia*

*Corresponding Author's E-mail: ana.knezovic@mef.hr

Alzheimer's disease (AD) is increasingly viewed as a brain-centered metabolic disorder involving insulin resistance, impaired glucose metabolism, and mitochondrial dysfunction [1]. Disrupted insulin signaling, marked by IRS1 serine phosphorylation and reduced Akt activity, contributes to tau pathology and amyloid accumulation [2]. Given the brain's high metabolic demand and region-specific glucose use, restoring insulin signaling is a promising therapeutic target. Intranasal (IN) insulin enables direct brain delivery and has shown cognitive benefits in individuals with mild cognitive impairment and AD [3]. In our study, rats received 2 IU of IN insulin and were sacrificed at 3, 7.5, 15, or 30 minutes post-administration, with untreated rats as controls. IRS, AMPK, and CAMK activity were measured indirectly as a ratio of phosphorylated/total protein levels using Western blot analysis in the olfactory bulb (OFB), striatum (S), frontal (FC), temporal (TC), and parietal (PC) cortices, as well as in the hypothalamus (HPT), hippocampus (HPC), cerebellum (CB), and brainstem (BS). Spearman's rank correlation was performed to analyze monotonic associations of the measured parameters in all observed brain regions. A strong positive correlation between p/IRS608 and p/tIRS307 ($p<0.001$) was observed in most regions, except in the TC and HPT, where no meaningful correlation was found. In the TC, there was a strong positive correlation between p/tAKT and insulin concentration and p/tIRS608, and negative correlation between insulin concentration and p/tCAMK. Additional region-specific correlations included strong positive associations between p/tCAMK and p/IRS307 in the HPC, p/tCAMK and p/tAKT in the S, p/tAMPK and IR in TC, and a strong negative correlation between insulin concentration and p/tIRS608 in the PC. These findings demonstrate that intranasally administered insulin modulates insulin signaling in a region-specific manner, offering insight into the brain's localized metabolic responsiveness.

Keywords: Intranasal insulin; Insulin signalling; IRS; AMPK; CAMK.

Acknowledgments: *This work was supported by the Croatian Science Foundation under the project number HRZZ-2022-10-1895 and DOK-NPOO-2023-10-3354, University of Zagreb project (10106-23-2516) and co-financed by the Scientific Centre of Excellence for Basic, Clinical and Translational Neuroscience (project "Experimental and clinical research of hypoxic-ischemic damage in perinatal and adult brain"; GA KK01.1.1.01.0007 funded by the European Union through the European Regional Development Fund).*

References:

[1] Morris JK, Wood LB, Wilkins HM. Front Neurosci. 2022;15:824145.

[2] Mullins RJ, Diehl TC, Chia CW, Kapogiannis D. Front Aging Neurosci. 2017;9:118.

[3] Hallschmid M. CNS Drugs 2021;35(1):21-37.

Synthesis, *in silico* ADMET properties, target prediction, docking studies, and *in vitro* antiproliferative activity of C19-oxime and -nitrile androstane aromatise inhibitors

Ivana Kuzminac[1,*], Edward Petri [2], Marija Sakač[1], Dimitar Jakimov[3], Maja Marinović[2]

[1] *Department of Chemistry, Biochemistry and Environmental Protection, Faculty of Sciences, University of Novi Sad, Trg Dositeja Obradovića 3, Novi Sad, Serbia*

[2] *Department of Biology and Ecology, Faculty of Sciences, University of Novi Sad, Trg Dositeja Obradovića 2, Novi Sad, Serbia*

[3] *Oncology Institute of Vojvodina, Sremska Kamenica, Serbia*

*Corresponding Author's E-mail: ivana.kuzminac@dh.uns.ac.rs

Cytochrome P450 aromatase (CYP19A1) plays a crucial role in the final step of estrogen biosynthesis. Therefore, inhibiting this enzyme is one of the key strategies in treating estrogen-dependent diseases, such as breast cancer [1]. Crystal structures of aromatase are very important for designing novel aromatase inhibitors. Natural substrates bind with their β-face oriented towards the heme group, positioning the C19 methyl group close to the heme iron atom in the active site [2]. This proximity, along with the fact that the C19 methyl group is eliminated during the aromatization process, suggests that C19 derivatives could serve as effective aromatase inhibitors. With this in mind, we have synthesized androstanes containing oxime or nitrile groups at C19, as well as a D-homo lactone moiety. Analysis using the SwissADME tool, predicts ideal ADME properties; while. ProTox 3.0 predicts a toxicity class of 5 or 4, with LD_{50} values of 5000 or 1185 mg/kg. All compounds underwent target prediction using the SwissTargetPrediction tool, and among the three most likely targets for each compound, CYP19A1 was identified. Molecular docking simulations predict binding of the investigated compounds to the aromatase active site via hydrogen bonding, salt bridges and a network of hydrophobic interactions with the steroid core. Among these interactions, polar contacts to Arg115 and Met374 were found; and these interactions are conserved in exestane binding to CYP19A1 [3]. Anti-proliferative activity against six tumor and one healthy cell line was also conducted *in vitro* using the MTT assay. One oxime derivative showed significant activity against estrogen-positive human breast adenocarcinoma cells (MCF-7), making it a promising candidate for further biological testing in breast cancer treatment.

