# EFMC-ASMC

International Symposium on Advances in Synthetic and Medicinal Chemistry

Zagreb, Croatia

September 3-7, 2023

# BOOK OF ABSTRACTS

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September 3-7, 2023
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# Content

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European Federation
for Medicinal Chemistry
and Chemical Biology

EFMC is an independent association founded in 1969, representing 30 societies from 26 European countries, and more than **9000 scientists**. It's main objective is to **advance the science of medicinal chemistry and chemical biology.**

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# Opening Lecture - Biography & Abstract

## Prof. Jeffrey BODE

***ETH ZURICH, Zürich, Switzerland***

Jeffrey Bode completed his studies in the United States, Switzerland, and Japan before beginning his independent scientific career in 2003. Since 2010, he has been Full Professor of Synthetic Chemistry and Chemical Biology at ETH Zurich, where his fantastic and fearless students and postdocs develop new methods for the construction of modified biological molecules and apply these collaborations on fundamental biology and the development of new therapeutic modalities. He is co-founder and Director of Studies for the new Biochemistry and Chemical Biology Curriculum at ETH. His group operates a satellite laboratory at the Institute of Transformative Biomolecules (ITbM) at Nagoya University and has founded three spin-off companies, including Synple Chem and Bright Peak Therapeutics.

# SYNTHETIC AND MEDICINAL CHEMISTRY FOR BIOLOGICS

**Jeffrey W. Bode**

*Department of Chemistry and Applied Biosciences*
*ETH Zürich*
*Zürich, Switzerland*

Modern pharmaceuticals are often perceived as being split into two tribes – small molecules and biologics. This distinction extents not only the size of the molecules – with biologics being large therapeutic proteins, antibodies, vaccines, and nucleic acids – but to their mode of production. Small molecules benefit from the power of organic synthesis to manipulate and tailor every atom in their structure, while biologics are largely limited to natural building blocks with few opportunities for modification and manipulation by organic reactions. Advances in synthetic method for de novo construction of biologics, including therapeutic proteins, and innovative new approaches for site-specific modification of recombinant biologicals increasingly blur this line. Advances in new chemical and enzymatic reactions will make possible a new generation of biologicals that can be precisely tailored with systematic changes to their their structure, making it possible to perform precise "medicinal chemistry" on biologics. With these advances, precisely turned therapeutic proteins with new receptor signalling, conditional activation, and novel targeting strategy can be produced and evaluated.

## *NOTES*

# Invited Lectures - Biographies & Abstracts

## Prof. Timothy NOËL

***UNIVERSITY OF AMSTERDAM, Amsterdam, The Netherlands***

Timothy Noël received in 2004 his MSc degree (Industrial Chemical Engineering) in Ghent. He then moved to Ghent University to obtain a PhD in synthetic organic chemistry (2005-2009). Next, he crossed the ocean to work at the Massachusetts Institute of Technology (MIT) as a Fulbright Postdoctoral Fellow with Professor Stephen L. Buchwald. Returning to Europe, he became assistant professor in 2012 and associate professor in 2017 at Eindhoven University of Technology. In 2020, he was promoted to Full Professor at the University of Amsterdam where he is the Chair of Flow Chemistry. His research interests are synthetic organic chemistry and technology, and especially the delicate synergy between these two fields. His research on flow chemistry was recognized with several awards, including the DECHEMA award (2017), the Hoogewerff Jongerenprijs (2019), the IUPAC-ThalesNano Flow Chemistry Award (2020), the KNCV Gold Medal (2021) and the ACS Sustainable Chemistry & Engineering Lectureship Award 2022. He is the editor in chief of Journal of Flow Chemistry.

# INNOVATION IN SYNTHETIC FLUORINE CHEMISTRY THROUGH USE OF FLOW

**Timothy Noel**

*University of Amsterdam, Van 't Hoff Institute for Molecular Sciences, Flow Chemistry Group, Amsterdam, The Netherlands*

Fluorine chemistry plays a crucial role in advanced material and drug development due to unique properties of fluorine-containing compounds. However, traditional synthesis methods face challenges in terms of selectivity, efficiency, and scalability. Flow chemistry presents a practical solution, offering precise reaction control and scalability through the use of flow reactors. In this lecture, we will examine the efficient synthesis of fluorinated moieties using flow chemistry and present case studies showcasing its effectiveness.

## Dr Cara BROCKLEHURST

***NOVARTIS, Basel, Switzerland***

Dr Cara Brocklehurst is currently Director and Head SynTech Basel within Global Discovery Chemistry at Novartis Institutes for Biomedical Research. After a PhD with Prof. Nick Turner in Edinburgh and a postdoc with Prof. Andreas Pfaltz in Basel, Cara joined Novartis Basel in 2005 in the Global Discovery Chemistry, Prep Labs. A group based in the Novartis research arm, Novartis Institutes for Biomedical Research (NIBR), primarily concerned with the optimisation of synthetic routes and scale up of intermediates and drug candidates. The group has evolved over time and become SynTech, and Cara now leads the group. The global SynTech team works across the portfolio, optimising synthetic routes for scale up, testing novel chemical transformations to open chemical space for project teams, applying chemical technologies such as flow chemistry, photo-chemistry and building automated workflows to catalyse drug discovery.

# MICROCYCLE: A MACHINE LEARNING DRIVEN, AUTOMATED AND INTEGRATED PLATFORM FOR DRUG DISCOVERY

**Eva Altmann (1), Corentin Bon (1), Holy Davis (1), Peter Ertl (1), Carol Ginsburg-Moraff (2), Daniel Gosling (1), Guillaume Lapointe (1), Heinrich Mues (1), Marco Palmieri (1), Sophie Racine (1), Richard Robinson (2), Kian Tan (2), William Ulmer (2), Rene Wyler (1), Cara Brocklehurst (1)**

*1) Global Discovery Chemistry, Novartis Institutes for Biomedical Research, Novartis Pharma AG., Basel, Switzerland*
*2) Global Discovery Chemistry, Novartis Institutes for Biomedical Research, Cambridge, MA, USA*

What if we could develop an integrated drug discovery platform to accelerate drug discovery, which utilises micro-scale chemistry, real-time biological and physicochemical characterisation and machine learning driven compound design? At NIBR this is a reality! Our automated medicinal chemistry design-make-test-analyse cycle is powered by machine learning, enables sequential multi-parameter optimisation and generates knowledge in a timeframe never before possible. We have built MicroCycle; a modular technology platform which benefits from recent advances in plate based micro-scale chemistry, micropurification, *in-situ* quantification and machine learning thus ensuring rapid access to high quality chemical matter already formatted for assay. Furthermore, by reorienting existing high throughput assay technology we can generate a full package of relevant data on each set of compounds in every learning cycle

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2) MicroCycle: An Integrated and Automated Platform to Accelerate Drug Discovery C. E. Brocklehurst E. Altmann, C. Bon, H. Davis, D. Dunstan, P. Ertl, C. Ginsburg-Moraff, J. Grob, D. J. Gosling, G. Lapointe, A. N. Marziale, H. Mues, M. Palmieri, S. Racine, R. Robinson, C. Springer, K. Tan, W. Ulmer and R. Wyler J. Med Chem 2023, manuscript submitted

## Dr Tatiana BESSET

***INSA OF ROUEN, Rouen, France***

Tatiana Besset obtained her PhD in chemistry (2009) at Grenoble University with Dr Greene. She then moved to the WWU Münster as a postdoctoral fellow in the group of Prof. Glorius. In 2011, she joined the group of Prof. Reek at Amsterdam University, as an industrial postdoctoral fellow (Eastman company). From 2012-2021, she was a CNRS Associate Researcher (habilitation in 2018) in the "Fluorinated Biomolecules Synthesis" group at the laboratory COBRA (UMR 6014, Rouen, France) and she was promoted Director of Research CNRS in 2022. Her research career has been recognized with different awards and honors such as ERC Starting Grant (2017), Bronze medal of the CNRS (Young Investigator Award 2018), Jean Pierre Sauvage 2018 Prize awarded by the Division of Organic Chemistry (DCO) of "Société Chimique de France" (SCF) and the Thieme Chemistry Journals Award (2020). In 2022, she was elected as Chemistry Europe Fellow Class 2020/21. Her research involves the design of new transformations involving transition-metal catalysis (C-H bond activation) and the development of new strategies in organofluorine chemistry.

# SYNTHESIS OF FLUORINATED MOLECULES USING MODERN SYNTHETIC TOOLS

**Tatiana BESSET**

*Normandie Univ, INSA Rouen UNIROUEN, CNRS, COBRA (UMR 6014), Rouen, 76000, FRANCE*

Organofluorine chemistry is a fascinating research field. Beyond the strong interest that represents fluorinated molecules in materials science, pharmaceuticals, agrochemicals, and modern drug design,[1] the quest for innovative approaches is still needed to achieve synthetic challenges and push further the boundaries of knowledge in this appealing research field. [2] In this talk, different synthetic approaches for synthesizing original fluorinated molecules will be presented including new tools based on transition metal-catalyzed C-H bond functionalization and the design of original reagents.[3]

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## Dr Uwe GRETHER

***F. HOFFMANN-LA ROCHE, Basel, Switzerland***

Uwe Grether received his Diploma degree in Chemistry at the University of Karlsruhe, Germany, where he subsequently earned his Ph.D. in Organic Chemistry with Professor Herbert Waldmann in 2000. After that, he joined Professor James D. White's group at Oregon State University for his postdoctoral research. In 2001, Dr Grether started in the Medicinal Chemistry department of the Pharma Research and Early Development unit of F. Hoffmann-La Roche Ltd. in Basel, Switzerland. He is Expert Scientist and team lead aiming on innovative treatments for inflammatory and CNS diseases. Dr Grether's research interests include medicinal chemistry, late stage functionalization and chemical biology focusing on holistic approaches toward a clinical end-goal.

# CHEMICAL PROBES AND THEIR RELEVANCE FOR DRUG DISCOVERY PROGRAMS – EXAMPLES FROM THE ENDOCANNABINOID SYSTEM

**U. Grether (1), S. M. Ametamey (2), J. Benz (1), B. Brennecke (3), E. M. Carreira (4), L. Collin (1), M. R. Edelmann (1), J. Fingerle (1), T. Gazzi (3), J. Gertsch (5), M. Giroud (1), L. Gobbi (1), W. Guba (1), M. Guberman (3), A. Haider (2), Y. He (2), D. Heer (1), A. Hentsch (3), M. Hilbert (1), R. Hochstrasser (1), M. Honer (1), B. Hornsperger (1), S. Huber (1), C. Keller (2), B. Kicin (4), C. Korn (1), M. Kosar (4), C. Kroll (1), B. Kuhn (1), M. Maccarrone (6,7), L. Mach (3), R. E. Martin (1), C. Meier (2), Y. Mostinski (3), A. Müller Herde (2), M. Nazaré (3), S. Oddi (7,8), F. O'Hara (1), A. Omran (3), P. Pacher (9), P. Pfaff (4), A. Postmus (10), H. Richter (1), M. Ritter (1), M. Rogers-Evans (1), D. Rombach (1), F. Steven (10), D. Sykes (11), M. F. Taddio (2), H. Wang (1), M. Wittwer (1), S. D. Krämer (2), L. Mu (2,12), R. Sarott (4), R. Schibli (2), R. Slavik (2), M. Soethoudt (10), J. Kretz (1), M. van der Stelt (10), C. Ullmer (1), Z. Varga (9), D. B. Veprintsev (11), M. Westphal (4)**

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*2) Institute of Pharmaceutical Sciences, ETH Zurich, 8093 Zurich, CH*
*3) Leibniz-Forschungsinstitut für Molekulare Pharmakologie FMP, Campus Berlin-Buch, 13125 Berlin, D*
*4) Laboratorium für Organische Chemie, ETH Zurich, Vladimir-Prelog-Weg 3, 8093 Zurich, CH*
*5) Institute of Biochemistry and Molecular Medicine, University of Bern, 3012 Bern, CH*
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*9) National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health, Rockville, MD 20852, USA*
*10) Leiden University, Einsteinweg 55, 2333 CC Leiden, NL*
*11) Faculty of Medicine & Health Sciences, University of Nottingham, Nottingham NG7 2UH, UK, United Centre of Membrane Proteins and Receptors (COMPARE), University of Birmingham and University of Nottingham, Midlands, UK*
*12) Department of Nuclear Medicine, University Hospital Zurich, CH-8091 Zurich, CH*

The endocannabinoid system (ECS) is a highly important lipid-signalling network.[1] It consists of endogenous molecules, which are termed endocannabinoids (eCBs), their processing enzymes and molecular targets. The ECS controls homeostasis and many highly critical bodily functions, which are e.g. of high relevance for the immunological system and CNS. In particular The G-protein coupled cannabinoid receptor type 2 ($CB_2R$) and the serine hydrolase monoacylglycerol lipase (MAGL) are relevant drug targets. $CB_2R$ agonism exerts anti-inflammatory and tissue protective effects in preclinical animal models and holds great therapeutic potential in major pathologies affecting humans including e.g. neurodegenerative disorders, kidney, liver and ocular diseases. MAGL is the key regulator of 2-arachidonoylglycerol (2-AG) brain concentrations. Its inhibition increases 2-AG while concomitantly reducing arachidonic acid and proinflammatory eicosanoids levels in the central nervous system. Thereby neuroinflammation is reduced and hence MAGL inhibition offers promising options for treating neurodegenerative diseases such as Alzheimer's disease or Multiple Sclerosis for which chronic neuroinflammatory processes are characteristic features.

Chemical probes are of utmost importance to bring drugs from the laboratory through the clinic and ultimately to market. They support and influence all research and development phases: target verification and validation; assay development; lead optimization; and biomarker engagement in the context of preclinical studies and human trials.[2] Because of this high relevance $CB_2R$ agonist and MAGL inhibitor drug discovery programs were accompanied by the generation of chemical probes. In particular, labelled chemical probes that consist of a target recognition element and an additional reporter group for specific applications were in the focus.

In this paper, we describe the discovery and evaluation of labelled $CB_2R$ and MAGL probes carrying a variety of different reporter groups. This includes e.g. fluorescent dyes and different radioisotopes. Structure-guided optimization work will be discussed. Key absorption, distribution, metabolism, and excretion properties such as lipophilicity, protein binding and rodent pharmacokinetic profiles relevant for the individual probe types will be highlighted. Successful utilizations such as the development of cellular assays based on fluorescently labelled ligands will be shown. Imaging studies in living cells and animals and proteomics studies will be highlighted. Furthermore, novel $^{3}H$, $^{11}C$ and $^{18}F$ labelled ligands and respective applications such as autoradiography and positron emission tomography studies will be disclosed to illustrate the relevance of chemical probes for deepening the understanding of target biology and their impact for facilitating drug discovery programs.

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2) M. Guberman, M. Kosar, A. Omran, E. M. Carreira, M. Nazaré, U. Grether, Chimia 2022, 76, 425, DOI: 10.2533/chimia.2022.425.

## Prof. Darren J. DIXON

***UNIVERSITY OF OXFORD, Oxford, United Kingdom***

Darren J. Dixon graduated and obtained his D. Phil from the University of Oxford, where he worked with Prof. S. G. Davies. In 1997 he moved to the University of Cambridge to carry out post-doctoral work with Prof. Steven V. Ley CBE, FRS. He began his independent career in 2000 at Cambridge before moving in 2004 to a Senior Lectureship at The University of Manchester. In 2007 he was promoted to Reader and in 2008 he moved to his current position at Oxford University as Professor of Chemistry where he holds the Knowles-Williams Tutorial Fellowship in Organic Chemistry at Wadham College.

His research focuses on the development of synthetically relevant catalyst enabled methodologies and their application to the synthesis of complex target molecules of biological and medicinal relevance. He has published over 200 papers and patents and delivered over 250 invited lectures. He has received several awards including an EPSRC Leadership Fellowship (2008-2013), the AstraZeneca Research Award in Organic Chemistry (2010), the Royal Society of Chemistry's inaugural Catalysis in Organic Chemistry Award (2010), the Scynexis Lectureship, Duke University, North Carolina (2010), Novartis Lectureship in Central Europe (2011), the A. S. Kende Distinguished Lectureship, University of Rochester, USA (2013), Fred Pattison Senior Lectureship, Western University, Ontario, Canada (2013), Visiting Professorship, University of Perugia, Italy (2015), Novartis Chemistry Lectureship, Basel-Boston-California (2016-2017), Alphora Lectureship, University of Toronto, Canada (2017); Distinguished Visiting Professorship, Kyoto University, Japan (2019); International Organic Chemistry Foundation Lectureship, Kyoto University, Japan (2019), Swiss Chemical Society Lectureship (2021), JSPS Fellowship (2022) and CINMPIS Lectureship, Pisa, Italy (2023).

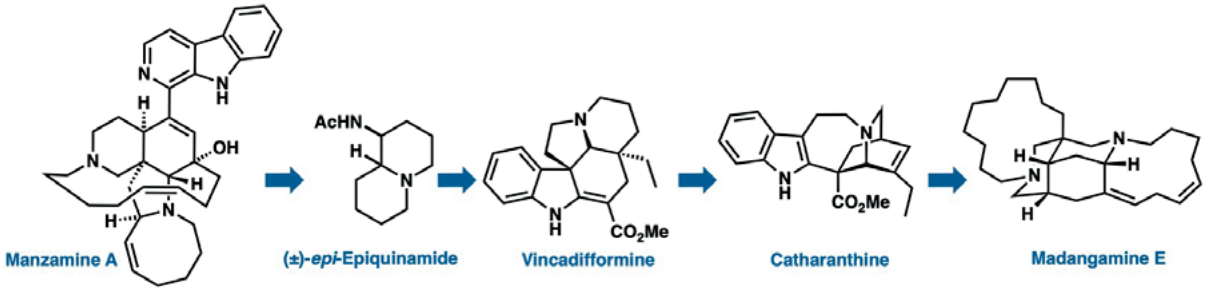
He serves on the Consulting Board of Editors of Tetrahedron/Tetrahedron Letters (2015-present), on the Board of Directors of the Medicinal & Bioorganic Chemistry Foundation, and on the Board of Editors of Organic Syntheses. He is co-founder, director and CSO of Cortex Organics Ltd, a spin-out Oxford University, specializing in new innovative route development and challenging molecular target synthesis.

# NEW CATALYTIC APPROACHES FOR SIMPLIFYING COMPLEX AMINE SYNTHESIS

**Darren J. Dixon**

*Chemistry Research Laboratory, Department of Chemistry, University of Oxford, UK*

In this presentation, new "synthesis inspired" user-friendly broad scope catalytic strategies for forging the key carbon-carbon bonds of key late stage synthetic intermediates en route to various complex alkaloid natural products will be described. In one strand, the use of the abundant and venerable tertiary amide as a precursor to reactive iminium and enamine intermediates via reductive activation will be explored as a new approach to manzamine, aspidosperma and vinca alkaloids. Furthermore, the strategic use of multifunctional metal free catalysts to rapidly construct the bicyclic core of madangamine E via enantioselective desymmetrization of readily constructed achiral precursors will be described. The presentation will include details of the newly arising methodologies as well as their provenance and applications in natural product total synthesis.



## Dr Frauke POHLKI

***ABBVIE, Ludwigshafen, Germany***

Dr Frauke Pohlki has 15 years of experience in drug research and development for various CNS indications. Dr Frauke Pohlki studied chemistry at the Leibniz University in Hanover and received her doctorate in 2004 at the Ruprecht-Karls-University Heidelberg in the field of organic chemistry on the topic "Studies on the intermolecular titanium-catalyzed hydroamination of alkynes". After a three-year postdoctoral stay at Boston University with a focus on natural product synthesis, she started working as a laboratory manager in medicinal chemistry at Abbott/AbbVie in 2008. Since that time, Dr Frauke Pohlki went through positions with increasing scientific and personal responsibility and accompanied and managed projects from the early phases of the preclinical phase to the development of clinical candidates. At the beginning of 2023, she took over the management of medicinal chemistry in Ludwigshafen. The focus of her team is in the indication area of neuroscience and also includes innovative approaches in the field of protein degradation and green chemistry.

# CHALLENGES AND OPPORTUNITIES IN CNS DRUG DISCOVERY

**Frauke Pohlki**

*Director, Global Medicinal Chemistry, Small Molecule Therapeutics and Platform Technologies,*
*AbbVie Deutschland GmbH & Co. KG, Ludwigshafen, Germany*
*Corresponding author: frauke.pohlki@abbvie.com*

The unmet medical need in the field of neuroscience, including many tough-to-treat disorders ranging from Alzheimer's and Parkinson's to migraine, schizophrenia, and stroke, remains high.

Delivery of most drugs across the blood-brain-barrier represents a significant hurdle for the development of novel therapeutics, molecular tracers, or novel modalities targeting the central nervous system (CNS). With a more holistic framework incorporating machine-learning models we aim to expand our toolbox of in silico predictions beyond known principles.

Opportunities and challenges associated with the drug discovery of CNS-therapeutics will be discussed.

## Dr Rebecca RUCK

***MERCK, SHARP & DOHME, Rahway, United States***

Rebecca T. Ruck (she/her/hers) is Associate Vice President, Merck Process Research & Development, where she leads the Enabling Technologies group, a role she proposed that has created an innovation incubator of biologists, chemists and engineers. Her team is making impressive contributions across all pipeline projects, including the application of an unprecedented biocatalytic cascade sequence to the manufacturing route of islatravir, a pioneering photochemical flow process for belzutifan and an immobilized enzymatic flow reaction for nemtabrutinib. Becky graduated summa cum laude from Princeton University, obtained her PhD in organic chemistry from Harvard University in the lab of Prof. Eric Jacobsen and conducted NIH-funded post-doctoral research at University of California-Berkeley with Prof. Robert Bergman.

# ENABLING TECHNOLOGIES TO DRIVE THE MSD PORTFOLIO

**Rebecca Ruck**

*Merck, Sharp & Dohme*
*NJ 07065 Rahway*
*United States*

MSD's chemistry strategy in Process Research & Development is to deliver synthetic solutions that afford the most efficient, sustainable and cost-effective processes. Invention of new capabilities is critical to achieving our audacious goals. This presentation will highlight how investments in enabling technologies allow us to invent novel methods on the critical path and to ultimately deliver on our mission of getting critical medicines and vaccines to patients across the globe.

## Prof. David SARLAH

***UNIVERSITY OF ILLINOIS, Urbana, United States***

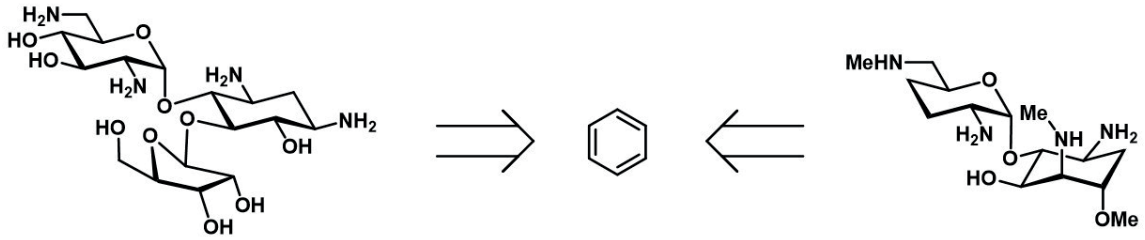
David was born and raised in Slovenia, where he obtained his Bachelor's Degree in Chemistry (University of Ljubljana). He carried out his undergraduate research with Prof. K. C. Nicolaou at Scripps and Prof. Samuel J. Danishefsky at Columbia. He obtained his Ph.D. in chemistry with Prof. K. C. Nicolaou involving the total synthesis of complex natural products. David then joined Prof. Erick M. Carreira's group at ETH as a postdoctoral fellow and explored the field of asymmetric catalysis. In the fall of 2014 David joined the faculty at the University of Illinois, Urbana-Champaign. His research interests span from the synthesis of complex, biologically active natural products and the related chemical biology to methodology development.

# EMPOWERING ORGANIC SYNTHESIS: FROM UNIQUE METHODS TO COMPLEX NATURAL PRODUCTS

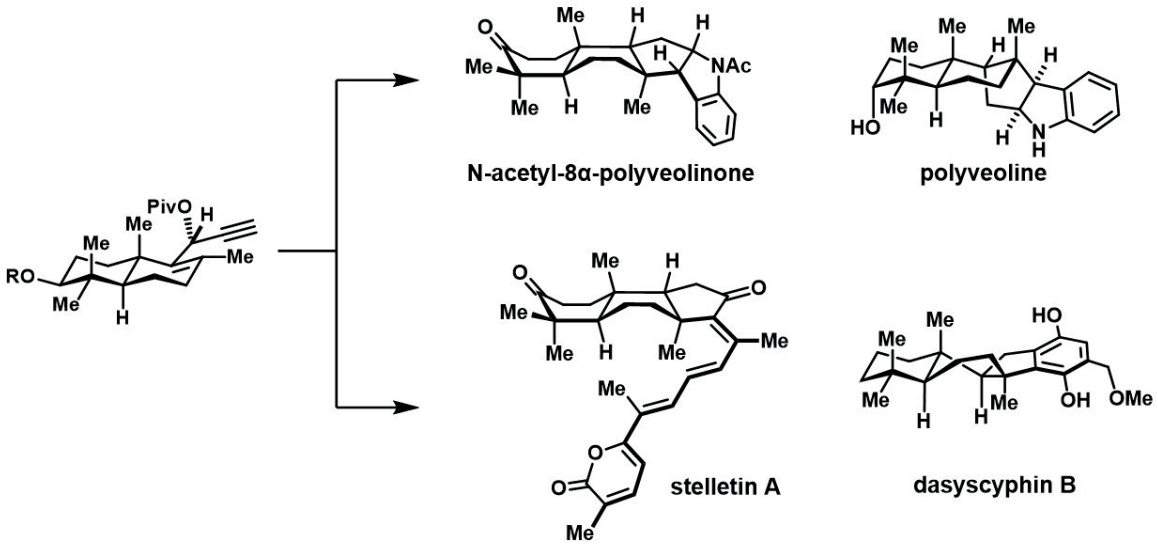
**David Sarlah**

*University of Illinois, Department of Chemistry*
*270 RAL, Box 107-5*
*600 South Mathews Avenue*
*IL 61801 Urbana*
*United States*

Inspired by complex natural products, we have been developing empowering disconnections, tailored strategies, and general methods for rapid and controlled formation of molecular complexity. This lecture will cover an overview of several recent and ongoing case studies from our laboratory, ranging from aminoglycosides to terpenoids. For example, we will show how an enantioselective hydroamination of benzene enables the synthesis of ribostamycin and opens access to additional aminoglycosides, such as sannamycins.




Moreover, we have been developing stereodivergent cycloisomerizations that can access all naturally occurring perhydrobenz[e]indene terpenoids, using a retrosynthetic analysis incorporating predetermined moments of stereochemical bifurcation. This strategy makes possible the divergent synthesis of all four naturally occurring trans-diastereomers of a common perhydrobenz[e]indene terpenoid scaffold with high stereocontrol, laying the foundations for the synthesis of any of the myriad natural products that contain this prevalent core structure, as showcased with stelletin A, polyveolinone, and dasyscyphin B.



## Prof. Nuno MAULIDE

***UNIVERSITY OF VIENNA, Vienna, Austria***

Nuno Maulide (*1979) is Professor of Organic Synthesis and Head of the Institute of Organic Chemistry at the University of Vienna, as well as an Adjunct Principal Investigator at the Research Center for Molecular Medicine (CeMM) of the Austrian Academy of Sciences. Nuno Maulide is also head of a Christian-Doppler Laboratory (Entropy-Oriented Drug Design, in collaboration with Boehringer Ingelheim), expert reporter for Organic Chemistry at the executive board of the Austrian Science Foundation (FWF) and Associate Editor for JACS Au. Amongst other funding, Nuno Maulide's research has been supported by an ERC Starting Grant (2011-2016), an ERC Consolidator Grant (2016-2022) and an ERC Proof of Concept Grant (2018).

# THE BEAUTIFUL SIMPLICITY OF REARRANGEMENTS? TOWARDS IDEAL REACTIONS?

**Nuno Maulide**

*Institute of Organic Chemistry, University of Vienna, Währinger Strasse 38, 1090 Vienna, Austria*

The turn of the century brought about a pressing need for new, efficient and clean strategies for the chemical synthesis of biorelevant compounds. Our group has studied the use of various molecular rearrangements and atom-economical transformations as particularly appealing means towards the streamlined synthesis of complex small molecule targets.

In this lecture, we will present an overview of our research in these areas focusing on the chemistries of sulfonium salts, keteniminium ions and small strained rings, and how they provide efficient solutions for the discovery of unusual reactivity or concise total synthesis. We furthermore will discuss innovative approaches to reactions that might come a step closer towards “ideality”.

References

1) Nature Chemistry 2022, 14 (11), 1306–1310

## Dr Maria MÉNDEZ PÉREZ

***SANOFI, Frankfurt, Germany***

Dr María Méndez Pérez is currently the head of SMD Medicinal chemistry and global IDD portfolio coordinator at Sanofi. Her team focuses on supporting drug-discovery programs in the areas of immunology and neurological diseases, focusing thereby on the implementation of novel technologies and workflows with the aim to shorten the DMTA cycle.

María obtained her Ph.D. in organic chemistry from the Universidad Autónoma of Madrid in 2001, followed by postdoctoral research in catalysis at the MPI in Mülheim an der Ruhr and in theoretical chemistry at the TU in Berlin. She started her industrial career at Sanofi in 2006, where as medicinal chemist, she has worked with several target classes, modalities and disease areas in all discovery phases.  As project leader or co-leader, she has contributed to the delivery of clinical candidates in the areas of diabetes and neuroscience.

# STRUCTURE BASED IDENTIFICATION OF NOVEL ALBUMIN BINDERS FOR HALF-LIFE EXTENSIONS OF PROTEINS AND PEPTIDES

**Maria Méndez Pérez (1), Thomas Boheme (1), Nis Halland (1), Michael Podeschwa (1), Stefan Guesrregen (1), Martin Will (1), Nils Rackelmann (1), Laurent Bialy (2)**

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*Industriepark Höchst, Bldg. G838, 65926 Frankfurt am Main (Germany)*
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*3) Current affiliation: Bayer,*

Prolonged *in-vivo* half-life of biomolecules is a desirable property for many injectable therapeutics, in order to increase patient compliance and satisfaction by reducing the burden of frequent injections. The principle of albumin protraction has been successfully applied for the *in-vivo* half-life extension of several now marketed insulins and peptides. At Sanofi, using a structure-based approach, novel albumin binders were identified and further modified for conjugation with peptides and insulins, leading to compounds with extended plasma half-life comparable to other publicly know acylating residues. Several aspects of the discovery and optimization strategy will be discussed in this presentation.

## Dr Daniel OEHLRICH

***JANSSEN PHARMACEUTICA, Beerse, Belgium***

Daniel Oehlrich is Senior Principal Scientist at The Janssen Pharmaceutical Companies of Johnson & Johnson

Daniel has more than fifteen years of pharmaceutical experience, focused on identifying novel compounds that could potentially offer therapeutics against neurodegenerative diseases, including Alzheimer`s disease. He is responsible for supporting the portfolio at all stages from target validation to NME declaration.

Specialties: Organic Synthesis, Medicinal Chemistry, Management, Total Synthesis, Heterogeneous catalysis, Organometallic chemistry, Robust route development.

His clinical research consists of co-authoring 35 peer reviewed articles in the past 15 years.

# PHENOTYPIC SCREEN TO NLRP3 LEAD

**Daniel Oehlrich (1), Michael Muratore (1), Xiaodi Yu (2), Rosalie Matico (2), Robyn Miller (2), Bertrand Van Schoubroek (1), Karolien Grauwen (1), Javier Suarez (2), Beth Pietrak (2), Yanting Yin (2), Gary Tresadern (1), Astrid Bottelbergs (1), Nina Van Opendenbosch (1), Sujata Sharma (1)**

*1) Therapeutics Discovery; Janssen Pharmaceutica, Beerse Belgium*
*2) Janssen Research and Development, Spring House, PA 19044, USA*

The NACHT-, leucine-rich-repeat-, and pyrin domain-containing protein 3 (NLRP3) is a critical intracellular inflammasome sensor and a highly important clinical target against inflammation-driven human diseases. Dysregulation of the NLRP3 inflammasome was shown to be involved in the pathogenesis of numerous human diseases eg. atherosclerosis, gout, multiple sclerosis, Alzheimer's disease and several cancers. Moreover, Cryopyrin-Associated Periodic Syndromes (CAPS) are caused by point mutations in the *nlrp3* gene leading to its aberrant activation.

Given the important contribution of NLRP3 to multiple pathologies, enabling therapeutic inhibition has become an area of focus for many researchers. The small-molecule MCC950 has been described as a highly potent and selective NLRP3 inhibitor in several preclinical models further highlighting the importance of this target. Since then, several other small-molecule inhibitors have been reported to target the NLRP3 inflammasome but so far none have reached the clinic.[1]

In this study, we describe the identification of a novel class of NLRP3 small-molecule inhibitors picked up from a cellular phenotypic high-throughput screen. During our exploration, we demonstrate target engagement via several techniques. The initial series was optimized which led to the identification of compound our pre-clinical lead, that exhibits high potency and selectivity over the other inflammasomes. Our lead was then evaluated extensively both in vitro and vivo, de-risking the compound for further profiling in the future.

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## Prof. Rebecca GOSS

***UNIVERSITY OF ST ANDREWS, St Andrews, United Kingdom***

Rebecca Goss grew up in the Isle of Man and studied toward a degree in Chemistry at the University of Durham. In 1997 she began researching toward her PhD under the guidance of Professor David O'Hagan. Inspired by a lecture that she had heard during the first year of her PhD, and in order to develop skills in molecular biology, in 2000 she moved to take up a postdoctoral position at the University of Cambridge with Professors Peter Leadlay (FRS) and Jim Staunton (FRS). In 2002 she moved to a one year teaching fellowship at the University of Nottingham where she began her independent research. In 2003 Rebecca was awarded a Royal Society BP Dorothy Hodgkin fellowship and a lectureship at the University of Exeter, this fellowship was transferred to the University of East Anglia in Norwich in 2005. She stayed in Norwich 'till 2012 progressing from Lecturer to Reader. In 2013 she was appointed to St Andrews as a Reader in Chemistry, being promoted to Full Professor in 2018.

She has won a few medals and awards including the RSC Meldola Medal (awarded to the most promising UK chemist under the age of 32), the RSC Corday Morgan Medal (for the most meritorious contributions to chemistry by a mid-career chemist), AccelerateHer 2021 and Converge Challenge 2022 for business development, a Thieme award, Natural Product Lectureship, an ERC consolidator award, and election to Fellowship of the Royal Society of Edinburgh.

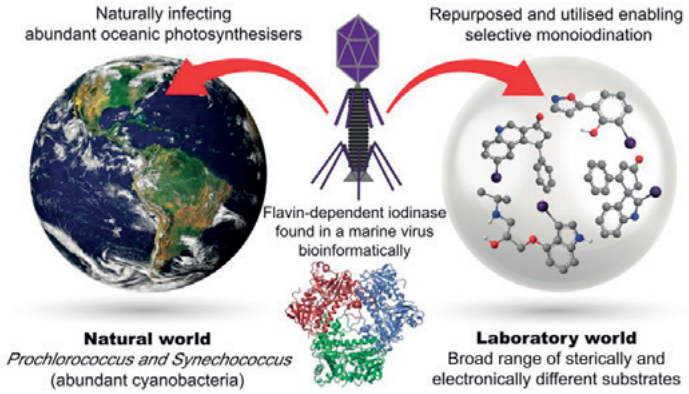
# GENOCHEMETIC DIVERSIFICATION OF NATURAL PRODUCTS

**Rebecca Goss**

*School of Chemistry, University of St Andrews, Fife, Scotland, KY169ST UK*
*rjmg@st-andrews.ac.uk*


**GenoChemetics:** gene insertion enables the installation of a reactive and chemically orthogonal handle into a natural product, permitting its selective functionalization and diversification

Though natural products represent a treasure trove of medicinally relevant compounds, they are commonly misperceived to be unsuitable for medicinal chemistry. We are developing new approaches to natural product analogue synthesis by blending together synthetic biology and synthetic chemistry. By complementing the biosynthetic machinery encoding an existing natural product with foreign genes we are able to introduce chemically orthogonal, reactive and selectable functionalisable handles into natural products.[1] We have been developing mild chemical methodologies to enable the chemical derivatization of these handles.[2,3]




Here we report new enzymatic tools for late stage halogenation of molecules including a viral encoded iodinase,[4] and mild and even cell compatible cross-coupling methodologies for use in GenoChemetic approaches

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## Dr Clara CHRIST

***BAYER, Berlin, Germany***

Dr Clara Christ is Vice President, Head of Molecular Design at Bayer AG where her team focuses on advancing drug discovery projects with structural insights and digital means. Clara obtained her diploma (2005) and doctorate (2009) in Chemistry from ETH Zürich where she focused on developing new algorithms for estimating binding affinities. She then joined Boehringer Ingelheim (BI) as a Post-Doc working on mining electronic notebooks for de novo design of synthetically accessible compounds. As an R&D scientist at BI she supported drug discovery project teams in the therapeutic areas cardiometabolic, respiratory, and neurological disease research. In 2013 Clara decided to join Bayer to dedicate herself to computational drug design in the therapy area oncology. In 2019 she took over the lead of Computational Molecular Design (CMD) in Berlin where the team focuses on supporting drug discovery projects with computational science applications and developments. Since June 2022 she heads Molecular Design with groups in Monheim (Structural Biology), Wuppertal, and Berlin (CMD).<br/><br/>

Clara is a globally renowned expert in free energy calculations, a frequent speaker at international conferences, a lecturer at the Freie Universität Berlin and Technische Universität Berlin, co-author on a multitude of patents and publications, and a Bayer Senior Science Fellow. She has played a vital role in establishing and refining binding free energy techniques, making us one of the very early adopters of these technologies. As a promoter of open science and software, Clara has initiated the Open Force Field Consortium, a large academic-industry collaboration which aims at improving the predictive power of computational drug discovery techniques.

# NEXT GENERATION DESIGN-MAKE-TEST-ANALYSIS-CYCLES

**Yannic Alber, Hans Briem, Clara Christ, Michael Hahn, Gary Hermann, Florian Koelling, Mario Lobell, Georg Mogk, Florian Mrugalla, Michael Schimeczek**

*Bayer AG*

Compound optimization has been characterized by iterative cycles of design, make, test, and analysis for decades. Although the basic steps of the cycle remain unchanged, the way scientists are supported by digital tools in these steps is changing at rapid pace. Scientists can now complement their own ideation with compound generators based on generative chemistry and cheminformatics approaches or through computational searches in huge virtual, on-demand libraries. To filter down the ever-growing ideation space, scientists can make use of machine-learning and physics-based property prediction to focus experimental efforts onto those virtual compounds that are predicted to lead to improvements in the property profile or the prediction models. Synthetic accessibility can be facilitated by synthesis-aware virtual compound generation and by combining generators with retrosynthesis prediction algorithms. This can be expanded to multi-compound synthesis plans that minimize the overall number of steps and intermediates and consider building block availability. With more and more digital tools and prediction models supporting drug discovery scientists, education on applicability domain and model performance is essential to ensure adequate usage and to avoid inflated expectations. We will share how virtual compound generators combined with our retrosynthesis platform CHAI ("Chemistry using AI") are used in practice to support scientists when navigating chemical space in their hunt for drug candidates.

## Dr Ivana FLEISCHER

***UNIVERSITY OF TÜBINGEN, Tübingen, Germany***

Ivana Fleischer studied chemistry at the Comenius University, Bratislava, Slovakia. She received her PhD from the University of Basel, Switzerland and then she joined the Leibniz-Institute for Catalysis in Rostock, Germany as postdoctoral fellow of the Swiss National Science Foundation. In 2013, she moved to University of Regensburg as a Liebig Fellow and junior group leader and in 2017 she was appointed as tenure track assistant professor at the University of Tübingen. In 2022, her tenure was positively evaluated and she accepted the position as full professor at the same institution.

# MAKING AND BRAKING OF C-S BONDS USING METAL CATALYSIS

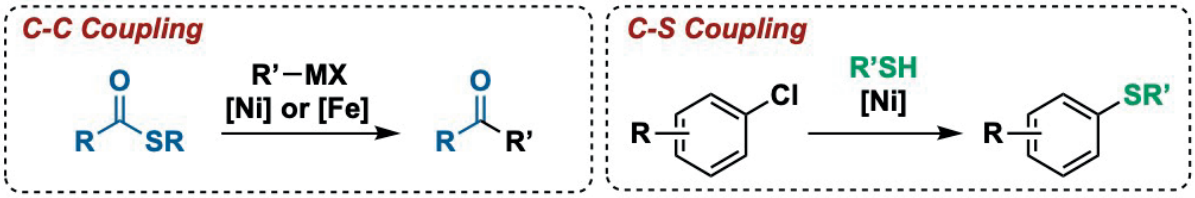
**Ivana Fleischer**

*University of Tübingen, Institute of Organic Chemistry, Auf der Morgenstelle 18, 72067 Tübingen, Germany*

Our research interests focus on development of new methods for the synthesis and use of sulfur-containing compounds, such as thioesters and thioethers. They constitute valuable synthetic intermediates and target compounds for material chemistry and pharmaceutical applications.1 Our aim is to develop efficient transformations employing non-precious metals as homogeneous catalysts.

We have demonstrated the usefulness of thioesters in cross coupling reactions with arylzinc reagents to generate ketones.2 A defined nickel complex was employed as catalyst and a series of functionalized ketones was successfully obtained. The scope was later expanded to the coupling of thioesters with more reactive organomanganese reagents upon iron catalysis.3

Furthermore, we developed nickel-catalyzed coupling reactions of challenging aryl chlorides with thiols, whereby max. TOF of 800 h-1 was achieved.4 A broad scope of substrates containing various functional groups and heterocyclic motifs was successfully converted. A systematic study of couplings of sterically hindered aliphatic thiols was conducted.

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## Dr Darryl McCONNELL

***BOEHRINGER INGELHEIM REGIONAL CENTRE, Vienna, Austria***

Darryl McConnell is currently Senior Vice President and Research Site Head at Boehringer-Ingelheim Regional Centre Vienna, Austria. His goal is to discover new chemical therapeutics for the “undruggable” cancer targets with the team at BI. Darryl’s current interests lie in TKI and KRAS signaling, pushing the frontiers of protein degradation and pioneering new strategies in medicinal chemistry. Darryl is author of over 100 peer reviewed publications and patents and likes to teach medicinal chemistry.

Darryl commenced his industrial career with Chiron Technologies in Melbourne in 1997. After jobs at Biota Holdings Ltd in Melbourne from 1999 in the area of respiratory viruses and Intervet in Vienna from 2001, in 2002 he joined Boehringer-Ingelheim as a Research Laboratory Head. He is in his current role since 2015.

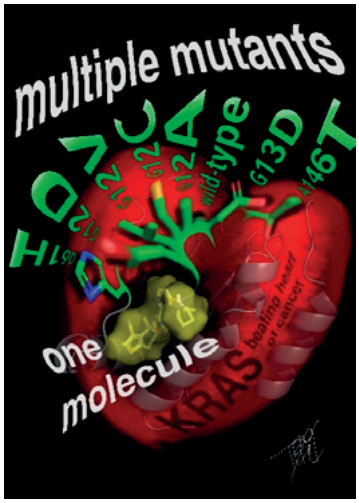
Darryl McConnell received his Bachelor of Science with First Class Honours in 1991 with Professor John Elix at the Australian National University in Canberra. He performed his PhD at the University of New South Wales in Sydney with Professor David Black for which he was awarded the Cornforth Medal for the best chemistry PhD thesis in Australia for that year. Following this he performed a 2-year Postdoctoral study at the University of Sydney with Professor Leslie Field in organometallic chemistry.

# FROM BRD4 TO KRAS: 12 YEARS OF ONCOLOGY SMALL MOLECULES

**Darryl B. McConnell**

*Boehringer Ingelheim Regional Centre Vienna, Austria*

Around 2010 we decided to focus our oncology drug discovery efforts around the most compelling yet undrugged targets. It became immediately clear that systematically being able to drug protein-protein interactions (PPI) would be necessary as many key oncogenic drivers, such as KRAS, b-catenin and MYC, function via PPIs. In contrast to today, in 2010 it was not clear whether small molecules could be generally discovered against PPI targets and it would be another 6 years before the Bcl2::Bax inhibitor Venetoclax was approved. The talk will replay highlights from our twelve year journey at Boehringer Ingelheim to drug PPI targets in oncology.