Keywords: Steroids; D-homo lactone; Bioavailability; Toxicity; Aromatase.

Acknowledgement: The authors gratefully acknowledge the financial support of the Provincial Secretariat for Higher Education and Scientific Research of the Autonomous Province of Vojvodina (Project No. 003056514 2024 09418 003 000 000 001/1).

References:

[1] Ram Yadav M, Barmade MA, Tamboli RS, Murumkar PR. Eur J Med Chem. 2015;105:1-38.

[2] Ghosh D, Griswold J, Erman M, Pangborn W. J Steroid Biochem Mol Biol. 2010;118(4–5): 197-202.

[3] Ghosh D, Lo J, Morton D, Valette D, Xi J, Griswold J, Hubbell S, Egbuta C, Jiang W, An J, Davies HM. J Med Chem. 2012; 55(19): 8464-76.

Synthesis, antiproliferative and antibacterial activity *in vitro* of new pyridine benzamides and acrylonitriles

Antonija Mamić[1,*], Kristina Butković[2], Leentje Persoons[3], Dirk Daelemans[3], Robert Vianello[4], Mihailo Banjanac[5], Marijana Hranjec[1]

[1]*Department of Organic Chemistry, University of Zagreb Faculty of Chemical Engineering and Technology, Trg Marka Marulića 19, Zagreb, Croatia*

[2]*Selvita Ltd., Prilaz baruna Filipovića 29, Zagreb, Croatia*

[3]*Department of Microbiology, Immunology and Transplantation, Rega Institute KU Leuven, Herestraat 49, Leuven, Belgium*

[4]*Laboratory for the Computational Design and Synthesis of Functional Materials, Ruđer Bošković Institute, Bijenička cesta 54, Zagreb, Croatia*

[5]*Pharmacology In Vitro, Selvita Ltd., Hondlova 2, Zagreb, Croatia*

*Corresponding Author's E-mail: amamic@fkit.unizg.hr

Pyridine derivatives exhibit a wide range of biological activities, making them promising scaffolds in medicinal chemistry and drug development. The presence of a nitrogen atom in the pyridine ring affects its lipophilicity and enhances aqueous solubility, metabolic stability, and the ability to form hydrogen bonds which is crucial for interactions with biological targets [1]. In this work, we synthesized hydroxy substituted *N*-(pyridine-2-yl)benzamides and pyridin-2-yl derived acrylonitriles to investigate their antiproliferative and antibacterial activities *in vitro* and the influence of the type and the number of substituents on the activity. The hydroxy substituted *N*-(pyridine-2-yl)benzamides were prepared from methoxy substituted benzoyl-chlorides and corresponding 2-aminopyridines in the first step of the synthesis followed by demethylation with BBr_3 in the second step [2]. On the other hand, pyridin-2-yl derived acrylonitriles were prepared via an addition reaction between differently substituted benzaldehydes and 2-pyridineacetonitrile in methanol, using $NaOCH_3$ as a base [3]. The structures of newly prepared compounds were confirmed by means of ^1H and ^{13}C NMR spectroscopy and mass spectrometry. All compounds will be evaluated for their antiproliferative activity *in vitro* on several human cancer cell lines and for antibacterial activity *in vitro* against a panel of Gram-positive and Gram-negative bacterial strains.

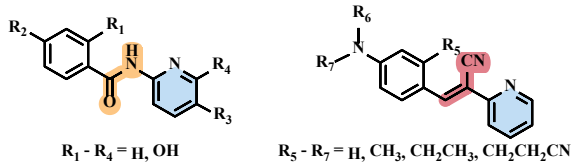


Figure 1. Structures of prepared pyridine derived benzamides and acrylonitriles

Keywords: Pyridine; Benzamide; Acrylonitrile; Biological activity.

Acknowledgement: This work has been supported by the Croatian Science Foundation under the project BenzHetPot IP-2024-05-7208.

References:

[1] Villa-Reyna A-L *et al*. Int J Mol Sci. 2024;25(14):7640.

[2] Beč A, Zlatić K, Banjanac M, Radovanović V, Starčević K, Kralj M, Hranjec M. Molecules 2024;29(9):2138.

[3] Boček Pavlinac I, Persoons L, Daelemans D, Starčević K, Vianello R, Hranjec M. Int J Biol Macromol. 2024;26(2):131239.

Lactone-based derivatives induce apoptosis via Caspase-3 activation in drug-resistant K562 cells

Ana Petrović[1], Marija Hefer[1], Sanja Đokić[2], Jelena Kesić[2], Robert Smolić[3], Vesna Kojić[4], Jovana Francuz[2], Srđan Bjedov[2], Martina Smolić[1,*]

[1]*Department of Translational Medicine, Faculty of Dental Medicine and Health Osijek, J. J. Strossmayer University of Osijek, Crkvena 21, Osijek, Croatia*

[2]*Department of Chemistry, Biochemistry and Environmental Protection, Faculty of Sciences, University of Novi Sad, Trg Dositeja Obradovića 3, Novi Sad, Serbia*

[3]*Department of Pharmacology and Biochemistry, Faculty of Dental Medicine and Health Osijek, J. J. Strossmayer University of Osijek, Crkvena 21, Osijek, Croatia*

[4]*Oncology Institute of Vojvodina, Faculty of Medicine, University of Novi Sad, Put Dr. Goldmana 4, Sremska Kamenica, Serbia*

*Corresponding Author's E-mail: martina.smolic@fdmz.hr

Introduction: Multidrug resistance (MDR) remains a significant barrier in the effective treatment of hematologic malignancies such as chronic myelogenous leukemia (CML). Resistance mechanisms often involve impaired apoptosis, which contributes to cancer cell survival despite chemotherapy [1-3]. In this study, we investigated the potential pro-apoptotic effects of three lactone-based derivatives in a drug-resistant CML model. Methods: The compounds were tested on human chronic myelogenous leukemia K562 cells and human fetal lung fibroblast MRC-5 cells (negative control) for 72 hours. To assess the activation of the apoptotic pathway, cells were treated with half-maximal inhibitory concentrations (IC_{50}) for 72 hours and Caspase-3 levels were measured using enzyme-linked immunosorbent assay (ELISA). Results: All three lactone-based derivatives exhibited no effects on MRC-5 cells, while Caspase-3 levels were markedly increased in treated K562 cells, indicating induction of apoptosis as a potential mechanism of selective cytotoxicity. Conclusion: These results suggest that the lactone-based derivatives exert selective cytotoxicity on drug-resistant K562 cells and promote apoptosis through Caspase-3 activation. Therefore, these compounds show a potential in overcoming drug resistance in CML via pro-apoptotic mechanisms. However, further studies are required to elucidate their full therapeutic potential.

Keywords: Multidrug resistance; K562; Caspase-3; Apoptosis; Lactone derivatives.

Acknowledgement: This work has been supported by Interreg IPA project CRO-SRB (DAADRAC, HR-RS00053).

References:

[1] Rinaldi I, Winston K. J Blood Med. 2023;6(14):261-77.
[2] Talati C, Pinilla-Ibarz J. Curr Opin Hematol. 2018;25(2):154-61.
[3] Häselbarth L, Gamali S, Saul D, Krumbholz M, Böttcher-Loschinski R, Böttcher M, Zou D, Metzler M, Karow A, Mougiakakos D. BMC Cancer. 2023;23(1):1153.

How reliable are *in silico* predictions? A comparative study of ML models

Marina Punčec, Tomislav Šmuc, Višnja Stepanić*

Division of Electronics, Ruđer Bošković Institute, Bijenička cesta 54, Zagreb, Croatia

*Corresponding Author's E-mail: Visnja.Stepanic@irb.hr

Predictive quantitative structure-activity relationship (QSAR) models are widely used by medicinal chemists to optimise physico-chemical and ADME properties of novel compounds. However, a key challenge is: how reliable are the model predictions for a given molecule?

One of the most important aspects of machine learning (ML) models is their robustness, defined as the ability of the model to generalize across diverse chemical structures and maintain consistent performance. Robust models should maintain low prediction error and, ideally, provide a quantitative estimate of uncertainty - especially for structurally novel compounds. If a model lacks generalisation, its performance may vary drastically: exhibiting high accuracy for compounds similar to those in the training set (*i.e.* within its applicability domain) [1], but poor predictivity for structurally dissimilar molecules.

Given the public health and environmental concern posed by endocrine disrupting chemicals (EDCs), we evaluated several ML models developed to predict the activity of small molecules (MW<850) on the human androgen receptor (AR) and estrogen receptor (ER). We compared predictions from multiple ML-based tools, including CERAPP and CoMPARA (which predict activity on ER and AR, respectively) [2,3], PASS (bioactivity spectra) [4], and ADMET Predictor (toxicity profiling) [5]. For this purpose, we used a curated set of approved small-molecule drugs. These compounds offer a valuable reference point – many have well-documented pharmacological targets and known toxicity profiles, allowing partial validation of model predictions. Moreover, approved drugs are generally well represented in the training data of most predictive models, making them suitable for assessing how reliable such models are within their intended chemical space.

In this work, we examine prediction agreement across models and discuss what it reveals about their predictive power and reliability. We also highlight practical strategies for improving the reliability and interpretability of predicted outcomes, illustrated by a representative example.

Keywords: Machine learning; QSAR; Prediction reliability; Model applicability domain.

References:

[1] Sheridan RP, Feuston BP, Maiorov VN, Kearsley SK. J Chem Inf Comput Sci. 2004;44(6):1912-8.