The following highlights will be covered in the talk. The discovery of BI 907828 an oral inhibitor of Mdm2::p53 capable of intermittent dosing. The technical learnings for drugging PPIs gained from the BET inhibitor program which lead to BI 894999. From PPIs to glues: the serendipitous discovery of BCL6 degrading glues (BI-3802). One atom makes all the difference: turning SOS1 activators into SOS1::KRAS PPI inhibitors (BI 1701963). Leveraging a non-functional pocket on SMARCA2 to make orally bioavailable VHL-based PROTACs (ACBI2). And last but certainly not least, the discovery of pan-KRAS inhibitors (BI 2865 & BI-2493) and beyond.

## Dr Stéphanie NORSIKIAN

***INSTITUT DE CHIMIE DES SUBSTANCES NATURELLES, Gif-sur-Yvette, France***

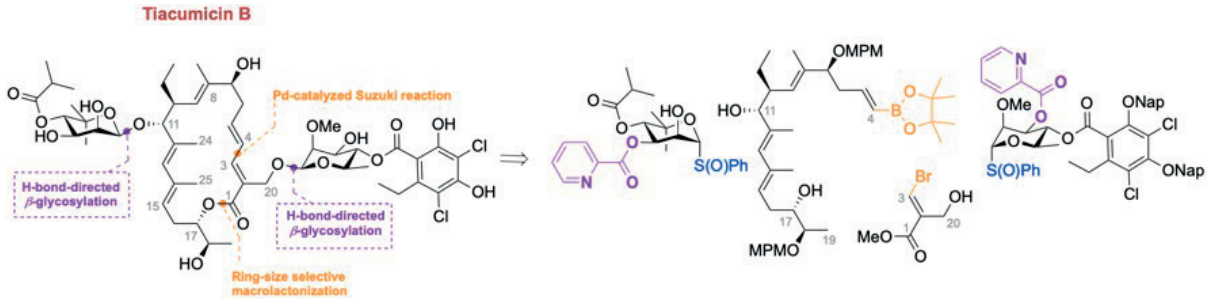
Stéphanie Norsikian received a Ph.D. degree from the Université of Paris VI in 1999, under the supervision of Professors J.-F. Normant and I. Marek. After post-doctoral trainings in the groups of Professor D.M. Hodgson (Oxford, U.K), Professor G. Guillaumet (Orléans, France) and Professor H. Kagan (Orsay, France), she was appointed by the CNRS as Chargée de Recherche in 2002 in the group of Professor A. Lubineau (Orsay). In January 2007, she joined Professor J.-M. Beau's group at the Institut de Chimie des Substances Naturelles (ICSN in Gif sur Yvette). Since January 2015, she has been working in the team "Probes and Modulators for Biological Targets" (Chemical-Biology Department-ICSN). In 2022, she has been promoted Research Director and became assistant coordinator of the Chemical Biology department of ICSN. Her research interests focus mainly on glycochemistry and synthesis of biomolecules but also on organometallic chemistry, multicomponent reactions and fluorescent probes.

# SYNTHESIS OF GLYCOSYLATED NATURAL PRODUCTS OR ANALOGUES OF BIOLOGICAL INTEREST

**Stéphanie Norsikian**

*Institut de Chimie des Substances Naturelles*
*Bâtiment 27, Avenue de la Terrasse*
*91198 Gif-sur-Yvette*
*France*

We have been interested for several years in the preparation of natural glycostructures or their analogues. Given their important biological activities, low bioavailability and structural complexity, the preparation of such compounds represents a major challenge in organic chemistry. Glycosylation is a modification present in many natural molecules and more than half of proteins. It involves reacting a glycosyl acceptor with a glycosyl donor using a promoter under appropriate conditions to selectively form a glycosidic bond of alpha or beta stereochemistry.[1] Despite the considerable progress already made on the glycosylation reaction, it is sometimes difficult to obtain a fully stereoselective reaction, especially with sensitive and/or complex aglycone structures. My presentation will focus mainly on the total synthesis of tiacumicin B, a complex glycosylated natural macrolide with antibiotic properties. Our strategy is based on developing novel synthetic strategies and methodologies and draws on our experience with synthesizing the tiacumicin B aglycone.[2] In addition, we will show how we were able to solve the problem of the difficult steps of 1,2-*cis*-glycosylation relying on H-bond-mediated Aglycone Delivery (HAD).[3]

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## Dr Marwin SEGLER

***MICROSOFT RESEARCH, Cambridge, United Kingdom***

Marwin Segler is a team lead at Microsoft Research AI4Science, working at the intersection of chemistry, drug discovery, catalysis, and machine learning. Prior, he was Lead Researcher at BenevolentAI (London), and received his PhD in chemistry from the University of Muenster.

Marwin pioneered modern machine learning for molecular design, and chemical synthesis planning. His research interests are in computer-assisted scientific discovery, algorithm development in computational chemistry and AI, and applications in organic synthesis and drug discovery.

# MACHINE LEARNING TO ACCELERATE DESIGN-MAKE-TEST CYCLES

**Marwin Segler**

*Microsoft Research AI4Science*
*21 Station Road*
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*United Kingdom*

Modern machine learning has become a powerful tool of computational chemistry over the past five years, allowing to tackle previously very hard problems in organic and medicinal chemistry. In this talk, we will highlight recent developments in automated synthesis planning, which allows addressing the crucial make-step in computer-aided molecular design and discovery workflows, in generative models for molecules, which allow the design of bespoke molecules with desirable properties, and knowledge-driven approaches to support medicinal and synthetic organic chemistry.

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**Prof. Thomas POULSEN**
***AARHUS UNIVERSITY, Aarhus C, Denmark***

Marwin Segler is a team lead at Microsoft Research AI4Science, working at the intersection of chemistry, drug discovery, catalysis, and machine learning. Prior, he was Lead Researcher at BenevolentAI (London), and received his PhD in chemistry from the University of Muenster.

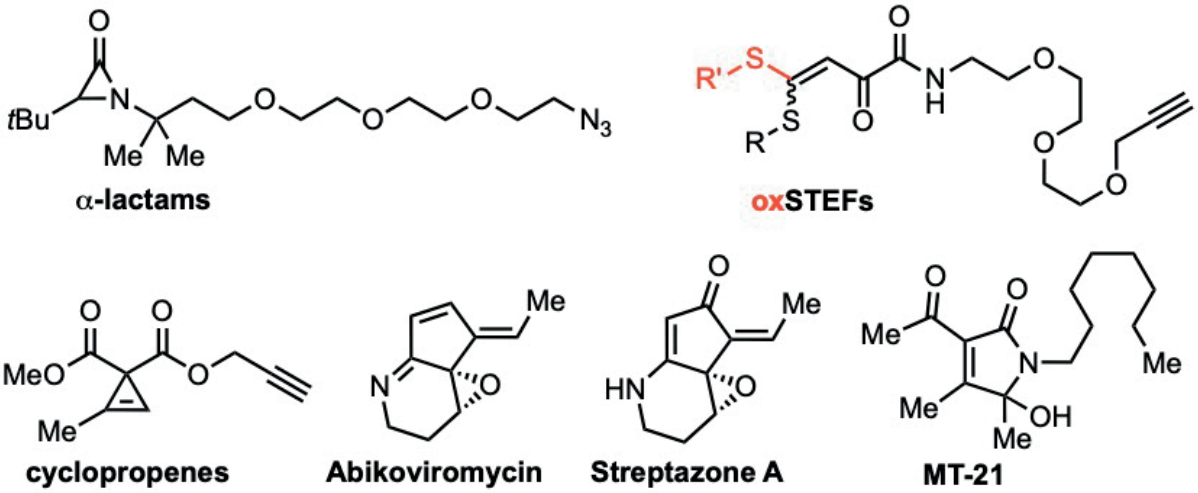
Marwin pioneered modern machine learning for molecular design, and chemical synthesis planning. His research interests are in computer-assisted scientific discovery, algorithm development in computational chemistry and AI, and applications in organic synthesis and drug discovery.

# COVALENT CHEMICAL BIOLOGY WITH NATURAL PRODUCTS AND NOVEL SYNTHETIC WARHEADS

**Thomas Poulsen**

*Department of Chemistry, Aarhus University, Langelandsgade 140, 8000 Aarhus C, Denmark*
*thpou@chem.au.dk*

Electrophiles are subject to strong interest within chemical biology and biomedicine. The discovery of novel, biologically relevant electrophiles can enable e.g. bioconjugation to native proteins for the preparation of biopharmaceuticals or provide new warhead-classes for targeted covalent inhibitors or fragments for screening. In this talk, I will provide examples from our most recent projects focused on electrophilic compounds [1,2,3,4,5,6] which span from fully synthetic constructs, e.g. based on strained ring systems, to natural products (Figure 1). I will e.g. discuss the different synthetic strategies involved and assessment of biological performance in different biochemical and cellular contexts.

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**Dr Ming Joo KOH**
***NATIONAL UNIVERSITY OF SINGAPORE, Singapore, Singapore***

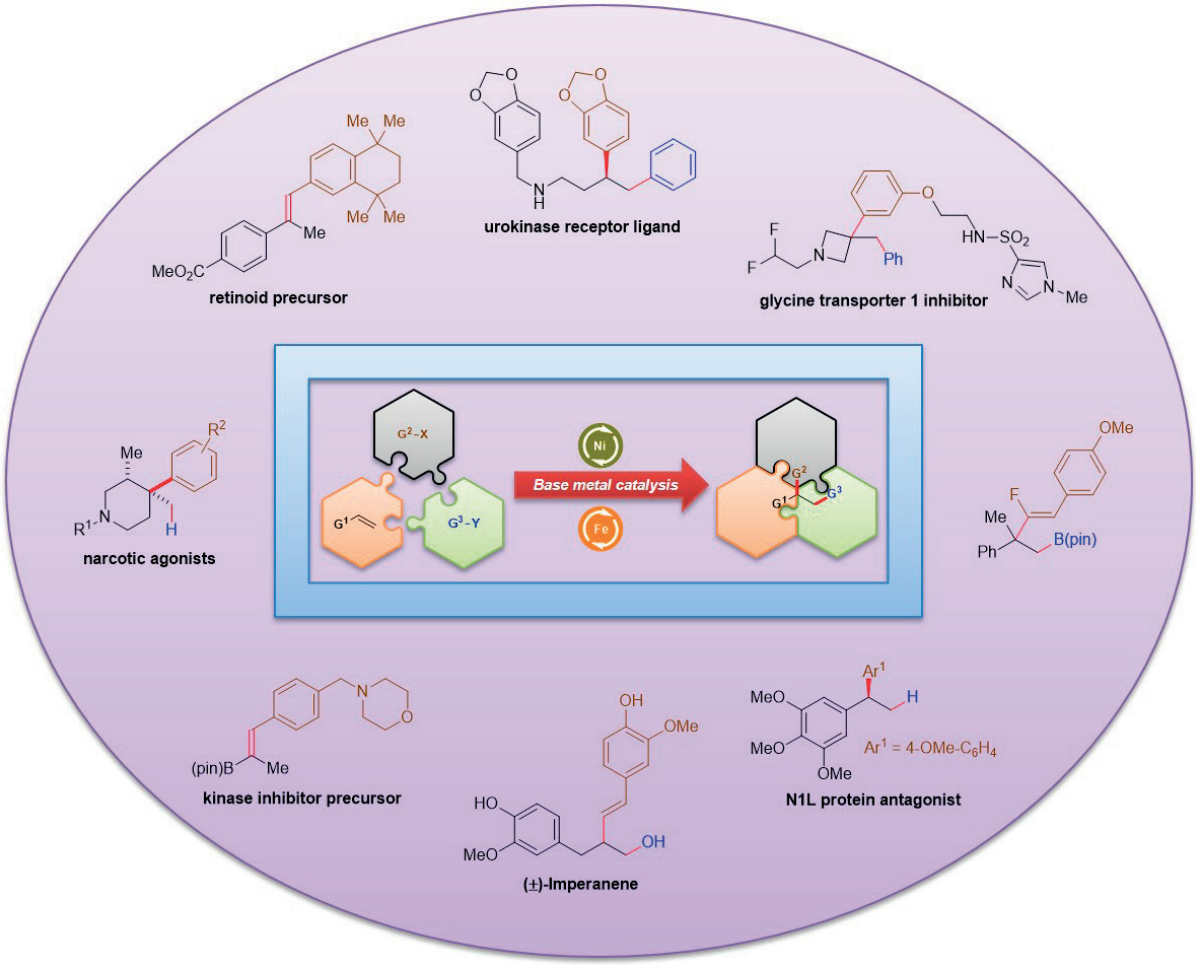
Ming Joo (MJ) Koh was born and raised in Singapore. He received his BSc. degree (First Class Honors) in Chemistry & Biological Chemistry from Nanyang Technological University in 2012, before heading to Boston College for his Ph.D. and post-doctoral studies from 2012 2018. In 2018, MJ joined the Department of Chemistry at the National University of Singapore as the first President's Assistant Professor. His current research focuses on developing sustainable and practical catalytic solutions that address critical challenges in chemical synthesis through base metal catalysis and radical chemistry.

# MULTICOMPONENT ALKENE CROSS-COUPLING BY BASE METAL CATALYSIS

**Ming Joo Koh**

*Department of Chemistry, National University of Singapore, 4 Science Drive 2, Republic of Singapore, 117544*

Multicomponent alkene functionalization has emerged as an attractive class of transformations that enable the cross-coupling of alkene feedstocks with readily available reagents for the expeditious generation of molecular complexity and diversity. However, its widespread adoption in organic synthesis is thwarted by a number of challenges related to reactivity, selectivity and cost. In this talk, we will describe our recent efforts in the development of nonprecious base metal catalytic systems for alkene cross-coupling to generate high-value building blocks with simultaneous control of regio-, diastereo- and/or enantioselectivities. Mechanistic design principles and applications to shrink synthetic chemistry's environmental footprint will be discussed.

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## Dr Martin SCHRÖDER

***NOVARTIS, Basel, Switzerland***

Martin Schröder is a structural biologist with several years of experience in chemical biology and drug discovery. He is currently working as an Innovation Fellow in the Chemical Biology and Therapeutics department at Novartis with a focus of structure-guided drug design. While working in his PhD with the SGC, he contributed to the development and characterization of several chemical tool compounds. At Novartis he presently supporting several projects by providing biophysical assays and structural insights in ligand-protein complexes.

## Dr Anna VULPETTI

***NOVARTIS, Basel, Switzerland***

Anna Vulpetti has over 25 years of experience in drug discovery in Pharma, at Pharmacia & Upjohn, Pharmacia, Pfizer, and since 2006 at Novartis. She is an Associate Director and Senior Principal Data Scientist in the Global Discovery Chemistry department at Novartis with experience from hit finding technologies to hit-to-lead optimization. By working interdisciplinary across different disease areas, she impacted multiple pipeline projects through molecular designs and knowledge in structural chemistry and biophysics.

# NOVEL, REVERSIBLE DCAF1 LIGANDS EXPAND THE TPD-TOOLBOX

**Martin Schroeder (1), Anna Vulpetti (2)**

*1) Chemical Biology & Therapeutics, Novartis Institutes for BioMedical Research, Basel 4002 (Switzerland)*
*2) Global Discovery Chemistry, Novartis Institutes for BioMedical Research, Basel 4002 (Switzerland)*

Targeted protein degradation (TPD) with proteolysis targeting chimeras (PROTACs) has emerged as a key modality in exploring new biology as well as designing new drug candidates. TPD is mediated through harnessing E3 ligases and redirecting them to ubiquitinate de novo target proteins for subsequent proteasomal degradation. Until now however, discovery of E3 ligase chemical matter mediating TPD has been limited to few ligases considering that over 600 E3 ligases are encoded by the human genome. In addition, CRBN has been observed to be downregulated in emerging clinical resistance settings against factors immunomodulatory inhibitory drugs (IMiDs), molecular glues that target IKZF transcription factors to CRBN for degradation. Resistance is potentially accelerated by non-essential cellular functions of CRBN in cells.

Here, we describe the rapid discovery of new potent binders for the WD40 domain of the essential E3 ligase receptor DCAF1 (1). The binder is selective for the $CRL4^{DCAF1}$ E3 ligase complex and can be functionalized into an efficient DCAF1-BRD9 PROTAC. Chemical and genetic rescue experiments confirmed specific degradation via the $CRL4^{DCAF1}$ E3 ligase and proteasomal pathway.

We further showcase the versatility of the DCAF1 for TPD by developing a DCAF1-Dasatininb PROTAC targeting multiple cytosolic and membrane bound Tyrosine Kinases. We expanded these results towards potent and selective DCAF1-BTK PROTAC. With this molecule, we showed rescue of BTK degradation in a BTK-sensitive CRBN-degradation-resistant cell line and thereby provide a rationale for E3 ligase ligand swap to overcome CRBN mediated resistance (2).

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## Dr Nicolas SOLDERMANN

***NOVARTIS, Basel, Switzerland***

Nicolas Soldermann studied chemistry at Ecole Nationale Supérieure de Chimie de Mulhouse, France, where he obtained his chemical engineer diploma, majoring in medicinal and bioorganic chemistry and a MSc. in organic and macromolecular synthesis from the University of Haute-Alsace, France. He then moved in 1998 to Switzerland to the University of Neuchatel for a Ph.D. in organic chemistry with Prof. R. Neier. In 2002, he moved to Stanford University, CA in the US for a Postdoc with Prof. P.A. Wender. Nicolas joined Novartis in 2003 in the Global Discovery Chemistry department as an investigator in medicinal chemistry and since then worked on projects in neurosciences, autoimmunity & transplantation and oncology disease areas. Nicolas led several drug discovery projects from exploratory to late-stage lead optimization contributing to numerous development candidates yielding several clinical compounds. Nicolas is currently Director, medicinal chemistry in Oncology and Immuno-Oncology.

# DISRUPTING THE YAP-TEAD PROTEIN-PROTEIN INTERACTION WITH SMALL MOLECULES - DISCOVERY OF DRUG CANDIDATE NVP-IAG933

**Nicolas Soldermann**

*Novartis Pharma AG*
*Forum 1, Novartis Campus*
*WSJ-386/13/10*
*4002 Basel*
*Switzerland*

The inhibition of the YAP-TEAD protein-protein interaction constitutes a promising approach for the treatment of cancers associated with a dysregulation of the Hippo pathway. The extended interaction surface of the two proteins represents a substantial challenge for a small molecule inhibitor approach. By virtual screening we were able to identify a weakly active hit based on a dihydrobenzofurane scaffold binding to one of the two main sites of interaction of YAP at the surface of TEAD. Guided by structure-based and compound property-based design we managed to improve the potency of this hit class by several orders of magnitude and to identify inhibitors with cellular activity which displayed efficacy in tumor-bearing mice after oral administration. The main features of this work will be presented finally leading to the discovery of our clinical compound NVP-IAG933.

## Prof. Leroy CRONIN

***UNIVERSITY OF GLASGOW, Glasgow, United Kingdom***

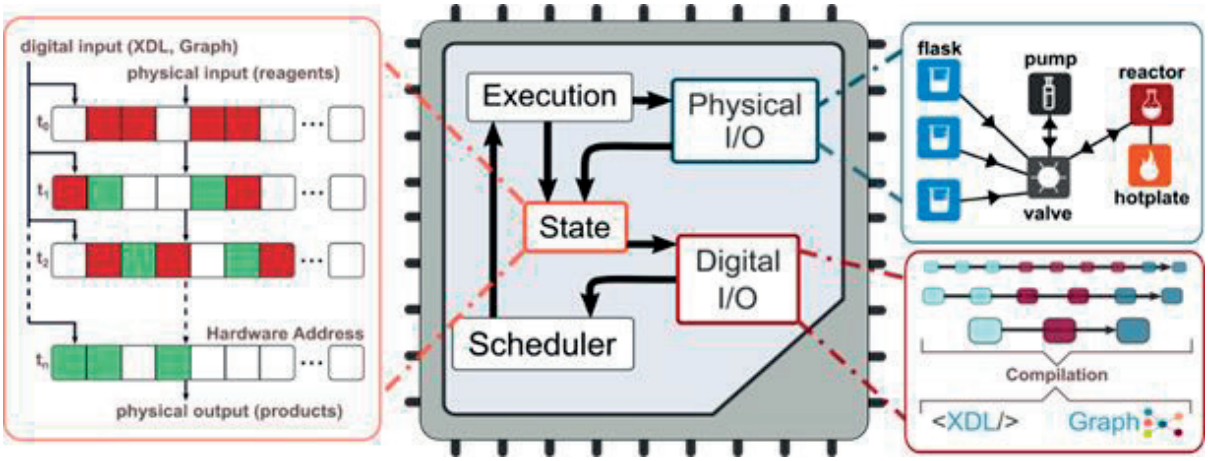
Lee Cronin was born in the UK and was fascinated with science and technology from an early age getting his first computer and chemistry set when he was 8 years old. This is when he first started thinking about programming chemistry and looking for inorganic aliens. He went to the University of York where he completed both a degree and PhD in Chemistry and then on to do post docs in Edinburgh and Germany before becoming a lecturer at the Universities of Birmingham, and then Glasgow where he has been since 2002 working up the ranks to become the Regius Professor of Chemistry in 2013 aged 39. He has one of the largest multidisciplinary chemistry-based research teams in the world, having raised over $35 M in grants and current income of $15 M. He has given over 300 international talks and has authored over 350 peer reviewed papers with recent work published in Nature, Science, and PNAS. He and his team are trying to make artificial life forms, find alien life, explore the digitization of chemistry, understand how information can be encoded into chemicals and construct chemical computers.

# WILL CHEMPUTERS DREAM OF ELECTRIC DRUGS

**Lee Cronin**

*School of Chemistry, The University of Glasgow, Glasgow, UK*

I will explain why 'Chemputation' is a universal approach to explore chemical reactivity, discovery of new reactions, and molecules, as well as program chemical synthesis that allows us to translate all procedures, manual or automatic, into a executable chemical programming language that can run the processes on a chemputer. This code is written in the world's first universal programming language for chemistry: χDL (pronounced Chi-DL). This new approach maps into a universal programming language for chemistry that is accessible to ALL synthetic chemists and will work on ALL robotic systems (subject to suitable specification). We demonstrate that the process is universal, and by analogy with computation, we call systems capable of universally turning code into reliable chemistry and materials processes *Chemputation*, see Figure.

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## Dr Etienne DONCKELE

***MONTE ROSA THERAPEUTICS, Basel, Switzerland***

Etienne performed his undergraduate research with Prof. Barry M. Trost at Stanford University and carried out one-year placement in medicinal chemistry at the Broad Institute of MIT and Harvard. He obtained his Ph.D. in organic chemistry at the ETH Zürich under the supervision of Prof. François Diederich. After a postdoctoral stay at Caltech in the group of Prof. Brian Stoltz in natural product synthesis, Etienne gained experience across multiple phases of drug discovery throughout Philochem AG (a Swiss subsidiary of the Philogen group) first as a research scientist before taking on the role of Deputy Head of Chemistry. He worked on the development of the DNA-encoded chemical library platform and on multiple medicinal chemistry projects. Etienne joined Monte Rosa Therapeutics in 2021 and is currently an Associate Director in chemistry where is thriving to develop novel molecular glue degraders on various indication areas. Etienne is a named inventor on 6 patent applications and has authored more than 20 scientific publications.

# TAKING MOLECULAR GLUE DEGRADERS TO NEW HEIGHTS

**Owen Wallace, Etienne Donckele**

*Monte Rosa Therapeutics*
*40 Guest St., MA 02135 Boston*
*United States*

Targeted Protein Degradation is an attractive modality to deeply modulate protein pathways, employing the body's natural mechanisms to selectively eliminate therapeutically relevant proteins. Monte Rosa Therapeutics is a biotechnology company developing a portfolio of novel molecular glue degrader (MGD) medicines, using its proprietary protein degradation platform, called QuEEN™ (Quantitative and Engineered Elimination of Neosubstrates). The platform combines diverse and proprietary chemical libraries of small molecule protein degraders with in-house capabilities in proteomics, structural biology, AI/machine learning-based target selection, and computational chemistry. This has resulted in a growing pipeline of drug discovery programs that are progressing in/towards the clinic. This presentation will cover the QuEEN platform and our programs, including the discovery of our GSPT1 degrader, MRT-2359, for solid tumors.

The development of MRT-2359 began with the combination of our CRBN-targeted library of molecular glue degraders and the hypothesis that targeting protein translation in MYC-driven cancer cells could uncover a vulnerability. Through optimization efforts, we achieved differential toxicity between MYC- and non-MYC-driven cancer cell lines, optimized kinetics and depth of degradation, enhanced oral bioavailability, and demonstrated promising in vivo efficacy. To evaluate the anti-tumor activity of MRT-2359, we conducted studies using over 80 patient-derived xenografts (PDXs) from lung cancer patients. The results confirmed the preferential anti-tumor activity of MRT-2359 in N- and L-MYC high non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC) PDXs when administered orally on a daily or intermittent basis. Currently, oral MRT-2359 is being investigated in a Phase 1/2 clinical trial involving selected cancer patients with MYC-driven cancers.

**Dr Susannah COOTE**
***UNIVERSITY OF BATH, Bath, United Kingdom***

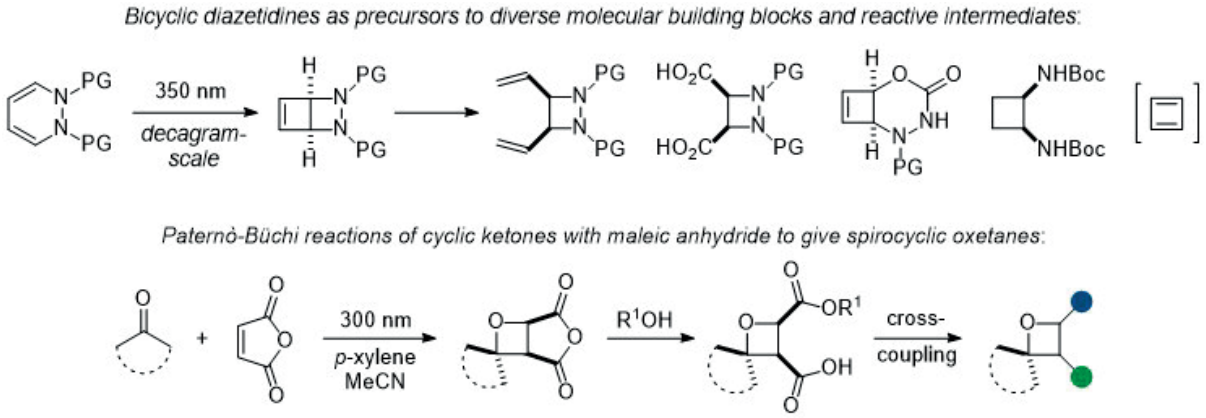
After undergraduate studies at the University of York (UK) and the Université Joseph Fourier (France), Susannah Coote received her Ph.D. from the University of York in 2007, working under the supervision of Prof. Peter O'Brien. After postdoctoral positions at the Université Paris-Sud (France) in the group of Prof. Cyrille Kouklovsky, and at the University of Manchester (UK) in the group of Prof. David Procter, she was awarded an Alexander von Humboldt postdoctoral fellowship to join the group of Prof. Thorsten Bach at the Technische Universität München (Germany). Susannah started her independent career as a Lecturer in 2014 at Lancaster University, focusing on developing synthetic organic photochemical methodology for the synthesis of interesting strained molecules. Susannah was promoted to Senior Lecturer in 2021, and in 2023 she moved to her current position at the University of Bath.

# MAKING DIFFICULT-TO-MAKE MOLECULES: PHOTOCHEMISTRY AS AN ENABLING TOOL

**Susannah Coote**

*Department of Chemistry, University of Bath, Claverton Down, BA2 7AY, UK*

Research in our group focuses on the development of photochemical methodology for the synthesis of molecules that cannot be accessed (or are accessed only with great difficulty) by using standard synthetic transformations, with a view to applications within medicinal chemistry. In general, photochemical reactions have been under-exploited by synthetic chemists but allow complementary reactivity to ground state processes, and access to highly strained molecular frameworks. In particular, we focus on the preparation of four-membered rings, and case studies will be presented based on our recent work on bicyclic diazetidines[1,2] and spirocyclic oxetanes,[3] as summarised in the graphic below:

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## Dr Anders JOHANSSON

***ASTRAZENECA, Molndal, Sweden***

Dr Anders Johansson is a director of chemistry at the research unit for cardiovascular, renal and metabolic disorders (CVRM) within AstraZeneca Biopharmaceuticals R&D. In his current role, he is a chemistry lead and project lead for discovery projects in all phases up to CD selection. Dr Johansson has 20+ years experience of drug discovery and has been involved in numerous projects directed towards thrombosis, IBS, IBD, obesity, diabetes and kidney disease. He has been leading chemistry on most of the major target classes, incuding soluble enzymes, GPCR's and transcription factors. Anders also has a long experience of CNS drug discovery. Before joining AstraZeneca, Dr Johansson worked at a small biotech focused on diagnostics for thrombosis and heamostatis. Dr Johansson is an author of multiple publications and a named inventor on several patents. He holds a PhD in organic chemistry from Chalmers University of Technology in Gothenburg.

# NOVEL MODES OF ENZYME INHIBITION

**Anders Johansson (1), Jakob Danielsson (1), Martin Bauer (1), Bertrand Arnaud (1), Tomas Akerud (2), Claudia De Fusco (3), Peter Brandt (4), Fredrik Bergstrom (5), Patrik Johansson (2), Margareta Ek (2), Ulf Borjesson (6), Maria Stromstedt (7), Birgitta Rosengren (7), Frank Jansen (2), Linda Fredlund (2)**

*1) Medicinal Chemistry, Research and Early Development, Cardiovascular, Renal and Metabolism (CVRM), BioPharmaceuticals R&D, AstraZeneca, Gothenburg, Sweden*
*2) Mechanistic and Structural Biology, Discovery Sciences, R&D, AstraZeneca, Gothenburg, Sweden*
*3) BenevolentAI, Cambridge, UK*
*4) Beactica Therapeutics, Uppsala, Sweden*
*5) DMPK, Research and Early Development, Cardiovascular, Renal and Metabolism (CVRM), BioPharmaceuticals R&D, AstraZeneca, Gothenburg, Sweden*
*6) Hit Discovery, Discovery Sciences, R&D, AstraZeneca, Gothenburg, Sweden*
*7) Bioscience, Research and Early Development, Cardiovascular, Renal and Metabolism (CVRM), BioPharmaceuticals R&D, AstraZeneca, Gothenburg, Sweden*

Methyl transferases are a big class of enzymes involved in various important biological processes. These enzymes are intracellular and rely on the same co-factor, S-adenosyl methionine (SAM), for transfer of a methyl group to the substrate in question. Our interest is focussed towards N-nicotinamide methyl transferase (NNMT). NNMT plays an important role in the degradation of NAD+ by methylation of nicotinamide to the N1-methylnicotinamide, 1-MNA. Altered NNMT activity has been linked to a number of diseases from metabolic and cardiovascular to oncology and neurodegenerative disease.[1] Hence, a small molecule NNMT inhibitor could play an important role in the management of associated disorders. Efforts towards the development of an efficient NNMT inhibitor have followed several different approaches.[2] For several of the published NNMT inhibitors, we found that combining oral bioavailability and high potency can be a challenge. During the course of our program, we discovered a novel mode of inhibition, allowing for the optimization of permeability and bioavailability, but at the same time achieving high potency inhibitors of the NNMT enzyme. The presentation will unravel of the mechanism of inhibition, detail the mechanistic basis for achieving high potency and will also present *in vivo* assessment of the chemical series.

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## Prof. Ryan SHENVI

***SCRIPPS RESEARCH, La Jolla, United States***

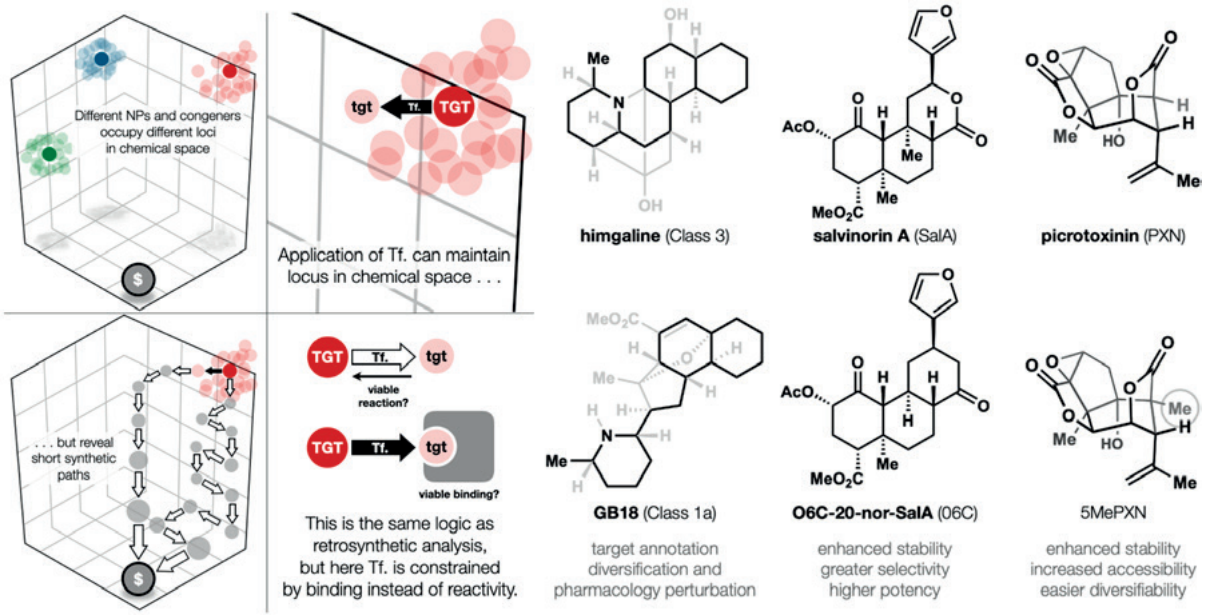
Ryan Shenvi is a Professor in the Department of Chemistry at Scripps Research, where his lab develops new chemistry to synthesize complex molecules and explore their functions. He obtained his BS degree with distinction in chemistry as a Schreyer Honors Scholar at Penn State University and earned his PhD as an NDSEG predoctoral fellow at Scripps Research, followed by NIH-funded postdoctoral studies at Harvard University.

# NATURAL PRODUCT SYNTHESIS THROUGH THE LENS OF INFORMATICS

**Ryan Shenvi**

*Scripps Research*
*Department of Chemistry*
*10550 North Torrey Pines Road, BCC 420*
*La Jolla, CA 92037*

Natural products (NPs) often populate areas of chemical space that are remote from commercial compounds and thus challenging to access, study and modify. Therefore, our group develops new chemistry to accelerate access to nodes in NP space. These syntheses can be leveraged to assign mechanism of action, remove structural liabilities and perturb target selectivity. Recently, we developed new cross-coupling methods to access two alkaloids from *Galbulimima*. These syntheses led to the identification of potent ligands for the κ- and μ-opioid receptors, and optimization of their pharmacology.[1,2] This work extended our research in naturally occurring psychotropics,[3] including salvinorin A (SalA).[4] Here, we identified two scaffold mutations that were predicted to stabilize the SalA scaffold, maintain target affinity, maintain gross physicochemical properties yet increase our ability to diversify and optimize the natural product,[4] recently delivering analogs with increased potency, selectivity, stability and functional bias for G protein signaling.[5] An identical workflow led to 5-methyl-picrotoxinin, a more complex analog of picrotoxinin (PXN) that simplified synthetic access, stabilized the scaffold and allowed diversification to probe selectivity among ligand-gated ion channels (LGICs).[6]

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## NOTES

# EFMC Prize for a Young Medicinal Chemist or Chemical Biologist - Biographies & Abstracts

## Dr Georg WINTER

***RESEARCH CENTER FOR MOLECULAR MEDICINE OF THE AUSTRIAN ACADEMY OF SCIENCES, Vienna, Austria***

### EFMC PRIZE FOR A YOUNG MEDICINAL CHEMIST OR CHEMICAL BIOLOGIST IN ACADEMIA

Georg Winter, PhD, performed his graduate studies at CeMM, working on elucidating the mechanism of action of cancer drugs. He specialized on proteomics- as well as chemical genetics approaches. He continued his training in chemical biology, working as a postdoctoral fellow with Dr. James Bradner the Dana Farber Cancer Institute. Supported by an EMBO fellowship, he published the first paper reporting on in vivo target protein degradation (Winter et al., Science 2015). He was recruited as a CeMM Principal Investigator in June 2016. Thematically, his lab works at the interface of chemical biology, cancer, and gene control. His group aims to innovate novel pharmacologic strategies that allow us to probe, understand and eventually disrupt aberrant transcriptional circuits in cancer. Dr. Winter's research strategy is inspired and driven by high-throughput and unbiased technologies such as quantitative proteomics, (nascent) transcriptomics and functional genomics. Connecting these technologies with synthetic chemistry empowers the understanding of the mechanism of action of proteins, protein complexes or small molecules both on a holistic but also mechanistic level.

# TARGETED PROTEIN DEGRADATION VIA MOLECULAR GLUES

**Georg Winter**

*CeMM - Center for Molecular Medicine of the Austrian Academy of Sciences, 1090 Vienna*

Targeted protein degradation is a pharmacological paradigm that is based on small molecules that induce molecular proximity between disease-causing proteins and effectors of the cellular degradation machinery.

Here, I will discuss our efforts in employing multi-omics approaches in order to identify and characterize small-molecule degraders that target previously undruggable proteins for degradation by the proteasome or for clearance via macroautophagy. Additionally, I will discuss how we are using deep mutational scanning to experimentally infer functional hotspots in E3 ubiquitin ligases and review the associated implications for the emergence of resistance mechanisms for small-molecule degraders.

## Dr Teresa DE HARO GARCIA

***UCB, Braine l'Alleud, Belgium***

### EFMC PRIZE FOR A YOUNG MEDICINAL CHEMIST OR CHEMICAL BIOLOGIST IN INDUSTRY

Teresa de Haro received her M. Sc. Chemistry degree in Madrid (Spain) from Autonoma University in 2007. After a medicinal chemistry internship at Eli&Lilly (Madrid), she joined the lab of Prof. Nevado at the University of Zurich (Switzerland) where she completed her PhD in Organic Chemistry in 2012. Then, she joined the group of prof. Fürstner at Max-Planck-Institute (Mulheim, Germany) studying total synthesis of natural products. In 2013 she joined Eli&Lilly in Surrey (UK) before moving to UCB in Slough (UK) in 2014 as senior scientist. Since 2018, Teresa is working as principal scientist at UCB in Braine l'Alleud (Belgium).

# DISCOVERY AND CHARACTERIZATION OF POTENT, EFFICACIOUS AND ORALLY AVAILABLE ANTIMALARIAL PLASMEPSIN X INHIBITORS

**Teresa de Haro Garcia**

*UCB, Medicinal Chemistry*
*Chemin du Foriest 1*
*1420 Braine l'Alleud*
*Belgium*

Malaria is a serious mosquito-borne disease with an estimated 247 million cases and 619 thousand deaths worldwide in 2021, predominantly in small children in Africa.[1] There is a constant need for novel antimalarial medicines to complement existing artemisinin-dependent therapies, which are under pressure from resistance conferring mutations. Plasmepsin X (PMX), an essential aspartyl protease of malaria parasite, was recently identified as new potential multistage drug target to fight against malaria. PMX controls malaria parasite egress and invasion of erythrocytes, development of functional liver merozoites (prophylactic activity) and blocking transmission to mosquitoes.[2] In this talk, we will present the discovery of potent, orally available, PMX inhibitors. We will describe the *in vitro* and *in vivo* characterization of our lead molecules, efficacy data in a SCID mouse model of *Plasmodium* falciparum malaria and in a prophylactic liver mouse model for rodent *Plasmodium berghei* malaria.[3] In addition, we will present the results obtained on liver stage Chemovaccination Studies.

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## *NOTES*

# Oral Communications - Biographies & Abstracts

## Prof. Alexandros ZOGRAFOS

***ARISTOTLE UNIVERSITY OF THESSALONIK, Thessaloniki, Greece***

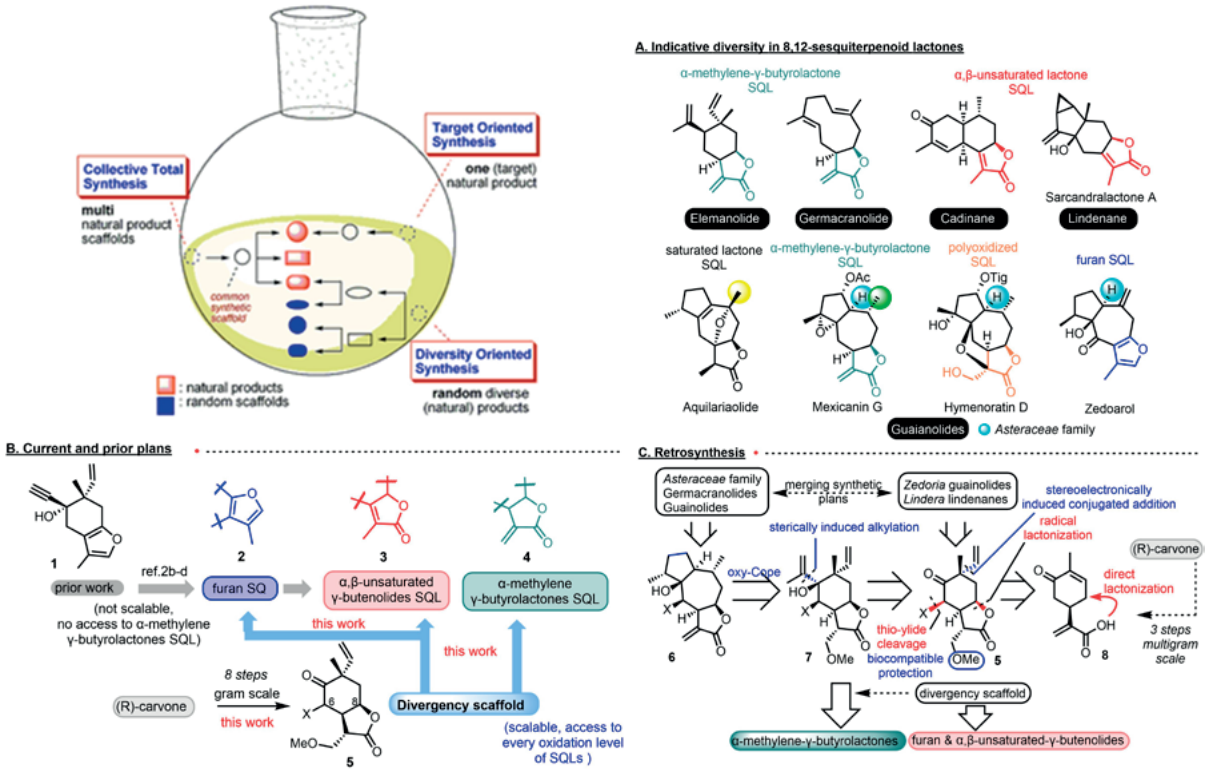
Alexandros L. Zografos graduated as a chemist in 1996 from National University of Athens. After earning his PhD in 2001 under the supervision of Prof. Olga Igglessi-Markopoulou at National Technical University of Athens, he pursued postdoctoral studies first at The Scripps Research Institute, under the guidance of Prof. Phil S. Baran and then at Columbia University with Prof. Scott Snyder, before moving back to Greece to work as a senior researcher at National University of Athens and at NCRS Demokritos Institute. In 2009, he began his independent career at Aristotle University of Thessaloniki where currently he is Associate Professor. He is recipient of Hildegrad award of the National Academy of Athens and the Fulbright Visiting Scholar Award. His group is working on the exploration of divergent total synthesis of natural products and the development of biomimetic methodologies for the aerobic oxidation of complex substrates.