[2] Mansouri K, Abdelaziz A, Rybacka A, Roncaglioni A, Tropsha A, *et al*. Environ Health Perspect. 2016;124(7):1023-33.

[3] Mansouri K, Kleinstreuer N, Abdelaziz AM, Alberga D, Alves VM, *et al*. Environ Health Perspect. 2020;128(2):027002.

[4] Filimonov DA, Lagunin AA, Gloriozova TA, Rudik AV, Druzhilovskii DS, *et al*. Chem Heterocycl Compd. 2014;50:444-57.

[5] Lawless MS, Waldman M, Fraczkiewicz R, Clark RD. New Approaches Drug Discov. 2016:139-68.

Phenolic profile, AKR1C inhibition, and steroid receptor binding of *Cyclocybe aegerita* extracts: Insights into anticancer and metabolic therapeutic potential

Milena Rašeta[1,*], Sofija Bekić[1], Sanja Berežni[1], Gordana Gojgić-Cvijović[2], Anđelka Ćelić[3], Edward Petri[3]

[1]*Department of Chemistry, Biochemistry and Environmental Protection, University of Novi Sad, Faculty of Sciences, Trg Dositeja Obradovića 3, Novi Sad, Serbia*

[2]*Department of Chemistry, Institute of Chemistry, Technology and Metallurgy, University of Belgrade, Njegoševa 12, Belgrade, Serbia*

[3]*Department of Biology and Ecology, Faculty of Sciences, University of Novi Sad, Trg Dositeja Obradovića 2, Novi Sad, Serbia*

*Corresponding Author's E-mail: milena.raseta@dh.uns.ac.rs

The edible fungal species *Cyclocybe aegerita* (V. Brig.) Vizzini 2014 remains underexplored regarding the mycochemical composition and bioactivity of its fruiting body components. This study investigated the chemical profile and anticancer potential of extracts derived from wild-grown *C. aegerita* collected in Novi Sad (Northern Serbia), using solvents of varying polarity—80% methanol (MeOH), 70% ethanol (EtOH), chloroform ($CHCl_3$), hot water (H_2O), and a polysaccharide-enriched (PSH) fraction—applied separately to caps, stems, and whole fruiting bodies.

Fourier-transform infrared (FTIR) spectroscopy of the PSH extract revealed β-glycosidic linkages, proteins, and aromatic structures, suggesting the presence of bioactive polysaccharide–polyphenol complexes. LC-MS/MS analysis identified quinic acid and phenolic acids, notably *p*-hydroxybenzoic and cinnamic acids, with the highest total phenolic content (71.77 ± 1.43 mg GAE/g d.w.) found in the 80% methanol extract of the caps.

In vitro assays demonstrated strong inhibitory effects of the extracts on human aldo-keto reductase 1C3 (AKR1C3) and 1C4 (AKR1C4) isoforms, enzymes implicated in steroid metabolism and cancer progression. The H_2O extract showed the highest AKR1C3 inhibition (81.86%), surpassing the effect of the reference inhibitor ibuprofen (70.32%). Furthermore, polar extracts (MeOH, EtOH, and H_2O) exhibited moderate binding affinity for the ligand-binding domain (LBD) of glucocorticoid receptor (GR), while showing negligible interaction with estrogen receptors (ERα, ERβ) and the androgen receptor (AR). This selectivity could suggests a favorable therapeutic profile without hormone-like side effects.

Molecular docking simulations supported these findings, showing that phenolic and quinic acids could form hydrogen bonds with catalytically crucial residues (Y55 and H117) in AKR1C3, resembling the binding mode of ibuprofen and suggesting a competitive inhibition mechanism.

Taken together, the findings suggest that *C. aegerita* may serve as a valuable natural source of bioactive polysaccharides and polyphenolics with potential utility in cancer treatment.

Keywords: Edible mushroom; Cyclocybe aegerita; Polysaccharides; Quinic acid; Aldo-keto reductase 1C.

Acknowledgement: The authors gratefully acknowledge the financial support of the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Grant Nos. 451-03-137/2025-03/200125 and 451-03-136/2025-03/200125).

Synthesis and immunological propeties of cholesterol derivatives of mannosylated desmuramyl peptide

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

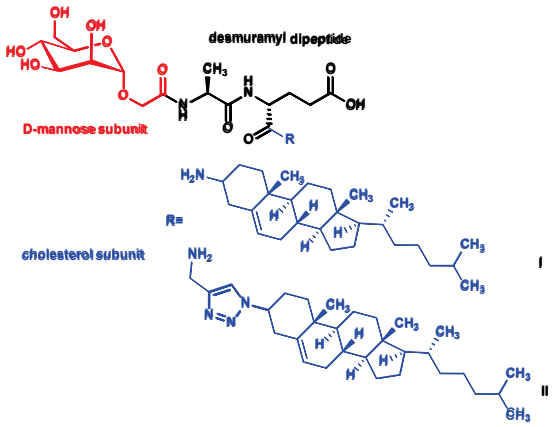
[1]*Department of Chemistry, Faculty of Science, University of Zagreb, Horvatovac 102a, Zagreb, Croatia*

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

[3]*University North, University Center Varaždin, Jurja Križanića 31b, Varaždin, Croatia*

*Corresponding Author's E-mail: rribic@unin.hr

Muramyl dipeptide (MDP, *N*-acetylmuramyl-L-alanyl-D-isoglutamine) and its analogue lacking the hydrophilic N-acetylmuramyl moiety, desmuramyl peptide (DMP, L-Ala-D-*iso*Gln), are well-known immunological adjuvants [1]. Studies on the structure-activity relationship of DMP derivatives have shown that incorporating lipophilic groups into the DMP pharmacophore, as well as its subsequent mannosylation, can increase its adjuvant activity. In our recent work, we synthesized mannosylated DMP derivatives containing lipophilic units such as adamantyl, adamantylethyl, and a C12 alkyl chain. These were attached to the DMP molecule via a triazole ring at the C-terminal (either α- or γ-position of D-*iso*Gln). The in vivo evaluation of their adjuvant activity revealed that the α-position of D-*iso*Gln is most favorable for the attachment of lipophilic moieties [2].
In this work we described the synthesis of mannosylated DMPs with cholesteryl (I) and cholesteryl-triazole (II) subunit attached to α-position of D-isoGln through an amide bond, and immunological properties of derivatives I and II. Immunomodulating properties were evaluated in vivo in well-established mice model using ovalbumin as antigen. Overall anti-OVA IgG antibodies, as well as anti-OVA IgG1 and IgG2a subtypes, were quantified in order to determine the immunostimilating potential of compounds and the type of the immune reaction (Th1 or Th2).



Keywords: Desmuramyl peptide; Mannose; Cholesterol; Immunomodulating activity.

Acknowledgement: This work has been supported by UNIN-BIOMED-25-1-2.

References:

[1] Ribić R, Paurević M, Tomić S, Croat Chem Acta 2019;92:153-61.
[2] Petrović Peroković V, Car Ž, Bušljeta M, Mihelec D, Paurević M, Ivanković S, Stojković R, Ribić R, Int J Mol Sci. 2022;23:8628-48.

Synthesis and structural characterization of new nitro(triazole/imidazole)-appended quinolines with potential antitrypanosomal activity

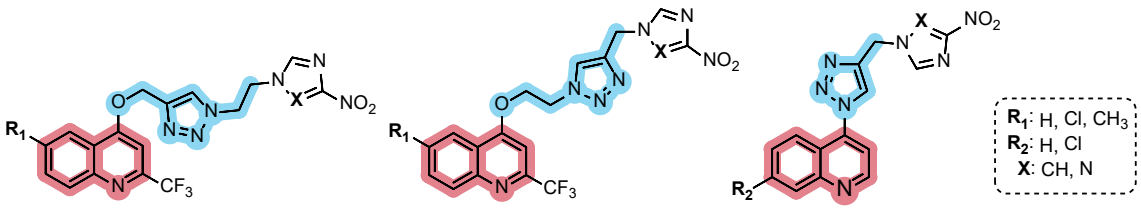
Leon Sačer[1], Marina Ter[1], Doris Babić[1], Martin C. Taylor[2], John M. Kelly[2], Silvana Raić-Malić[1,*]

[1]*University of Zagreb Faculty of Chemical Engineering and Technology, Zagreb, Croatia*

[2]*Department of Infection Biology, London School of Hygiene and Tropical Medicine, London, UK*

*Corresponding Author's E-mail: sraic@fkit.unizg.hr

Human African trypanosomiasis (HAT), commonly known as sleeping sickness, is a neglected tropical disease caused by the protozoan parasite, Trypanosoma brucei. Current treatment options are limited in their efficacy across different stages and subspecies of the parasite, drawing attention to develop more effective antitrypanosomal therapy [1-3]. In this work, novel quinoline derivatives containing 3-nitrotriazole or 4-nitroimidazole connected by different 1,2,3-triazoline linkers are designed, synthesized and structurally characterized. The design of novel quinoline derivatives was focused on exploring diverse linker types, alongside modifications to nitro-substituted heterocycles, with the aim of identifying compounds exhibiting optimal biological activity. The synthesis of new derivatives was carried out by Huisggen 1,3-dipolar cycloaddition from previously synthesized alkynes and azides. Structurally suitable compounds were used as ligands in the subsequent synthesis of rhenium(I) tricarbonyl complexes to improve the physicochemical properties of compounds and increase their potency.



Keywords: Quinolines; Triazole; Imidazole; Antitrypanosomal activity.

Acknowledgements. This work was supported by the Croatian Science Foundation under the project number (HRZZ-IP-2022-10-9420).

References:

[1] Bistrović Popov A, Krstulović L, Koštrun S, Jelić D, Brokulić A, Radić Stojković M, Zonjić I, Taylor MC, Kelly JM, Bajić M, Raić-Malić S. Eur J Med Chem. 2020;207:112802-21.