# DIVERGENT SYNTHESIS AS AN EMERGENT TOOL FOR DRUG DISCOVERY

**Alexandros L. Zografos**

*Aristotle University of Thessaloniki, Department of Chemistry, Laboratory of Organic Chemistry, University Campus, Thessaloniki, 54124, Greece*

The emergence of preparing diverse natural product scaffolds is firmly associated with the need of our society for more potent and selective biomodulators. In response, nowadays, divergent synthesis utilizing common synthetic scaffolds that can be readily transformed to an array of diverse natural compounds is progressively gaining ground in drug discovery.[1]
The impressive machinery of complexity as exemplified by the biosynthesis of terpenoids, highlights the ability of Nature to perform highly selective transformations by utilizing enzymes and rather simple common scaffolds. This divergent protocol empowered by the iterative use of primary metabolism reactions (IPP, DMAPP, cationic reactions) produce the carbocyclic frameworks (cyclase phase) that finally reach the oxidative enzymes (oxidase phase). The common motives of reactivity used by Nature permits, in some extent, the identification and application of selective biosynthetic-like reactions in modern total synthesis.
The lecture will focus on drawbacks and solutions towards the development of a unified synthetic plan for accessing highly cytotoxic sesquiterpene lactones inspired by the two-phases synthesis of Nature.[2-5]

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***UNIVERSITY OF RIJEKA, Rijeka, Croatia***

Dean Marković studied at the Faculty of Science, University of Zagreb and received his PhD at EPF-Lausanne (2005) mentored by Prof. dr. sc. Pierre Vogel. After postdoctoral studies at Yale University (2006) and UIUC Urbana-Champaign (2007) in the group of prof. dr. sc. John Hartwig as a fellow of the Swiss National Science Foundation and F. Hoffmann-La Roch Foundation, he held the position of scientific associate at EPFL (2008-2011). He completed his habilitation at the University of Paris Descartes (2012). As an associate professor, he teached at the University of Osijek (2015-2016) and at the University of Rijeka (2016-2021), where he was promoted to full professor in 2021. He was a visiting professor at the University of Rijeka (2010-2012) and at Riga Technical University (2016) and at the University of Picardy Jules Verne (2017). His research interests include the chemistry of natural compounds, novel CO2 catalytic methodologies, mechanistic studies and physical-organic aspects of metal-catalyzed reactions, and CO2 chemistry.

# ISOLATION AND BIOLOGICAL ACTIVITIES OF NATURAL PRODUCTS AND ANALOGUES FROM THE ADRIATIC CORAL EUNICELLA CAVOLINI

**Dario Matulja (1), Gabrijela Matijević (2), Sanja Babić (2), Petra Grbčić (1), Krunoslav Bojanić (2), Sylvain Laclef (3), Ozren Jović (2), Višnja Stepanić (2), Tomislav Šmuc (2), Iris Car (4), Mirela Sedić (4), Rozelindra Čož-Rakovac (2), Sandra Kraljević Pavelić (5), Dean Marković (1)**

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*Eunicella cavolini* (Koch, 1887) generally recognized as yellow gorgonian, is a species of colonial soft coral in the family Gorgoniidae. Although it is common into Mediterranean basin, the evaluation of biological potential of *E. cavolini* from the Adriatic Sea has not been performed yet. Taking this into account, as a part of our Center of excellence for Marine Bioprospecting of Adriatic Sea BioProCro, we have studied the chemical composition and biological activities of this gorgonian.[1] In this talk, we will discuss the results of screenings of the antiproliferative, antibacterial, antioxidant, and anti-inflammatory activities of organic extracts and corresponding semipurified fractions. Additionally, we will show data concerning antiproliferative activity against 4 cancer and one non-transformed cell line, as well as its anti-inflammatory potential concerning of successfully isolated pregnane type steroid by employing dereplication methods.

In final, we will present the synthesis of granulatamide B[2] isolated from the coral species of the Eunicella genus and its 12 derivatives by employing Fürstner's Fe-catalyzed C-C coupling reaction[3]. The obtained small compound library of these *N*-acyl tryptamines differing in the number of C-atoms, saturation degree and conjugation of double bonds, were evaluated towards the formation and development of zebrafish *Danio rerio* embryos[4].

**ACKNOWLEDGEMENTS** We would like to thank to Croatian Government and the European Union (European Regional Development Fund—the Competitiveness and Cohesion Operational Program - KK.01.1.1.01) for funding this research through project Bioprospecting of the Adriatic Sea (KK.01.1.1.01.0002) granted to The Scientific Centre of Excellence for Marine Bioprospecting - BioProCro. We also acknowledge the project "Research Infrastructure for Campus-based Laboratories at University of Rijeka", co-financed by European Regional Development Fund (ERDF) and the University of Rijeka research grants UNIRI-prirod-18-102 and UNIRI-biomed-18-133. We would like to thank Croatian Science Foundation project 'Career Development of Young Researchers-Training of New PhDs' for funding PhD of D. Matulja and research project IP-2019-04-8846.

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**Dr Anne-Sophie REBSTOCK**
***VINCERX, Monheim am Rhein, Germany***

Anne-Sophie Rebstock graduated from INSA Rouen, a french engineering school, in 2001. She obtained a PhD in organic chemistry in 2004 in Pr. Quéguiner Group under the supervision of Prof. Mongin. After 2 postdocs in medicinal chemistry in Johnson & Johnson and the Wolston institute for Biomedical Research, she worked in Bayer for 14 years, both in the Cropscience and Pharma divisions. In 2022, she joined Vincerx Pharma as head of medicinal chemistry where she is thriving to develop Vincerx' innovative bioconjugation platform to further improve safety in both the small molecule and antibody drug conjugates fields. Anne-Sophie has contribued to 13 papers and 55 patents.

# DESIGN AND OPTIMIZATION OF HIGH PERFORMANCE KSPI ADCS WITH INCREASED TUMOR SELECTIVITY: ENHANCING THE THERAPEUTIC WINDOW BY LEGUMAIN-MEDIATED ADC ACTIVATION

**Anne-Sophie Rebstock (1), Hans-Georg Lerchen (1), Beatrix Stelte-Ludwig (1), Anette Sommer (3), Sven Wittrock (3), Sandra Berndt (3), Sarah Johannes (3), Yolanda Cancho-Grande (3), Christoph Mahlert (3), Simone Greven (3), Leo Marx (3), Mareike Wiedmann (1), Raquel Izumi (2), Ahmed Hamdy (2)**

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*3) Bayer AG, Pharmaceuticals R&D*

With the approval of 13 antibody-drug conjugates (ADCs) for hematologic and solid tumor malignancies, ADCs are becoming increasingly prominent in the oncology landscape. So far, the number of cytotoxic payload classes successfully employed in antibody-drug conjugates is still rather limited. Contrary to the dogma that ADCs increase the maximum tolerated dose (MTD) of their cytotoxin payloads, the mounting clinical evidence suggests that the tolerated doses of ADCs are not significantly different from those of related small molecules[1].

In an effort to enhance the therapeutic index over current ADCs, we have developed ADCs utilizing kinesin spindle protein inhibitors (KSPi) as a novel payload class[2]. In addition to the amino group present in potent KSP inhibitors and suitable for the attachment of cleavable linkers, we identified further positions in the KSPi molecule where the installment of non-cleavable linkers for antibody attachment was tolerated and provided highly potent ADCs. Such conjugates demonstrate high efficacy in various tumor models with a novel mode of action.

The therapeutic window of ADCs is limited by either premature cleavage of the payload or by unspecific or on-target/off-tumor ADC uptake into healthy cells. Therefore, we strived to achieve an additional safety level by preferential activation in tumor versus healthy cells and an otherwise high stability of the ADCs. We successfully designed novel linkers in KSPi ADCs, which are efficiently and selectively cleaved by the tumor-associated lysosomal endopeptidase, legumain[3]. Due to the high expression of legumain in tumors and its unique cleavage site after asparagine, those ADCs demonstrate high specificity for legumain-mediated activation in tumors as compared with healthy tissue. We discovered an interesting structure-activity relationship (SAR) of the linker peptides allowing us to adapt the linker design for optimal performance in different tumor indications while sparing healthy tissues.

In addition, the SAR of KSPi payloads allowed installation of chemical entities, which modify the physicochemical properties of the active metabolites cleaved from the ADC without interfering with its potent binding to the KSP target. In particular, the attachment of a Celltrapper™ moiety to reduce membrane permeability of the active metabolite increases efficacy (due to intracellular accumulation) and reduces damage to healthy cells (due to low membrane permeability of free payload).

In conclusion, we developed an innovative, broadly applicable KSPi-ADC platform combining high potency with a new mode action, optimizing tumor selectivity vs healthy tissues and providing long-lasting intracellular exposure by using tailor-made active metabolites. VIP943, our frontrunner-ADC based on this technology, is targeting CD123 for the treatment of acute leukemias and myelodysplastic syndromes. It has potent efficacy in cell line and patient-derived mouse models of CD123+ hematologic malignancies and a favorable safety profile in monkeys. VIP943 is on track to enter clinical trials this year.

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Afjal joined GSK in 2006 and has worked on multiple hit-to-lead and lead optimisation projects in respiratory, immuno-inflammation and cancer. Afjal has been working in the targeted protein degradation field since 2012, where he has gained extensive experience leading projects in discovery and lead optimisation of PROTACs and E3 ligases. He helped design and manage the development of GSK's proprietary PROTAC monomer library, and more recently, Afjal has collaborated with GSK's Discovery High Throughput Chemistry (DHTC) group, to develop an integrated biology and chemical screening platform (D2B) for the synthesis of PROTACs. This is now routinely used by all PROTAC projects in an HTC fashion and has had significant impact on reducing cycle times.

His research interests include new modalities in protein degradation such as molecular glues, novel E3 Ligases and the ubiquitin proteasome system.

# HIGH-THROUGHPUT PROTAC SYNTHESIS AND DIRECT TO BIOLOGY ASSAYING FOR RAPIDLY EVALUATING TARGETED PROTEIN DEGRADATION

**Afjal Miah**

*GlaxoSmithKline, Medicine Research Centre, Gunnels Wood Road, Herts, SG1 2NY Stevenage, United Kingdom*

Over the last decade, PROTAC has received immense attention as a new modality for therapeutic intervention. It provides promising opportunities to overcome some of the limitations faced with small molecule inhibitors, and to target previously classed 'undruggable' proteins. Yet synthesis and optimization of PROTACs via traditional iterative synthesis for drug discovery program can be resource intensive and very time-consuming.

We have developed a highly efficient High-Throughput Chemistry Direct-to-Biology platform to conduct miniaturized reactions in 1536-well plates to make large set of PROTACs and directly assess these crude reactions in a cellular degradation assay. Case studies will be presented to showcase how the platform has impacted timelines for identifying new PROTACs and supported lead optimisation projects.

**Dr Chiara BORSARI**
***UNIVERSITY OF MILAN, Milan, Italy***

Chiara Borsari obtained her PhD in Medicinal Chemistry from the University of Modena and Reggio Emilia (Italy) in 2017. During her PhD, two fellowships allowed her to join the NHRF in Athens (Greece) and the State University of New York at Albany (USA). From 2017 to 2022, she was a postdoctoral fellow in the group of Prof. M. Wymann at the University of Basel (Switzerland). In September 2022, she moved to the University of Milan supported by a L'Oréal UNESCO for Women in Science and a Fondazione Umberto Veronesi Fellowship. Since January 2023, she is chair of the EFMC Young Scientists Network. Her main research focus is the development of innovative chemical strategies for cancer therapy.

# DEVELOPMENT OF SELECTIVE mTOR INHIBITORS FOR THE TREATMENT OF CANCER AND NEUROLOGICAL DISORDERS

**Chiara Borsari (1,2)**

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The mechanistic target of rapamycin (mTOR) is activated downstream of phosphoinositide 3-kinase (PI3K) and is a key regulator of cell growth and survival. The mTOR pathway is dysregulated in many diseases, including cancer and neurological disorders.[1]

In 2018, we discovered PQR620, the first-in-class brain-penetrant ATP-competitive mTOR inhibitor (TORKi) able to attenuate epileptic seizures in a mouse model of Tuberous Sclerosis Complex (TSC).[2] Despite promising results in rodent disease models, the limited stability of PQR620 in human hepatocyte assays and short half-live in pharmacokinetic studies in Cynomolgus monkeys, prevented its entry into clinical development. Aiming to develop follow up compounds for PQR620, we have disclosed a conformational restriction strategy and discovered the first pyrimido-pyrrolo-oxazine highly selective TORKi (PQR617).[3] While the first-generation tricyclic compounds displayed a limited brain penetration, investigation on the heteroaromatic ring led to second generation pyrimido-pyrrolo-oxazines with predicted BBB permeability.[4] In parallel, we combined pharmacophore features of PQR620 and PQR617, and discovered PQR626. PQR626 displayed an excellent brain penetration, very good tolerability in mice and was able to significantly prevent mortality in *Tsc1*$^{GFAP}$CKO mice.[5] Very recently, we explored 3,6-dihydro-2*H*-pyran (DHP) and tetrahydro-2*H*-pyran (THP) as isosteres of the morpholine moiety to unlock a novel chemical space for TORKi generation.[6]

Overall, we exploited a variety of chemical strategies to identify metabolically stable mTOR inhibitors for the treatment of cancers and neurological disorders driven by mTOR deregulation, and requiring drug distribution also to the central nervous system.

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## Dr Alexandros VAKALOPOULOS

***BAYER, Wuppertal, Germany***

Alexandros studied chemistry at the University of Hannover in Germany and received his PhD in 2000 in the field of marine natural product synthesis. He began his industrial career at Bayer in the field of semi-synthetic pharmaceutical active compounds based on natural products in 2001. He then worked as a medicinal chemist on various indication areas in Drug Discovery and made numerous contributions to various development assets in the field of cardiovascular and oncological diseases. In addition to his experience in the hit-to-lead process and late research projects, he also dealt in depth with DMPK topics and DEL chemistry. Alexandros has contributed to 87 scientific papers and patents.

# DISCOVERY OF IMIDAZO[1,2-A]PYRIDINE CARBOXAMIDE BAY 1165747, A LONG-ACTING SOLUBLE GUANYLATE CYCLASE STIMULATOR FOR RESISTANT HYPERTENSION

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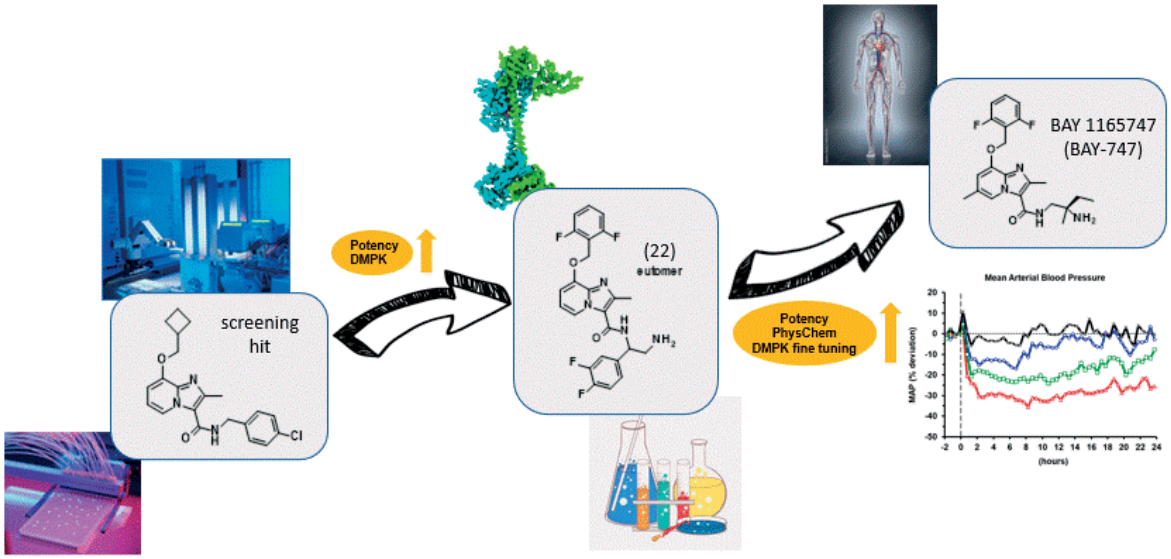
We describe the identification, chemical optimization and preclinical characterization of novel soluble guanylate cyclase (sGC) stimulators. sGC stimulators have shown clinical benefit in cardiopulmonary diseases (riociguat), and chronic heart failure (vericiguat). However, given the very broad therapeutic opportunities for sGC stimulators, more tailored molecules for distinct indications with specific pharmacokinetics, tissue distribution and physicochemical properties will be required in the future. Here, we report the ultra-high-throughput (uHTS)-based discovery of a new class of sGC stimulators from a imidazo[1,2-a]pyridine lead series. Through the extensive and staggered optimization of the initial screening hit liabilities such as potency, metabolic stability, permeation and solubility could be substantially improved in parallel. These efforts resulted ultimately in the discovery of the new sGC stimulators **22** and BAY 1165747 (BAY-747, **28**). With their differentiated and long-acting pharmacological effects these compounds could turn out to be optimal for indications and tailored uses where a very low peak/trough ratio is desired. Thus, BAY-747 (**28**) could be an ideal treatment alternative for patients with hypertension, especially those not responding to standard anti-hypertensive therapy (resistant hypertension). BAY-747 (**28**) demonstrated sustained hemodynamic effects up to 24 hours in phase 1 studies.

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## Prof. Olga GARCIA MANCHENO

***UNIVERSITY OF MÜNSTER, Münster, Germany***

Prof. Dr. Olga García Mancheño is professor for organic chemistry at the University of Münster (WWU). She obtained her PhD in Chemistry in 2005 at the University Autonomous of Madrid under the supervision of Prof. J.C. Carretero. After her postdoc in the group of Prof. C. Bolm at RWTH Aachen, she carried out her habilitation at the WWU Münster. In 2013, she was appointed as professor for organic chemistry at the University of Regensburg and, in 2017, to her current position at the University of Münster. Her research aims at developing new, efficient synthetic methodologies in organic chemistry, with especial focus on the design of novel catalytic systems and their application in catalysis, including photocatalysis and asymmetric anion-binding catalysis, and late-stage functionalization towards valuable small and drug building-blocks.

Selected Awards: International Isotope Society IIS-CED Prize 2022, ORCHEM EJOC Lecture Award 2022, ERC Consolidator Grant (2017), ORCHEM Prize 2016, Young Researcher Award WWU Münster (2013), Thieme Chemistry Journal Award 2012, Extraordinary Doctorate-Award 2006 (U.A.M.), Lilly Investigation Award, Spanish II Edition (2004).

# DIRECT ACCESS TO UNNATURAL DRUG-LIKE MOLECULES BY PHOTOCATALYTIC LATE-STAGE FUNCTIONALIZATION

**Prof. Dr. Olga García Mancheño, Martin Stinglhamer, Tobias Brandhofer, Jan Kuhlmann**

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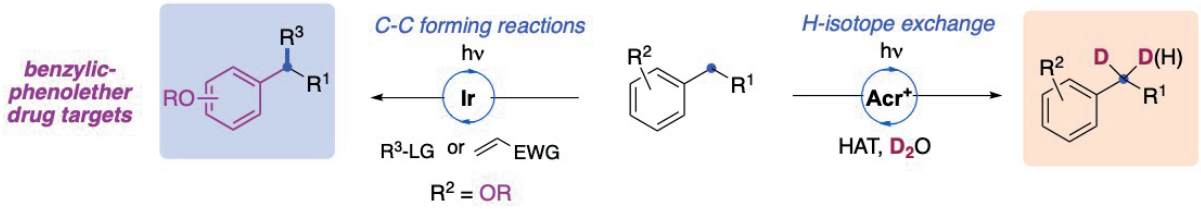
The direct modification of readily available molecules is a highly valuable emerging field in organic synthesis and drug discovery.[1] The rapid access to structurally diverse chemical series has a significant impact on the time and cost consuming optimization of clinical candidates. Nevertheless, chemo- and site-selective functionalization are still major challenges. In this regard, visible light photoredox catalysis has recently gained great attention due to its mild character,[2] leading to the development of various selective methodologies.[3] In particular, the direct and modular modification of electron rich compounds containing a phenolic functional group and related drug-like derivatives is highly appealing, since these moieties are ubiquitous in biomolecules and pharmaceuticals.

Based on our previous expertise in photoredox benzylic functionalization,[4] we herein present our recent visible light-mediated photocatalytic methodologies towards site-selective, late-stage benzylic functionalization[5] / hydrogen isotope labelling of complex molecules and drug candidates.[6] Competitive experiments and mechanistic investigations will also be discussed.

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## Prof. Dmytro VOLOCHNYUK

***ENAMINE LTD, Kyiv, Ukraine***

Prof. Dmitriy Volochnyuk at present shares his time as senior scientific advisor of ENAMINE LTD, Head of Medicinal Chemistry Department at the Institute of Organic Chemistry NAS of Ukraine and Professor of Chemistry at the Institute of High Technologies of Taras Shevchenko National University of Kyiv. He received his PhD in Organic Chemistry in 2005 and Dr.Sci in Organic and Organometallic Chemistry in 2011. Having been previously working at the key positions in CRO industry (2006–2010: Director of Chemistry at Enamine Ltd; 2010–2014: Executive Director at Curplyx–Macrochem; 2014 – 2017: New business development director at Life Chemicals), Prof. Volochnyuk has over 15-year experience in managing chemical out-sourcing projects and is an expert in fluoroorganic, organophosphorus, heterocyclic, combinatorial and medicinal chemistry as well as in cheminformatics. He is an author of more than 180 scientific publications and 3 monograph chapters.

# COCKTAILS FOR 19F NMR FRAGMENT SCREENING: FROM THEORY TO APPLICATIONS USING ENAMINE'S STOCK CHEMICAL SPACE

**Dmitriy Volochnyuk (1,2,3), Oleksandr Oksiuta (2,4), Yaroslav Filatov (4), Kostantyn Melnykov (1,3), Yurii Moroz (3,4), Sergey Ryabukhin (1,2,3)**

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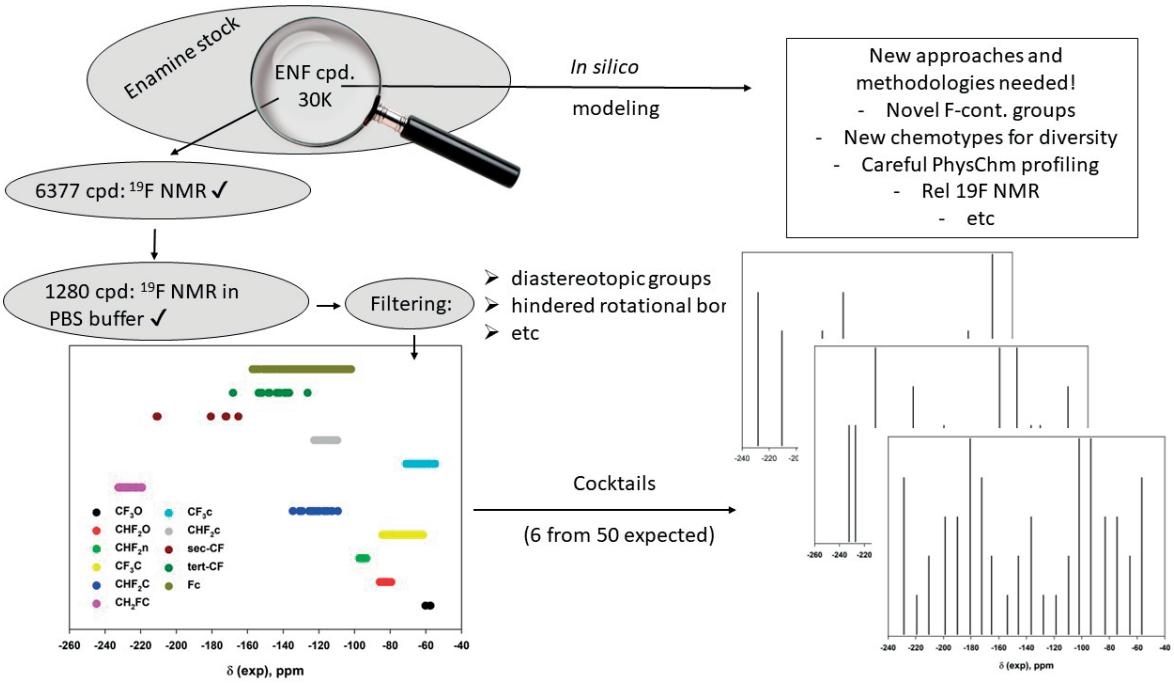
Despite the simplicity and attractiveness of the FAXS method (Fluorine Chemical Shift Anisotropy and Exchange for Screening) in competitive screening for the identification of bioactive fluorinated compounds concerning various targets, it has not become widespread due to the difficulty of forming mixtures (or "cocktails") of the corresponding compounds. Historically, fluorine-containing compounds from screening collections were synthesized for other purposes. Accordingly, their structural and spectral properties were not considered at the library design step, synthesis, and possible usage in cocktails for competitive screening. [1-3] This report will consider the problems of creating cocktails based on Enamine's stock chemical space. As a starting point, a subspace of Enamine fluorinated fragments with experimentally measured $^{19}F$ NMR shifts was chosen [4]. Considering the lack of measured fluorine chemical shifts, the first step was to create and validate a theoretical model for the prediction of such data. The analysis of the theoretical model showed the overpopulation of the region containing fluoroaromatic fragments and the underpopulation of the regions in "strong" and "weak" fields. During the transition to experimental validation of the theoretical model, several fundamental "features" which significantly complicated the task were found. As a result, only 6 cocktails (from 50 expected) of 20 compounds, out of 1.2k were selected. The principles of the design and the synthesis of the new fragments will be discussed in this report.

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Floriane Eshak graduated with a Bachelor's degree in Pharmacy from the German University in Cairo, Egypt in 2018. Passionate about research and development in the field of drug discovery, she graduated with a Master's degree in In Silico Drug Design from University of Paris, France in 2020. In 2021, she started her Ph.D. at the university Paris Cité, working on analyzing and developing nanobodies binding to metabotropic glutamate receptors using in silico tools. Floriane's Ph.D. is supervised by Dr. Bruno Gasnier (University Paris Cité), Dr. Anne Goupil (Biovia, Dassault Systèmes), and Dr. Francine Acher (University Paris Cité), and funded by the National research agency.

# EPITOPE IDENTIFICATION USING IN-SILICO APPROACHES, A CASE STUDY: NANOBODIES BINDING TO mGlu5 RECEPTOR

**Floriane Eshak (1), Francine Acher (1), Anne Goupil (2)**

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*2) Biovia Science Council, Dassault Système, Vélizy-Villacoublay, France*

Schizophrenia is a mental disorder that affects approximately 1% of the worldwide population. Scientists finally developed a hypothesis that may explain the pathophysiology behind this disorder, which is related to the deficiency of glutamate in the brain. Among glutamate targets, in this study we have focused on metabotropic glutamate receptor 5(mGlu5). However, targeting mGlu receptors selectively is challenging due to their highly conserved orthosteric binding site. To overcome this, an alternative approach was developed which is the use of nanobodies as allosteric modulators[1] . A nanobody is defined as the variable fragment of the heavy chain-only antibody. Its small size and stability under extreme physical conditions make it an interesting therapeutic agent.

This study is based on previous biological data indictating that nanobody Nb5A is a positive allosteric modulator of rat mGlu5 . The aim of the study is to identify the epitope of NB5A and rat mGlu5 followed by re-epitoping or re-designing of the nanobody. Molecular modeling techniques and artificial intelligence algorithms were used such as homology modeling[2], AlphaFold, IgFold, and ImmuneBuilder to predict the structures of NB5A and rat mGlu5. This was followed by blind rigid-rigid[3] and flexible docking to generate docking poses where two possible epitopes were selected. Among these, only one epitope was identified using molecular dynamics, which was validated through biological experiments.

Finally, the re-eptoping of this nanobody was performed aiming to target both rat mGlu5 and human mGlu5 for therapeutic purposes.

Our study provides insights into the capacity of *in-silico* approaches and novel artificial intelligence algorithms for epitope identification and re-designing nanobodies for therapeutic purposes.

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## Dr Birgit WILDING

***BOEHRINGER INGELHEIM, Vienna, Austria***

Birgit Wilding is a Scientific Director in Medicinal Chemistry at Boehringer Ingelheim in Vienna, Austria. Since joining Boehringer Ingelheim in 2018, Birgit has led and impacted several drug discovery programmes in oncology research, and successfully delivered three development candidates. Birgit obtained her PhD in organic synthesis and biocatalysis at Graz University of Technology, in collaboration with the Austrian Centre of Industrial Biocatalysis (acib), followed by a position as postdoctoral researcher at acib. She then moved to the CRUK Drug Discovery Unit at the Institute of Cancer Research in London, UK as Postdoctoral Fellow in Synthetic Medicinal Chemistry. As medicinal chemist, Birgit has worked across modalities, including small molecule reversible and covalent inhibitors, as well as degraders, in early as well as late-stage projects. At both, the Institute of Cancer Research and Boehringer Ingelheim, Birgit contributed to drug discovery programmes which resulted in compounds now investigated in clinical trials.

# FIRST TIME DISCLOSURE - DISCOVERY OF THE CLINICAL CANDIDATE BI 1810631 (ZONGERTINIB), A SELECTIVE HER2 INHIBITOR FOR THE TREATMENT OF HER2 EXON 20 INSERTION DRIVEN TUMORS

**Birgit Wilding, Dirk Scharn, Anke Baum, Lydia Woelflingseder, Valeria Santoro, Julian E. Fuchs, Matthias Treu, Stephan Zahn, Gerd Bader, Thomas Gerstberger, Paolo Chetta, Dietrich Böse, Peter Ettmayer, Martin Augsten, Darryl B. McConnell, Norbert Kraut, Flavio Solca, Ralph A. Neumüller**

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Oncogenic mutations in human epidermal growth factor receptor 2 (HER2) occur in 2-3% of patients with non-small cell lung cancer and are predominantly found in exon 20 of the ERBB2 gene. The HER2 exon 20 insertion mutations were found to be least sensitive to HER2 inhibitors from literature. Many known HER2 inhibitors are in addition limited by adverse events from inhibition of EGFR wild type. We therefore initiated a drug discovery program aiming at finding novel, HER2 selective inhibitors, sparing EGFR wild type.

Here, we report the medicinal chemistry optimization, identification, and pharmacological characterization of novel selective HER2 exon 20 mutation inhibitors. The presentation will focus on the lead optimization of a covalent inhibitor series. The optimization efforts resulted in the clinical candidate BI 1810631, a new, orally bioavailable inhibitor that shows excellent potency on HER2, including hard-to-hit mutations.

Upon treatment with BI 1810631, cancer cell survival and proliferation were reduced, which translated into tumor regressions in preclinical xenotransplantation models of HER2 exon 20 mutant driven cancers. Our results suggest that HER2 exon 20 insertions can be effectively treated by a potent and highly selective HER2 inhibitor that spares EGFR wild type. Our clinical candidate BI 1810631 is now in investigated in clinical trials.

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**RSC MEDICINAL CHEMISTRY LECTURESHIP**

## Prof. Christoph NITSCHE

***AUSTRALIAN NATIONAL UNIVERSITY, Canberra, Australia***

Christoph Nitsche is Associate Professor at the Australian National University (ANU). He completed his PhD at Heidelberg University in 2014 and received a Feodor Lynen Research Fellowship to work at the ANU from 2015 to 2018. After a short period as Rising Star Fellow at the Free University of Berlin, he was awarded an ARC Discovery Early Career Research Award (2019) to return to the ANU where he was appointed to a faculty position in 2020. He recently received an ARC Future Fellowship (2022), the John Wade Early Career Researcher Award (2022), the Peter Schwerdtfeger Award (2022), the Australian Research Award as top researcher in the field of Medicinal Chemistry (2023), the Thieme Chemistry Journals Award (2023), and the RSC Medicinal Chemistry Emerging Investigator Lectureship (2023). His research program focuses on drug discovery against infectious diseases, biocompatible chemistry, and peptide and protein modification.

# TIME TO SHINE FOR CONSTRAINED PEPTIDES IN MEDICINAL CHEMISTRY

**Christoph Nitsche**

*Research School of Chemistry, Australian National University, Canberra, ACT, Australia*

Constrained peptides fill an important area of chemical space between small molecule therapies and larger antibodies. Noncanonical modifications such as (bi)cyclisation can (i) enhance metabolic stability by greater resistance towards proteolysis, (ii) promote biological uptake across cell membranes, and (iii) decrease the entropic penalty of binding by locking the peptide in the active conformation.

We developed various unnatural amino acids functionalized with cyanopyridine and 1,2-aminothiol groups that can be directly incorporated into peptides using solid-phase peptide synthesis.[1,2] Cyclisation and stapling reactions proceed under biocompatible conditions in presence of protein drug targets to identify high-affinity peptide ligands. Importantly, these amino acids can also be charged onto tRNA enabling their use in selective protein modification and genetically encoded (bi)cyclic peptide libraries.[3,4]

Bicyclic peptides offer even greater conformational rigidity, metabolic stability, and antibody-like affinity and specificity. We explored the reaction between 1,2-aminothiols and 2,6-dicyanopyridine to establish a biocompatible, selective, and catalyst-free pathway to access bicyclic peptides, which displayed plasma stability, conformational preorganisation, and high target affinity.[5] We are even able to selectively construct tricyclic peptides using this biocompatible toolbox.

Recently, we introduced bismuth as a selective, stable, rigid, and green reagent for peptide modification. Bismuth represents the smallest "scaffold" ever explored and allows *in situ* access to bicyclic peptides for biochemical screening assays.[6] We also developed peptide-bismuth bicycles that are able to penetrate mammalian cell membranes as a new type of efficient cell-penetrating peptides.[7]

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## NOTES

# Posters Communications - Abstracts

# MULTIDRUG RESISTANCE-ASSOCIATED PROTEIN 1 (MRP1) INHIBITORS BASED ON 2-AMINOPYRIMIDINE CORE

**Marijo Čičak, Jaroslav Kozák, Viktor Kolman, Helena Mertlíková Kaiserová, Zlatko Janeba**

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To this day, drug resistance remains one of the biggest obstacles to achieving an efficient cancer treatment. Numerous studies have shown that overexpression of multidrug resistance-associated protein 1 (ABCC1/MRP1) has the main role in the development of drug resistance in many types of cancers. MRP1 is a member of the ATP-binding cassette (ABC) transport proteins family. The physiological function of MRP1 is to protect cells from endobiotic and xenobiotic organic anions and maintain an optimal ratio of reduced glutathione (GSH) to oxidized glutathione (GSSG) in cells under oxidative stress by active cellular efflux. Various anticancer drugs such as etoposide, vincristine, and doxorubicin, are well-known substrates for MRP1 decreasing their antitumor efficacy. Studies in ABCC1$^{-/-}$ knockout mice suggest that MRP1 may be safely inhibited. This makes MRP1 a relevant therapeutic target in certain types of cancers.[1,2]

We designed a library of compounds with 2-aminopyrimidine as the central core, and developed and optimized their syntheses to study the structure-activity relationships with respect to their ability to inhibit drug efflux. Biological evaluation was performed with use of U-87MG-doxorubicin-resistant cancer cells overexpressing MRP1 (ABCC1 mRNA expression >100-fold increased compared to wt cells). Calcein acetoxy methylester (calcein-AM) was used as a cell-permeable probe to release calcein, a well-established fluorescent MRP substrate. Its intracellular accumulation is proportional to an inhibition of MRP1-mediated efflux. The capability of tested compounds to block calcein efflux was expressed as a percentage of the effect of a reference MRP1 inhibitor, MK-571, which was arbitrarily set to 100% inhibition. Several compounds showed superior inhibition of calcein efflux compared to reference inhibitor MK-571.[3]


**Figure 1.** The general structure of novel MRP1 inhibitors

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# ASYMMETRIC SYNTHESIS AND THEORETICAL INVESTIGATION OF 4,5-DIHYDRO-1H-[1,2,4]-TRIAZOLE USING DFT CALCULATION AND X-ray CRYSTALLOGRAPHY

**Raid Abdel-Jalil, John Husband, Anwaar Al Maqbali, Nawal Al Rasbi**

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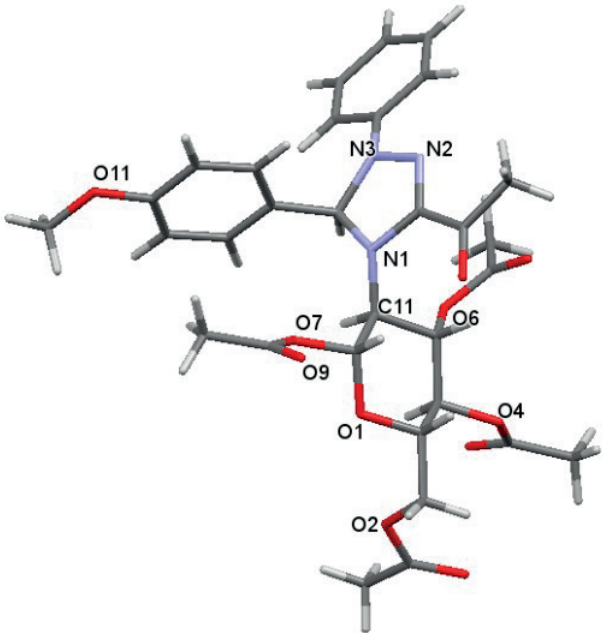
A novel series of stereo-selective 4,5-dihydro-1*H*-[1,2,4]-triazole were asymmetrically synthesized *via* 1,3-dipolar cycloaddition reaction. The structure of synthesized compounds and the stereo-chemistry were proved by x-ray crystallography where the ( R ) configuration was assigned for all the new compounds. Density Functional Theory (DFT) was used for conformational theoretical study, where it confirm the preference of $^4C_1$ conformation in the glucopyranose ring *via* aug-cc-pVDZ basis set and WP04 functional. The anti-tumour activity was examined for the products by National Institute of Cancer (NCI) in USA and shows no significant growth inhibition of the tumor cell-line. However, **R045**, **R102** and **R0416** shows the highest anti-cancer activity compared to other selected compounds.



# DEVELOPMENT OF A NEW CARBON-FOR-NITROGEN SWAPPING PARADIGM AS A RAPID AZA-ANALOGING TOOL

**Bram Ach, Prof. Dr. Johan M. Winne**

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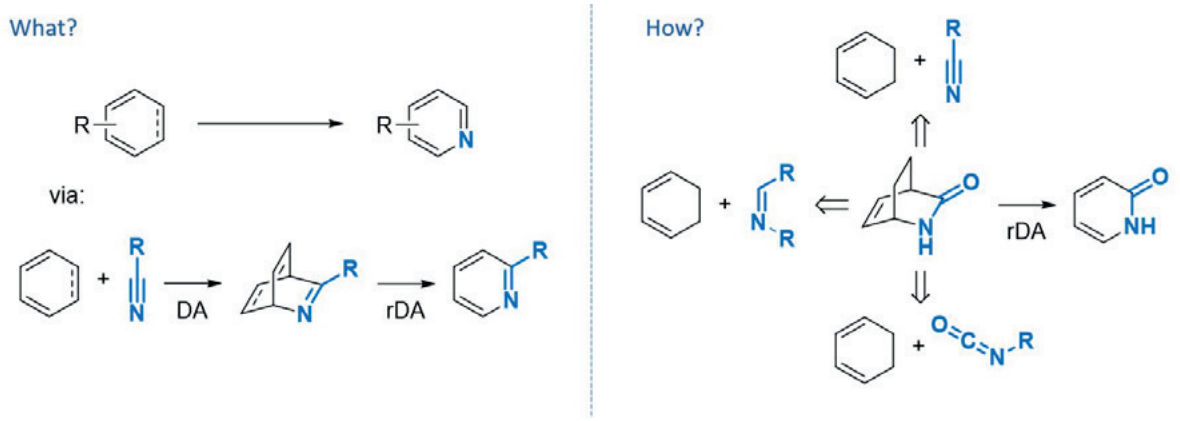
Nitrogen heterocycles are quite literally fundamental building blocks of life. Within the realm of the different types of molecules of primary metabolism, they can be considered the champions of molecular recognition, making them part of the backbone of the vast majority of all small molecule drugs.[1] Today this heterocyclic scaffold is usually formed in the first few steps the synthesis of a specific scaffold or pharmacophore. Taking this active scaffold and decorating its periphery with different substituents is a powerful and often also quite feasible strategy in small molecule design and SAR studies.[2]




This work aims to find a way of performing one and/or two step, single point substitutions not around the scaffold, but within the scaffold itself as a powerful complementary method for biology-driven compound synthesis. We are focusing on using a double substitution reaction which requires the controlled breaking of two carbon-carbon bonds, so that this atom can be excised and replaced. Especially the selective substitution of a carbon for a nitrogen within a broad range of carbocyclic scaffold via a aza-Diels-Alder (DA)/retro-Diels-Alder (rDA) pathway is showing promise. This two-step aza-analoging method is a nice counterpoint to the current methods to construct N-heterocycles via atom swapping strategies, which typically require numerous consecutive reaction steps.

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# DEAROMATIVE GOLD(I)-CATALYZED (3+2) CYCLOPENTANNULATION OF INDOLES

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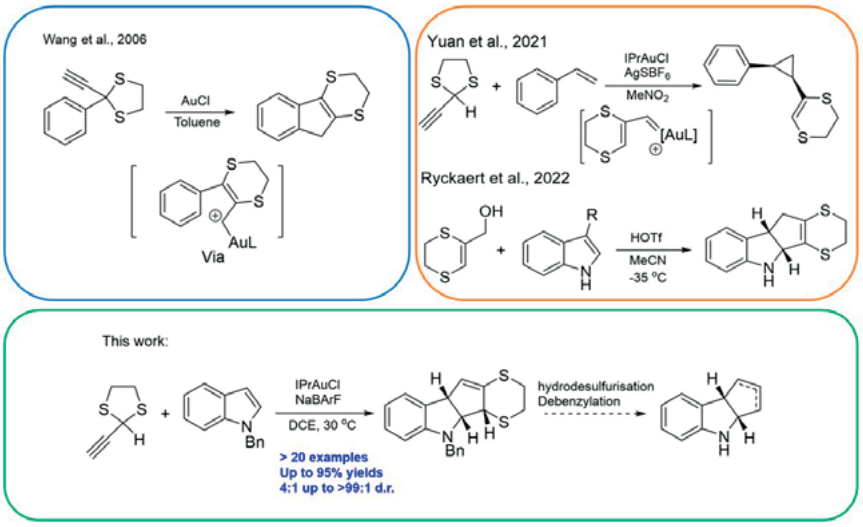
Despite their wide presence in natural products and pharmaceuticals, all-carbon five membered rings remain rather challenging to synthesize compared to other small size rings. Leveraging sulfur chemistry, our team has recently developed promising synthetic pathways to three and five-membered rings[1,2]. Drawing inspiration from our group's previous work and the research conducted by Wang and colleagues[3] we envisaged an access to cyclopentannulation of indoles using gold(I) catalysis. This research outlines the successful (3+2) dearomative cycloaddition of benzylated indoles and propargyl 1,3-dithiolane, resulting in a range of cycloadducts with yields varying from 40% to 95%. The method demonstrates broad applicability across various protected indoles, accommodating substitution at C2 and C3, as well as substitutions on the indole's benzene moiety. Electron withdrawing groups at C3 led to a complete loss of reactivity, while electron donating groups resulted in certain side products due to electrophilic aromatic substitution side reactions. Our research introduces a versatile synthetic tool that allows the introduction of a 'naked' all-carbon cyclopentene-fused indoles, following hydrodesulfurization and debenzylation.

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# A NEW SERIES OF HYDRAZONE-BASED ANTI-INFLAMMATORY AGENTS ENDOWED WITH SELECTIVE COX-1 INHIBITORY POTENCY

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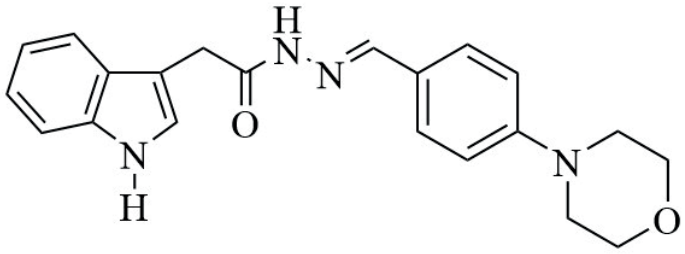
Cyclooxygenase-1 (COX-1) has received less attention than cyclooxygenase-2 (COX-2) as a biological target for the development of selective inhibitors because of the paradigm stating that the constitutively expressed COX-1 serves a homeostatic function in most tissues and it is responsible for the synthesis of prostaglandins, thus exerting cytoprotective action along with the regulation of platelet activity, gastric and renal functions under normal physiological conditions. Recent data highlighting the involvement of COX-1 in the pathogenesis of cancer, inflammation, cardiovascular diseases and pain have changed this paradigm and therefore the development of selective COX-1 inhibitors has come into prominence.[1,2]

In an effort to identify selective COX-1 inhibitors, herein, new hydrazone derivatives carrying an indole scaffold were synthesized. These compounds were subjected to *in vitro* studies, which were conducted to assess their inhibitory effects on COX-1 and COX-2 using COX inhibitor screening assay. Compound **2** (Fig. 1) ($IC_{50}$= 8.90 µM for COX-1; 71.00 µM for COX-2) was found as a selective COX-1 inhibitor in this series as compared to indomethacin ($IC_{50}$= 0.12 µM for COX-1; 0.58 µM for COX-2). The *in vitro* cytotoxic activity of compound **2** towards L929 mouse fibroblast cells was also evaluated. Based on this assay, compound **2** did not exert cytotoxicity towards L929 cells at its effective concentration. Lipopolysaccharide-induced sepsis model was used to assess its *in vivo* anti-inflammatory activity. Compound **2** decreased serum myeloperoxidase (MPO) and nitric oxide (NO) levels pointing out its anti-inflammatory action. Furthermore, compound **2** diminished serum aminotransferase (particularly aspartate aminotransferase (AST)) levels.