[2] Bistrović Popov A, Stolić I, Krstulović L, Taylor MC, Kelly JM, Tomić S, Tumir L, Bajić M, Raić-Malić S. Eur J Med Chem. 2019;173:63-75.

[3] Racané L, Ptiček L, Koštrun S, Raić-Malić S, Taylor MC, Delves M, Alsford S, Olmo F, Francisco AF, Kelly JM. J Med Chem. 2023;66:13043-57.

New steroid hydrazones as potential ligands for estrogen receptors

Marina Savić[1,*], Tijana Kovačević[1], Sofija Bekić[1], Anđelka Ćelić[2]

[1]*Department of Chemistry, Biochemistry and Environmental Protection, Faculty of Sciences, University of Novi Sad, Trg Dositeja Obradovića 3, Novi Sad, Serbia*

[2]*Department of Biology and Ecology, Faculty of Sciences, University of Novi Sad, Trg Dositeja Obradovića 2, Novi Sad, Serbia*

*Corresponding Author's E-mail: marina.savic@dh.uns.ac.rs

Steroid molecules are pharmacologically important bioactive agents, and their interaction with numerous nuclear receptors is often exploited in drug design, especially in diseases associated with impaired steroid receptor expression and function. Molecular hybrids with improved biological activity can be obtained by combining privileged pharmacophores into a single entity. Thus, by modifying the parent steroid molecule, novel hybrid molecules containing a lactam ring and a hydrazone function as a linker to an additional nucleus attached at position C-17 are created. New steroid hydrazones **1-6** were tested in order to evaluate their relative binding affinities for the ligand-binding domain (LBD) of estrogen receptor α (ERα), estrogen receptor β (ERβ) and androgen receptor (AR), using a fluorescent assay in yeast [1,2]. The results indicate that derivative **4** exhibited the highest binding affinity for ERα, higher than that for natural ligand estrone, while showing no binding for ERβ isoform. None of the tested hydrazone derivatives showed significant binding to AR. Design of compounds that specifically bind to ERα subtype, which is responsible for proliferation of breast cancer cells, is of great importance. Further, *in silico* testing of their physicochemical properties and evaluation of their pharmacokinetics and toxicity (ADMET) were conducted. Based on predicted **physico-chemical properties,** druglikeness, bioavailability, and toxicity, it can be concluded that all tested compounds meet the given criteria with small deviations [3].

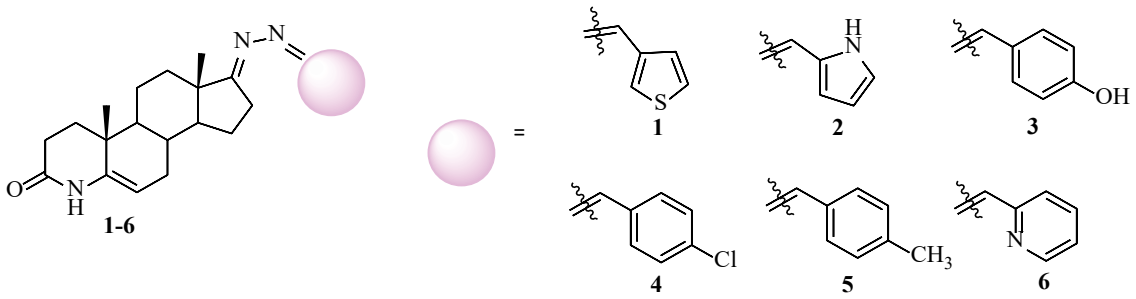


Figure 1. Structures of new steroid hydrazones **1-6**

Keywords: Lactam; Biological activity; ADMET; Estrogen receptor α and β; Androgen receptor.

Acknowledgement: The authors acknowledge the financial support of the Provincial Secretariat for Higher Education and Scientific Research of the Autonomous Province of Vojvodina (Project No. 003056514 2024 09418 003 000 000 001/01) and the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Grants No. 451-03-137/2025-03/ 200125 & 451-03-136/2025-03/ 200125).

References:

[1] Bekić S, Marinović M, Petri E, Nikolić A, Kojić V, Ćelić A. Steroids 2018;130:22-30.

[2] Savić M, Ajduković J, Plavša J, Bekić S, Ćelić A, Klisurić O, Jakimov D, Petri E, Djurendić E. MedChemComm. 2018;9:969-81.

[3] Daina A, Michielin O, Zoete V. Sci Rep. 2017;7:42717.

Yellow K2 – potential next generation NSAID

Gjino Šutić*

UR Institute, Frana Supila 27, Dubrovnik, Croatia

Corresponding Author's E-mail: gjino.sutic@gmail.com

One of the main imperatives of medicine and pharmacy is the treatment of pain, inflammation, and fever. The relatively recent development of precise *in silico* bioinformatic and chemoinformatic tools that can substantially optimize and fast-track the process of drug development, along with exponentially increasing knowledge of molecular mechanisms behind pathological states, have enabled proper ground for engineering and development of better NSAIDs with better – more precise targeting without undesired side effects.

A newly developed compound "Yellow K2" with potential use as an NSAID with a functional group ofheightened cyclooxygenase-2 (COX-2) affinity has been engineered and developed using stated contemporary chemoinformatic and bioinformatic methodologies of *in silico* molecular engineering. The lead compound development and optimization included *in silico* design, pharmacokinetic and pharmacodynamic studies, molecular docking simulations, and structural studies with the use of Virtual reality (VR), followed by a computer-assisted exploration of reverse synthesis – possible pathways of organic synthesis from economically viable constituent reagents. The project was wrapped up by base *in vitro* cytotoxicity studies on human embryonic kidney 293 cell lines using MTT assay to test the *in silico* obtained projections.

Building upon promising results of this small economically viable molecule, the research is scheduled to proceed into the second phase of preclinical trials – *in vitro* immunofluorescence pharmacokinetic and pharmacodynamic studies on a wider array of mammalian cell cultures – traditional liquid cultures and novel 3D cell cultures (organs-on-a-chip and organoids).

Keywords: Better painkillers; Medicinal chemistry; Synthetic chemistry.

Acknowledgement: Developed at UR Institute labs under supervision of dr.sc. Antonija Šakić, and mentorship of dr.sc. Clare Sansom of The Open University.

References:

[1] Yaksh TL, Hunt MA, dos Santos GG. Trends Pharmacol Sci. 2018;39(12):1000-2.

[2] McCarberg B, Gibofsky A. Clin Ther. 2012;34(9):1954-63.

[3] Praveen Rao PN, Knaus EE. J Pharm Pharm Sci. 2008;11(2):81s-110s.

Selection of promising hit candidates through an automated filtering workflow for structure-based virtual screening: a case study on TMPRSS2

Luca Tongiorgi*, Simone Albani, Francesco Musiani

Department of Pharmacy and Biotechnology, University of Bologna, Via Piero Gobetti 93, Bologna, Italy

*Corresponding Author's E-mail: luca.tongiorgi@studio.unibo.it

Virtual screening is a powerful computational approach for streamlining drug discovery in its early stages. Its objective is to select a subset of potential hit molecules from large digital molecular databases for subsequent *in vitro* and *in vivo* testing. Achieving satisfactory hit-list enrichment often requires time-consuming visual inspections which, while remaining crucial, rely heavily on individual experience and intuition, thus making them poorly reproducible [1].

Here, we propose a fully automated and reproducible Python-based workflow designed to filter molecular docking results, generating a reduced list of high-quality hit candidates which enables for a more reliable visual inspection. The filtering process consists of four stages. First, if multiple poses of the same compound are available, root-mean-square deviations within the group of poses are computed, and compounds with the highest pose coherence are kept. Next, only molecules with a docking score better than a chosen threshold are retained. In the third step, a reference protein-ligand interaction fingerprint is generated based on a specified list of key target residues, and only molecules interacting with at least one of these residues are retained. The final stage involves clustering the remaining molecules using Butina's algorithm [2], with the centroids of the most populated clusters being selected for the final compound list.

We applied this workflow to a structure-based virtual screening on two groups of four structures of human transmembrane serine protease 2 (TMPRSS2), one identified through atomistic molecular dynamics simulations [3], the other comprising the latest published crystallographic structures [4]. An initial docking of 475,770 compounds was performed, from which the top 10% scoring molecules of each group of targets were selected for a second, high-resolution docking, which saved the 10 best poses per compound. The resulting two sets of 47,577 molecules were further processed through our filtering workflow, yielding for each a final list of 500 compounds. These were subsequently subjected to visual inspection, leading to the identification of 10 final candidates for *in vitro* testing. The entire workflow is open source, simple to use and customisable by the user. The code base is publicly available as a downloadable tool by the working name of "FilterFiesta" in the github repository at the following address: https://github.com/LBIC-biocomp/filterfiesta/.

Keywords: Virtual Screening; Molecular Docking; Drug Discovery; TMPRSS2.

References:

[1] Fischer A, Smieško M, Sellner M, Lill M A. J Med Chem. 2021;64(5):2489-500.
[2] Butina D. J Chem Inf Comput. 1999;39(4):747-50.
[3] Frumenzio G, Chandramouli B, Besker N, *et al*. Proteins. 2023;91(9):1288-97.
[4] Wang H *et al*. Nat Commun. 2023;14:7574.

SARS-CoV-2 spike protein interference with monoamine oxidase B: Potential links to neurodegenerative diseases

Lucija Vrban*, Robert Vianello

Division of Organic Chemistry and Biochemistry, Ruđer Bošković Institute, Bijenička cesta 54, Zagreb, Croatia

*Corresponding Author's E-mail: lvrban@irb.hr

COVID-19, caused by the **SARS-CoV-2** virus, is best known for its respiratory symptoms; however, growing evidence points to notable neurological complications as well. One potential mechanism involves the virus's interaction with monoamine oxidase (**MAO**) enzymes [1]. In our research, we explore the binding of the SARS-CoV-2 spike protein to the **MAO-B** enzyme, proposing that this interaction may disturb the finely regulated monoaminergic system and contribute to the onset or progression of neurodegenerative disorders [2].