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# PRECLINICAL STUDIES AND DRUG COMBINATION OF LOW-COST MOLECULES FOR CHAGAS DISEASE AND LEISHMANIASIS

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Chagas disease and Leishmaniasis are neglected protozoan diseases recognized as public health problems by the World Health Organization. These diseases affect millions of people around the world however, efficient and low-cost treatments are not available. Hundreds of new compounds with trypanosomicidal action have been identified from different sources such as synthetic or natural molecules, but they have been deficient in several stages of drug development (toxicology, scaling-up, and pharmacokinetics). Previously, we described a series of compounds with simple structures, low cost, and environmentally friendly production with potent trypanosomicidal activity in vitro and in vivo [1-4]. These molecules are from three different families: thiazolidenehydrazines, diarylideneketones, and steroids. From this collection, we explored their capacity to inhibit the triosephosphate isomerase and cruzipain of T. cruzi. Then, the mechanism of action was explored using NMR metabolomics and computational molecular dynamics. Moreover, the mechanism of death was studied by flow cytometry. Consequently, five compounds, 314, 793, 1018, 1019, and 1260, were pre-clinically studied and their pharmacologic profiles indicated low unspecific toxicity. Interestingly, synergetic effects of diarylideneketones 793 plus 1018 and 793 plus 1019 were evidenced in vitro and in vivo. In vivo, the combination of compounds 793 plus 1018 induced a reduction of more than 90% of the peak of parasitemia in the acute murine model of Chagas disease [5]. Also using a drug repurposing approach in Leishmania, we found a low-cost drug for the treatment of visceral Leishmaniasis.

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# DESIGN, SYNTHESIS, IN-VITRO CARBONIC ANHYDRASE-II / IX, CHOLINESTERASE AND MONOAMINE OXIDASE INHIBITION STUDIES OF C-3 PREGNENOLONE- SULFAMOYLPHENYL THIOUREA DERIVATIVES

**Bandar A. Alyami (1), Abdul Sadiq (2), Muhammad Saeed Jan (3), Umer Rashid (4), Mater H. Mahnashi (1), Ali O. Alqarni (1), Yahya S. Alqahtani (1)**

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In continuation of our research on pregnenolone, we have synthesized a series of sulfonamide derivatives by modification of C-3 (-OH) and C-17 (-$COCH_3$). Cholinergic system direct role in pathophysiology and as drug target has not yet been implicated, although in animal's models cholinergic activation leads to development of seizures in mice. In current study, we evaluated the synthesized pregnenolone derivatives against cholinesterases and monoamine oxidases for Alzheimer's disease treatment. However, the presence of sulfonamide nucleus is considered as key structural feature for inhibition of a number of CA isozymes. Hence, all the synthesized derivatives were also evaluated or their inhibition potential against human carbonic anhydrase-II / IX isoforms. Compound **34** emerged as potent compound against AChE and MAO-B with IC50 value in low submicromolar concentration. While compound **31** with di-sulfonamide with $IC_{50}$ value of 0.67 ± 0.09 μM emerged as the most potent inhibitor of *h*CA-II. Ligand-CA-II binding analysis showed that all the compounds confined in the active site coordinating with zinc center and forming hydrogen bond interactions to key amino acid residues.

# THE TREATMENT OF VIRUS INFECTION-INDUCED INFLAMMATIONS WITH NOVEL PIEZO1-AGONISTS

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From the beginning of the coronavirus pandemic, it was reported that *severe acute respiratory syndrome coronavirus 2* (SARS-CoV-2, COVID-19) patients present not only various severe acute respiratory symptoms but also neurological complications. These neurological complications have been estimated to occur with 35-57% of COVID-19 patients. SARS-CoV-2 induces an abnormal anti-inflammatory response and infections that lead to many metabolic disorders and coagulation activity. These events in turn increase the risk of severe encephalopathies and encephalitis. and cerebrovascular accidents. However, the mechanisms behind neurological manifestation in virus-infected patients are poorly understood. We have identified a novel target, mechanosensitive ion-channel Piezo1, from the surface of microglia. The research data shows that activation of Peizo1 with highly selective agonist (Yoda-1) increases microglia phagocytosis, reduces microglia pro-inflammatory activation, and provides neuroprotection against ischemic damage. In addition, the data shows Piezo1 to protect against ventilator-induced lung injury. Therefore, Piezo1 ion-channel is a potential multitarget druggable protein, by which COVID-19 induced lung injuries accompanied by neurological complications can be treated.

The challenge is that Yoda-1, like other current Piezo1-agonists, is poorly water soluble and metabolizes rapidly in the target tissues. Thus, the aim of this study was to develop novel more soluble and stable compounds that can interact with Piezo1 to treat acute respiratory syndromes and infection-related neurological complications.

With the help of computational models, compounds that can interact with Piezo1 were screened and structural features that are required to activate Piezo1 were recognized. According to the initial *in silico* screening, over 60 novel Piezo1-agonists were synthesized and their physicochemical properties (e.g., solubility and chemical/enzymatic stability) were studied along with *in vitro* $Ca^{2+}$-imaging assay with Piezo1-transfected cell line. Based on these preliminary data, a novel lead molecule being more potent than Yoda-1 in Piezo1 activation was identified.

# DISCOVERY OF SMALL-MOLECULE INHIBITORS OF MICRORNA-21 BIOGENESIS

**Michele Anselmi (1), Francesco Bellina (1), Riccardo Quaia (1), Marc Panosetti (3), Marina Veronesi (2), Debora Russo (1), Stefania Girotto (2), Fabio Bertozzi (1), Maria Duca (3), Tiziano Bandiera (1)**

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*3) Université Côte d'Azur, CNRS, Institute of Chemistry of Nice (ICN), 06100 Nice, France.*

MicroRNAs (miRNAs) are small, non-coding RNA molecules implicated in the regulation of gene expression. The miRNAs' biogenesis starts with the transcription of a long primary miRNA (pri-miRNA), which is subsequently cleaved by the nuclear ribonuclease *Drosha* into a shorter stem-loop-structured precursor (pre-miRNA). The latter is then exported to the cytoplasm where it is further processed by another ribonuclease, called *Dicer,* to yield the mature miRNA duplex. The mature miRNA is loaded onto the *RNA induced silencing complex* (RISC), which separates the two strands and guide the interaction of a single strand with a complementary sequences on mRNAs thus inducing their degradation.[1]

Given their engagement in numerous biological processes, it is not surprising that the dysregulation of miRNAs biogenesis has been linked to several human diseases, including cancer. Notably, the oncogenic miRNA-21 (miR-21) is one of the most extensively studied, since it has been found to be upregulated in nearly all cancers, acting as inhibitor of tumor suppressor proteins.[2] Therefore, its downregulation represents a potential therapeutic approach for the treatment of many tumors.

Currently, promising approaches for downregulating miR-21 production include antisense oligonucleotides (ASOs), miR sponges, CRISP/Cas9 genome editing, and small-molecules.[3,4] The latter approach offer an important alternative to the others, which suffer from well-known administration and pharmacokinetic limitations. However, targeting RNA with small molecules remains a challenge in terms of potency and selectivity due to their highly flexible structures and lack of hydrophobic pockets.

Here, we report the discovery of new, drug-like small molecule modulators of the miR-21 biogenesis. In the search for new chemotypes capable of selectively interacting with the secondary structure of RNA targets, we applied two well-known approaches: Fragment-Based Drug Discovery (FBDD)[5] and High-Throughput-Screening (HTS).[6] By applying a $^{19}$F-NMR-based FBDD we screened a small library of about 600 fluorinated fragments that allowed us to easily discriminate binders from nonbinders of pre-miR-21.[7] The selected fragments can be further optimized to enhance affinity and selectivity through merging, linking, or fragment growing. Moreover, through a HTS of about 4000 compounds from our library we were able to identify new chemotypes with good affinities to pre-miR-21 in preliminary *in vitro* assays. This workflow has led us to discover new small molecules that represent a good starting point for the development of novel miR-21 modulators, potentially useful in cancer therapy.

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# OaAEP1 LIGASE-ASSISTED CHEMOENZYMATIC TOTAL SYNTHESIS OF CYANOBACTERIAL METALLOTHIONEIN SmtA

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The field of protein semisynthesis is rapidly advancing in the realm of biochemistry, and the development of new and efficient strategies for the production of functional biomolecules is of great cognitive and practical importance. Nowadays, the enzymatic method recently has gained great popularity among other synthetic approaches due to its high selectivity, excellent compatibility, and ability to conduct reactions under mild conditions. Recent advances in enzymatic engineering have led to the discovery of an improved variant of ligase from the Asx family, asparaginyl endopeptidase (OaAEP1 C247A)[1]. Its 160 times higher activity compared to the wild-type, as well as its short C-terminal recognition motif -NGL, forms an excellent alternative to other enzymes, especially for cysteine-rich sequences.

The molecular target of this work is low molecular weight metallothionein (MT) from the cyanobacteria Synechococcus elongatus (SmtA). Due to the presence of numerous cysteine residues, it binds with Zn(II), Cd(II), and Cu(I) ions and is responsible for their storage, transport, and homeostasis in the cell[2]. As a consequence, it makes SmtA an interesting object of research. Therefore, we applied recently discovered by genetic engineering a new mutant of asparaginyl endopeptidase for the semisynthesis of metallothionein from the cyanobacteria Synechococcus elongatus (SmtA). Two peptide substrates were synthesized according to the SPPS strategy and then enzymatically ligated. Independently, the mentioned metallothionein has also been overproduced in E. coli as a reference. MS and HPLC analyses confirmed the compliance of the final products obtained by different strategies, and titration experiments presented their identical Zn(II) and Cd(II) binding properties. As a result, this experimental work presents a fast, selective, and efficient synthetic strategy for metallothionein production by recombinant ligase. It may also provide an excellent path for producing other proteins for biomedical applications, which obtain via other pathways is not effective or still remains impossible.

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# BIOISOSTERIC REPLACEMENTS IN DEVELOPMENT OF SECOND-GENERATION 2,6-SUBSTITUTED 2H-PYRAZOLO[4,3-c]PYRIDINES

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Fused aza-polycycles have attracted the interest of organic and medicinal chemists because of their abundance in natural products and due to their significant biological and pharmacological activities. Among other fused systems pyrazolopyridines were investigated for their antidepressant, anti-inflammatory, antihyperglycemic, antitumor, antibacterial, anxiolytic activities [1]. Also, these derivatives are used for the treatment of Alzheimer disease, drugs addiction, and infertility [2].

We have previously investigated the synthesis and potential application of variously substituted 2*H* -pyrazolo[4,3-*c*]pyridines as anti-mitotic agents against K562 and MCF-7 cell lines [3]. From the compounds prepared, 2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine displayed the lowest micromolar $GI_{50}$ values in both cell lines.

In this work we present synthesis and further investigation of 2*H*-pyrazolo[4,3-*c*]pyridines as anticancer agents. Following the bioisosteric replacement strategy pyridin-2-yl, 3-yl or 4-yl motifs were introduced to the 6$^{th}$ position of 2*H*-pyrazolo[4,3-*c*]pyridine system. Also, the effect on biological activity was investigated with variously substituted benzene rings at the 2$^{nd}$ position of the fused compound. The strongest antiproliferative activities in the panel of selected cancer cell lines displaying compound was chosen for further evaluation of its biological effects *in vitro*. Flow cytometry analysis of K562 cells showed a massive enrichment of G2/M cell population and increased number of subG1 cells undergoing the cell death. Further evaluation at the protein level by immunoblotting and analysis of BrdU-pulse labelled K562 confirmed that the most active compound induces massive M phase arrest followed by endoreduplication caused by disruption of proper cytokinesis.

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# A SYSTEMATIC APPROACH TOWARDS POTENT CK1δ INHIBITORS

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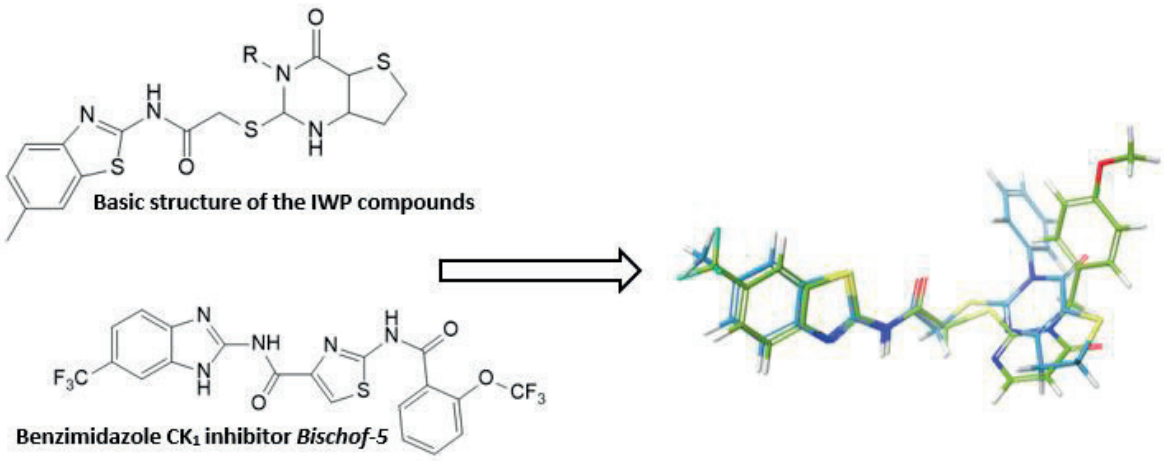
The $CK_1$ family consists of highly conserved serine/threonine kinase isoforms, which are involved in the regulation of numerous cell functions, such as DNA damage response, cell cycle progression, mitosis, apoptosis or control of the circadian rhythm.[1] The $CK_{1\delta}$ isoform e.g. is part of several cellular developmental pathways, in particular Hippo and Wnt/ß-Catenin mediated signaling, rendering $CK_{1\delta}$ a potential drug target.[2]

Since known benzimidazole-based $CK_1$ inhibitors, such as Bischof-5, show high structural similarity with reported inhibitors of Wnt production (IWP's) that target the membrane bound O-acyltransferase porcupine (Porcn), we hypothesized that IWP's could also inhibit $CK_1$ isoforms. Molecular modeling demonstrated plausible binding modes for IWP's within the ATP binding pocket of $CK_{1\delta}$. Furthermore, an initial *in vitro* activity screening on $CK_1$ family members confirmed the inhibition, especially of the $CK_{1\delta}$ isoform.

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# DESIGN AND SYNTHESIS OF NOVEL RNA LIGANDS AS INHIBITORS OF ONCOGENIC MICRORNAS PRODUCTION

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Sequencing of the human genome has highlighted that the main part of the genome, approximately 70%, is occupied by non-coding RNAs [1]. During the last decade, stunning progress in the elucidation and understanding of the authentic functions played by these molecules has been established with a particular focus on microRNAs. With an average size of 23 nucleotides, these small RNA molecules play a crucial role in the negative regulation of translation of several genes through a complex and specific mechanism.

Unsurprisingly, altered regulation of microRNAs is strongly linked to the emergence and development of a wide range of lethal pathologies such as cancers. A relationship between tumor proliferation and microRNAs was quickly identified. Effectively, each type of cancer has a specific microRNAs fingerprint marked by the over-expression or deletion of certain miRNAs ensuring the development of significant resistance to treatment via oncogene promotion and stem cell maintenance (CSC) [2]. Being a true tumor biomarker, miRNAs are increasingly attracting attention as a key target in therapeutic interventions and drug development [3].

Our work focused on the design and the synthesis of small molecules that interfere with the biogenesis of oncogenic microRNAs playing a key role in cancer development, especially in cancer stem cells proliferation. Thanks to previously developed structure-activity relationships within the team, we were able to identify a hit based on a bithiazole heterocyclic backbone with two side chains that induces very promising biological results. Starting from this pharmacophore, we synthesized various modifications on both extremities to optimize the affinity and the selectivity for the target. Biological evaluations of the new derivatives are in progress, with intracellular testing planned as the next step. The results of these evaluations will allow us to better define the mechanism of action of these derivatives and to confirm their cell-based activity

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# TARGETING PROTEIN-PROTEIN INTERACTIONS INVOLVED IN OXIDATIVE STRESS USING FRAGMENT-BASED DRUG DISCOVERY

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The production and elimination of reactive oxygen species (ROS) are tightly regulated to prevent damaging oxidative stress and maintain redox homeostasis. However, in many diseases, ROS play a central role for example by inducing inflammation or cellular degeneration. To generate new chemical probes and drug leads we target protein-protein interactions involved in oxidative stress by using fragment-based drug discovery (FBDD). We focus on the superoxide-generating multi-subunit enzyme complex NADPH oxidase 2 (NOX2) and the adaptor protein Keap1, which regulates the endogenous antioxidant response.

For NOX2, we have made a unique series of bivalent inhibitors that bind to the p47phox subunit, thereby blocking the interaction with the p22phox subunit (Ki < 0.5 μM) and assumingly prevent assembling and activation of the NOX2 complex [1–2]. Our work illustrates an alternative pharmacological approach of targeting NOX2, which have promise, but also comes with certain challenges.

Keap1 is an adaptor protein of Cullin3-based ubiquitin E3 ligase and serves as an oxidative stress sensor. Initially, we did a comparative assessment study of all known Keap1 inhibitors and found that only about half of the published compounds were genuine Keap1 binders [3]. We then deconstructed the known compounds into a target-biased library of 77 fragments and tested them for Keap1 binding using four orthogonal biophysical assays. The binding modes of key fragment hits where determined by X-ray crystallography, which allowed us to merge two fragments into novel compounds with high affinities to Keap1 [4]. More recently, we screened and validated 2,500 commercially available fragments, which led to 28 high-priority hits and 13 fragment-Keap1 costructures. Subsequent fragment-to-lead (F2L) efforts led to a 1700-fold improvement in affinity producing potent lead compounds that exploit new parts of the Keap1 binding pocket [5]. Finally, a crystallographic screen of 768 XChem fragments revealed 80 novel fragment hits binding in the Keap1 Kelch pocket. One hit is now converted to a drug-like series of high-affinity (Ki = 5–30 nM) and cell active Keap1-Nrf2 inhibitors that are currently being further developed.

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# DARPINS AS BIOLOGICAL TOOLS TO REACTIVATE WILD-TYPE AND MUTANT P53 IN CANCER CELLS

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The transcription factor p53, also known as the guardian of the genome, is inactivated in virtually every tumor, either by mutation in the *TP53* gene or by dysregulation of its regulatory pathways. Most of the p53 cancer mutations are missense mutations in the DNA-binding domain (DBD), and many of them reduce the conformational stability of the protein, resulting in rapid unfolding, followed by aggregation (1). Therapeutic efforts to restore p53 conformational stability and transcriptional activity involve diverse strategies that aim to either protect p53 from its negative regulators or restore the functionality of mutant p53 proteins (1,2). Here we propose Designed Ankyrin Repeat Proteins (DARPins) (3) as a potential novel therapeutic strategy to reactivate both wild-type and mutant p53 DBD.

Through ribosome display, we identified DARPin C10 that selectively binds to the p53 DBD with an affinity of 180 nM. Our structural and transactivation assay data demonstrate that C10 stabilizes and restores the transcriptional activity of wild-type p53 in papilloma virus infected HeLa cells by blocking the HPV-E6 mediated degradation of p53. Analysis of the p53-C10 interface suggested that DARPin C10 may also be used as molecular chaperone to stabilize and reactivate conformationally unstable mutants. In a proof-of-concept study, we therefore performed systematic dose-response thermal stability and transactivation assays with a series of cancer hotspot mutants, complemented by high-resolution co-crystal structures of the mutant p53 DBD-C10 complexes, showing that C10 can stabilize and potentially reactivate conformationally unstable p53 mutants in cancer cells. Future studies will now focus on (i) improving the binding affinity of DARPin C10 for the p53 DBD, (ii) screening the p53 cancer mutome to identify and classify targetable mutants, and (iii) the development of cellular delivery systems for therapeutic exploitation of our generic p53-stabilizing DARPins.

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# QUINUCLIDINIUM BASED O-ALKYL OXIMES AS POTENTIAL ANTICHOLINESTERASE AGENTS IN SYMPTOMATIC TREATMENT OF ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is a neurodegenerative disease and a leading cause of dementia globally. It is manifested through progressive loss of memory and cognitive functions which is later accompanied by difficulties in speech and deterioration of visuospatial abilities. AD develops and progresses with age, and with prolonged human lifespan in developed countries and general aging of world population, it is estimated that the number of AD-related dementia cases worldwide will triple by 2050. Half of currently approved FDA drugs for treatment of AD are cholinesterase inhibitors that act to elevate concentrations of neurotransmitter acetylcholine which is depleted due to loss of cholinergic neurons. The rest of approved drugs are aimed against other hallmarks of the disease: excitatory neurotoxicity effect of glutamate and deposition of amyloid plaques in brain. Because of the multifactorial nature of the disease, development of multi-target directed ligands is a welcome approach to tackle multiple hallmarks of disease at once.

In our study, we have determined inhibition potency of novel *O*-alkyl oximes against acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) and evaluated their ability to elevate production of toxic AChE-amyloid plagues as well as ability to pass the blood-brain barrier. Oximes are well known as AChE reactivators, but also may poses other useful biological activities like antibacterial, anticancer, anti-inflammatory activity, and inhibition of AChE as well. Our series comprises of *O*-alkyl oximes with quinuclidine base substituted by benzyl group containing electronegative atom or group on either *meta*- or *para*- position. Majority of tested compounds show inhibitory activity against both cholinesterases, with $K_i$ values in micromolar range. Generally, compounds preferentially inhibit BChE over AChE with $K_i$ values ranging from 20 to 60 micromolar concentrations. All compounds inhibit cholinesterases in either competitive or mixed-type manner, with latter being a significant trait in the case of AChE for inhibiting peripheral active site of AChE could prevent AChE-induced amyloid aggregation. According to our results, *O*- alkyl oximes show potential to be further investigated as a structural motif that could act against some of major AD hallmarks.

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# SYNTHESIS AND PHOTOCHEMISTRY OF BODIPY COMPOUNDS WITH ANTIPROLIFERATIVE ACTIVITY AND POTENTIAL THERANOSTIC APPLICATIONS

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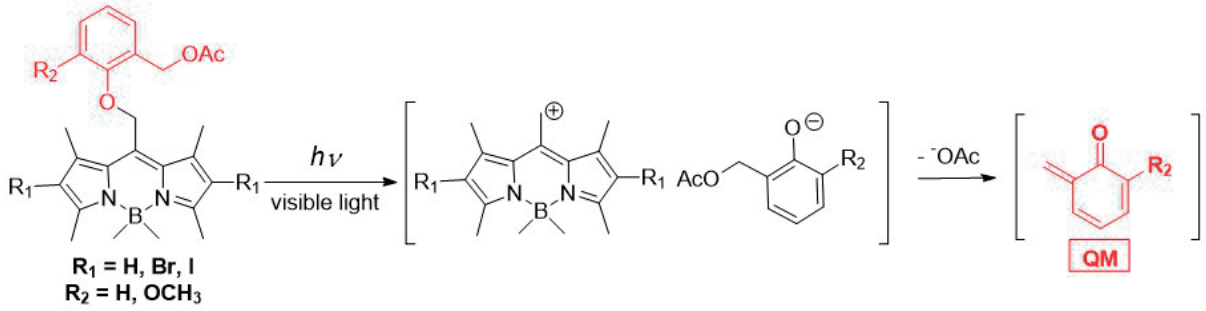
BODIPY is a tradename for the class of heterocyclic fluorescent dyes, 4,4-difluoro-4-bora-3a,4a-diaza-s-indacene derivatives, that are characterized by exceptional spectroscopic and photophysical properties [1]. Therefore, they were often used as fluorescent dyes in biology or different sensing applications [2], as well as in photodynamic therapy [3]. Moreover, BODIPY derivatives were used as photo-cleavable protective groups, which are also known as photocages [4].
We have recently demonstrated the generation of quinone methides (QMs) from BODIPY compounds in the anti-Kasha photochemical reaction (UV irradiation), which can be used for the photo-labeling of proteins [5]. Such a reaction may also be applicable in anticancer photo-therapy, as QMs are known to exhibit antiproliferative activity [6]. However, for the therapeutic applications, excitation by visible light is required (650-800 nm). Consequently, we designed new line of BODIPY QM precursors that are anticipated to undergo described photo-cleavage chemistry of BODIPY photocages (Scheme 1). Herein we present the synthesis of BODIPY compounds and investigation of their photophysical properties and photochemical reactivity. We found out that the anticipated photo-cleavage proceeds with some competing photochemical processes on the boron atom. Nevertheless, the investigated compounds showed significant enhancement of antiproliferative activity upon treatment of human cancer cells with the compounds and irradiation with visible light. The reasons for the enhancement of the antiproliferative activity will be discussed.




**Scheme 1.** Anticipated formation of QMs in the photoreaction of BODIPY compounds.

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# NEW ISOQUINOLINEQUINONE ANALOGS AS POTENTIAL ANTICANCER AGENTS

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Clinical trials and subsequent phase studies using mitoxantrone, an anthraquinone derivative, in 1979 demonstrated that mitoxantrone has significant clinical activity in patients with breast cancer, acute leukemia, and lymphoma [1]. Due to the high cardiotoxicity problem of mitoxantrone, BBR2778 (Pixantrone) was developed, which exhibits less cardiotoxicity [2].This effect is due to the replacement of the 5,8-dihydroxy phenyl moiety in Mitoxantrone with a pyridine ring [3]. For this reason, in recent years, designing and synthesizing new organic compounds on the basis of Pixantrone type compounds and examining their biological effects has become a very interesting subject where organic chemists and medicinal chemists intersect.

Therefore, the rationale for the synthesis reported in this study was to obtain a series of novel potential cytotoxic amine-substituted isoquinolinequinone derivatives based on Pixantrone-type compounds, and then seek the answer to the question of whether the addition of the differentially substituted aromatic amine ring to the quinone core would lead to enhanced cytotoxic activities.

In this direction; the isoquinolinequinone compound produced *in situ* from the treatment of isoquinolin-5-ol with iodobenzene bis(trifluoroacetate) (PIFA) was then reacted with aromatic amines containing different substituents and the anticancer properties of the obtained new analogues were investigated.

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# PALLADIUM-CATALYZED HIGH PRESSURE-ASSISTED STRATEGY FOR SYNTHESIZING DIAZA-DIBENZO[A,E]AZULENE AND DIAZA-BENZO[A]FLUORENE DERIVATIVES VIA CROSS-DEHYDROGENATIVE COUPLING AND THEIR CYTOTOXIC ACTIVITY

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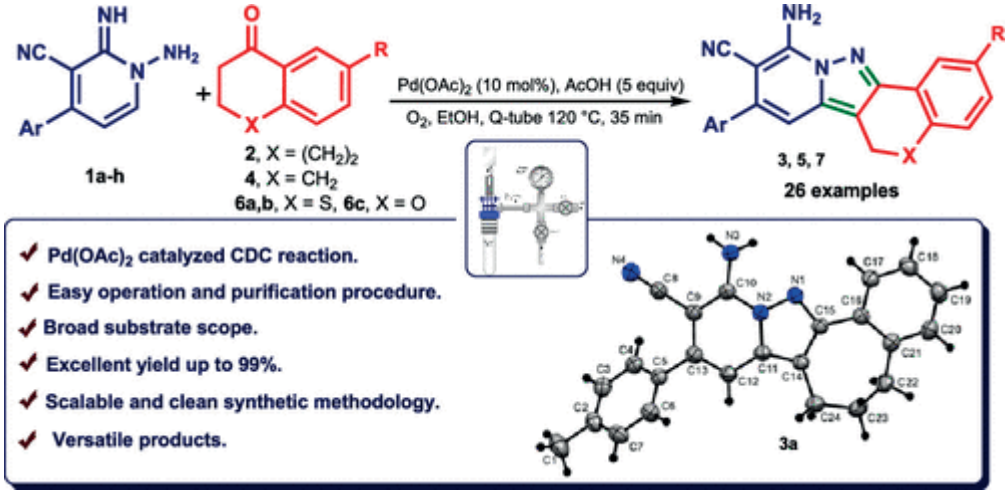
An appropriate and efficient Q-tube-assisted palladium- catalysed strategy for the synthesis of novel, unparalleled diaza-dibenzo[a,e]azulene and diaza-benzo[a]fluorene derivatives has been sophisticated, which includes oxygen and AcOH-induced oxidative C($sp^3$)–C($sp^2$) cross-dehydrogenative coupling reactions of 1-amino-2-imino-4-arylpyridine-3-carbonitriles with benzocyclic ketones such as benzosuberone, tetralone, thiochromone, and chromone, respectively. This Q-tube gas purging kit assisted protocol features safe due to easy pressing and sealing, a wide substrate scope, easy workup and purifying phases, and the use of $O_2$ as a benign oxidant, in addition to being scalable and having a high atom economy. The suggested mechanistic pathway includes a formal dehydrative step followed by palladium AcOH-induced CH($sp^3$)–CH($sp^2$) oxidative cross-coupling. In this study, X-ray crystallographic analysis has been used to authenticate the targeted products. The cytotoxicity of the newly synthesized compounds was preliminary examined toward three cell lines of human cancer; lung cancer (A549), breast cancer (MCF-7) and colon cancer (HCT-116) and shown that members from the synthesized compounds exhibited promising cytotoxic activity.

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# LIGHTING UP THE BAD BUGS: FLUORESCENT LABELLING OF THE BACTERIAL RESISTANCE FACTOR ARNT WITH COMMERCIAL FLUOROPHORES

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Antimicrobial resistance (AMR) is a major threat to global health predicted to lead to 10 million deaths per year by 2050 if no action is taken [1]. Operationally simple technologies for the diagnostic labelling of bacterial resistance factors are therefore of great interest to study molecular, cellular, and environmental factors driving AMR development.

One such resistance factor is the enzyme 4-amino-4-deoxy-l-arabinose transferase (ArnT). ArnT is involved in membrane lipopolysaccharide remodelling of Gram-negative pathogens such as *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Burkholderia cenocepacia*, resulting in resistance to polymyxins, last-resort antibiotics employed to treat multidrug antimicrobial resistant infections [2].

In this project, we have investigated the use of commercially available fluorophores as chemical tools for the diagnostic labelling of ArnT.

Analysis of sequence and structural data for ArnT from *K. pneumoniae*, *P. aeruginosa*, *B. cenocepacia* and *Cupriavidus metallidurans* identified several lysine residues as potential target sites for the covalent attachment of fluorescent probes. To exploit this opportunity, we assembled a small library of commercially available fluorophores with different lysine-reactive electrophiles and established a workflow for protein labelling. Relevant experimental parameters such as incubation time and fluorophore concentration were optimised using bovine serum albumin as a model protein. Application of our protocol to the *B. cenocepacia* ArnT [3] allowed the desired in-gel fluorescent detection of ArnT after electrophoretic separation. Protein mass spectrometry confirmed the covalent attachment of selected fluorophores and identified their attachment sites. Finally, we rationalised the observed reactivities using a recently developed algorithm for the site and sequence prediction of protein modifications with lysine-reactive reagents [4].

Our results demonstrate that ArnT can be readily labelled with commercially available fluorophores, providing a basis for the rational development of bespoke labelling reagents for this enzyme as novel diagnostic tools in the fight against antimicrobial resistance.

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# 2-(BENZYLOXY)ARYLUREA INHIBITORS OF MPTP DO (NOT) TARGET HUMAN CYCLOPHILIN D

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Cyclophilin D (CypD) is a mitochondrial enzyme that regulates opening of the mitochondrial permeability transition pore (mPTP). This pathophysiological phenomenon is manifested in several diseases associated with mitochondrial dysfunction including ischemia-reperfusion injury or neurodegeneration.[1] Suppression of mPTP opening through CypD inhibition represents a promising approach for treatment of above-mentioned diseases. However, only limited number of CypD inhibitors are currently available - mostly macrocyclic compounds derived from cyclosporin A, which suffer from undesirable physico-chemical properties and low selectivity for CypD over other cyclophilins.[1] Dynamic development of much more preferable selective small molecule inhibitors is expected in this regard.

In this study we have synthesized 10 compounds (Fig. 1) similar or identical to previously published mPTP inhibitors [2,3,4,5] that were supposed to act through CypD inhibition but their mechanism of action has never been confirmed. Unlike the original reports, we have tested the inhibitors directly on purified CypD enzyme using a novel RNAse T1 refolding assay.[6] Additionally, we have tested the selectivity of inhibition towards CypD using CypA as an off-target.

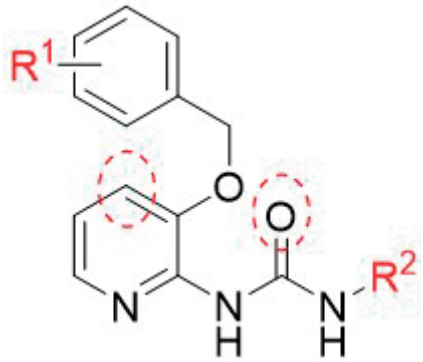


Figure 1: General structure of prepared mPTP inhibitors.

*This work was supported by the Ministry of Health of the Czech Republic (no. NU22J-02-00006).*

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# SITE SELECTIVE GOLD(I)-CATALYSED BENZYLIC C-H AMINATION VIA AN INTERMOLECULAR HYDRIDE TRANSFER TO TRIAZOLINEDIONES

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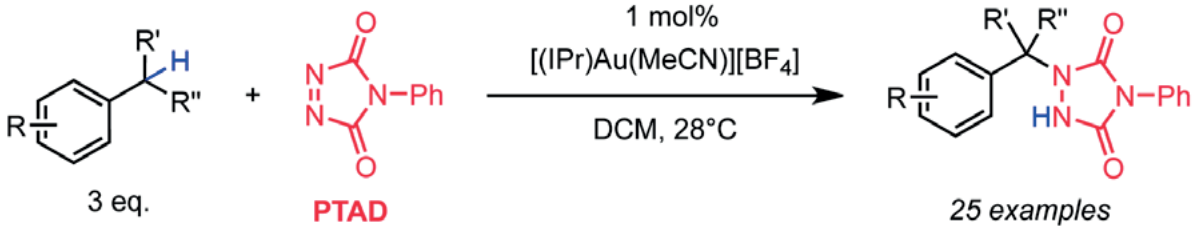
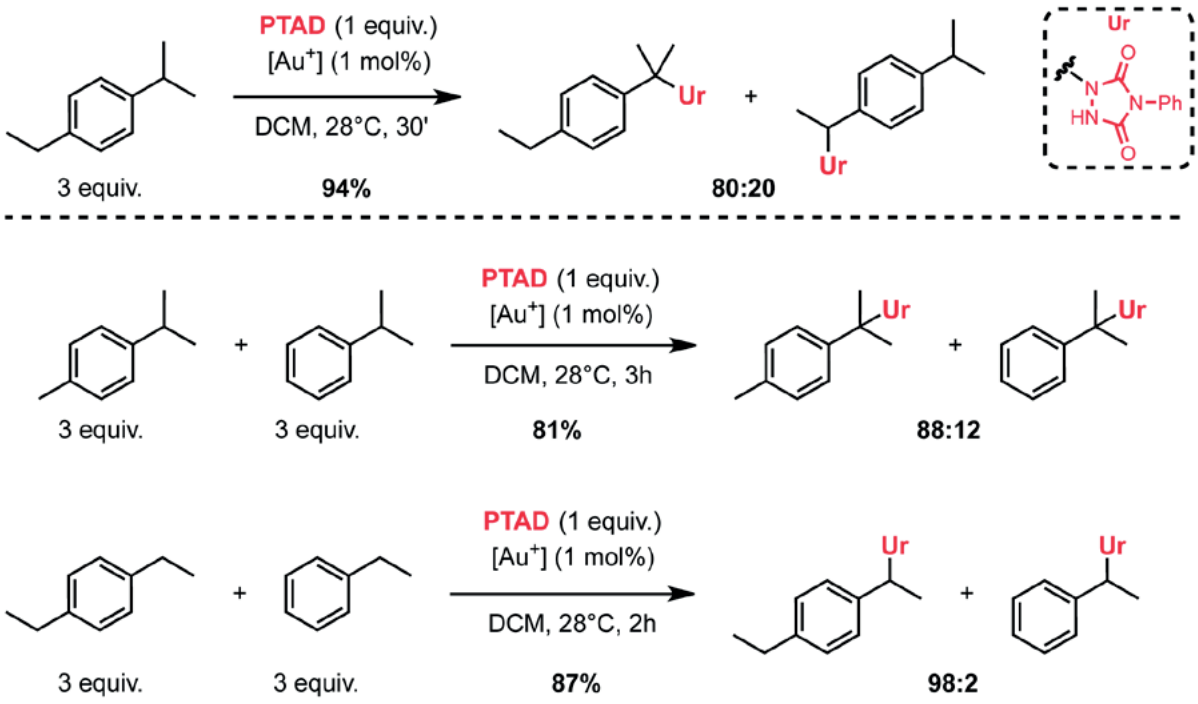
Direct amination of hydrocarbons is an outstanding challenge in catalysis. Whereas electrophilic oxygen-based species that can activate C-H and C=C bonds are common reagents (e.g. hydroxonium ion, singlet oxygen, peroxy acids…), related electrophilic nitrogen-based species are less common and the known examples are typically much less reactive electrophiles.[1] Here, we report a benzylic CH-amination using 4-phenyl-1,2,4-triazoline-3,5-dione (**PTAD**) under **mild reaction conditions** using a well-defined cationic gold complex as catalyst.




During the investigation of substrates, unexpected site-selectivity patterns were observed. In particular, **discrimination between very similar benzylic positions is possible** with this novel method, stemming from relatively **remote differences** in substitution.

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# THE EFFECT OF A NOVEL 4-THIAZOLIDINONE DERIVATIVE IN COMBINATION WITH ANTI-HER2 ANTIBODIES ON AUTOPHAGY IN HUMAN AGS GASTRIC CANCER CELLS

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Combining chemotherapy with immunotherapy still remains a regimen in anticancer therapy. Novel 4-thiazolidinone-bearing hybrid molecules possess well-documented anticancer activity, and together with anti-HER2 antibodies, may represent a promising strategy in treating patients with gastric cancer with confirmed human epidermal growth factor receptor 2 (HER2) expression. The aim of the study was to investigate the effect of a novel 4-thiazolidinone derivative (Les-4367) in combination with trastuzumab or pertuzumab on autophagy in human AGS gastric cancer cells. To assess how Les-4367 alone and in combination with anti-HER2 antibodies (trastuzumab and pertuzumab) affect the expression of proteins involved in autophagy processes (ATG5 and LC3B), the Western blot technique was used. Additionally, Beclin-1, LC3A, and LC3B concentrations were measured. The obtained results showed that Les-4367 alone, as well as in combination with trastuzumab or pertuzumab, decreased Beclin-1 concentrations in the gastric cancer cell line. The combination of Les-4367 with pertuzumab was also effective in reducing LC3A and LC3B concentration in cell lysates. Western blot analysis was performed to confirm the results obtained by the ELISA technique. Monotherapy and the combination of Les-4367 and anti-HER2 led to a reduction in ATG5 and LC3B expression in AGS cell lysates compared with the untreated control cells. Based on the obtained results, it can be concluded that the molecular mechanism of action of the analyzed combinations is not related to the induction of autophagy in AGS gastric cancer cells.

The abstract has been supported by the Polish National Agency for Academic Exchange under the “Strategic Partnerships” programme (Grant agreement no. BPI/PST/2021/1/00002/U/00001).

# THE IN VITRO CYTOTOXIC AND APOPTOTIC ACTIVITY OF NEW THIOPYRANO[2,3-d]THIAZOLES

**Krzysztof Bielawski (1), Olga Szewczuk (1), Anna Bielawska (2), Piotr Roszczenko (2), Iryna Ivasechko (3), Rostyslav Stoika (3), Roman Lesyk (4), Robert Czarnomysy (1)**

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Thiazole and its structure-related analogs thiazolidinone derivatives constitute a known class of compounds that can became the basis for the creation of novel lead compounds, since they have a broad spectrum of the biological activities and great potential for further chemical modification. A series of 11-substituted 9-hydroxy-3,5,10,11-tetrahydro-2H-benzo[6,7]thiochromeno[2,3-d][1,3]thiazole2,5,10-triones were synthesized via hetero-Diels-Alder reaction of 5-ene-4-thioxo-2-thiazolidinones and 5-hydroxy-1,4-naphthoquinone. The structure of newly synthesized compounds was established by means of spectral data and a single-crystal X-ray diffraction analysis. Synthesized compounds were tested on their potential antitumor activity toward a panel of cancer cell lines. These compounds demonstrated strongest cytotoxic effect toward all used tumor cell lines, and the IC 50 ranged from 0.6 μM to 31.16 μM. For quantitative determination of the apoptotic effects of the most active compound in MDA-MB-231 cells, flow cytometric assay was performed using double staining Annexin V-FITC and Propidium iodide (AV/PI). Considering the important role of caspase 8 in the initiation of apoptosis via the extrinsic pathway, we evaluated the effects of the most active compound (11-(Furan-2-yl)-9-hydroxy-3,11-dihydro-2H-benzo[6,7]thiochromeno[2,3-d]thiazole-2,5,10-trione) (1) on the activation of this protein in MDA-MB-231 breast cancer cells after 24 h exposure. The results of evaluating caspase 8 activities were consistent with those obtained in the AV/PI assay, indicating that apoptosis induced by the tested compound 1 may proceed via an extrinsic pathway mediated by cell death receptors. The initiation of the intrinsic apoptosis pathway results in the activation of the caspase 9. Therefore, we have evaluated the effect of the tested compound 1 on the activation of this protein in MDA-MB-231 breast cancer cell lines. The 24 h treatment of cells with the compound 1 induced the elevation in active caspase 9, compared to control. After activation of the initiator caspases (caspases 8 and 9), both apoptotic pathways (intrinsic and extrinsic) converge into a common one and the executive phase of apoptosis begins. During this stage, active executioner caspases, mainly caspase 3, and also caspase 7, are formed. Thus, we evaluated caspase 3/7 activity in the MDA-MB-231 breast cancer cells using flow cytometry after 24 h treatment of cells with the tested compound. Changes in caspase 3/7 activity were also detected at the action of the compound, in compare with control. It was demonstrated, that the compound 1 induced apoptosis in the MDA-MB-231 breast cancer cells proceeding through two pathways, extrinsic and intrinsic.

The abstract has been supported by the Polish National Agency for Academic Exchange under the "Strategic Partnerships" programme (Grant agreement no. BPI/PST/2021/1/00002/U/00001).

# VOCs ART : VOC-BASED PROBES FOR EXPLORATION, DETECTION AND MONITORING OF PATHOLOGIES

**Estelle Blochouse (1), Rony Eid (1), Elsa Cannoni (1), Remi Châtre (1), Claude Geffroy (1), Isabelle Opalinski (1), Sébastien Papot (1,2), Pauline Poinot (1)**

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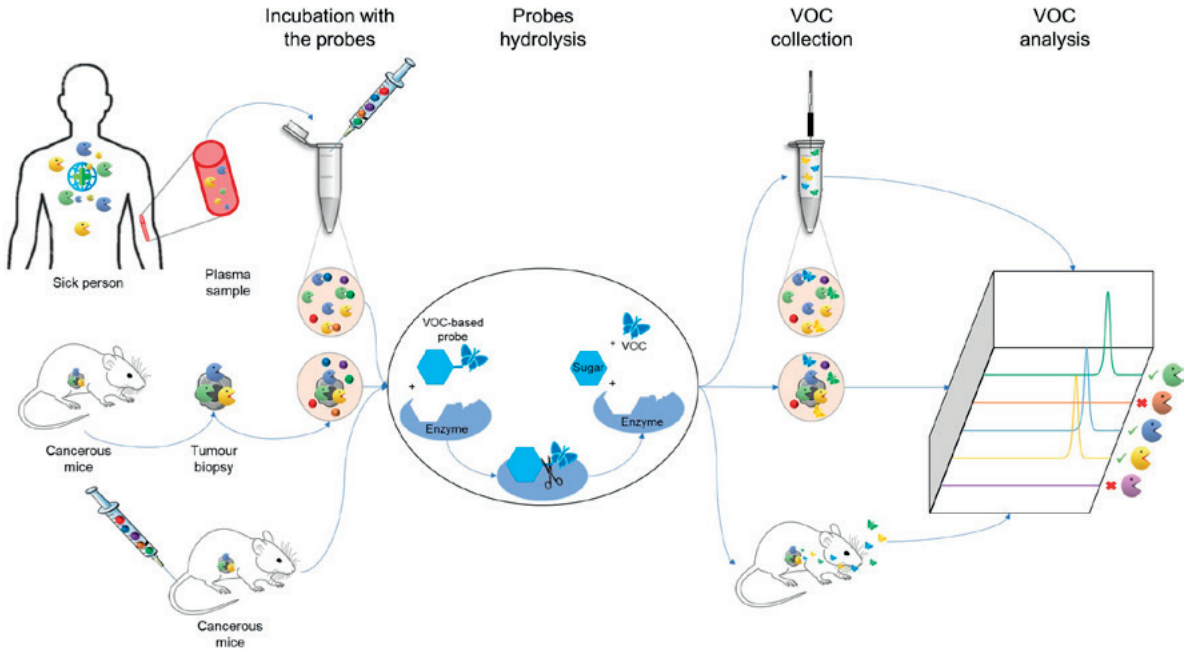
The volatolome corresponds to the whole volatile organic compounds (VOCs) produced by the metabolic activity of a biological system. A change in the volatolome can be linked to the development of a disease and its study, the volatolomics, may be a simple, fast, accessible, and safe diagnostic approach. However, the identification of endogenous volatile markers specific to a pathology has encountered difficulties due to the high interindividual variability and the disparities between laboratories in sample preparation and analysis. In this context, we have proposed a novel strategy, “induced volatolomics”[1], which relies on the use of off-on VOC-based probes that can be converted into exogenous volatile compounds through a metabolic stimulus. The targeted enzymes can be linked to specific disease as cancer, viral or bacterial infection, or to inflammation processes.
In this proposal, the concept of off-on VOC-based probes will be presented. Their value for the in vivo diagnosis of tumors will be described. Also, their use to survey and improve the efficacy of novel chemotherapeutic agents will be demonstrated[2]. Then, cocktails of off-on probes will be presented as tools for the evaluation of enzymes dysregulation in cancer[3] or viral or bacterial infection. Within this framework, pilot results will be presented on COVID-19 infection severity[4].

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# NOVEL FAR-RED FLUORESCENT 1,4-DIHYDROPYRIDINES FOR L-TYPE CALCIUM CHANNELS IMAGING

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L-type calcium channels (LTCCs) are key players in physiological processes such as excitation-transcription coupling, excitation-contraction coupling and in the release of neurotransmitters and hormones.[1] In fact, they activate downstream processes mediated by $Ca^{2+}$ flowing into the cytoplasm. Extensive investigations have linked changes in LTCC function and expression to all important human diseases[1], however, LTCCs are extremely complex and the development of new imaging tools could shed light on the lingering questions.

In particular, in the last decades, the number of small-molecule fluorescent ligands has increased in tandem with high-precision optical techniques, becoming a prominent tool to study the role of proteins both in physiological and pathological processes.[2] Nevertheless, the development of fluorescent ligands for voltage-gated ion channels (VGICs), including LTCCs, is still in its infancy and we still lack far-red imaging probes applicable to live-cell imaging of LTCCs.

In this work, we unlocked a library of thirteen racemic and homochiral 1,4-dihydropyridines (1,4-DHP) tagged with commercially available fluorophores. The library was based on different pharmacophores linked to the far-red fluorophores via linkers of variable nature and length. Pharmacological evaluation via live-cell calcium imaging and automated patch-clamp showed retention of the antagonist-like activity on LTCCs with μM potency for eleven out of thirteen compounds and led to the identification of the most potent LTCC ligand having sub-μM potency, FluoDiPine.

Finally, FluoDiPine was tested in confocal microscopy to evaluate the specificity of the ligand and its applicability to fluorescence imaging in a heterologous expression system. The specificity of FluoDiPine was confirmed via both GFP-colocalization and pre-blocking using a nonfluorescent 1,4-DHP. Later experiments in SH-SY5Y cells showed the applicability of FluoDiPine in live confocal microscopy in a cell line that expresses LTCCs endogenously.

In conclusion, we report herein the first far-red fluorescent ligand for LTCCs applicable at a very low concentration (50 nM) in live-cell fluorescence imaging. Currently, experiments are being carried out to probe single-molecule association.

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# N-OCTYLAMINO DERIVATIVES OF 4-AMINOQUINOLINE AS POSSIBLE MULTIFUNCTIONAL AGENTS FOR ALZHEIMER'S DISEASE TREATMENT

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Alzheimer's disease (AD) is the most prevalent neurodegenerative disease which, according to WHO Global status report, in 2019 affected about 55.1 million people worldwide (1). So far, the treatment of AD was exclusively symptomatic and affects mainly the alleviation of symptoms, rather than the course, development and final outcome of the disease. It was based mainly on increasing the level of neurotransmitter acetylcholine in the brain by inhibiting the action of enzymes responsible for its hydrolysis; acetylcholinesterase (AChE) and since recently also of butyrylcholinesterase (BChE) whose selective inhibition was connected to low occurrence of side-effects and beneficial effect on cognitive abilities of rodents (2). In the last two years a step forward was made with the introduction of two drugs whose mode of action is inhibition of accumulation of benign amyloid-β plaques in the brain of patients with AD. However, AD is a multifactorial disease characterized by multiple pathophysiological factors which induce the loss of neurons and their connections in brain of AD patients (deficiency of the neurotransmitter acetylcholine, accumulation of beta-amyloid (Aβ) plaques, changes in the homeostasis of biometals, oxidative stress, hyperphosphorylation of tau protein, overstimulation of N-methyl-D-aspartate receptor and increased MAO-B enzyme activity) calling for use of multitarget-directed ligands (3).
In our study, we have shown that 4-aminoquinolines with eight methylene groups as a linker between basic terminal amino group and quinoline moiety are promising structural scaffolds for the design of novel central nervous system active drugs, particularly AD, due to their simple structure, high inhibitory potential toward both cholinesterases, potential to cross the blood-brain barrier and nontoxicity to neuronal, kidney and liver cells. This is additionally supported by the fact that majority of the tested compounds displayed the ability to bind simultaneously into the AChE peripheral (PAS) and catalytic anionic site (CAS) presuming the possibility to interfere with the formation of an AChE-Aß complex, They are also able to chelate biometal ions Cu2+, Zn2+ and Fe2+ presuming compound's ability to reduce ROS production and formation of toxic metal-Aβ plaques, and to inhibit the action of BACE1, enzyme involved in production amyloid-β aggregates.

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# NOVEL CHEMOTYPES OF ANGIOTENSIN-CONVERTING ENZYME 2 BINDERS VIA SUCCESSIVE IN SILICO SCREENING AND IN VITRO EVALUATION APPROACHES

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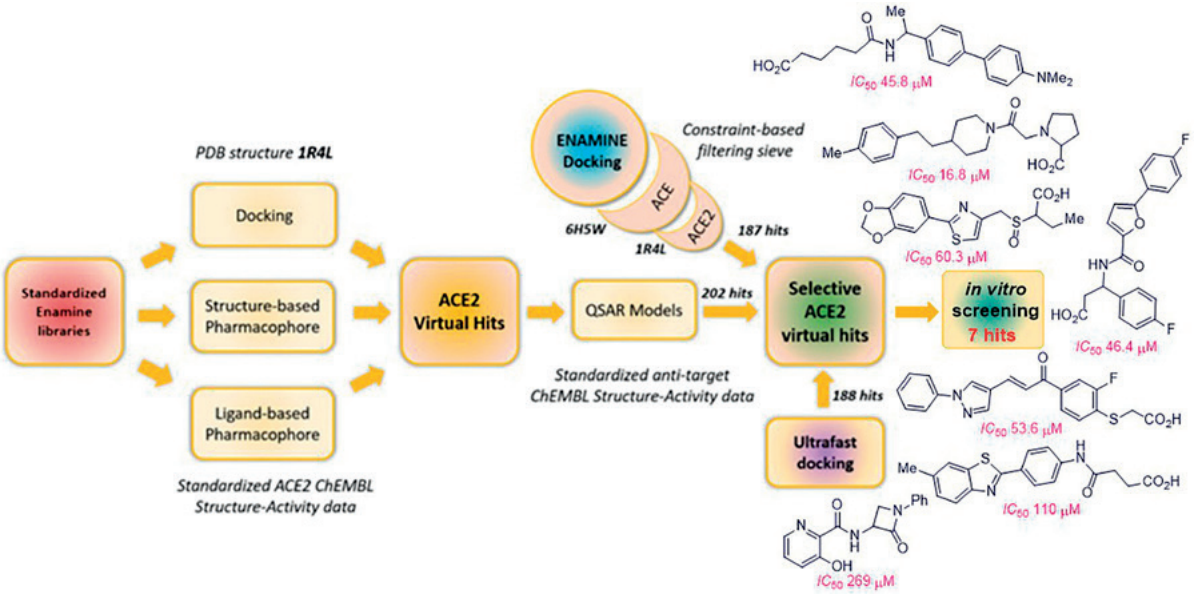
Angiotensin-converting enzyme 2 (ACE2) is a protein that can exist in both bound-to-membrane and free soluble forms. It is being expressed in the intestines, kidney, testis, gallbladder, lungs and heart. Both of the ACE2 types are integral parts of the renin-angiotensin-aldosterone system maintaining normal blood pressure. Owing to high importance of ACE2, it is crucial to understand clearly its functions and figure out biochemical pathways involving this protein. The most straightforward way to achieve this is exploitation of chemical probes efficiently and selectively binding the enzyme. However, we noticed that there are very few ACE2-binder discovery projects in comparison with its homologue ACE. Thus, by the current project we intended to identify ACE2-targeting chemical probes to provide effective tool for biochemical investigations. In our recent work, we reported 188 potential hits identified by means of ultrafast docking.[1] Now we have complemented the previous *in silico* screening result with 389 newly selected potential ACE2 binders detected *via* joint application of classical docking, pharmacophore-based search, and QSAR ACE2/ACE and ACE2/NEP selectivity filtering following experiments for *in vitro* validation. The combined set of the most promising molecules, (overall 577 compounds from the Enamine screening collection), were tested in HTS mode in an optimized and validated enzymatic assay using the fluorescence method (Abcam ACE2 Inhibitor Screening Kit, ab273373). Seven compounds out of 577 screened were selected according to hit criteria “Inh% > Avg + 3SD” for dose-response assay and demonstrated micromolar activity.[2] Those compounds represent novel ACE2-binding chemotypes that could be used for further structural optimization. The general workflow of *in silico* search for selective ACE2 inhibitors as well as detected *in vitro* hits are given below.

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# ASYMMETRIC ENE-REDUCTION OF ALPHA,BETA-UNSATURATED COMPOUNDS BY F420-DEPENDENT OXIDOREDUCTASES A (FDOR-A) ENZYMES FROM MYCOBACTERIUM SMEGMATIS

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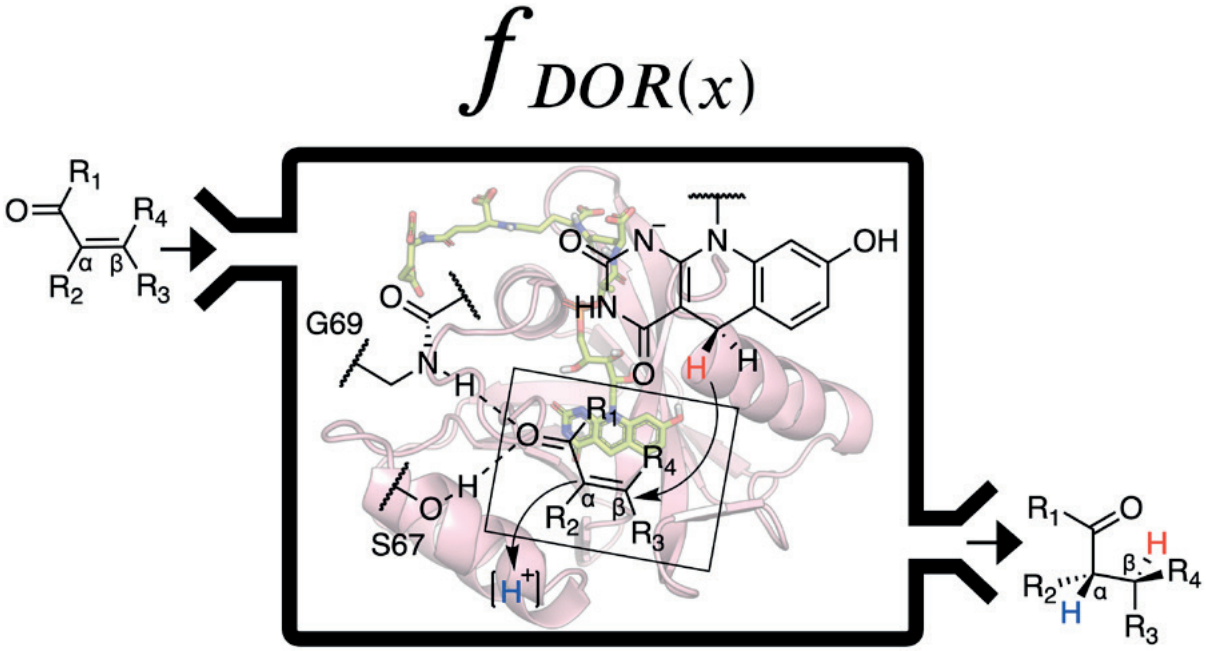
The stereoselective reduction of alkenes conjugated to electron-withdrawing groups by ene-reductases has been extensively applied to the commercial preparation of fine chemicals. Although several different enzyme families are known to possess ene-reductase activity, the Old Yellow Enzyme (OYE) family has been the most thoroughly investigated. Recently, it was shown that a subset of ene-reductases belonging to the flavin/deazaflavin oxidoreductase (FDOR) superfamily exhibit enantioselectivity that is generally complementary to that seen in the OYE family. These enzymes belong to one of several FDOR subgroups that use the unusual deazaflavin cofactor $F_{420}$. Here, we explore several enzymes of the FDOR-A subgroup, characterizing their substrate range and enantioselectivity with 20 different compounds, identifying enzymes (MSMEG_2027 and MSMEG_2850) that could reduce a wide range of compounds' stereoselectivity. For example, MSMEG_2027 catalyzed the complete conversion of both isomers of citral to *(R)*-citronellal with 99% *ee*, while MSMEG_2850 catalyzed complete conversion of ketoisophorone to (*S*)-levodione with 99% *ee*. Protein crystallography and computational docking have allowed the observed stereoselectivity to be mechanistically rationalized for two enzymes. These findings further support the FDOR and OYE families of ene-reductases being generally stereocomplementary to each other and highlight their potential value in asymmetric ene-reduction.



# OPTIMIZATION OF SELECTIVE AND POTENT $A_{2A}R$ NEGATIVE ALLOSTERIC MODULATORS AS IMMUNOTHERAPY FOR HIGH ADENOSINE CANCERS' TREATMENT

**Margot Boujut (1,2), Margaux Héritier (1,2), Aurélie Gouiller (1,2), Camille Süess (1,2), Alessandro Scapozza (1), Maxime Guibert (1), Hesham Hamed (1,2), Sébastien Tardy (1,2), David Pejoski (1,2), Leonardo Scapozza (1,2)**

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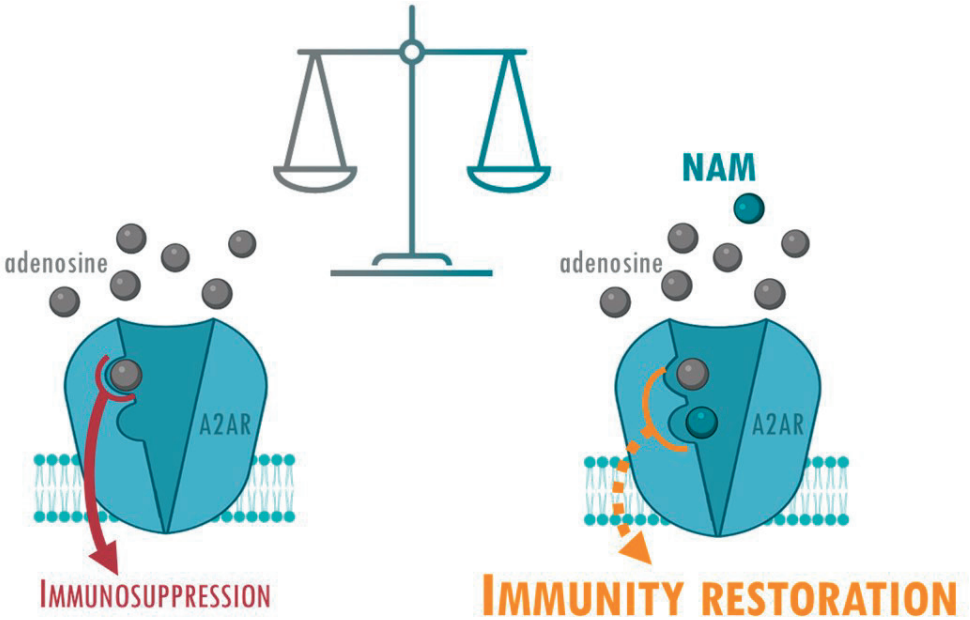
Adenosine exerts its immunosuppressive action in a microtumor environment (TME) *via* the adenosine 2A receptor ($A_{2A}R$)[(1)], hence $A_{2A}R$ has become an emerging alternative immune checkpoint to be targeted by cancer immunotherapies.[(2)] Due to the adenosine rich TME, orthosteric drugs must have very low potencies to compete, which can in turn generate unwanted off-target side effects. Allosteric modulators will overcome both these drawbacks thanks to their unique endogenous ligand-independent mode of action and their localized action enabling minimum-function signaling of the drug target.[(3)]

Small molecule library screenings with a proprietary assay allowed the hit identification and the molecular mode-of-action confirmation of novel $A_{2A}R$ negative allosteric modulators (NAMs). Assisted by molecular docking tools, the hit optimization was efficiently tackled, and a SAR model was developed. The most promising leads showed nanomolar potencies, in both adenosine low and rich environments, and the potential to be the first $A_{2A}R$ allosteric drug immunotherapy by restoring inflammatory cytokines responses in translational human immunoassays that recapitulate high-adenosine TME.

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# REDOX-SENSITIVE PROBES FOR THE ACTIVITY-BASED PROTEIN PROFILING OF OXIDOREDUCTASES

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Activity-based protein profiling (ABPP) is a unique proteomic tool for measuring the activity of enzymes in their cellular context, which has been well established for enzyme classes exhibiting a characteristic nucleophilic residue (e.g. hydrolases). In contrast, the enzyme class of oxidoreductases has received less attention, as its members rely mainly on cofactors instead of nucleophilic amino acid residues for catalysis. ABPP probes have been developed for specific oxidoreductase subclasses, which rely on the oxidative conversion of the probes into strong electrophiles. Here we describe the development of ABPP probes for the simultaneous labeling of various subclasses of oxidoreductases. The probe warheads are based of hypervalent diarylhalonium salts, which show unique reactivity as their activation proceeds via a reductive mechanism resulting in aryl radicals leading to covalent labelling of proteins.

# BIAS AND THE MEDICINAL CHEMIST'S RELATIONSHIP WITH ADDITIVITY

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Given the importance and complexity of ligand-protein interactions in drug discovery, medicinal chemists continuously rely on structure activity relationships to design molecules which improve binding affinity. Today the medicinal chemistry literature is dominated by papers which optimize using Linear Analoging, (A single R group is optimized, followed by a second R group sequentially.) rather than creating a matrix of compounds altering multiple R groups simultaneously (Combinatorial Analoging). This presentation will highlight the advantages of Combinatorial Analoging in combination with quantitative additivity determinations which provides distinct SAR understandings not available in Linear Analoging efforts. The technique provides distinct structural insights on ligand binding and property determinations which greatly aid the SAR effort. The key example presented is the optimization of PDE2 inhibitors for the treatment of cognitive disorders.

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# HETEROBIFUNCTIONAL E3 LIGASE RECRUITERS ENABLE PAN-DEGRADATION OF INHIBITOR OF APOPTOSIS PROTEINS

**Aleša Bricelj (1), Yuen Lam Dora Ng (2), Jacqueline Jansen (2), Arunima Murgai (2), Jan Krönke (2), Kirsten Peter (2), Katherine A. Donovan (3), Michael Gütschow (4), Christian Steinebach (4), Izidor Sosič (1)**

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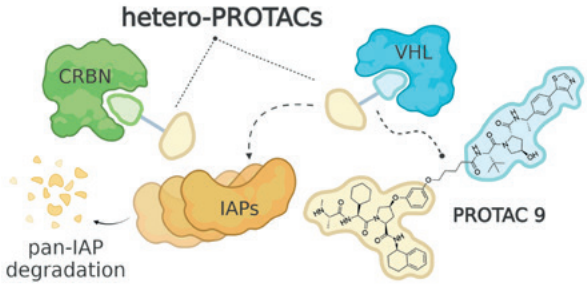
Cellular IAP1 (cIAP1), cellular IAP2 (cIAP2), and X-chromosome-linked IAP (XIAP) are members of the inhibitor of apoptosis (IAP) protein family and have been extensively studied due to their crucial role in the regulation of apoptosis, where they act as proto-oncogenes by inhibiting cell death. Deregulation and overexpression of IAPs is frequently observed in various cancers and correlates with tumour progression, resistance to anticancer therapies, and poor prognosis.[1] Due to its clinical significance, numerous small-molecule mimetics of the IAP-binding motif of the endogenous IAP antagonist, second mitochondria-derived activator of caspases (SMAC), have been developed. Several monovalent and bivalent antagonists have entered clinical trials, but demonstrated low efficacy as single agents.[2] Importantly, these IAP antagonists have profound effects on cIAPs levels, as their binding leads to autoubiquitination and degradation of cIAP1 and cIAP2, whereas such effects are rarely observed with XIAP.[3]

Through significant advances in the field of targeted protein degradation, proteolysis-targeting chimeras (PROTACs) are considered as one of the most promising modalities in medicinal chemistry. Consisting of two distinct ligands connected by a linker, PROTACs can facilitate the formation of a ternary target complex protein–PROTAC–E3 ligase, followed by ubiquitination of the target protein and its subsequent degradation by the proteasome.[4] The concept has also been utilized in so-called homo- and hetero-PROTACs in which E3 ligases were directed against each other, resulting in successful depletion of cereblon (CRBN),[5,6] von Hippel-Lindau (VHL)[7], murine double minute 2 (MDM2),[8] and Keap1.[9] Encouraged by previous successful attempts and the fact that IAPs are validated anticancer targets, we systematically designed three series of bifunctional molecules by cross-linking VHL- and CRBN-targeting ligands with an IAP antagonist to apply the heteroPROTAC approach to IAP modulation. Our efforts produced compounds that resulted in strong, rapid and preferential degradation of cIAP1, cIAP2, and even XIAP and, in one case, also to concentration-dependent selective XIAP degradation. Notably, our pan-IAP degraders outperformed IAP antagonists and showed potent inhibition of cancer cell proliferation and could translate to degraders with significant therapeutic benefits in the battle against cancer.[10]

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# ACTIVITY AND ENANTIOSELECTIVITY IN BIOCATALYSIS – INFLUENCE OF ORGANIC SOLVENTS AND ACTIVE SITE MUTATIONS

**Zlatko Brkljača (1), Nevena Milčić (2), Irena Dokli (3), Petra Švaco (3), Lixia Tang (4), Ivo Crnolatac (3), Zvjezdana Findrik Blažević (2), Višnja Stepanić (3), Maja Majerić Elenkov (3)**

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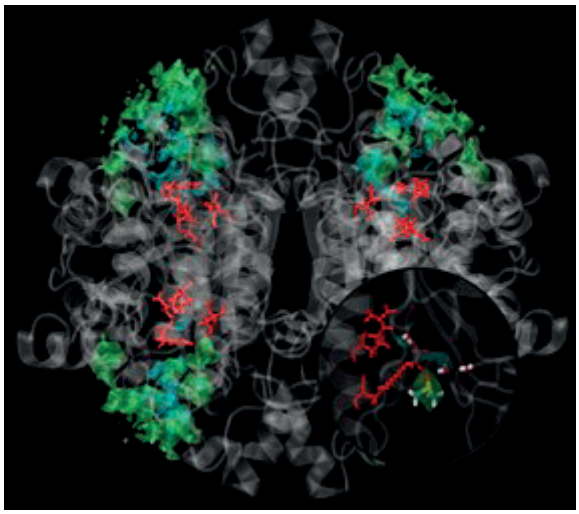
Enantiopure compounds often play a pivotal role in the pharmaceutical, agrochemical, and chemical industries. Among the methods used for their production, biocatalytic approaches, are generally considered a green and effective synthetic alternative due to their mild reaction conditions and remarkable enantioselectivity.[1] In this respect, we will focus on two different, yet intertwined biocatalysis topics - potential benefits and drawbacks of organic/non-aqueous solvents in biocatalysis, as well as the potential of active site mutations as a mean to guide both activity and enantioselectivity of enzymatic assays.



This is going to be exemplified on a model system, namely homotetrameric halohydrin dehalogenase (HHDH) from Agrobacterium radiobacter AD1, i.e., HheC. This enzyme naturally catalyses reversible dehalogenation of vicinal haloalcohols, but it is utilized with a whole range of unnatural nucleophiles in epoxide ring-opening reactions. Using complementary experimental and computational techniques we firstly investigated the influence of DMSO-water solvent mixtures on the activity of HheC enzyme.[2] DMSO, one of the most prevalent co-solvents for biocatalytic transformations, was found to act as a mixed-type inhibitor with a prevalent competitive contribution.[2] Secondly, HheC enzyme was investigated for the azidolysis of fluorine-substituted styrene-oxide derivatives. Preliminary investigation confirmed HheC to be predominantly (*R*)-enantioselective, and moreover, sterically challenging compounds were not accepted by the enzyme. Further investigation pointed to the active mutant P84V/F86P/T134A/N176A (named HheC-M4), showing both high activity and inverted enantioselectivity towards *o*-$CF_3$-styrene oxide. The observed (*S*)-enantioselectivity is ascribed to formation of the additional space by introduced mutations in HheC-M4, which is also confirmed by classical MD simulations. The successive molecular docking demonstrated that this newly formed tunnel located close to the protein surface is a critical feature of HheC-M4, representing a novel binding site.[3]

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# REGENERATIVE CARDIAC PHARMACOLOGY – DESIGN, SYNTHESIS, AND BIOLOGICAL EVALUATION OF NOVEL SMALL MOLECULE COMPOUNDS

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Ischemic heart diseases are the leading cause of death worldwide. In an acute myocardial infarction, up to 25% of the heart muscle cells i.e. cardiomyocytes (CMs) die. Due to the limited renewal capacity of CMs, the infarction often leads to fibrosis and heart failure. However, some lower vertebrates and neonatal rodents have an intrinsic regenerative capacity to fully repair the injured heart muscle, which in rodents quickly declines after birth due to irreversible cell cycle arrest of CMs. It also seems that humans possess this ability at the time of birth. By reactivating the CM cell cycle, the damaged area can potentially be regenerated and fatal heart failure prevented. There is an urgent need for more effective therapies to treat heart failure, since current alternatives have only a limited effect on prognosis.

Signaling pathways mediated by protein kinase B (PKB or AKT) are known for antiapoptotic and cell proliferation-promoting activities in various cell types, including CMs. AKT activators could thereby enhance the proliferation of CMs after myocardial infarction. **SC79** is a known small molecule AKT activator which, however, is chemically and metabolically labile for degradation. Moreover, **SC79** has several stereoisomers and tautomeric forms, which further complicate the identification of binding interactions with its target AKT. The aim of the study is to find more stable **SC79** derivatives to activate AKT, and to study their effects on CM viability and cell cycle.

In this project, we have designed and synthesized **SC79** derivatives with alternative core structures and substituent patterns. The effect of these compounds on AKT activation has been evaluated on a cell-based phosphorylation assay (AlphaScreen®). Also, the binding to AKT has been evaluated in microscale thermophoresis assay. Furthermore, the proliferative and hypertrophic effect of compounds on human induced pluripotent stem cell (hiPSC) CMs were evaluated with high content analysis.

**Keywords:** Protein kinase B, AKT, **SC79**, drug design, cardiac regeneration, heart failure

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# TOTAL SYNTHESIS AND BIOLOGICAL PROFILING OF N-METHYLATED MARINOAZIRIDINE A

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As a result of marine bioprospecting, chiral alkaloids marinoaziridines A and B were recently isolated from marine sediment Gram-negative bacteria of the order *Cytophagales* (Figure 1).[1] In the structure, they contain aziridine and quinolin-2(1H)-one rings, which are frequent pharmacophore fragments in bioactive natural and synthetic molecules.[2,3] Natural products represent continuous inspiration for medicinal chemists and are still the main source of novel small molecular weight drugs, including their semi-synthetic derivatives or scaffolds inspired by them.[4]

Herein, we report the first total synthesis of racemic *N*-methyl-marinoaziridine A derivative along with its *in silico*, *in vitro* and *in vivo* bioactivity assessment. Target *N*-methylated marinoaziridine A was prepared in eight synthetic steps. The route begins with the synthesis of two achiral fragments, which undergo a convergent coupling reaction of the achiral sulfur ylide derived from the sulfonium salt and the corresponding aldehyde to form the chiral epoxide as the key intermediate in our strategy.[5] Regioselective ring-opening of the epoxide with sodium azide proceeded under acidic conditions to give the azidoalcohol which was then subjected to Staudinger reaction to yield marinoaziridine B. Final step includes *N*,*N*-dimethylation of marinoaziridine B yielding desired *N*-methyl- marinoaziridine A. *In silico* profiling was performed considering physicochemical and ADMET features as well as biological activity spectrum. *In vitro* screening of *N*-methyl-marinoaziridine A derivative was done by MTT test on three cell lines (MCF-7, breast cancer; H-460, lung cancer; HEK293T, embryonic kidney) and on bacterial cultures of *E. coli* and *S. aureus*. Toxicological tests were performed using a test on zebrafish embryos *Danio rerio*.

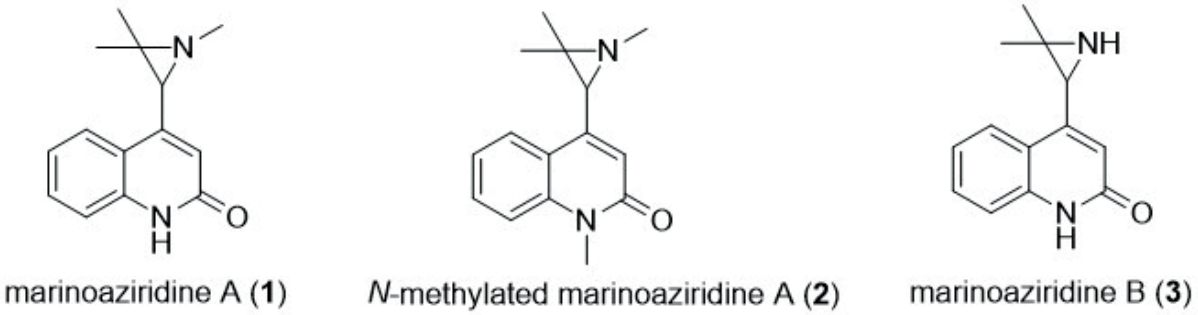


**Figure 1.** Chemical structures of marinoaziridine A (**1**), *N*-methylated marinoaziridine A (**2**), and marinoaziridine B (**3**).

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# PREPARATION OF DISUBSTITUTED FERROCENYL DESMURAMYL PEPTIDES AND THEIR MANNOSYLATED ANALOGUES - NOVEL POTENTIAL ADJUVANTS

**Željka Car (1), Vesna Petrović Peroković (1), Monika Kovačević (2), Rosana Ribić (3), Lidija Barišić (2)**

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Muramyl dipeptide (*N*-acetylmuramyl-L-alanyl-D-isoglutamine, MDP) is the smallest structural unit of peptidoglycans with immunostimulatory activity.[1] MDP analogues lacking the hydrophilic *N*-acetylmuramyl moiety are referred to as desmuramyl peptides (DMP) and have been extensively studied by our group as potential new adjuvants.[1] This work is a continuation of our ongoing SAR study on amphiphilic mannosyl DMP analogues and their adjuvant activity, where the lipophilic subunits previously used were adamantyl, adamantyl triazolyl, dodecyl.[2] Herein, we are preparing diamide (Fig.1a) and ester-amide (Fig.1b) derivatives of disubstituted ferrocenes with DMP and mannose subunit. Appropriately protected DMP pharmacophore will be linked to the prepared disubstituted ferrocene precursors with various alkyl chain lengths. Protected mannose subunit will be incorporated next. After protecting group removal, the targeted mannosylated molecules as well as their precursors, the DMP ferrocenyl analogues, will be screened for their *in vitro* adjuvant activity which will be performed on HEK-Blue NOD2 cells. The most potent compounds will be evaluated on peripheral blood mononuclear cells (PMBC).

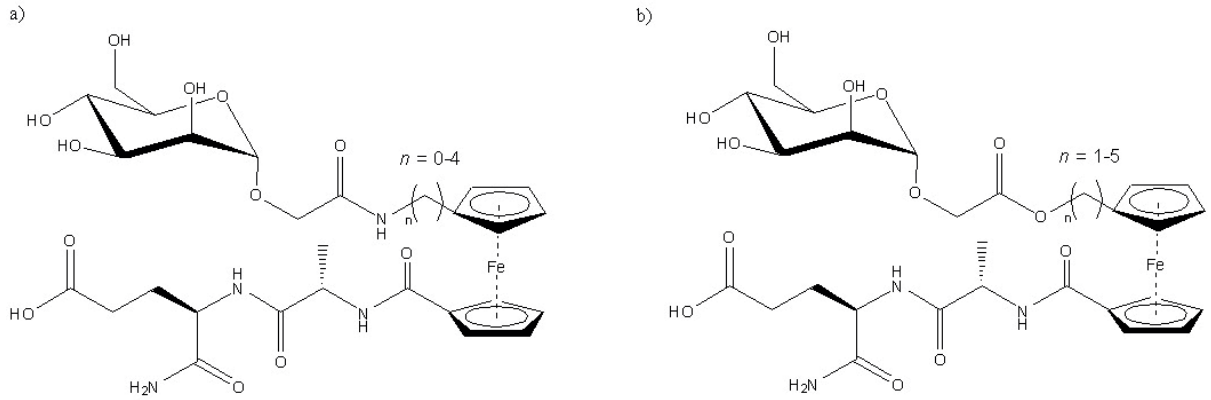


Fig. 1: Target a) diamide b) ester-amide mannosyl DMP analogues.

**ACKNOWLEDGEMENTS:** This work has been fully supported by Croatian Science Foundation under the project IP-2020-02-9162.

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# DEVELOPMENT OF NOVEL DRUG CANDIDATES FOR SELECTIVE INHIBITION OF BCL-2 PROTEIN–PROTEIN INTERACTIONS

**Jairo Paz (1), César Guerrero (1), Ramón Rodríguez (1), Jacobo Cruces (1), Monica Carreira (1), Mati Cohen (2), Roberto Olender (2), Rakefet Rosenfeld (2), Tal Parnes (2)**

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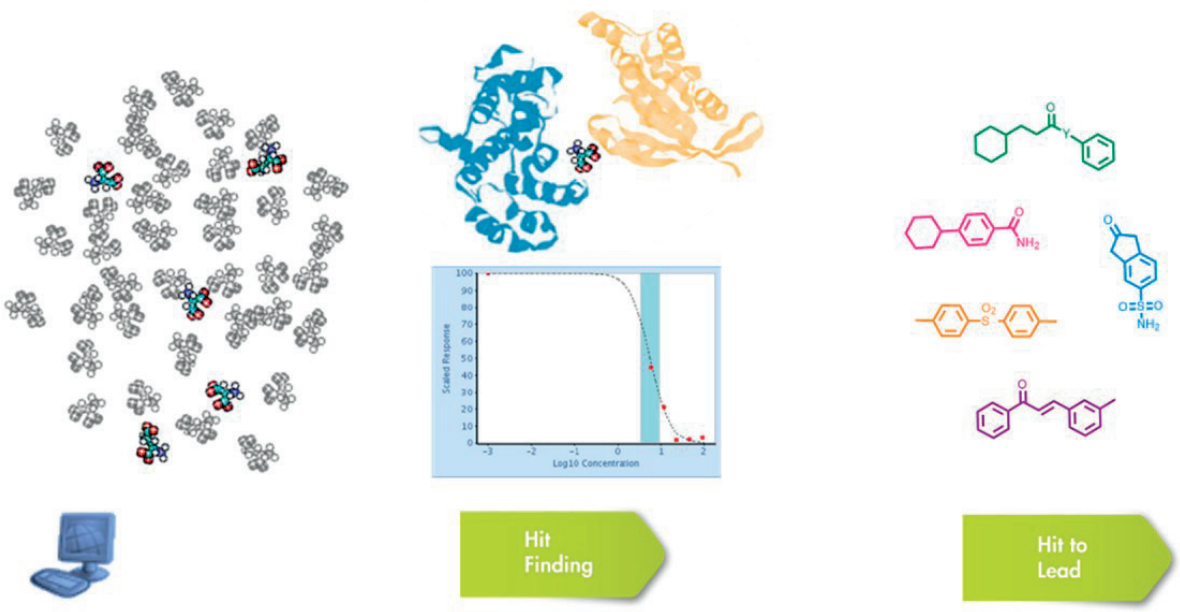
*2) HQL Pharmaceuticals, 4th Hulda Hanevia st., 44343 Kfar-Saba (Israel)*

The Bcl-2 family of proteins is central to the regulation of apoptosis, and their overexpression has been linked to certain types of lymphoma and carcinoma [1], as well as resistance to conventional antitumor treatments. In particular, the interactions between pro- and anti-apoptotic Bcl-2 family proteins control the integrity of the outer mitochondrial membrane. The aim of this project was to develop a set of novel anti-cancer drug candidates that function by selectively inhibiting such protein–protein interactions (PPI) [2].

In collaboration with HQL Pharmaceuticals (Israel), a virtual library of potential candidates was designed and further refined following ADME, stability, structural complexity, and patentability criteria. The virtual hits were clustered into families and selected compounds were chosen for synthesis. The activity of the compounds, that is, their capability to inhibit Bcl-2 PPI, was evaluated by Surface Plasmon Resonance (SPR) measurements. After the initial hit-finding stage, the structures were further optimized (hit-to-lead) to improve the activity down to the nanomolar range.

This work was carried out within the projects ONCOGALFARMA and NEOGALFARM, funded by the Galician Innovation Agency (GAIN) and FEDER through the CONECTA-PEME Programme.

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# DEVELOPMENT OF FIRST-IN-CLASS DUAL LSD1-PRMT5 INHIBITORS FOR THE TREATMENT OF ACUTE MYELOID LEUKEMIA

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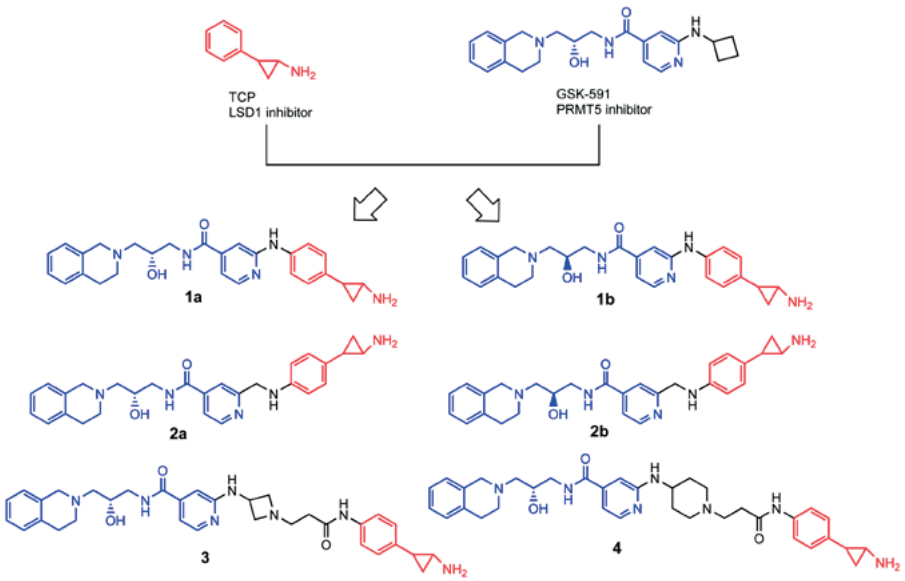
The lysine-specific demethylase 1 (LSD1) catalyses the removal of mono- and dimethyl modifications of Lys4 of histone H3 (H3K4me1/2), which are essential marks of transcriptional activation [1]. LSD1 has been shown to play a central role in the insurgence of solid and blood cancers. In particular, it is highly expressed in acute myeloid leukemia (AML), where LSD1 is crucial for the maintenance of cancer cell stemness, inhibition of cell differentiation, and prevention of apoptosis [2]. Similarly to LSD1, the protein arginine methyltransferase 5 (PRMT5), a methyltransferase that catalyses the symmetric dimethylation of arginine residues [3], acts as an oncoprotein in AML. Indeed, PRMT5 activity was shown to support AML growth *in vitro* and *in vivo [4]*. Given the involvement of both LSD1 and PRMT5 in AML, the simultaneous inhibition of these enzymes may represent a successful approach to treating this malignancy. Notably, we have identified a synergistic interaction between a LSD1 inhibitor and a PRMT5 inhibitor in multiple AML cell lines. The two inhibitors combined promote AML differentiation and eventually growth inhibition and apoptosis. To leverage on this synthetic lethal interaction, we developed a series of dual-targeting LSD1/PRMT5 inhibitors (Figure 1) that could inhibit both enzymes *in vitro* in the submicromolar to nanomolar range, while being selective over PRMT1 and PRMT7. Among the prepared compounds, two of them impaired leukemic cell viability with higher potency compared to single-target inhibitors and induced apoptosis and myeloid differentiation. In addition, we were able to solve the X-ray co-crystal structure of one of the designed inhibitors with LSD1, thus elucidating its binding mode and providing a structural basis for the rational design of further inhibitors.

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# AUTOMATED WORKFLOW TO STUDY MICROSOMAL CLEARANCE AND ANALYSIS OF METABOLITES USING COLLISION INDUCED DISSOCIATION AND ELECTRON ACTIVATED DISSOCIATION MS/MS DATA

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## Introduction

Studies of *in vitro* metabolism of drugs in human and animal tissues help to predict the metabolic clearance rate of compounds and identify major metabolism pathways ("soft spots"). For such studies, metabolite identification is critical and is often software-aided to ensure proper metrics are used for confident identification and prediction of the metabolism site. A software-aided methodology was developed to quantitatively study microsomal clearance and qualitatively identify the soft-spots for metabolism, aiding in the acceleration of the early drug discovery process. Datasets from collision induced dissociation (CID) and electron activated dissociation (EAD) were applied to predict the sites of metabolism.

## Methods

Various drugs were incubated at 37°C in human hepatocytes and rat liver microsomes at starting concentrations of 1-5µM. Samples were quenched with acetonitrile at 0-, 30-, 60-, 90-, 120- and 240-minute intervals. Separation was performed on a Phenomenex Kinetex Polar C18 column (2.1 x 100 mm, 2.6 µm, 100 Å) operated at 40°C. Mobile phase A was 0.1% formic acid in water and mobile phase B was 0.1% formic acid in acetonitrile. The sample injection volume was 5 µL. Analysis was performed using a data-dependent acquisition (DDA) method with traditional fragmentation (Zeno CID) and orthogonal fragmentation (Zeno EAD) on the ZenoTOF 7600 system. The Molecule Profiler software was used for the analysis of microsomal clearance and prediction of biotransformation sites.