We applied an integrated computational strategy to explore the interaction between the SARS-CoV-2 **spike protein** and the MAO-B enzyme. Using flexible molecular docking, we predicted the binding poses and affinities of the spike protein, which were further examined through **molecular dynamics (MD) simulations** to assess the stability and behavior of the resulting complexes. Our analysis revealed several high-affinity binding sites on MAO-B for the spike protein, with MD simulations confirming the persistence of these interactions over time. Subsequent **MM-GBSA** calculations demonstrated a strong binding affinity between the spike protein and MAO-B, accompanied by notable alterations in the binding free energies of both inhibitors and endogenous substrates. These findings suggest that the presence of the spike protein may disrupt the normal catalytic function of MAO-B. To gain deeper insight into its effect on enzymatic activity and neurotransmitter metabolism—key factors in neurodegenerative pathways—we plan to perform **quantum mechanics/molecular mechanics (QM/MM) simulations**.

Our results indicate a possible molecular mechanism by which SARS-CoV-2 may disturb monoaminergic signaling, potentially contributing to the neurological manifestations observed in COVID-19 patients. This study underscores the importance of further experimental validation and therapeutic development, as understanding these interactions could be crucial for addressing the neurodegenerative effects linked to COVID-19 [3,4].

Keywords: MAO-B; SARS-CoV-2; Selegiline; Dopamine; MD simulations.

Acknowledgement: This project is funded by The Croatian Science Foundation grant IP-2024-05-3953 to HF.

References:

[1] Hok L, Rimac H, et. al. Comput Struct Biotechnol J. 2022;20:1254-63.
[2] Ellul MA, Benjamin L, *et al*. Lancet Neurol. 2020;19(9):767-783.
[3] Cuperlovic-Culf M, Cunningham EL, *et al*. Sci Rep. 2021;11(1):10629.
[4] Pileggi CA, Parmar G, *et al*. Curr Res Neurobiol. 2023;5:100112.

Preparation of A_3B *meso*-(*N,N*-diethylaminophenyl)porphyrin with a long alkyl chain and *N*-quaternisation

Dejana Vujnović, Martina Mušković Lukić, Nela Malatesti*

Faculty of Biotechnology and Drug Development, University of Rijeka, Radmile Matejčić 2, Rijeka, Croatia

*Corresponding Author's E-mail: nela.malatesti@biotech.uniri.hr

Photodynamic therapy (PDT) is a type of selective therapy against cancer and it involves three key factors: photosensitizer (PS), light and oxygen. Porphyrins are known to be good PSs; upon excitation with light, porphyrin molecule can adopt a triplet excited state that converts surrounding oxygen molecules into singlet oxygen ($^1O_2$), initiating oxidative stress in tumor, and, consequently, tumor's destruction [1].

In this study, we synthesized three AB_3-*meso*-(*N,N*-diethylaminophenyl)porphyrins with acetamidophenyl, aminophenyl and tetradecanamidophenyl group as an A substituent. The incorporation of the alkyl chain with 14 C atoms aims to increase membrane affinity and improve interactions with hydrophobic environments, facilitating the passage through the cell membrane bilayer [2].

The compounds were synthesized by modified Adler-Longo synthesis and stepwise substitution at the A *meso*-position of the porphyrin core. Modified porphyrins were purified using the column chromatography multiple times and their structures were confirmed by ^1H and ^{13}C NMR, as well as high resolution mass spectroscopy (HRMS). Spectroscopic properties, such as UV-Vis and fluorescence spectra and fluorescence quantum yields were obtained for all compounds. Singlet oxygen production was measured using the photodegradation of 1,3-diphenylisobenzofuran (DPBF), and both amino- and tetradecanamido-functionalized porphyrins exhibited high $^1O_2$ production. Among the tested derivatives, the amino-functionalized porphyrin showed the highest singlet oxygen quantum yield, followed by the analogue with a long alkyl chain.

Preliminary cellular studies using two melanoma (non-pigmented A375 and highly pigmented B16F10) cell lines revealed limited phototoxicity, probably due to the minimal cellular internalization which was shown by phase-contrast microscopy. This is likely caused by suboptimal solubility of the compounds in biological media.

Keywords: Photodynamic therapy; *meso*-substituted porphyrins; Adler-Longo synthesis; Melanoma cells.

References:

[1] Chilakamarthi U, Giribabu L, Chem Records 2017;17:1-29.

[2] Malatesti N, Munitic, I, Jurak, I. Biophys Rev. 2017;9:149-68.

LIST OF AUTHORS

NOTES

ISBN 978-953-8334-17-7