## Results

The DDA data provided excellent MS/MS coverage of TOF MS peaks of interest for both Zeno CID and Zeno EAD acquisitions. Automatic prediction of metabolites based on MS1 data and structural elucidation using the precursor and metabolite-specific fragment ions were performed with the Molecule Profiler software.

Several phase 1 and phase 2 metabolites were identified and studied using Zeno CID and Zeno EAD MS/MS data. The Molecule Profiler software enabled processing and analysis of both Zeno CID and Zeno EAD data in a single file. The interpretation of the site of metabolism was enabled by the automated assignment of the structures by the software based on the relative weighting of Zeno EAD and Zeno CID MS/MS spectra on the scale of 1-100%. A correlation analysis was performed for drugs and metabolites by processing result table files from various time points to visualize a decrease in drug concentration relative to an increase in various metabolite concentrations.

Correlation analysis and DDA acquisition enabled parent and metabolite product ion analysis to find the most prominent metabolites and provide a visual summary of metabolism. Metabolite and fragment identification were performed with less than 5 ppm error. The mass accuracy enabled the confident prediction of metabolites present in an *in vitro* metabolism study.

## Novel aspect

A quick software-aided analysis of microsomal clearance and identification of metabolites using collision-induced dissociation and electron-activated dissociation MS/MS data

# DISCOVERY OF A SERIES, NOVEL OLEOCANTHAL - BASED COMPOUNDS AS POTENT ATP CITRATE LYASE INHIBITORS

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A key ingredient of EVOO, Oleocanthal, has attracted considerable scientific attention in recent years due to its diverse biological activities and its potential contributions to human health. To address this growing interest in this valuable natural compound, we present a concise and scalable synthesis procedure for various Oleocanthal analogues. This biomimetic and stereo-controlled approach utilizes oleuropein, a readily available raw material found abundantly in olive leaves [1].

All synthesized compounds were evaluated for their anticancer activity against nine cancer cell lines with interesting activities. Among these compounds, GS27 demonstrated remarkable activity against all tested cancer cell lines, exhibiting favorable ADME properties. Notably, GS27 effectively modulated the phosphorylation profile of ACLY, AMPK, and p70S6 in diverse cell types. ACLY serves as a pivotal enzyme that regulates de novo liposynthesis in cells, making it a promising target to disrupt the glucose-dependent lipid synthesis necessary for cancer cell proliferation. By decreasing the phosphorylation of both ACLY and p70S6, GS27 effectively inhibits two major metabolic pathways: de novo liposynthesis and protein synthesis.

In summary, our study highlights the compelling potential of Oleocanthal analogues, synthesized through our developed procedure, as potent anticancer agents. The remarkable activity of GS27 against a range of cancer cell lines, coupled with its ability to modulate key metabolic pathways, underscores its promising role in combating cancer growth and progression.

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# A REMARKABLE NEW MODALITY FOR THE ANTI-INFLAMMATORY PROPERTIES OF THE NATURAL PRODUCT ANDROGRAPHOLIDE VIA THE DEGRADATION OF MAPK-ACTIVATED PROTEIN KINASE 2

**Quy Thi Ngoc Nguyen (1,2), Phyllis X. L. Gan (2), Wupeng Liao (2), W. S. Fred Wong (2), Christina LL Chai (1)**

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Andrographolide, a labdane diterpene isolated from the plant *Andrographis paniculata*, has been demonstrated to exhibit potent anti-inflammatory effects in various inflammatory disease models. Despite continuous efforts to elucidate the anti-inflammatory mechanisms of Andrographolide, its specific action is not entirely clear. Among the diverse signaling pathways being investigated, NF-κB inhibition has generally been accepted as the main mechanism responsible for the anti-inflammatory action of Andrographolide. However, our studies suggest that that this is unlikely to be a significant contributing mechanism due to the high concentrations of Andrographolide that is needed for the inhibition of NF-κB. We and others have observed the modulatory effects of Andrographolide on the $p38^{MAPK}$ pathway but its precise link to the anti-inflammatory properties of Andrographolide remains elusive. In this presentation, we report our discovery that Andrographolide modulates MAPK-activated protein kinase 2 (MAPKAPK2 or MK2) activity, a direct downstream substrate of $p38^{MAPK}$, by targeting the protein for post translational degradation. MK2 is a validated target for inflammatory diseases, though current strategies for anti-inflammatory drug development focus on developing inhibitors of MK2. Our discovery paves the way for the development of novel anti-inflammatory agents targeting MK2 for degradation by harnessing the privileged scaffold of andrographolide. In addition, these studies suggest a new modality that may account for the observed anti-inflammatory effects of Andrographolide.

# SYNTHETIC STUDIES TOWARDS ARTATROVIRENOLS A AND B

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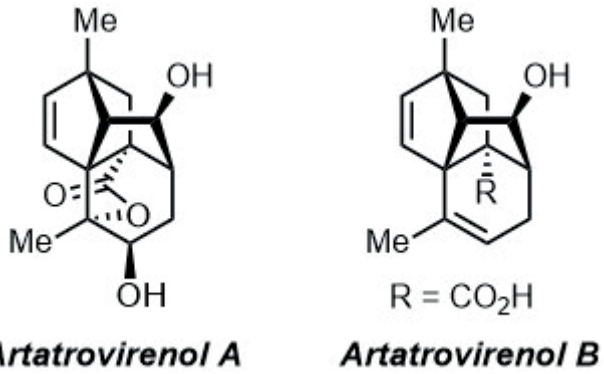
The *Artemisia* genus, one of the largest genera within *Asteraceae* family, comprises about 380 species worldwide. *Artemisia* plants are rich in sesquiterpenoids with wide pharmacological activities, including antitumor, antimalarial, anti-inflammatory, antimicrobial, antibacterial, immunomodulatory effects, etc. Artemisinin, arglabin, and ludartin are only few to mention among many other bioactive compounds. In 2020 Chen and co-workers isolated artatrovirenols A and B, structurally novel sesquiterpenoids from *Artemisia atrovirens*.[1] Artatrovirenols A and B have unprecedented strained cage-like tetracyclo[5.3.1.1.$^{4,11}$0$^{1,5}$]dodecane scaffolds, which makes them rather synthetically challenging targets. Their unique molecular architecture combined with bioactive properties render these compounds worth investigating.




***Artatrovirenol A*** ***Artatrovirenol B***

In this study, we aim to prepare both sesquiterpenoids for the subsequent biological testing in a divergent fashion.

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# ISOSTERIC REPLACEMENT OF CARBOXYLIC ACID MOIETY TARGETTING IMPROVED PERMEABILITY AND STABILITY

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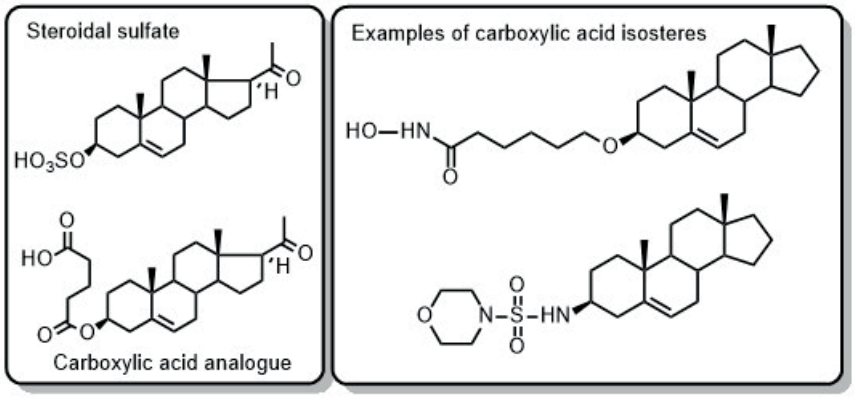
Excitatory glutamate transmission in the neuronal system is mediated mainly by the NMDA receptor. It is crucial for neuronal development, long-term potentiation, memory, and learning, in physiological conditions. Nevertheless, elevated glutamate concentrations contribute to excitotoxicity connected with for instance traumatic brain injury, stroke or some chronic disorders like neurodegenerative diseases. Endogenous steroidal sulfates allosterically modulate this important pharmacological target. Unfortunately, sulfate moiety is quickly deactivated by brain enzymes. Synthetic analogues with carboxyl instead sulfate moiety exert similar activity. Carboxylic functionality is known to be essential in many drugs. However, this moiety is often associated with some disadvantages such as poor pharmacokinetic properties like insufficient passive membrane permeability, metabolic instability, toxicity etc. The bioisosteric replacement of carboxylic acid is a modern strategy to avoid these problems.

We have prepared steroidal compounds with carboxylic acid isosteres in position C-3 of the steroidal skeleton. Results of the evaluation of membrane permeability, stability, pKa values and activity toward the NMDAr will be presented.




*This work was supported by the Technology Agency of the Czech Republic TACR Personalized Medicine: Translational research towards biomedical applications, No. TN02000109 and by the Academy of Sciences of the Czech Republic (AS CR) (grant RVO 61388963).*

# SYNTHESIS OF CARBOCYCLIC SINEFUNGIN ANALOGUES AS METHYLTRANSFERASE INHIBITORS

**Hongseok Choi, Sushil Kumar Tripathi, Lak Shin Jeong**

*Research Institute of Pharmaceutical Sciences, College of Pharmacy, Seoul National University, Gwanak-gu, Seoul, 08826, Korea*

Sinefungin is a natural product that acts as an S-Adenosyl-L-Methionine analog and has shown antiviral activity in previous studies. However, its use is limited due to its cytotoxicity and metabolic instability. To overcome these limitations, carbocyclic analogs of sinefungin were designed and synthesized. Various functional groups, including cyanide, amide, and amines, were installed at the C5 position to explore the chemical space. Starting from d-ribose, Mitsunobu condensation, Horner–Wadsworth–Emmons (HWE) reaction, and Hofmann rearrangement were key steps in the synthesis. The synthesized carbocyclic sinefungin analogs are currently undergoing biological assays to evaluate their antiviral activity and methyltransferase inhibition. The results from these assays will provide insights into the potential of these analogs as antiviral agents.

# SYNTHESIS AND SYNERGISTIC EVALUATION OF DECURSIN AND 4-AMIDODECURSINOL DERIVATIVES WITH GEFITINIB ON A549 NSCLC CELLS

**Hye Min Choi (1), Hyun-Ha Hwang (2), Min Ju Gwon (1), Min Ji Kim (1), Heungmo Kang (1), Jae Kyun Lee (3), Seong-Gyu Ko (2), Jae Yeol Lee (1)**

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*3) Brain Science Institute, Korea Institute of Science and Technology (KIST), Seoul 02792, Republic of Korea*

Based on the microstructure, lung cancer can be classified into two types: non-small cell cancer cell (NSCLC) and small cell lung cancer (SCLC). NSCLC makes up 85-90% of lung cancer patients. There are various treatment options of NSCLC and each treatment has its own advantages, but there are also limitations. To overcome such limitations, this study aims to develop a new NSCLC treatment agent through semi-synthesis using decursinol isolated from *Angelica gigas* Nakai (AGN, Cham Dang Gui). (*S*)-Decursin and its isomer (*S*)-decursinol angelate as a pyranocumarin scaffold are the principal bioactive components of AGN, exhibiting extensive cytotoxic effects on various human cancer cell lines. We synthesized and biologically evaluated a series of new decursin including both enantiomers and 4-amidodecursinol derivatives for anti-cancer effect on A549 lung cancer. Among the synthesized derivatives, compound **(*S*)-2d** showed better cytotoxicity ($IC_{50}$: 30.28 μM) than both natural product (*S*)-decursin ($IC_{50}$: 43.55 μM) and its enantiomer **(*R*)-2d** ($IC_{50}$: 151.59 μM) against A549 lung cancer cells. However, cytotoxicity of **(*S*)-2d** was not sufficient to be used as a single treatment and thus the synergy analysis with gefitinib was performed using SynergyFinder Web. As a result, co-treatment with **(*S*)-2d** (6.25 μM) and gefitinib (10 μM) showed the best ZIP synergy score (δ = 15.2), causing strong synergistic anti-cancer activity against A549 lung cancer cells.

# UNVEILING THE THERAPEUTIC POTENTIAL OF MINUTUMINOLATE ANALOGUES AS MULTI-TARGETING ANTI-INFLAMMATORY AGENTS

**Malcolm Z.Y Choo (1), En Tong Lim (1), Quy T.N. Tran (2), W.S. Fred Wong (2), Christina L. L. Chai (1)**

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Natural products have long been considered to be a treasure trove for bioactive compounds. Minutuminolate ( **MNT**), a coumarin natural product isolated in 2016, was reported to exhibit moderate anti-inflammatory activity. However, no further work has been done to evaluate the potential of **MNT** or its analogues as anti-inflammatory drugs. In this study, a series of **MNT** analogues and derivatives were designed and synthesised. Their anti-inflammatory activities were then evaluated in an *in-vitro* model of inflammation, and key structure-activity relationships (SARs) were established. Following pharmacophore hybridisation, lead compound **MC-1** was derived, exhibiting potent anti-inflammatory activities with low cytotoxicities. Mechanistic investigations revealed that **MC-1** is able to modulate multiple pathways (iNOS, PI3K/Akt and NF-κB) to achieve its anti-inflammatory effects. These findings allude to the multi-targeting nature of **MC-1** and accentuate the importance of natural products or natural product derived compounds for the management of complex conditions like inflammation

# AUTOMATED SYNTHESIS PROVIDES RAPID HIT VALIDATION OF VIRTUAL SCREENING HITS

**Guillaume Coin (2), Angus E. McMillan (1), Esperanza Pearl (1), Oh-Hyeon Choung (1), Wilson W. X. Wu (1), Samuele Bordi (2), Paula L. Nichols (1,2), Jeffrey W. Bode (1), Benedikt M. Wanner (2),**

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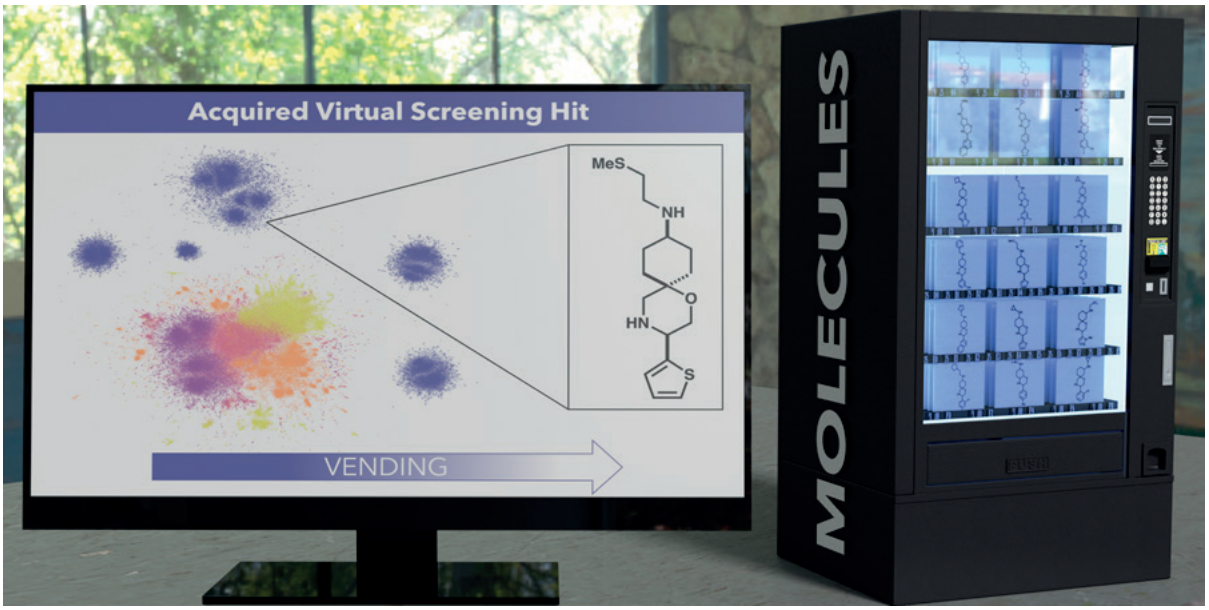


As a result of high false positive rates in virtual screening campaigns, prospective hits must be synthesised for validation. When done manually, this is a time consuming and laborious process. Large “on-demand” virtual libraries (>7 × $10^{12}$ members), suitable for preparation using capsule-based automated synthesis and commercial building blocks, were evaluated to determine their structural novelty. One sub-library, constructed from iSnAP capsules, aldehydes and amines, contains unique scaffolds with drug-like physicochemical properties. Virtual screening hits from this iSnAP library were prepared in an automated fashion for evaluation against Aedes aegypti and Phytophthora infestans. In comparison to manual workflows, this approach provided a 10-fold improvement in user efficiency. A streamlined method of relative stereochemical assignment was also devised to augment the rapid synthesis. User efficiency was further improved to 100-fold by downscaling and parallelising capsule-based chemistry on 96-well plates equipped with filter bases. This work demonstrates that automated synthesis consoles can enable the rapid and reliable preparation of attractive virtual screening hits from large virtual libraries.

# BIOLOGICAL ACTIVITY OF NOVEL ENVIRONMENTALLY COMPATIBLE 3-AMIDOQUINUCLIDINE QUATERNARY AMMONIUM COMPOUNDS

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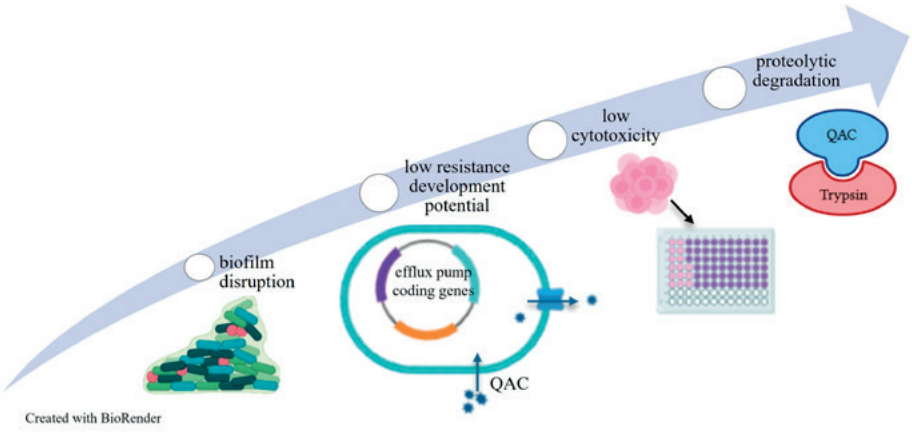
Since the early discovery of benzalkonium chloride in 1935, quaternary ammonium compounds (QACs) have been indispensable ingredients in antiseptic formulations, exhibiting a broad spectrum of antibacterial activity. Despite the wide variety of structurally diverse QACs, a notable drawback is their long-term chemical stability, which causes their accumulation in the environment and consequent initiation of bacterial resistance mechanisms. Therefore, scientists have turned to a new synthetic approach that leads to the preparation of soft QAC variants containing hydrolysis-prone functional groups incorporated into the QAC scaffold of interest.[1]

Our natural product-guided synthesis involved the functionalization of the quinuclidine backbone with a hydrolyzable amide bond containing long chains in its extension.[2] The synthesized 3-amidoquinuclidine QAC candidates showed potent antibacterial activity. When compared to commercially available QACs, they were less prone to induce bacterial resistance while displaying lower toxicity to healthy human cell lines. Docking study of the most potent compound suggests that the selected QAC may be a substrate for trypsin, implying possible enzymatic degradation of an amide bond. A comprehensive biological evaluation study of novel 3-amidoquinuclidine QACs points out eminent candidates as effective *soft* antimicrobial agents that do not compromise the environment or human health.

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# RUTHENIUM(II) COMPLEXES TARGETING AND PHOTO-OXIDIZING G-QUADRUPLEX DNA IN CANCER CELLS FAMILY

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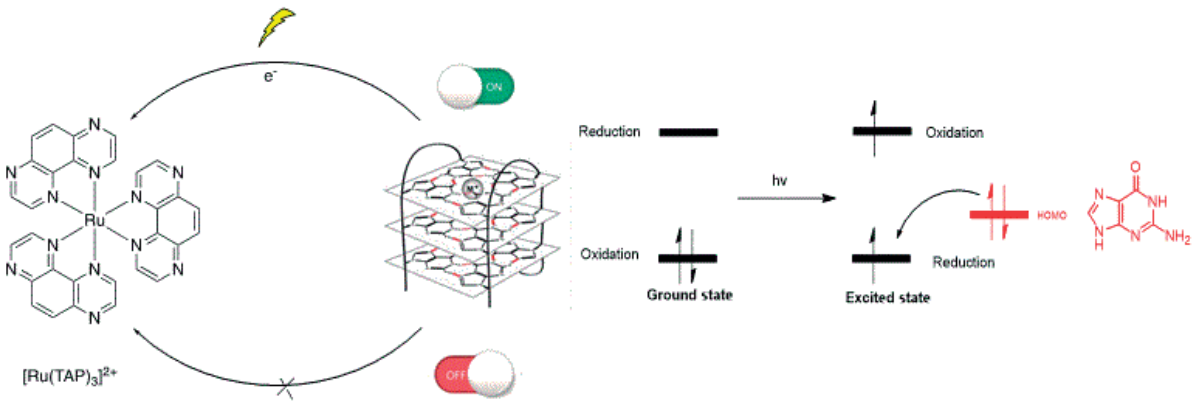
Cancer is a major issue of the XXIst century. It represents the major cause of death in developped countries, after cardiovascular diseases. The development of cancer cells generally starts by one (or more) lesion(s) in the genetic material. A number of anti-cancer drugs target the structure of DNA. However, most of these substances have lots of side effects. Therefore, the major challenge in research against cancer is to selectively target cancer cells without harming healthy ones. To achieve this goal, different strategies are used, and one of them is to use the light to selectively activate the molecules.

The purpose of this research project concerns the design, synthesis and study of photoactive compounds (organometallic complexes of iridium (III) and ruthenium (II)), which are able to interact and photoreact with DNA. In particular, the goal is to design molecules that selectively target G- quadruplex DNA, that is currently of great interest. Some complexes of iridium (III) and ruthenium (III) have the ability to be sufficiently oxidizing in the excited state, to extract an electron to a DNA base [1]. This electron transfer between the complex and the DNA can lead to irreversible DNA damages, in turn leading to cell death [2]. This research project is implemented between the Université catholique de Louvain (UCL - Prof. B. ELIAS), Université Grenoble Alpes (UGA - Prof. E. DEFRANCQ) and Institut De Duve (UClouvain - Prof. A. Decottignies) in order to benefit from mutual expertise of each laboratory

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# NEW METHODS OF SYNTHESIS OF SELF-IMMOLATIVE LINKERS OF CAMPTOTHECIN, FOR THE DEVELOPMENT OF hY1R SPECIFIC THERANOSTIC PEPTIDE-DRUG CONJUGATES

**Luke Darveniza (1,2), Kellie Tuck (1), Annette Beck-Sickinger (2), Brett Paterson (1,3)**

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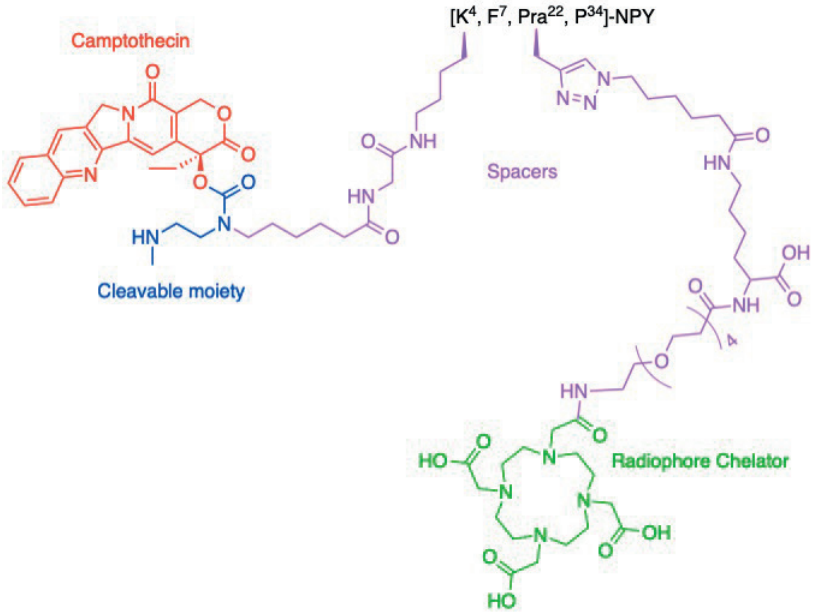
Camptothecin is a potent anti-tumour drug, however poor aqueous solubility and a lack of selectivity have hampered its use in modern clinical settings.[1] A lack of reactive functional groups has also severely limited its viability for the development of drug conjugates, a class of drugs that are attached to a targeting moiety, such as an antibody or a peptide. Peptide-drug conjugates involve the conjugation of a small-molecule drug to a receptor specific peptide via a cleavable linker.[2] This peptide acts as a shuttle system for the drug, delivering it to the receptor-abundant cancer site, avoiding the severe side effects caused by conventional therapy on healthy cells. Current methods of conjugation to camptothecin involve the formation of a carbamate or carbonate to a tertiary alcohol that is vital to the activity of the drug, requiring a traceless or 'self-immolative' cleavable linker in peptide-camptothecin conjugates. We aim to develop new synthetic methods for common self-immolative linkers, utilising solid-phase synthesis, in order to make these linkers more accessible for researchers worldwide. We then aim to attach camptothecin via these linkers to [$F^7$, $P^{34}$]-NPY, a modified variant of neuropeptide Y that is highly specific to $hY_1$ receptors in breast cancer.[3,4] These new water-soluble, highly specific peptide-drug conjugates will be tested on common breast cancer cell lines, with the aim to see minimal impact on the overall potency compared to pure camptothecin. We aim to also attach a DOTA conjugated linker to the peptide, in order to radiolabel the drug for animal studies, as well as to allow for potential theranostic capabilities during treatment.

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# DISCOVERY OF TRIAZOLO[4,5-d]PYRIMIDINES AS ANTAGONISTS OF THE HUMAN CC CHEMOKINE RECEPTOR 7 (CCR7)

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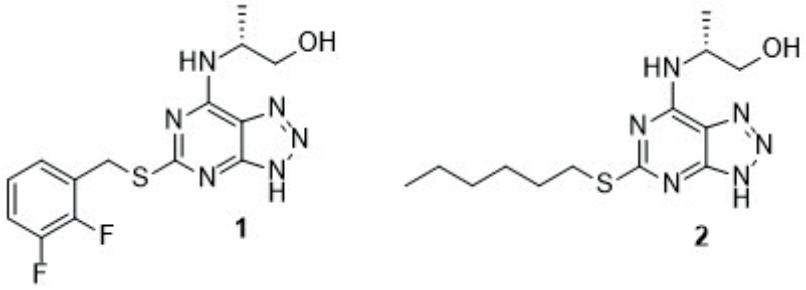
The human CC chemokine receptor 7 (CCR7) system is responsible for lymphoid organogenesis and for orchestrating the interaction between naïve T-cells and activated dendritic cells in the lymph nodes. Hence, CCR7 signaling is crucial for maintaining immune tolerance and mounting the adaptive immune response.[1] Dysregulation of CCR7 signaling has been implicated in various cancers,[2] where the tumor cells undergo CCR7-mediated metastasis to the lymph nodes. For example, in colorectal cancer elevated CCR7 expression correlates with increased lymph node metastases and poor survival. Leukemic T-cell infiltration in the central nervous system has also been shown to be CCR7 dependent. In addition, CCR7 signaling is involved in the onset and development of various inflammatory diseases (e.g. multiple sclerosis, rheumatoid arthritis and psoriasis).[2]

Despite the fact that CCR7 is involved in different human diseases, potent, selective and drug-like small molecule CCR7 antagonists remain elusive. Here, we will present our strategy towards the discovery of CCR7 antagonists. Several human chemokine GPCRs (e.g. CCR2, CCR7, CCR9, CXCR1 and CXCR2) share a conserved intracellular allosteric binding site in the G-protein and β-arrestin binding domain. Therefore, to identify new CCR7 antagonist chemotypes, we screened a proprietary compound library of CXCR2 antagonists that are known to target this intracellular pocket of CXCR2.[3] It led to the discovery of a triazolo[4,5-*d*]pyrimidine analogue (compound **1**) as a dual CXCR2/CCR7 antagonist, endowed with an $IC_{50}$ value of 2.43 µM against CCR7 and 0.66 µM against CXCR2. A systematic exploration of the structure-activity relationship (SAR) of the 3-, 5- and 7-position substituents of this scaffold resulted in an optimized triazolo[4,5-*d*]pyrimidine derivative (compound **2**), with improved potency and selectivity, displaying $IC_{50}$ values of 0.43 µM and 11.02 µM against CCR7 and CXCR2, respectively. The experimental data were rationalized by docking these triazolo[4,5-d]pyrimidines in the intracellular binding site of CXCR2 and CCR7.[4]

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# HIGH-THROUGHPUT SAMPLING OF CHEMICAL SPACE TO EXPLORE INHIBITION OF A CANCER-ASSOCIATED PROTEASE

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Fibroblast activation protein (FAP) is an important tumor antigen suitable for cancer tissue targeting *in vivo*. In our previous work, we established the most potent FAP inhibitors up-to-date and broadened the explorable chemical space within the P1' position by using an α-ketoamide warhead (Fig. 1).[1]

Despite high inhibition potency towards FAP, the ketoamide inhibitors lose selectivity over another homologous serine protease, prolyl endopeptidase (PREP). We decided to address that by exploiting the structure and activity relationship (SAR) of FAP inhibitors by a high-throughput approach.

We have validated direct testing of crude reaction mixtures as a suitable strategy to determine inhibition potency using two independent *in vitro* assays. This enables us to bypass compound isolation as the main bottleneck in SAR studies.

The synthetic approach with the modular last step allowed us parallel synthesis of several compounds on a small scale, providing concentrations still sufficient for inhibition assays. Parallel testing of the broad series of reaction mixtures gives us an opportunity to build up a complex SAR profile of FAP inhibition.

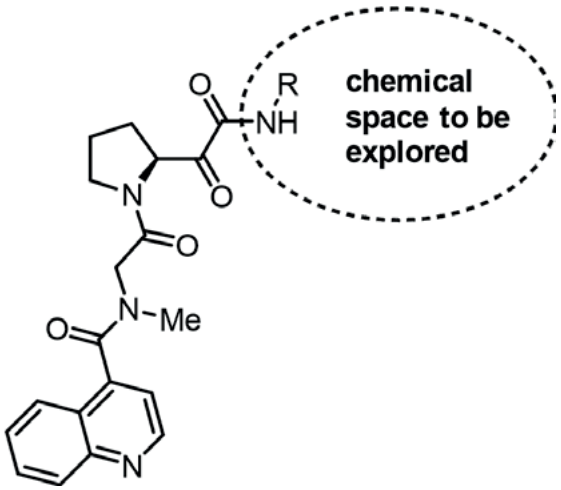


Fig. 1. General representation of FAP inhibitor series with varied P1´ position of the peptidomimetic scaffold.

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# DISCOVERY OF SMALL MOLECULES THAT DIRECTLY BIND THE HUMAN GERM CELL NUCLEAR FACTOR AND MODULATE ITS FUNCTION

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The germ cell nuclear factor (GCNF; NR6A1) is an orphan nuclear receptor that regulates reproduction, embryonic development and neuronal differentiation. GCNF is a known transcriptional repressor of pluripotency genes and was recently identified as a novel regulator of lipid metabolism in HepG2 cells. Here, we report synthetic ligands identified in a nuclear receptor-focused library screening that directly bind to GCNF, enhance corepressor peptide interaction, and act as agonist of GCNF-mediated transcriptional repression. Furthermore, the ligands modulate the expression of GCNF target genes in BeWo choriocarcinoma cells, HepG2 cells and i3 neurons differentiated from induced pluripotent stem cells (iPSCs). Neurons treated with these ligands have longer neurite outgrowth and more branching. Homology modeling, accelerated molecular dynamics simulations and docking of ligand hits reveal that the ligand binding pocket (LBP) of GCNF has the ability to expand to accommodate ligands and restrict the movement of the C-terminal helix when bound to ligand. Mutagenesis scanning of the LBP confirmed crucial residues that interact with ligands. The shape of the LBP in the GCNF model combined with mutagenesis scanning guided the design and synthesis of more potent ligand analogs. In aggregate, we have identified GCNF-specific ligands and synthetic analogs that may hold utility for modulating stem cell differentiation and neuronal function, as well as for developing potential treatments for cancer and neurodegenerative diseases.

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# THIO-ALLYL CATIONS AS VERSATILE REAGENTS TO CONSTRUCT FUNCTIONAL AND DIVERSE CYCLOPENTANOIDS

**Frederick Degroote, Bram Ryckaert, Brenda Callebaut, Jan Hullaert, Bram Denoo, Johan M Winne**

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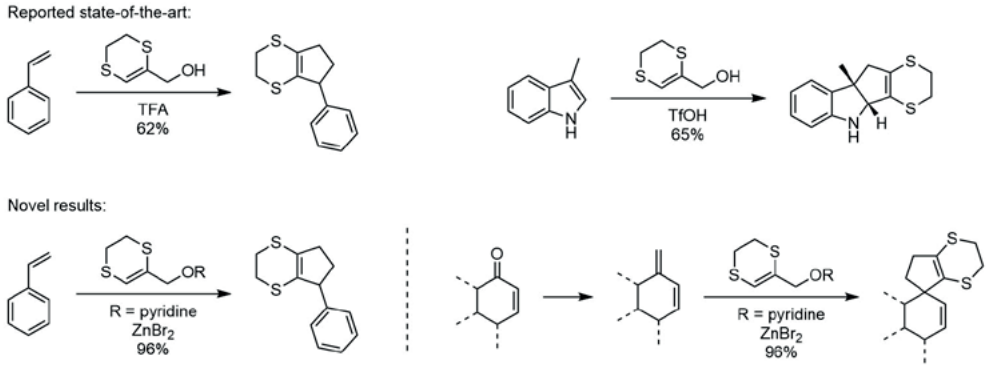
Although **all-carbon five-membered rings** are prevalent motifs found in both natural products and pharmaceutical compounds, their synthetic access routes remain scarce when compared to the amount of options towards all-carbon six-membered rings. In recent years, our research group has expanded this synthetic toolbox by leveraging **sulfur-heterocycles** as a key workhorse to prepare different odd-membered carbocycles, including seven- and three-membered ring products. Employing **cationic cycloadditions as a key cyclisation step**, the reported procedures exhibit high regio- and diastereoselective control over the formed products.




Herein, we present research that expands upon the substrate scope of the novel cationic cycloadditions. By using "traceless" heterocycles as a synthetic handle, recent insights have expanded upon the established reaction scope to include **all-carbon spirocycles**, which are not available using the normal reaction conditions. This spirocyclisation protocol provides an interesting decoration vector for the development of pharmaceutical compounds.

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# BIOINSPIRED SCAFFOLD ASSEMBLY VIA HETEROCYCLE-MEDIATED CYCLIZATIONS

**Ellen Demeyere, Prof. Dr. Johan Winne**

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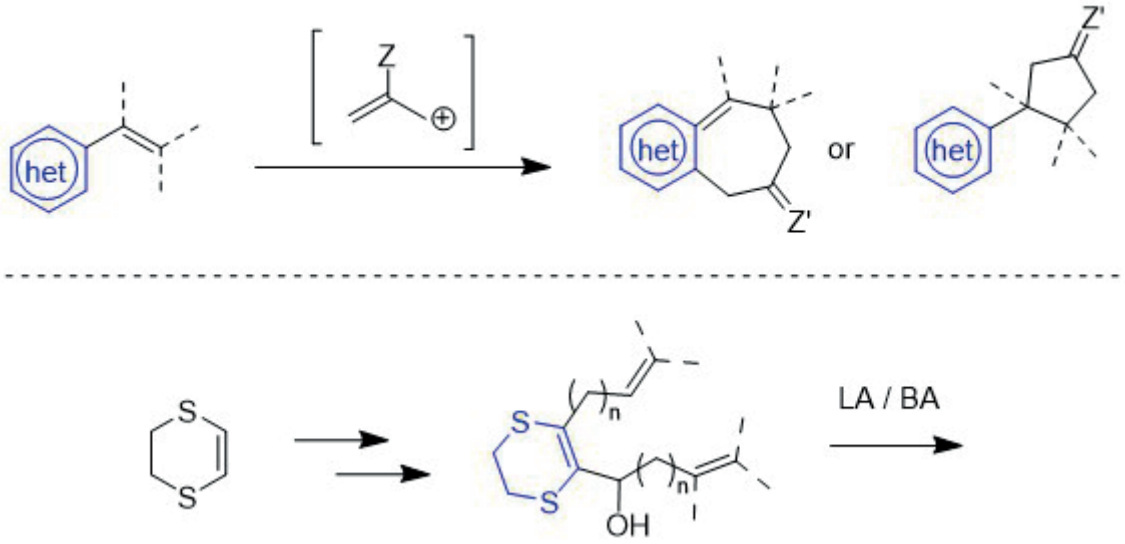
Terpenes all share a similar biosynthesis involving cationic cascade reactions, stabilized by enzymes, resulting in structurally well-defined three-dimensional scaffolds. Performing a biomimetic cyclization is however challenging since various side reactions can occur without the presence of an enzyme. Here, we report the stepwise development of novel reagent types and reactive heterocyclic building blocks that would allow controlled cationic carboannulation reactions. 2,3-dihydro-1,4-dithiine was selected as the main heterocycle since it has been previously employed in cationic (3+2) cycloadditions and because of its straightforward derivatization potential [1-2].

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# SULFONAMIDE-BASED AZANAPHTHOQUINONE HYBRID MOLECULES: PROMISING ANTIMICROBIAL AGENTS

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*2) İstanbul University-Cerrahpasa, Faculty of Engineering, Department of Chemistry, Avcılar, 34320, Istanbul, Turkey*
*3) Gebze Technical University, Chemistry Department, Gebze, 41400, Kocaeli, Turkey*
*4) İstanbul University, Pharmacy Faculty, Pharmaceutical Microbiology Department, Beyazit, Istanbul, Turkey*

Molecular hybridization strategy containing biologically active two or more pharmacophores generates novel hybrid molecules with strong biological activities.[1] Structural modifications of the hybrid molecules have led to promising leads in the chemotherapeutic field and, for that reason, it remains an attractive way to accelerate drug development processes. Nitrogen-containing 1,4-quinones constitute common structural motifs in a wide range of complex compounds. Among them, azanaphthoquinone are highly important organic molecules because of their broad spectrum of biological activity and wide spread natural occurrence. They are often used as anticancer, antidepressant, anti-inflamatory, antiviral, and antibacterial agents as well as acyltransferase inhibitors. Design, synthesis, and spectroscopic characterization of aminated azanaphthoquinones were carried out. In continuation of our earlier works,[2,3] the exploration of *in vitro* antimicrobial activity of sulfonamide-based azanaphthoquinone hybrid molecules *via* molecular hybridization strategy along with *in silico* evaluation has been reported for the first time. In addition, the extensive antimicrobial activity assessment of the sulfonamide-based azanaphthoquinone hybrid molecules was determined. The evaluation of antimicrobial activity indicated that most of the synthesized hybrid molecules exhibited superior biological potency against tested Gram-positive bacterial strains, including *Staphylococcus aureus* (ATCC® 29213), *Staphylococcus epidermidis* (ATCC® 12228), and *Enterococcus faecalis* (ATCC® 29212). Moreover, detailed antibiofilm activity of the selected hybrid molecules was further addressed against both *S. epidermidis* and *S. aureus*.

This work was financially supported by the Scientific Research Projects Coordination Unit of İstanbul University (Project number: FYO-2021-38298) for supplying the equipment and materials.

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# ANTIPLASMODIAL ACTIVITY OF NOVEL 7-CHLORO-4-AMINOQUINOLINE-BENZIMIDAZOLE HYBRIDS

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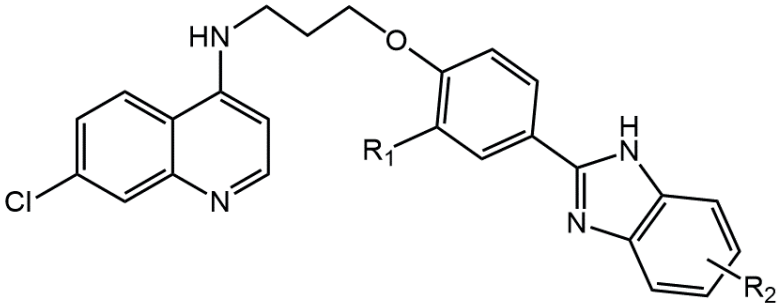
Due to the increasing resistance to currently available antimalarial drugs, there is an urgent need for more effective and safer agents [1]. One of the strategies used to search for new antimalarial compounds is molecular hybridization of two moieties possessing the desired biological activity, into a single compound [2]. As a part of our ongoing efforts to prepare novel quinoline hybrid compounds [3,4] herein we present synthesis of novel quinoline-benzimidazole hybrids **1**-**6**, and evaluation of their antiplasmodial activity *in vitro* against the erythrocytic stage of the *Plasmodium* life cycle (Figure 1). Non-amidine compounds, **1**, **2**, **4** and **5** exerted higher activity than their amidine counterparts **3** and **6.** Most active compounds (**1** and **2**) had $IC_{50}$ values between 1 and 5 nM.




| Comp. | 1 | 2 | 3 | 4 | 5 | 6 |
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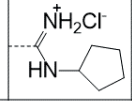
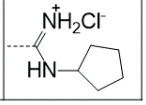
| $R_2$ | H | Cl |  | H | Cl |  |

Fig. 1: Novel quinoline-benzimidazole hybrids.

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# REPURPOSING ATP-COMPETITIVE TOPOISOMERASE IIα INHIBITORS FOR SELECTIVE Hsp90β INHIBITION

**Jaka Dernovšek (1), Nace Zidar (1), Janez Ilaš (1), Dunja Urbančič (1), Asta Zubriene (2), Daumantas Matulis (2), Tihomir Tomašič (1)**

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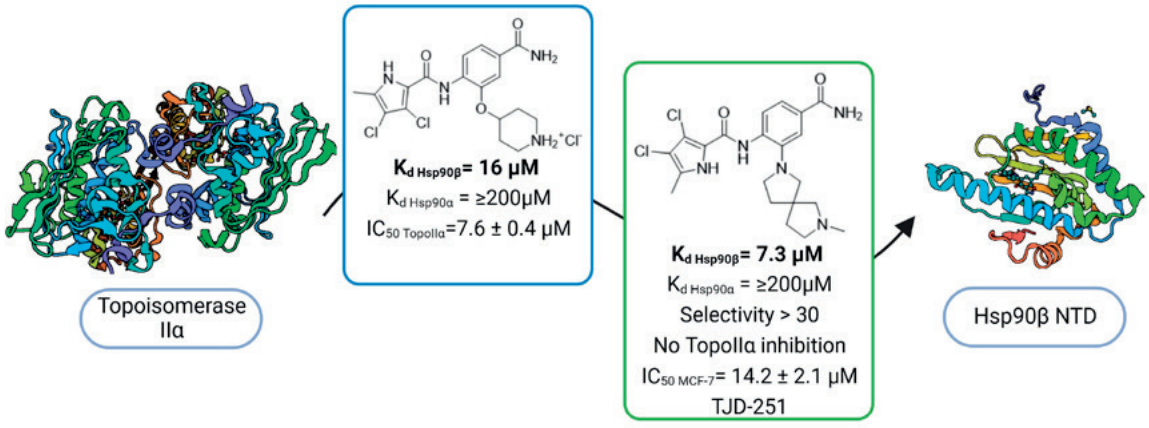
Since the discovery of the first N-terminal ATP-competitive inhibitor of Hsp90, geldanamycin, the Hsp90 family of proteins has been investigated as a potential target for the treatment of cancer and many different inhibitors have entered clinical trials. However, it has been found that equal effect on all four members of the Hsp90 family (the mitochondrial TRAP-1, the endoplasmic reticulum localised Grp94, and the cytoplasmic Hsp90α and Hsp90β) can lead to an induction of the heat shock response (HSR). This in turn may attenuate the anticancer effect of pan-Hsp90 inhibitors. To circumvent the HSR induction of pan-Hsp90 inhibitors, a switch was made to the design of inhibitors selective for only one of the four paralogues. However, the structure of the entire Hsp90 family is highly conserved. In particular, the cytoplasmic isoforms Hsp90α and Hsp90β are approximately 85% homologous and show an astonishing 95% identity when considering only the N-terminal ATP-binding site. Therefore, it is not surprising that only two structural classes of Hsp90β and one structural class of Hsp90α have been described so far and consequently new approaches to develop these inhibitors are in great demand. Looking at the binding sites of the GHKL (**G**yrase, **H**sp90, Histidin **K**inase and Mut**L**) protein family, a very distinct Bergerat fold makes the pockets susceptible to the binding of similar inhibitors.[1]

For this reason, the aim of our research was to repurpose known inhibitors of topoisomerase IIα prepared by our group[2] and redesign them into Hsp90β selective compounds. Indeed, our hit compound ($IC_{50}$ (TopoIIα) = 7.3 ± 0.4 μM) from the "in-house" inhibitor library was shown to bind Hsp90β with a $K_d$ value of 16 μM (CI:[11 μM;24 μM]). Simultaneously, the binding affinity to Hsp90α was significantly weaker ($K_d$ ≥ 200 μM). Therefore, we prepared a focused library of analogues to explore the structure-activity relationship and attempt to improve the affinity and the selectivity of the hit compound. Our efforts resulted in an inhibitor TJD-251 with no binding affinity for topoisomerase IIα, with an increased affinity for Hsp90β ($K_d$= 7.3 μM – CI:[4.8 μM;11 μM]) and with more than 30-fold selectivity for this isoform when compared to Hsp90α. Additionally, the compounds that inhibited either of the two cytoplasmic isoforms were evaluated for their antiproliferative activity against breast cancer cell line MCF-7 and many were shown to inhibit growth in the low micromolar range.

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# DISCOVERY OF FULLY SYNTHETIC FKBP12-MTOR MOLECULAR GLUES

**Robin C.E. Deutscher (1), Christian Meyners (1), Sabine C. Schäfer (1), Maximilian L. Repity (1), Wisely Oki Sugiarto (1), Tim Heymann (1), Thomas M. Geiger (1), Felix Hausch (1,2)**

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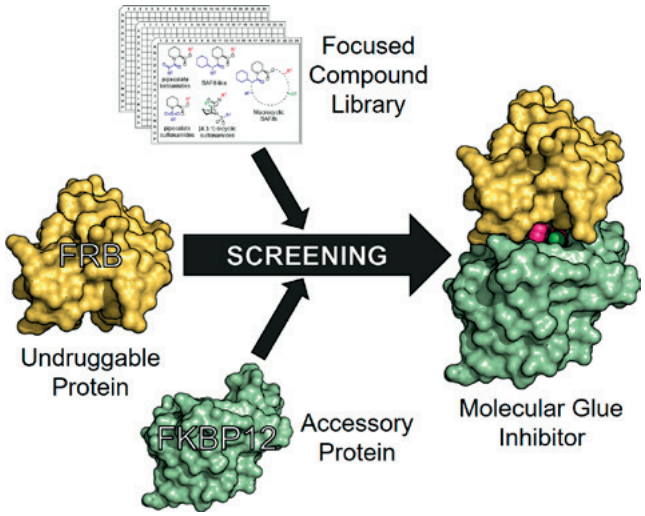


Molecular glues are a class of drug modalities with the potential to engage otherwise undruggable targets. However, the targeted discovery of molecular glues for desired targets is a major challenge and most known molecular glues have been discovered by serendipity.[1,2,3] Here we present the first fully synthetic FKBP12-mTOR molecular glues, which were discovered from a FKBP-focused ligand library. Our biochemical screening of >1000 in-house FKBP ligands, using a HRTF-assay, yielded one hit that dimerized FKBP12 and the FRB domain of mTOR. The cocrystal structure of the ternary complex revealed the binding mode. The hit bound to the same surface area as rapamycin, but with radically different interaction pattern. As the inital hit was very weak with a complex initiation IC50 of 93 μM we improved potency 500-fold (to 180 nM), aided by the crystal structure. The resulting substances initiated FKBP12-FRB complex formation and pS6 kinase inhibition in cells. Our results show that molecular glues targeting flat surfaces, although deemed rare, can be discovered by screening of a small focused ligand library. Weak initial hits can be optimized substantially by structure guided design as shown by our example. Our findings highlight the advantages of relying on FKBP12 as accessory protein and support the use of FKBP12 as a versatile accessory protein for molecular glues.

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# THE C9ORF72 HEXANUCLEOTIDE REPEAT EXPANSION AGGREGATES BY MEANS OF MULTIMOLECULAR G-QUADRUPLEX FORMATION

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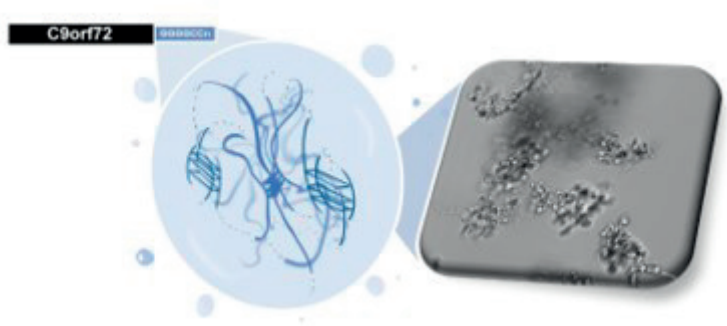
Amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) are just two of the neurodegenerative diseases characterised by the presence of pathological aggregates[1]. 40% of familiar cases in ALS and FTD have been correlated to expansion of the hexanucleotide repeat $(GGGGCC)_n$, which has been previously shown to assemble into G-quadruplexes (G4s) structures[2]. In this talk I will discuss our recent work where we investigated the role of nucleic acids and secondary structure formation in the generation of ALS/FTD aggregates, something that has often been relegated as a peripheral effect in the protein-led aggregation[3]. We showed a correlation between the emergence of multimolecular G4s (mG4s) formed by the DNA $(GGGGCC)_n$ repeats and the formation of protein free insoluble aggregates. Aggregation is dependent on $K^+$ concentration and repeat-length, indicating that G4-formation is essential to observe aggregates. G4-structures were detected in the aggregates by staining with the G4-specific fluorescent dye NMM. G4-unfolding promoted by NMM-mediated guanine photo-oxidation led to prompt disassembly of the insoluble aggregates, further confirming a G4-based aggregation mechanism. To reinforce the physiological relevance of our observations, we characterised the aggregation of RNA $(GGGGCC)_n$, which is thought to contribute to pathological aggregation in ALS/FTD. We observed that RNA repeats can aggregate at significant lower concentrations compared to DNA, suggesting that under physiological conditions RNA repeats can aggregate in the absence of any protein. Our findings constitute the first evidence supporting the formation of multimolecular G4-structures to drive protein-free aggregation, highlighting their potential as therapeutic target to for the treatment of ALS and FTD and revealing the potential of RNA targeting for the treatment of neurodegenerative diseases.[3]

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# SYNTHESIS OF CIPROFLOXACIN-FERROCENE CONJUGATES AND ITS FORMULATION AS A THERAPEUTIC DEEP EUTECTIC SOLVENT

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The outstanding antibacterial activity of ciprofloxacin, its efficient pharmacokinetics, and negligible side effects have led to widespread use of that fluoroquinolone antibiotic. The excessive use of ciprofloxacin has led to the emergence of bacterial resistance. Modification of the structure of existing fluoroquinolones is a good and promising strategy to find an alternative drug with high efficiency and minimal toxicity[1]. The incorporation of 1,2,3-triazoles as attractive linker units between two pharmacophores leads to innovative bifunctional drugs. This is an increasingly useful and important approach for the construction of bioactive and functional molecules.[2] The introduction of carboxymethyl in the *N*4 position of the piperazine ring[3] as a bridge between ciprofloxacin and ferrocene may lead to an increase in its antibacterial activity. Herein, we have described a preparation of compounds type **I** and **II** (Figure 1) and the target products were confirmed by spectroscopic analysis (1D and 2D NMR, FTIR). After successful synthesis of novel compounds, the possibility of developing a formulation of the drug in the form of therapeutic deep eutectic solvents (THEDES)[4] will be examined. It is expected that such an approach will enable better solubility, permeability,and bioavailability of the active compound, with conceivably better stability and preserved or even enhanced activity[5].

**Acknowledgements:** The work was supported by the Croatian Science Foundation (Grant No. 7712)

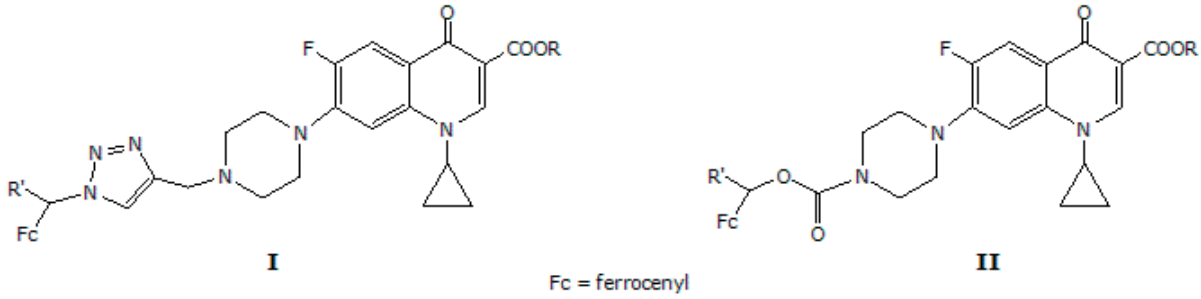


**Figure 1.** Ferrocenyl-ciprofloxacin conjugates **I** and **II**

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# SYNTHESIS AND INVESTIGATION OF ENZYME INHIBITION OF POTENTIAL DUAL COX-2 AND 5-LOX INHIBITORS

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The pathogenesis and progression of a various diseases, such as cancer, rheumatoid arthritis, autoimmune, cardiovascular and neurodegenerative diseases, are closely related to chronic inflammatory processes. Prostaglandins and leukotrienes are among the most important inflammation mediators, produced from arachidonic acid by the enzymes cyclooxygenases (COX) and lipoxygenases (LOX). COX-2 enzyme is expressed in many types of cancer and it has a role in the proliferation, angiogenesis, invasion, metastasis, inhibition of apoptosis of cancer cells, as well as in the occurrence of resistance to radiotherapy and chemotherapy. In addition, 5-LOX enzyme is related to cancer growth-promoting effects. The poor anticancer efficacy of COX-2 inhibitors against certain tumor types can be explained by the fact that the blockade of one enzyme pathway potentiates another, from which it can be concluded that blockade of both pathways (COX-2 and 5-LOX) is a good approach to effective anticancer therapy [1-3].

The aim of this study was synthesis of new dual COX-2 and 5-LOX inhibitors by modification of commonly used COX inhibitors (indomethacin, diclofenac, flurbiprofen, ibuprofen and naproxen) and investigation of their enzyme inhibition potential.

Compounds 1, 2 and 3 (Figure 1) were synthesized by Curtius rearrangement reaction of corresponding COX inhibitors, followed by the addition of hydroxylamine. Compounds 4 and 5 were synthesized by a four-step procedure, starting from ibuprofen- and naproxen-like ketones. Compounds 6 and 7 were synthesized by introduction of thiourea side chain into the structure of naproxen. COX-1, COX-2 and 5-LOX inhibitory potential was investigated using in vitro fluorometric enzyme inhibition kits. It can be concluded that compounds 1-5 possess dual COX-2 and 5-LOX inhibitory activity and low activity towards COX-1 ($IC_{50}$ > 100 μM), i.e. good COX-2/COX-1 selectivity. Modification of naproxen side chain (compounds 6 and 7) resulted in the occurrence of 5-LOX inhibitory activity, while COX-2 inhibitory activity was significantly reduced ($IC_{50}$ > 100 μM).

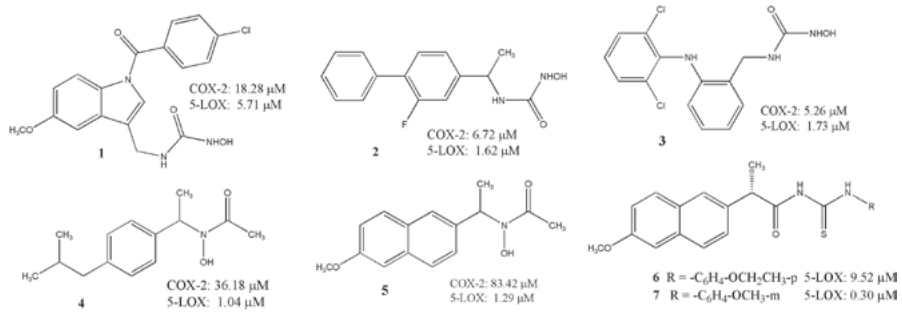


Figure 1. Chemical structures and enzyme inhibitory activity ($IC_{50}$ values) of synthesized compounds

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# [1,4]-SUBSTITUTED AZEPINES: OPTIMISING ACCESS TO BIOLOGICALLY ACTIVE TRICYCLICS

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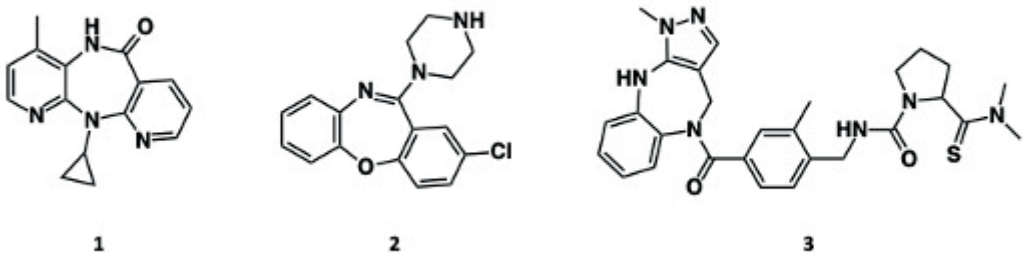
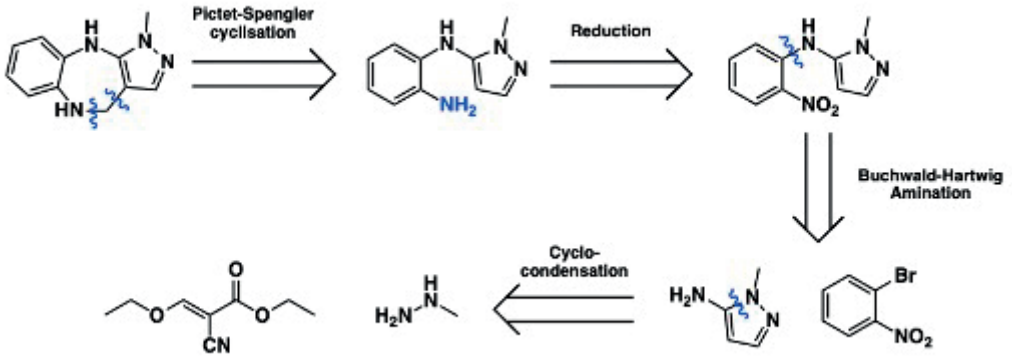
*University of Sydney*

Heteroatom containing 1,4-azepines are an exciting structural motif in medicinal chemistry due to their biological activity over a broad range of ailments in mammalian systems. Current pharmaceutical examples include the HIV-1 non-nucleoside reverse transcriptase inhibitor nevirapine (1) and tricyclic antidepressant amoxapine (2).[1,2] More recently, drug discovery programs have also identified LIT-001 (3) as a non-peptide agonist for the oxytocin receptor to treat depression and anxiety.[3]



1 2 3

A key feature of these molecules is the benzodiazepine motif, of which a five-step syntheses for pyrazolo[3,4]benzodiazepine has previously been reported.[4] Optimisation of the synthetic route to obtain this chemically diversifiable handle is of high interest and herein we report a new method of access to this interesting structural motif.

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# ENANTIOSELECTIVE SYNTHESIS OF 3-ARYL-3-HYDROXYPROPANOIC ESTERS AS SUBUNITS FOR CHIRAL LIQUID CRYSTALS

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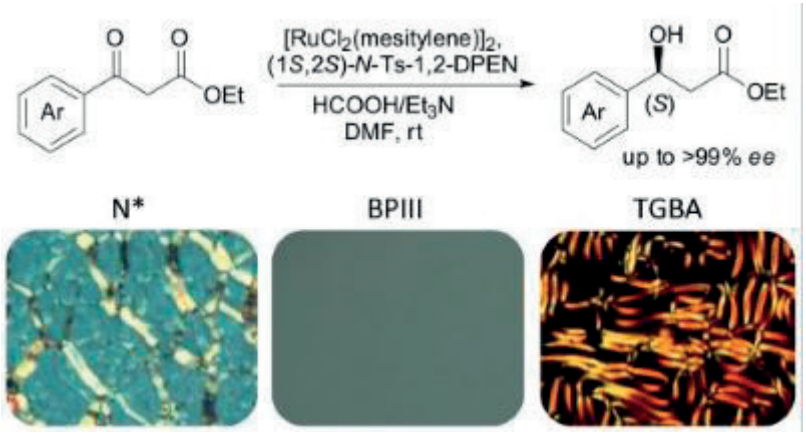
Liquid crystals (LCs) are dynamic functional soft materials that share the anisotropic properties of the crystalline state and the fluidity of a liquid. Chiral LCs with their unique optical and mechanical properties are perspective functional soft materials for fundamental science and advanced technological applications.[1,2] The introduction of chirality into LCs results in various chiral liquid crystalline phases such as the cholesteric, chiral smectic, twist grain boundary (TGB), and blue phases (BP), each with unique properties. [3,4] Herein, we introduce the chiral 3-aryl-3-hydroxypropanoic ester moiety as a versatile building block for the preparation of LC compounds. Three chiral 3-aryl-3-hydroxypropanoic ester subunits differing in aromatic part were obtained through asymmetric transfer hydrogenation using Ru(II) complexes in 98 - >99% ee.[5] These subunits were further used for preparation of chiral LC compounds of diverse topologies without deterioration of the ee during the synthesis. Mesomorphic behaviour of final LCs was investigated - chiral nematic and smectic phases were identified, as well as rarely observed twist grain boundary A and blue phases. The utilization of synthetic chiral building blocks offers the possibility of fine-tuning the intermolecular interactions by subtle changes in the molecular structure, as well as the preparation of corresponding racemic forms. This paves the way for the study of self-organization and the structure-property relationship in chiral soft materials.

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# 3CL PROTEASE, A PROMISING TARGET FOR ANTI-COVID-19 THERAPY. EXPRESSION, PURIFICATION, AND ASSAY DEVELOPMENT

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The prolonged global COVID-19 pandemic has prompted the development of new drugs against the SARS-CoV-2 virus. The so-called main protease of the virus, 3CLPro (3-chymotrypsin-like protease), is essential for the cleavage of polypeptides generated during viral RNA transcription. This enzyme is an ideal drug target for SARS-CoV-2 because of its different nature from cellular proteases. A major effort is underway worldwide to discover effective 3CLPro inhibitors by repositioning earlier antiviral agents (e.g. rupintrivir) and identifying novel agents (such as Pfizer's lufotrelvir and nirmatrelvir), which in combination with ritonavir was registered in 2021 as an orally active drug under the brand name Paxlovid.

Domestic research into the identification of 3CLPro inhibitors was also initiated early on, making it necessary to produce the protein for biological screening assays and to develop an *in vitro* assay capable of testing large numbers of molecules.

The 3CLPro enzyme was expressed in *E. coli* Rosetta 2 strain using pPAL7 plasmid expression vector. The recombinant 3CLPro protein was purified on an FPLC system using Mini Profinity eXact stationary phase for affinity chromatography.

For the enzyme activity assays, a FRET (Fluorescence Resonance Energy Transfer) assay was developed. For the measurements, a FRET peptide substrate and a covalent 3CLPro inhibitor (5-chloropyridine-3-ylbenzo-(*b*)-thiophene-2-carboxylate) were used at a final concentration of 10 μM as an inhibitor control. The signal was detected on a 96-well plate using a fluorescent microplate reader. Drug candidate compounds were first screened at a final concentration of 100 μM. Hits with greater than 50% inhibition were tested in a dose-response assay of 6-8 data points using a two-fold dilution series, and $IC_{50}$ values were determined by logistic curve fitting.

Biological screening of potential antiviral compounds synthesized at University of Debrecen, two carotenoid conjugates[1] of the pseudoaglycone teicoplanin showed promising 3CLPro inhibitory activities ($IC_{50}$: 14 and 34 μM). In addition, it was recently found that teicoplanin derivatives also showed good to excellent activity against Gram-positive bacteria resistant to all approved glycopeptide antibiotics.[2]

The authors would like to thank Ferenc Jakab and Henrietta Pap (National Virology Laboratory, Pécs) for the antiviral activity measurements.

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# DEVELOPMENT OF QUINOLINE PHOTOCAGES FOR BIOLOGICAL APPLICATIONS

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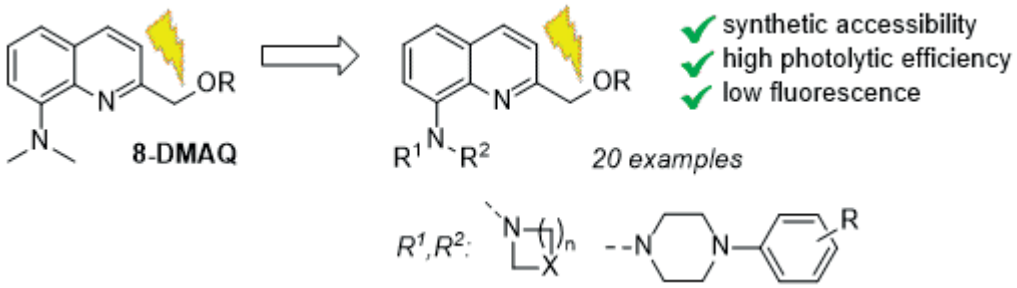
Photoremovable protecting groups („photocages") allow the precise, on-site as well as temporal/spatial selective release of biologically active agents, exploiting a light stimulus for the cleavage of a covalent bond between the protecting group and the substrate in question (1,2). Besides the original neuroscientific applications, different small molecule drugs could be the substrates (3). Moreover, photocages can be used for the design of more complex drug delivery systems, following different scenarios (4). Quinoline is a privileged scaffold in medicinal chemistry and for the design of fluorescent probes. Quinoline-derived photocages were first described by Dore et al (5). Importantly, these readily modifiable structures are characterized by efficient two-photon photolysis.




In the present work we aimed to prepare and characterize novel quinoline photocages. Inspired by the 8-dimethylaminoquinoline (8-DMAQ) caging chromophore, photocleavable 8-aminoquinoline derivatives were prepared with the aim of furnishing a robust synthetic pathway for novel cage scaffolds. Although efficient reference compounds of the same class were described in the literature (e.g. TMP-CyHQ, CyHQ, BHQ), their synthesis often necessitate several steps, offering modest overall yields. Our four-step, scalable synthesis could be therefore of significant practical relevance. In the applied synthetic pathway, the selenium dioxide oxidation step proved to be challenging, therefore other alternatives were tested. The novel photocages showed high photolysis efficiency and low fluorescence, comparable to literature reference compounds, demonstrating the utility of the probes for uncaging applications. Using amino groups allowing further functionalizations, the novel photocages could also serve as building blocks for the design of drug delivery systems, Moreover, to study the photolysis mechanism, density functional theory calculations were performed.

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# DEVELOPMENT OF A COUNTER ION DIRECTED STEREOSELECTIVE AZA-PRINS MULTICOMPONENT REACTION

**Lovisa Dybeck, Luke Odell**

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During the last decade there has been an increasing interest in multicomponent reactions as an atom economic tool to generate diverse bioactive compounds.[1] With a multicomponent strategy, it is possible to assemble several moieties in a simple cascade reaction without any additional reaction steps. In particular, nitrogen-containing heterocycles are well-known for their broad biological applications and desirable properties in drug development.[2] As a means to synthesize such *N*-heterocycles the aza-Prins cyclization reaction has for long been a useful synthetic pathway.[3] The aza-Prins reaction represents a powerful strategy to assemble piperidine derivatives from an aldehyde, butene amine and a nucleophile. Generally, strong Lewis or Brønsted acids are needed to catalyze this reaction. However, we have previously described the use of acetic acid as a promotor in the aza-Prins cyclization of an *o*-formyl aldehyde and a homoallylic amine, forming a dihydroquinazolinone scaffold. Additionally, acetic acid serves as a nucleophile in the reaction, constituting a convenient route to ester functionalized *N*-heterocycles.[4] Herein we present the synthesis of similar dihydroquinazolinones through an aza-Prins reaction which, on the contrary, can be conducted with the amine hydrochloride, directing the counter ion to act as a nucleophile. However, since acetic acid itself is too nucleophilic it will pose as a competing agent to the chloride ions. Through careful optimization, chloroacetic acid was discovered as a selective promotor, which prevents ester functionalization and further on enables the incorporation of other nucleophiles when using the neutral amine. Our work proves how this multicomponent reaction can be readily used to synthesize fused dihydroquinazolinone piperidine motifs in a diverse and highly stereoselective manner.

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# ANTI-ALZHEIMER PROPERTIES OF IMIDAZOLINE I2 RECEPTOR LIGANDS

**Carmen Escolano (1), Carla Barbaraci (1), Andrea Bagán (1), Christian Grinan-Ferre (2), Merce Pallas (2), Jose Angel Morales-Garcia (3), Katarina Nikolic (4), Jose Brea (5), Maria Isabel Loza (5)**

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Imidazoline $I_2$ receptors ($I_2$-IR) are heterogeneous entities that can be found in central nervous system and many other organs, and its pharmacological description relies in their modulation by high affine and selective ligands (1). Two $I_2$-IR ligands are progressing in clinical trials, BU99008, for PET in patients suffering from Alzheimer's disease (AD), and CR4056, for osteoarthritis. We described the potential of the $I_2$-IR ligand LSL60101, as a disease-modifying single therapy for the treatment of AD, and we also synthesized and pharmacologically characterized a family of congeners (2,3). The chemical structure of standard $I_2$-IR ligands was restricted to 2-heterocyclic-2-imidazolines (idazoxan, tracizoline, BU224, 2-BFI) until we open the structural chemical space by describing a family of (2-imidazolin-4-yl)phosphonates (4). The treatment with a representative compound MCR5 of an age-related cognitive decline murine model, the senescence-accelerate mouse prone 8 (SAMP8), resulted in cognitive improvement and amelioration of hallmarks of AD, including the behavioral and psychological symptoms of dementia. This work was the first experimental evidence that proposed $I_2$-IR as a new therapeutic target for AD (5,6). In addition, MCR5 also improved age-related endothelial dysfunction in an animal model (7). We provided a family of bicyclic iminophosphonates (8), with a selected compound B06 that ameliorated the cognitive decline and improved the behavior of two murine models, SAMP8 and the familial AD (5xFAD) (9). Furthermore, B06 showed beneficial *in vitro* ADME-Tox properties and neuroprotective/anti-inflammatory properties as well as beneficial effect in an *in vitro* model of PD (10). The 3D-QSAR studies of the new families led us to propose the pharmacophore.

Here we report a new family of bicyclic a-phosphoprolines that showed high affinity and selectivity upon $I_2$-IR and good BBB permeation. We evaluated three selected new compounds in dopaminergic neurodegeneration and neuroinflammation cellular models. The good results led us to take the challenge to carry out the first study of $I_2$ -IR ligands in *Caenorhabditis elegans* as an *in vivo* AD model organism.

Acknowledgements:

This work was supported by PDC2022-133441-I00 (MCIN/AEI/10.13039/501100011033 and the European Union NextGenerationEU/PRTR).

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# PHARMACOPHORIC ANALYSIS OF ANTICANCER ACTIVITY OF ISOPRENOIDS AND TERPENES ISOLATED FROM SCHINUS MOLLE L.

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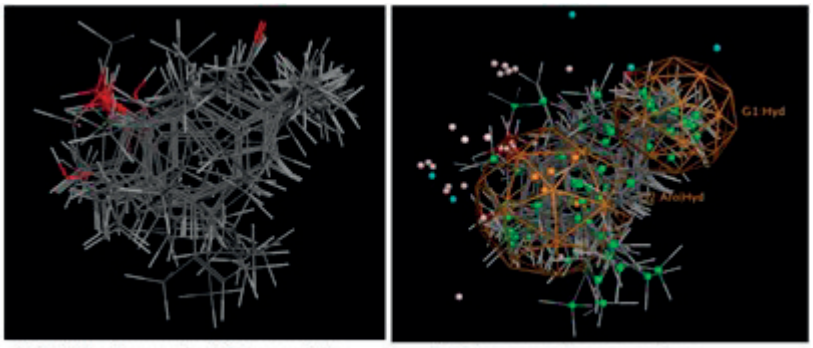
*Programa Centro de Investigaciones biomédicas y aplicadas (CIBAP) , Escuela de Medicina, Facultad de Ciencias Médicas, Universidad de Santiago de Chile , Santiago, Chile*

*Schinus molle L.* (Family: Anacardiaceae), is native species that found in Northern South America, Peru's Andean deserts and Central Chile and Argentina [1]. *S. molle* is recognized for its anti-rheumatic, antiseptic, anti-inflammatory, antifungal, antibacterial, cicatrizing activities, for the treatment of skin disorders and antitumoral properties [2-5]. The essential oils (EOs) from leaves and fruits are composed by terpenoids (monoterpenes and sesquiterpenes) and phenylpropanoids. The full and fractions of EOs and some isolated terpenoids inhibits tumor cell growth or induce cancer cells death by apoptosis [2-12]. The evidence in relation to the anticancer activity of EOs from *S. molle* is extensive but carried out under different experimental conditions. We recollect data from 20 isoprenoids and terpenes isolated from *S. molle* EOs [2-12], with known IC50 values for their cytotoxic activity against MCF-7 (human breast cancer cell line). Computational studies including energy optimization with MMFF94x forcefield and low energy conformers generation were done on MOE 2009. QSAR were performed using GA-MLR and AutoQSAR MOE-MLR. The flexible alignment and consensus pharmacophore were obtained using an unified scheme. The pharmacophore analysis of the compounds (Figure A flexible alignment and B consensus pharmacophore) showed that the common structural features presented in the compounds are the presence of one hydrophobic centroid and one aromatic/hydrophobic moieties. The best QSAR models for the anticancer activity were obtained using GA-MLR and this data will be used for virtual screening.




A. Flexible alignment of 20 terpenoids B. Consensus pharmacophore

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# FIRST-IN-CLASS SELECTIVE INHIBITORS OF THE LYSINE ACETYLTRANSFERASE KAT8

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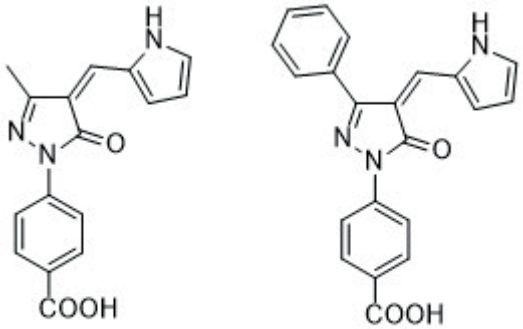
KAT8 is a lysine acetyltransferase primarily catalyzing the acetylation of Lys16 of histone H4 (H4K16). KAT8 dysregulation is linked to the development and metastatization of many cancer types, including non-small cell lung cancer (NSCLC) and acute myeloid leukemia (AML) [1-4]. Nonetheless, few KAT8 inhibitors have been reported so far, none of which displaying selective activity. Based on the structure of the KAT3B/KDAC inhibitor C646, we developed a series of *N*-phenyl-5-pyrazolone derivatives and identified compounds **19** and **34** as low-micromolar KAT8 inhibitors selective over KAT3B, KAT2B, and a panel of KDACs. Western blot and immunofluorescence experiments demonstrated that both inhibitors selectively target KAT8 in cells. Moreover, **19** and **34** exhibited mid-micromolar antiproliferative activity in different cancer cell lines, including NSCLC and AML, without impacting the viability of non-transformed cells. Overall, these compounds are valuable tools for elucidating KAT8 biology, and their simple structures make them promising candidates for future optimization studies.

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# DESIGN, SYNTHESIS AND EVALUATION OF NOVEL INDAZOLE BChE/p38α MAPK DUAL INHIBITORS FOR THE TREATMENT OF ALZHEIMER'S DISEASE

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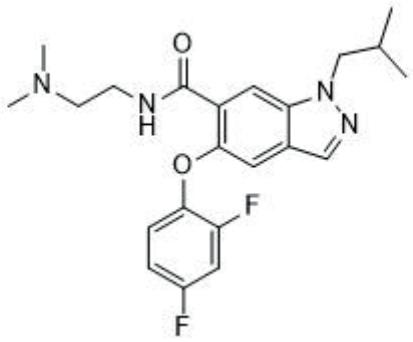
Alzheimer's disease (AD) is a progressive neurodegenerative disorder and represents a major cause of dementia. (1). Despite recent approval of amyloid beta (Aβ) targeting monoclonal antibodies aducanumab and lecanemab as novel therapeutics on the market, lack of convincing results and side effect such as vasogenic edema prevent the majority of biological drugs targeting AD to enter final stages of clinical trials. Design of novel small-molecule inhibitors responsible for slowing down early processes in AD therefore presents a major challenge in medicinal chemistry(2) .

Although a multitude of hypothesis tries to explain complex events in AD patophysiology, they all coalesce in the nowadays most extensively studied neuroinflammation hypothesis(3). Among many enzymes that are overexpressed in the process of microglial neuroinflammation p38α MAP kinase (p38α MAPK) recently gained more attention. This ubiquitous enzyme further up-regulates proinflammatory cytokines such as TNF-α and IL-1β, prevents BACE1 degradation which in turn promotes Aβ accumulation and drives the neurotoxic tau protein master site hyperphosphorylation(4–6). This in turn makes it an interesting pharmacological target to combat AD.

As trends in medicinal chemistry have moved towards multi-target directed ligands our aim is to design a dual inhibitor covering targets from both the neuroinflammation as well as the older cholinergic hypothesis by developing a dual BchE/p38α MAPK inhibitor. This way affect the patophysiological processes happening years before AD symptoms can be noticed as well as alleviate them when they appear. First, a library of small molecules with confirmed activity against p38α MAPK was prepared using ChEMBL and PDB (Of the 5490 compounds 172 were commercially available. These were further divided into 30 clusters and docked into the BChE active site gorge. 8 best small molecules were purchased and evaluated in vitro against BChE by the method of Ellman.

Of the eight compounds, very promising activity against BChE was exhibited by ARRY-371797 a p38α MAPK inhibitor of Pfizer Inc. This molecule was then subjected to further optimization.

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# A NOVEL COPPER-CATALYZED Csp3 -Csp3 CROSS-COUPLING REACTION USING READILY AVAILABLE STARTING MATERIALS

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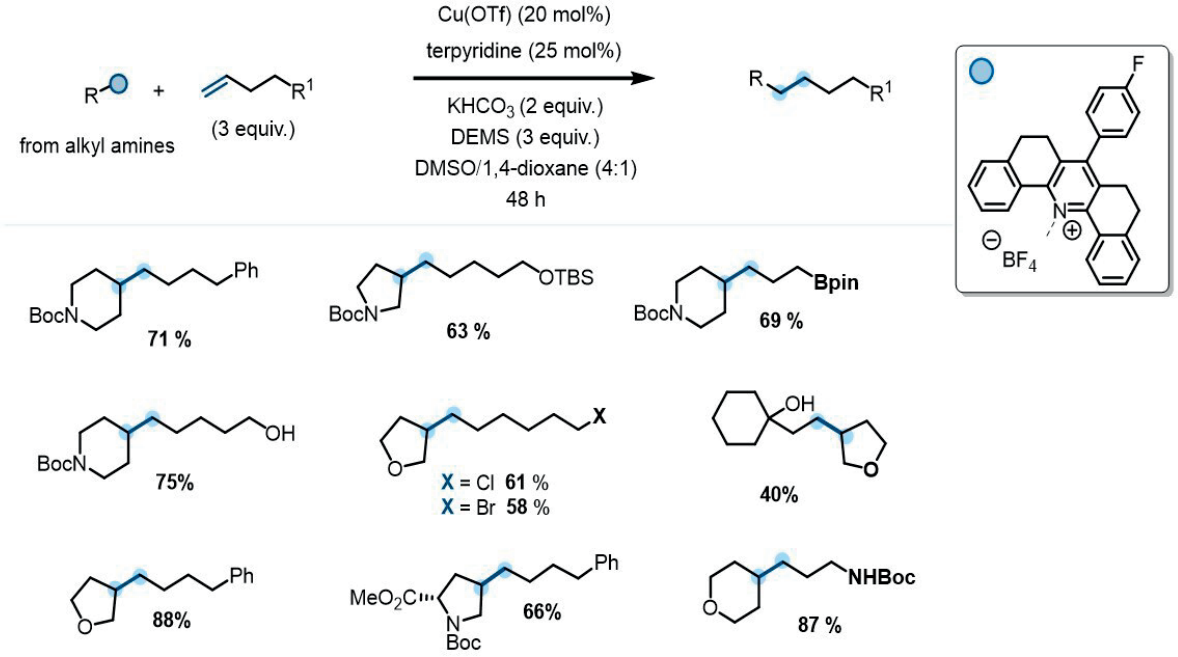
Transition metal-catalyzed cross-coupling reactions have emerged as one of the most reliable and powerful synthetic tools in organic chemistry. In contrast, advances in the development of general methods that form alkyl-alkyl bonds remain one of the main challenges in the field of cross-coupling chemistry.[1] These drawbacks are mainly due to the slow oxidative addition of alkyl electrophiles and the propensity of metal alkyl species to undergo β-hydride elimination.

Herein, we have established that, with the aid of alkyl Katritzky pyridinium salts, selectively prepared from aliphatic amines,[2] and terminal olefins, a copper-based catalyst derived from commercially available components can facilitate $Csp^3$–$Csp^3$ cross-coupling reactions in good yields.

In contrast to other electrophile-nucleophile methods described to date, which employ photoredox and a copper catalyst to effect radical and bond formation,[3] this copper-catalyzed hydroalkylation of olefins provides a unique disconnection where copper is responsible for the single-electron transfer and bond construction, simultaneously. To achieve this goal, the design and modulation of the alkyl pyridinium salt structure to enhance single electron transfer processes was a key component of our research. The method is operationally simple and uses mild reaction conditions. It has subsequently been used to synthesize a range of alkyl-alkyl bonds from a diverse array of alkyl amines and olefins with good outcomes.

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# SEMI-SYNTHESIS OF THIOGLYCOSIDE DERIVATIVES OF THE NATURAL PRODUCT ANTIBIOTIC FIDAXOMICIN

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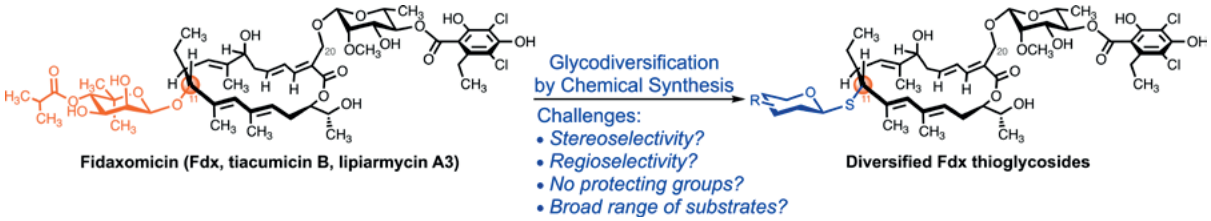
*Clostridioides difficile* infections (CDI) constitute one of the most frequent cases of nosocomial infections, with the severity and incidence rate increasing.[1,2] The most frequent symptom of a CDI is diarrhea, but in severe cases, patients encounter sepsis or death.[2] Treatment options are generally limited to mainly the antibiotics fidaxomicin (Fdx), vancomycin, and metronidazole. Of these, the polyketide macrolactone Fdx is the gold-standard treatment based on low recurrence-rates, and high selectivity for *C. diff*.[1–4] The first mutant *C. diff.* strain with decreased Fdx susceptibility in the clinics was described in 2018.[5] The limited treatment options and the potential for resistance emergence emphasize the importance of research on antibiotics targeting CDI.

Fdx exerts its bactericidal activity by targeting the bacterial RNA polymerase.[6,7] The binding site for Fdx is relatively narrow, which combined with the potential of resistance development sparked our interest in developing next-generation Fdx derivatives by semi-synthesis. Our aim is to access structural diversification of Fdx to slow down resistance development and to modify the PK/PD profile.

Fdx comprises a core aglycon with carbohydrate moieties attached at C11 and C20. The noviose-derived sugar at C11 binds deep within the binding pocket and constitutes the focus of this work. Herein, we will describe our strategy to access glycodiversification at C11 to access new Fdx derivatives not accessible by bioengineering. Further, the synthesis of non-classical thio-noviose derivatives and the biochemical characterization of new derivatives will be presented.

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# PRACTICALLY-ACCESSIBLE N-TRIFLUOROMETHYLATION METHODS FOR USE IN ORGANIC SYNTHESIS AND MEDICINAL CHEMISTRY

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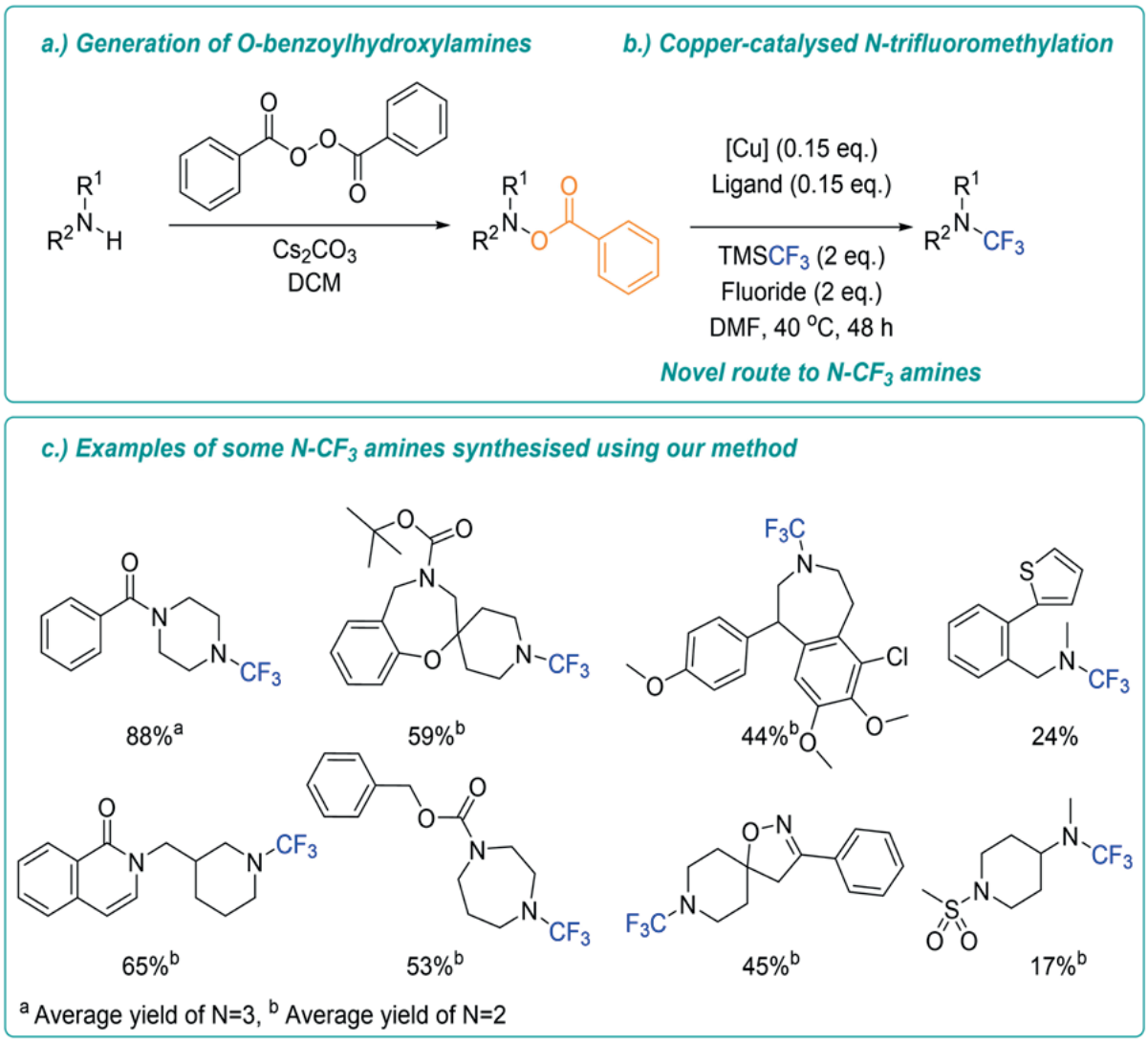
Whilst the use of trifluoromethyl containing compounds is well established within medicinal chemistry, with a range of approved drugs containing *C*-$CF_3$ and *O*-$CF_3$ units used,[i] the utilisation of *N*-$CF_3$ remains relatively unexplored. This may be attributed to the challenging synthesis of these units, with many current methods employing harsh conditions or expensive reagents.[ii] A robust methodology for the *N*-trifluoromethylation of amines has been developed which employs an umpolung strategy in the form of a metal-catalysed electrophilic amination. The method is operationally simple and uses mild, inexpensive reagents. It has subsequently been used to synthesise a range of novel, structurally complex *N*-$CF_3$ containing compounds.

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# DESIGN OF POTENTIATORS TARGETING THE ALLOSTERIC POCKET OF THE KAINATE SUBTYPE GLUTAMATE RECEPTORS

**Thomas Colson (1), Charline Leroy (1), Yasmin Bay (2), D.S. Pickering (2), A.S. Kristensen (2), Bernard Pirotte (1), J.S. Kastrup (3), Pierre Francotte (1)**

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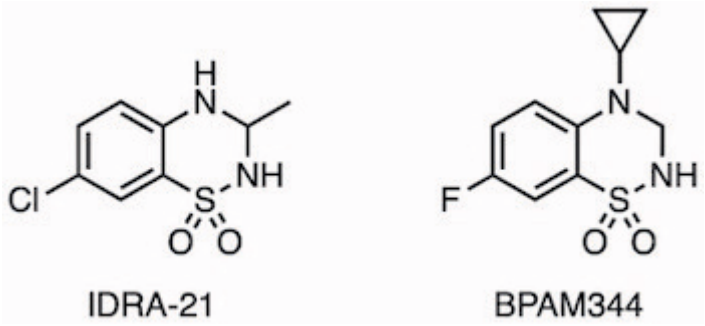
Based on the major role played by glutamate in our brain, the glutamatergic receptors always constitute interesting targets to develop therapeutics. Amongst all the pharmacological classes that have been investigated so far stand the AMPA receptor (AMPAR) positive allosteric modulators (AMPARpams).

Over the two last decades, our laboratory has been involved in the design of such compounds, namely preparing more than five hundred original 1,2,4-benzothiadiazine 1,1-dioxides and isosteres related to IDRA-21 acting as AMPARpams.

Amongst the lead compounds, some ligands such as BPAM344 were co-crystallized with the ligand-binding domain (LBD) of the GluK1 subunit of kainate receptors (KARs). BPAM344 was shown to interact at the allosteric site of the GluK1-LBD dimer interface supporting the view that this drug behaves as a KARpam. This breakthrough was confirmed after voltage-clamp experiments with homomeric GluK1b, GluK2a or GluK3 KARs expressed in HEK293 cells. BPAM344 was found to potentiate KARs, albeit at higher concentrations than required for potentiation of AMPA receptors [1].




Due to the structural analogy between AMPA and KA receptors and considering the recent knowledge of existing differences in residues at the level of their dimer interface after alignment of both receptors, a rational drug design leading to specific ligands for the KARs was explored.

Taking into account these data, the present work focused on the insertion of hydrocarbon chain bearing specific polar moieties on the nitrogen atom at the 4-position of BTDs with the aim to interact with specific amino-acid residues of the KAR allosteric site.

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# AUTOMATED CONTINUOUS FLOW CHEMISTRY APPLICATION IN THE PHARMACEUTICAL INDUSTRY: PHOTOCHEMICAL C-N, C-O AND C-C CROSS COUPLINGS REACTIONS

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Recent advances in the field of flow chemistry have resulted in it gaining considerable attention for application in the pharmaceutical industry over recent years. Flow chemistry is an innovative technology platform that presents many potential benefits over traditional batch procedures including higher selectivity**,** faster reactions times**,** access to a broader substrate scope or safe handling of highly reactive reagents among others.

The combination of flow chemistry with other enabling technologies such as electrochemistry or photochemistry, and the opportunity to automate these processes facilitates exquisite control over reaction performance. We disclose here the use of an automated flow process for the functionalisation of aryl halide building blocks by automated photoredox C-N, C-O and C-C cross-couplings, using commercially available flow reactors and a liquid handler.

This method has proven excellent for the rapid diversification of building blocks to access key intermediates that have been applied internally at GSK, accelerating exploration of Structure Activity Relationships on active drug discovery programmes.

Furthermore, other technologies such as photochemistry or electrochemisttry can be adapted to flow methodologies.The  development of an automated process enables the control over reaction performance and also the chance to achieve higher yields.

On the other hand, in GSK we have acquired a new liquid handler (like the one shown in the picture below) which has been combined with our flow capabilities, resulting on an automated platform which has allowed us to perform a photochemical C-N, C-O and C-C cross couplings reactions, using the same set up with similar conditions for the three approaches.

We have demonstrated that it is an excellent method for the rapid synthesis of a wide range of key intermediates that have been applied internally for the synthesis of more complex molecules.

Vapourtec system with liquid handler

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# DS42450411: A POTENT ORALLY ACTIVE HEPCIDIN PRODUCTION INHIBIROR FOR ANEMIA OF CHRONIC DISEASE

**Takeshi Fukuda, Kenjiro Ueda, Takashi Ishiyama, Takahiro Katagiri, Sumie Muramatsu, Masami Hashimoto, Anri Aki, Kengo Watanabe, Naoki Tanaka**

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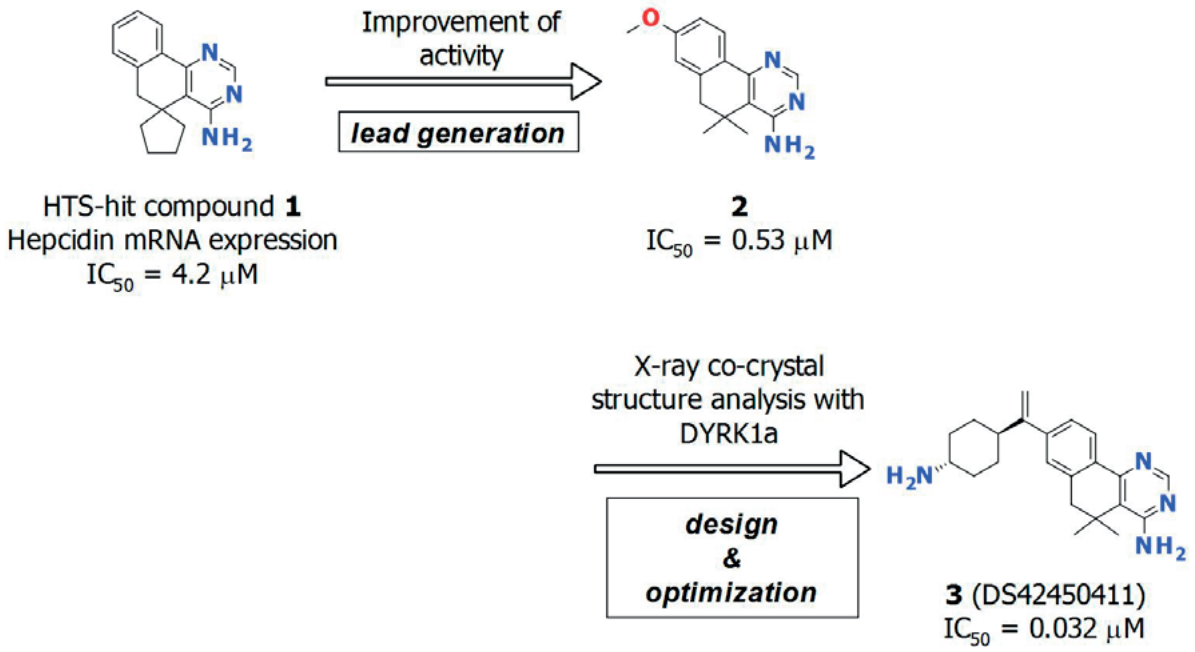
Hepcidin is a peptide hormone, and is known as the master regulator for systemic iron mobilization. Anemia of chronic disease (ACD), which includes anemia of inflammation, is a heterogenic anemic condition due to chronic inflammation from an underlying disease, such as rheumatoid arthritis. Some ACD patients are known to present iron deficiency despite abundant body iron store (termed *functional iron deficiency*). Recently, high hepcidin induction based on inflammatory status was recognized as the cause of functional iron deficiency. The controlling of hepcidin level would be a therapeutic option for treating hepcidin caused functional iron deficiency.

We report herein the lead generation from **1** to obtain an aminopyrimidine derivative **2**, enhanced inhibitory activity. In addition, we describe the optimization study of **2** led to the design of a potent and orally available inhibitor of hepcidin production, **3** (DS42450411), which showed serum hepcidin-lowering effects in a mouse model of interleukin-6-induced acute inflammation.

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# A NEW AI-DRIVEN APPROACH TO HIT IDENTIFICATION

**Rita S. Gaidar (1), Olga O. Tarkhanova (1), V. Joachim Haupt (2), Florian Kaiser (2), Yurii S. Moroz (1)**

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Exploration of giga-scale chemical spaces using *in silico* approaches has been the most effective way to identify new drug candidates[1,2].

The main goal of this study was to identify novel small-molecule inhibitors of Sirtuin-1 (SIRT1)[3,4,5]. We have utilized an AI-based[6,7] method that consists of an *in silico* pre-selection of the compound library followed by multi-stage *in vitro* validation and molecular dynamics simulation to identify the best hit molecules. The selection of the focused library (434 small molecules) and analysis of SIRT1 was performed by DiscoveryEngine technology created by PharmAI.

A multistage *in vitro* studies and validation identified nine hit compounds. One hit molecule was selected for a molecular dynamics simulation to study the mode of inhibition. Docking of the hit was applied to the substrate moiety of the reference X-ray structure of human SIRT1. The results of the study showed a hit rate of 2.1%. The approach shows a clear improvement over the known *in silico* methods. Application to nine hits a more rigorous label-free validation route resulted in a hit rate of 0.46%. The described approach demonstrates a hit count comparable to that of a classical high-throughput screening project while using significantly fewer compounds that must be screened. Furthermore, the technology is broadly applicable to any given small molecule screening library.

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# SYNTHESIS OF MODIFIED T-1106-5´-TRIPHOSPHATES AS POTENTIAL INHIBITOR OF THE SARS-CoV-2 RdRp

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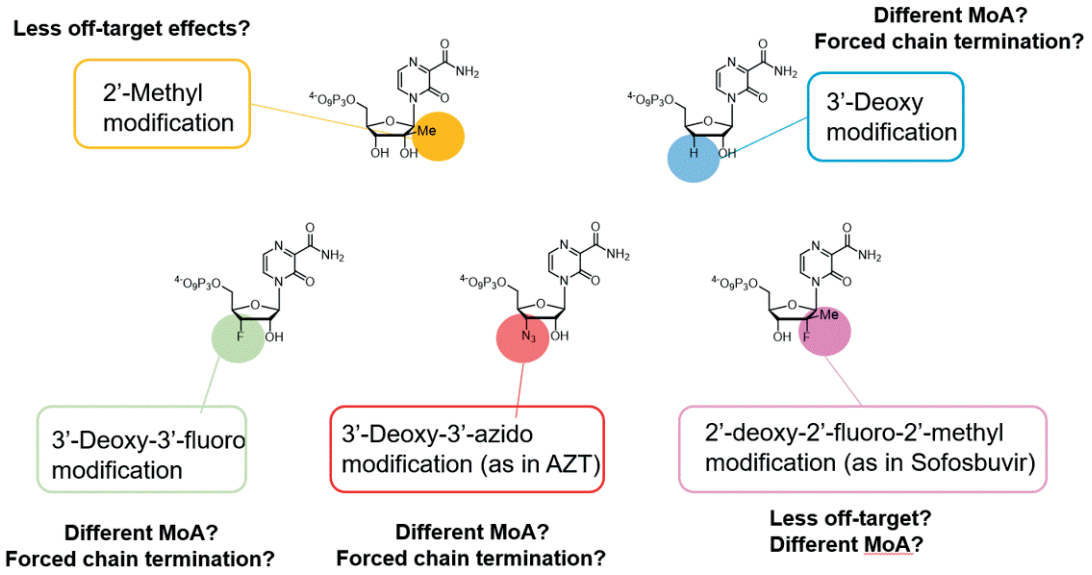
The current global pandemic, caused by the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), has clearly shown the urgent need for antiviral therapeutics. In the past nucleoside analogues were used successfully for the treatment of several viral diseases by inhibiting the viral RNA-dependent-RNA-polymerase (RdRp). Previous studies have shown that the nucleoside analogue T-1106 is rapidly incorporated into the viral genome by the SARS-CoV-2 RdRp. The incorporation of T-1106 triggers C-to-U and G-to-A transition mutations, causing an antiviral effect through lethal mutagenesis[1].
The aim of this work is the synthesis of modified T-1106-5´-triphosphates that will be tested for their antiviral potency. Modified T-1106 triphosphates might show different mechanisms regarding polymerase inhibition, or different *in vivo* properties that could enhanced the antiviral potency compared to T-1106. So far, it was possible to develop a reliable route for the synthesis of modified T-1106 triphosphates. Several T-1106 triphosphates are currently part of antiviral assays.

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# CHEMOTYPE EXPLORATION FOR POTENTIAL TAU AGGREGATION THERAPEUTICS

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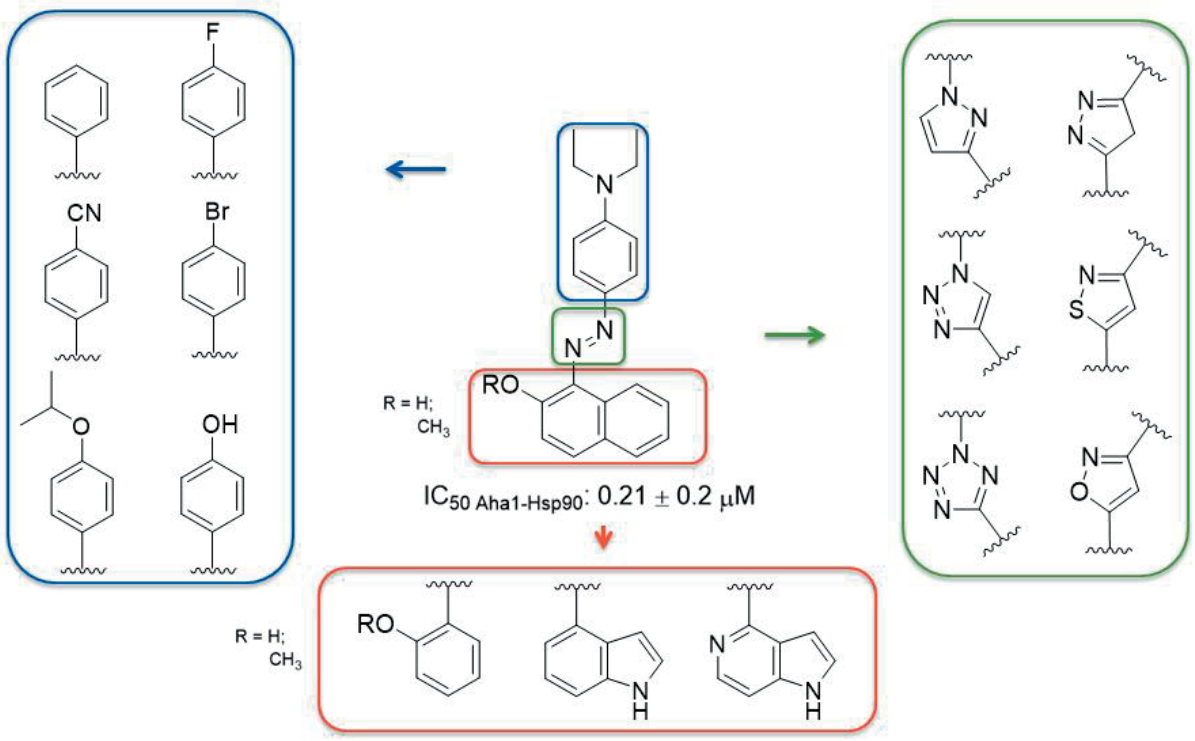
The defining pathologies of Alzheimer's disease are distinctive neurofibrillary lesions including neurofibrillary tangles, neuropil threads and dystrophic neurites that are a result of intracellular depositions of aggregated cortical tau protein.[1] Although tau-based therapeutics have mainly aimed to disrupt aggregation either directly or via kinase inhibition, tau-mediated neurodegeneration may potentially be a result of either soluble misfolded, hyperphosphorylated and/or mislocalised forms of tau.[2]

Heat shock protein 90 kDa (Hsp90) plays a fundamental role in homeostatic regulation, including mediation of tau.[3] Unfortunately, targeting of Hsp90 specifically has led to pharmacological issues due to the chemical structure of inhibitors, as opposed to their chemical target. Use of co-chaperones accompanying heat shock proteins could allow for more specific targeting while modulating substantially fewer processes, thus minimising off-target effects.[4]

To overcome this pharmacological barrier, assessment of small molecular scaffolds and their structure-activity relationships (SAR) is important to achieve a greater understanding of potential treatments for tau aggregation through novel molecular chaperone targets. A promising scaffold has been identified as an inhibitor of the Hsp90-Aha1 complex.[5] A library of compounds have been designed and synthesised to investigate the SAR surrounding these novel targets so as to determine their viability as potential tau aggregation therapeutics.




**Scheme.** Lead compound and proposed designs to probe structure-activity relationships (SAR) of direct tau protein and heat-shock protein inhibition.

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# SYNTHESIS, IN SILICO DRUG LIKENESS ESTIMATION, IN VITRO NEUROTOXICITY AND NEUROPROTECTION ON SUBCELLULAR LEVEL AND IN VITRO MAO-B INHIBITORY EFFECTS OF NEW THEOPHYLLINE-7-THIOSEMICARBAZIDES

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The current development of new biologically active molecules is often related to the synthesis either of hybrid molecules, combining different pharmacophores directly or through a linker.

Having in mind the pharmacological importance of both methylxanthines and thiosemicarbazides our study is pointed towards synthesis of five theophylline-7-thiosemicarbazide hybrids through an appropriate hybridization strategy with or without a catalyst. In addition, the corresponding drug like properties of the target molecules were evaluated through *in silico* web-based approaches, using free available web-servers Molinspiration Chemisnformatics and OSIRIS Property Explorer. The evaluations determined positive drug likeness for all synthesized compounds, where the introduction of the additional sulfur atom led to increased hydrophilicity. The target derivatives were further evaluated for their *in vitro* neurotoxic and neuroprotective effects on subcellular level, along with their possible MAO-B inhibitory activities. The results identified compound **5b** as the most promising structure for further *in vivo* evaluations with the lowest neurotoxicity and the highest neuroprotection profile and MAO B inhibitory activity of 28%.

# DESIGN, SYNTHESIS AND EVALUATION OF MACROCYCLIC DIAMINOPYRIMIDINES AS DUAL INHIBITORS FOR EPH AND GAK

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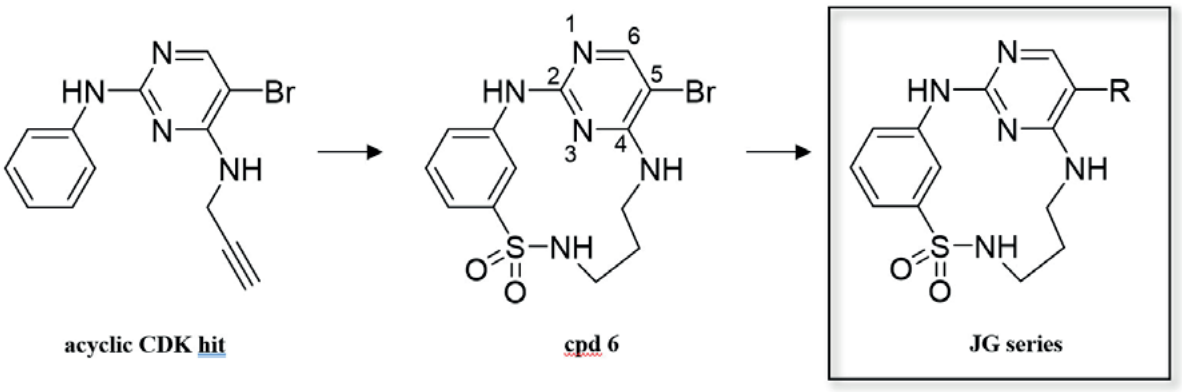


Diaminopyrimidines are a well-known chemotype in kinase drug discovery, but often lack selectivity. Macrocyclization represents a strategy to overcome selectivity problems and serves equally to improve binding affinity and pharmacokinetic properties.[1,2] In 2007, Lücking *et al.* published a macrocyclic diaminopyrimidine (cpd 6) derived from an acyclic hit, identified by high-throughput screening for potential CDK inhibitors.[3] We resynthesized cpd 6 and evaluated its selectivity in a panel of 100 kinases by differential scanning fluorimetry and observed promiscuous binding. Analysis of the co-crystal structure of cpd 6 in complex with cyclin-dependant kinase (CDK) 2 (pdb: 2j9m), allowed us to perform a structure-guided optimization. We saw that the bromine at position 5 of the pyrimidine ring points toward the back-pocket of the kinase. Late-stage functionalization allowed us to rapidly synthesize a series of 5-substituted diaminopyrimidine macrocycles. The corresponding macrocycles displayed greater selectivity in our in-house screening platform compared to the lead structure. The initial binding affinity for CDK2 was eliminated and both ephrin receptor kinases (EPH) and cyclin G-associated kinase (GAK) emerged as promising targets. Finally, we were able to demonstrate target engagement with low nanomolar $EC_{50}$'s in a cellular NanoBRET experiment. During this work, we could substantially improve the selectivity profile of the macrocyclic lead structure (cpd 6) by a structure-guided optimization. We were able to shift the selectivity profile, identify new target kinases and evaluate our series in various *in vitro* and *in cellulo* experiments.

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# SWITCHING AGONIST TO ANTAGONIST: STRUCTURE-BASED DESIGN AND SYNTHESIS OF NOVEL NUCLEOSIDE $A_{2A}$ ADENOSINE RECEPTOR ANTAGONIST AS POTENTIAL IMMUNE-ONCOLOGY AGENTS

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The A2A adenosine receptor ($A_{2A}AR$), a subtype of the adenosine receptor family, has emerged as a promising target for immune-oncology agents. While existing $A_{2A}AR$ agonists have a nucleoside scaffold, only modified purine or heterocyclic compounds have been discovered as $A_{2A}AR$ antagonists due to their structural similarity to adenosine. In this study, we employed structural drug design to switch a nucleoside-based $A_{2A}AR$ agonist to an antagonist. Vorbrüggen glycosylation and regio-selective Pd catalyzed cross-coupling reactions[1)] were used as key steps to synthesize 22 compounds for a structure-activity relationship (SAR) study. The most potent compound showed high binding affinity ($K_i$ = 7.7 nM) and a full antagonistic effect in the cAMP functional assay on $hA_{2A}AR$. Additionally, we evaluated the in vivo anti-cancer efficacy of the compound, which demonstrated a significant synergistic effect with the immune checkpoint inhibitor, Keytruda. Overall, this research discovered novel nucleoside-based $A_{2A}AR$ antagonists with C8-modification, which can serve as promising candidates for immune-oncology agents. Furthermore, we determined the X-ray co-crystal structure of the synthesized compound with the $A_{2A}AR$,[2)] which can provide deeper insights into receptor activation.

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# DEVELOPING pH-ACTIVATABLE RUTHENIUM (II) FLUORESCEIN PHOTOSENSITIZERS TOWARDS THERANOSTIC APPLICATIONS

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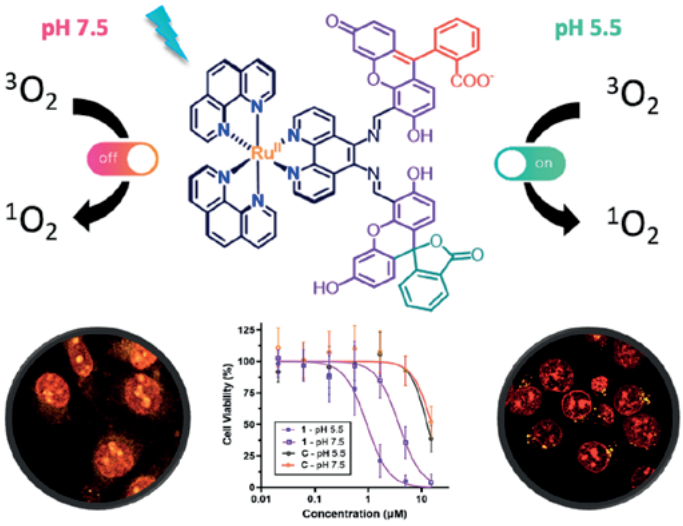
Having been extensively studied as effective photosensitizers for photodynamic therapy (PDT),[1,2] ruthenium (II) polypyridyl complexes still face common limitations of PDT agents due to their lack of selectivity towards cancer tissues. In this study, we present the utilization of these complexes as pH-controllable photosensitizers for molecular targeted PDT (mt-PDT). This innovative approach aims to enhance the specificity of PDT by leveraging the acidic nature of tumor tissues.[3] By incorporating a fluorescein moiety, known for its pH sensitivity, into a salphen Schiff base connected to a ruthenium center, we have successfully synthesized novel complexes capable of pH-sensitive $^1O_2$photogeneration. The photophysical properties of these photosensitizers, along with their ability to sensitize molecular dioxygen, were examined at different pH levels, revealing a significant increase in the quantum yield of $^1O_2$ under slightly acidic conditions. To assess their photo-cytotoxicity, the complexes were tested against U2OS osteosarcoma cells at pH 5.5 and 7.5, and their $IC_{50}$ values were determined. Notably, a substantial 5-fold enhancement in phototoxicity, reaching 0.88 µM, was observed at pH 5.5. Furthermore, in exploring the systems' potential for theranostic applications, we discovered that the cellular localization of the compounds was strongly influenced by pH.

The compounds showed higher photo-indexes compared to the previously reported pH-controllable BODIPY dyes, meaning they may lead to wider therapeutic windows. The developed compounds are also more water soluble and easier to prepare than the BODIPY and porphyrin dyes previously studied as pH activatable PS.[4,5] The results presented in this study underline the potential application of $Ru^{II}$ Schiff base complexes bearing a pH switchable moiety such as fluorescein as pH-controllable $^1O_2$-generating PS, which could lead to safer and more targeted PDT.

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# SYNTHESIS AND CHARACTERIZATION OF NEW ALKYL AMINES OF RE (I). STUDY AS A POSSIBLE OPTICAL MERCURY(II) SENSOR

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Optical chemosensors are generally of an organic nature, however, organometallic or coordination complexes have made it possible to improve detection limits, selectivity, etc. These systems contain a receptor or cavity, linker and a signaling unit. After the interaction of the analyte with the sensor, disturbances are produced, which can be measured using spectroscopic techniques such as Uv-Vis and/or Fluorescence.

In this work, we present the synthesis and characterization of new Re(I) complexes ($^{1}H$ and $^{13}C$ NMR; Mass spectrometry) and the study of complexation in the presence of $Hg^{2+}$ using Uv-Vis and fluorescence spectroscopy (Fig. **1**). Additionally, after the addition of one equivalent, the solution changed from orange to pale yellow. These types of systems are known as *naked eye* colorimetric sensors.

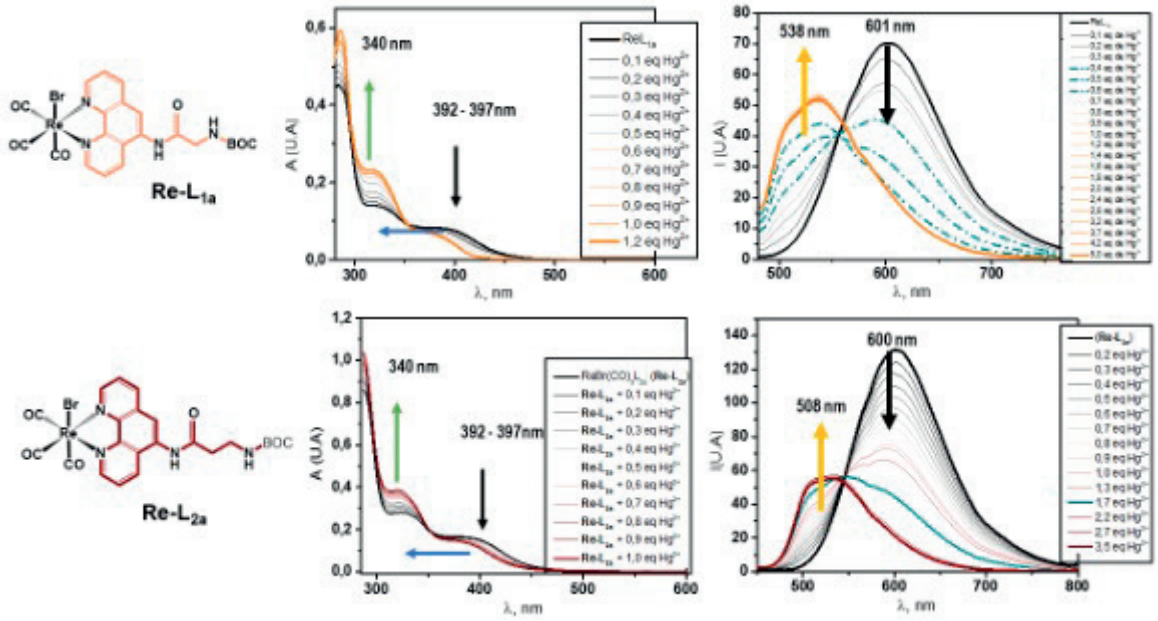


Figure 1. Spectrum Uv-Vis and Emission of $ReL_{1a}$ and $ReL_{2a}$ (in acetonitrile)

Acknowledgements

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# GSK-3β AND ROCK-1 KINASES UNDER THE MAGNIFYING GLASS - IDENTIFICATION OF DUAL INHIBITORS AGAINST TAUOPATHY AND NEUROINFLAMMATION IN ALZHEIMER'S DISEASE

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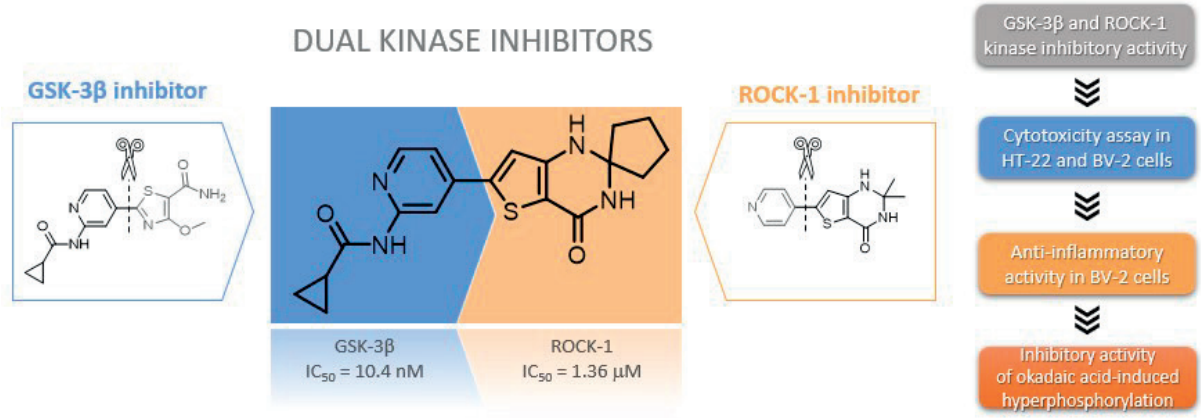
The phenomenon of abnormal protein aggregation underlies several diseases. One of them, particularly important due to the constantly growing number of cases is Alzheimer's disease (AD). In AD, there are two proteins in the spotlight – β-amyloid, which forms extracellular deposits of amyloid plaques, and microtubule-associated tau protein, which neurofibrillary tangles (NFTs) consist of. Accumulated protein aggregates are highly toxic, disrupting cellular homeostasis and activating an inflammatory response, ultimately leading to neurodegeneration [1]. The culprits for the proteinopathies include GSK-3β and ROCK-1 kinase enzymes, which activity is overexpressed in AD. GSK-3β causes excessive phosphorylation of the tau protein resulting in its detachment from microtubules and neurite degeneration. Unbound hyperphosphorylated tau undergoes further oligomerization to NFTs [2]. In the second case, ROCK-1 kinase enhances the production of β-amyloid by modulating the degradation of its precursor - amyloid precursor protein through the amyloidogenic pathway [3]. Moreover, ROCK-1 can activate microglia by binding to specific receptors on its surface and thus intensify the secretion of pro-inflammatory mediators. Based on the evidence, simultaneous GSK-3β and ROCK-1 inhibition, may allow effective modulation of the AD course and constitute a novel therapeutic approach.

In our research, we focused on the development of dual GSK-3β/ROCK-1 kinase inhibitors. To explore the structure-activity relationship, we evaluated their inhibitory potency *in vitro* in the Kinase-Glo™ luminescence assay. Then, we determined their safety profile *in cellulo* using hippocampal neuronal cells HT-22 and mouse microglial cells BV-2, followed by an evaluation of the release of LPS-induced pro-inflammatory mediators. In addition, we assessed the compounds' ability to inhibit okadaic acid-induced hyperphosphorylation.

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# SULFATASES IN CHEMICAL BIOLOGY

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Sulfatases play an important biological role by catalyzing the hydrolysis of sulfate esters. Sulfate esters are widely observed in biomolecules, with diverse structures and functions, ranging from small signaling molecules to complex structural biopolymers[1,2]. The sulfate ester functionality is highly hydrophilic, that presents interesting opportunities for the medicinal chemist to significantly improve aqueous solubility of compounds/prodrugs/linkers. The impact of sulfatases in human disease remain poorly understood[3,4] yet several STS inhibitors have entered clinical studies for the treatment of hormone dependent breast cancer[5]. Furthermore, bacterial and fungal sulfatases are largely unexplored, however, recent studies show that gut microbiome sulfatases impact the interaction between bacteria and the intestinal epithelium[6–8]. Thus, exploring the sulfatase space could reveal important discoveries within the field of chemical biology and medicinal chemistry.

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# SELECTIVE C3 FUNCTIONALIZATION OF N-PROTECTED TROPANE

**Beata Gryzło, Anna Czopek, Joanna Śniecikowska, Krzysztof Więckowski, Olga Ostrowska, Marcin Kołaczkowski**

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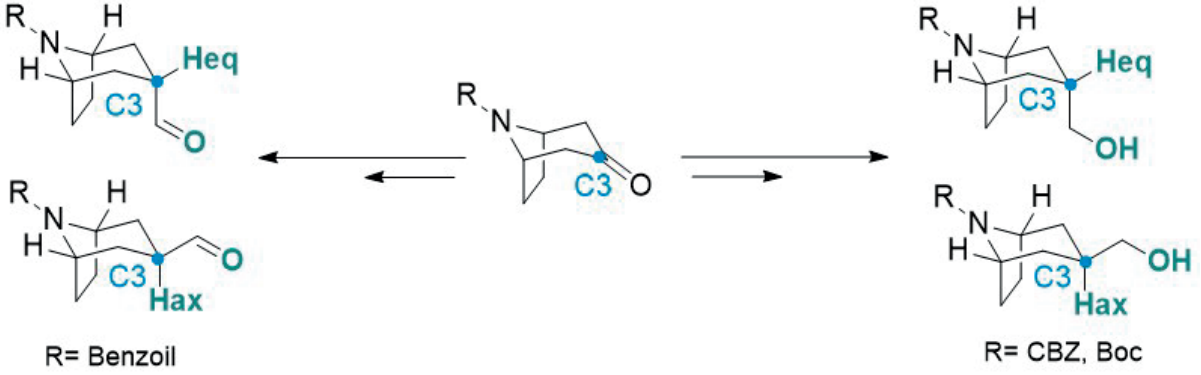
The tropane core (8-azabicyclo[3.2.1]octane) is found in diverse types of bioactive molecules like cocaine (monoamine reuptake inhibitors), atropine or scopolamine (muscarinic receptors antagonists) and serves as an important scaffold in organic and medicinal chemistry.[1,2] Biological activities of tropane derivatives often depend on the stereochemistry of the bicyclic framework. Therefore, understanding its reactivity and stereochemistry is essential for efficient synthesis planning in medicinal chemistry projects.




In the course of our studies, we have established conditions for stereoselective functional group interconversions at the C3 position. Commercially available *N*-nortropinone carbamates (*N*-Boc-nortropinone and *N*-CBZ-nortropinone) afforded tropanmethanol via alkene intermediate and selective hydroboration of the double bond. In another approach, *N*-benzoilnortropinone stable in acidic conditions was easily converted to aldehyde, although the stereoselectivity was lower in this case. The spatial arrangement of the substituents at the C3 position was thoroughly analyzed based on 1D and 2D NMR analysis and literature data. [3–5]

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# ORGANOCATALYTIC, HOMO-SYNERGISTIC ACCESS TO ENANTIOPURE 6,7-DIHYDROBENZIMIDAZOLES

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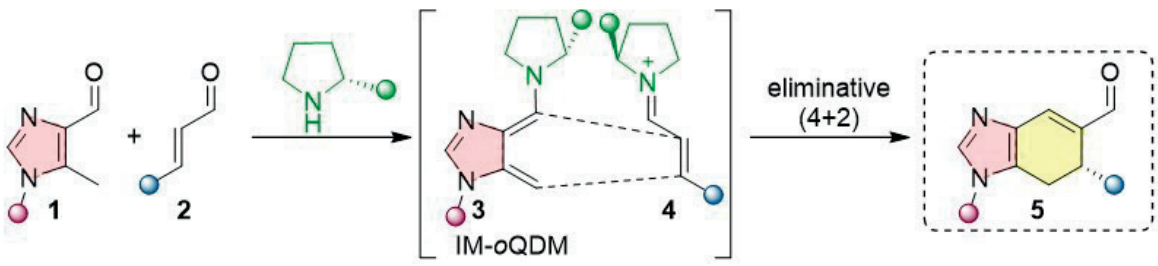
The construction of cyclohexene-fused heterocycles generating highly reactive *ortho*-quinodimethane (*o*-QDM) species as activated dienophiles in organocatalyzed [4+2] cycloadditions is a sought-after objective of recent research.[1] Nevertheless, this synthetic approach has scantly been applied before to the imidazole core,[2] probably due to remarkable challenges posed by the imidazole motif, comprehending its high degree of aromatic character and the interference of the heterocyclic ring with the reaction system.In the current work, we were able to perform, for the first time, the asymmetric, organocatalyzed cross [4+2] cycloaddition between remotely enolizable imidazole carbaldehydes **1** and enal dienophiles **2** to achieve [d]-fused imidazole products **5** (Scheme 1). The reaction is carried out through a homo-synergistic approach,[3] in which the enantiopure organocatalyst covalently activates both pronucleophile **1**, providing fleeting *ortho*-quinodimethane **3**, and dienophile **2**, to afford a series of slightly represented, chiral 6,7-dihydrobenzo[*d*]imidazoles **5**.




**Scheme 1.** The synthetic strategy performed in this work.

Despite an almost total regio- and enantiocontrol, the reaction suffered of scarce efficiency, a fact that poses several mechanistic issues that will be properly described in this presentation.

The development of a convenient method to access these scaffolds may serve the wide community of chemists and medicinal chemists interested in the discovery of biologically/pharmaceutically active compounds.

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# SYNTHESIS, BIOLOGICAL EVALUATION AND IN SILICO DMPK STUDIES OF LUTEOLIN DERIVATIVES CONTAINING SULFONAMIDE GROUP AS ANTICANCER AGENTS

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Luteolin is a flavonoid found in a wide range of plant materials such as vegetables, fruits, and medicinal herbs. It displays a wide range of biological activities and acts as an anticancer agent against various types of human malignancies but is known to have poor DMPK profiles. In this study, sulfonamide group was introduced to luteolin derivatives to improve antiproliferative activity against A549 lung cancer cell lines and DMPK profiles. The compounds were assayed for antiproliferative activity by MTT method. Among the compounds evaluated, **MRC-F-004** containing methylsulfonamide group at 4'-position of luteolin and **MRC-F-014** containing 2-chloro-4-fluorophenylsulfonamide group at 6'-position of luteolin displayed significant antiproliferative activity against A549 lung cancer. The $IC_{50}$ values of **MRC-F-004** and **MRC-F-014** were 22.45 and 22.09 μM, respectively, implying better anticancer activity compared to luteolin ($IC_{50}$: 46.22 μM). Using the biological results, a structure–activity relationships (SAR) were conducted. *In silico* drug metabolism and pharmacokinetic (DMPK) assessment of two active compounds and luteolin was also reported.

# HARNESSING SOLID PHASE SYNTHESIS IN THE PURSUIT OF PROTEIN DEGRADERS

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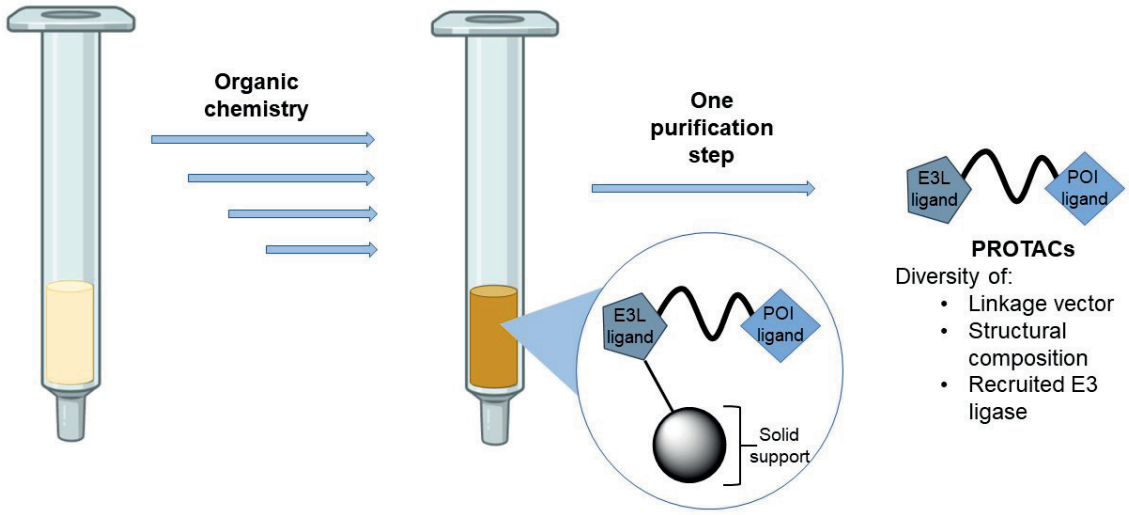
Targeted protein degradation utilising PROTACs is a field of ever-increasing interest within the drug discovery space. Numerous clinical candidates now exist which exhibit this modality.[1] An often-forgotten aspect of PROTACs is their developmental history rooted in peptides. The first published PROTAC was peptide-based, and one of the most commonly used E3 ligase recruiting ligands (VH032) is a peptidomimetic.[2] Consequently, well-established peptide synthesis strategies are suited to application in PROTAC discovery. Our goal is to employ the efficient and adaptable methodology of solid phase synthesis (SPS) in the context of PROTAC development. Key to this approach is the ability to rapidly synthesise diverse derivatives, due to high rates of conversion and minimal purification when compared to traditional solution phase methods. Furthermore, solid phase methodology lends itself well to parallel synthesis- particularly attractive for PROTAC optimisation given the importance of linker diversification.

Using SPS methods, a series of degrader candidates have been synthesised including the BET bromodomain targeting PROTACs MZ1 and AT1. This work has also involved manipulation of the structural composition and linkage location of the VH032 scaffold, a crucial element in the optimisation of VHL-recruiting PROTACs.[3] Consequently, a diverse set BRD4 targeting PROTACs have been synthesised, requiring only a single purification step. Additionally, we have adapted the solid-phase approach to prepare multiple degrader candidates which recruit IAP, an alternative E3-ligase. This highlights the adaptability and scope of this approach. These methodologies are well suited to application in development campaigns targeting other proteins of biological interest.

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# FLIPPING THE SWITCH: CONVERTING ACTIVATORS INTO DEGRADERS TO TARGET GLUCOSE METABOLISM

**Alex Hallatt (1), Brian Ford (2), Nikol Athina Zografou-Barredo (1), Shruti Chachra (2), Si kei Lai (2), Celine Cano (1), Loranne Agius (2)**

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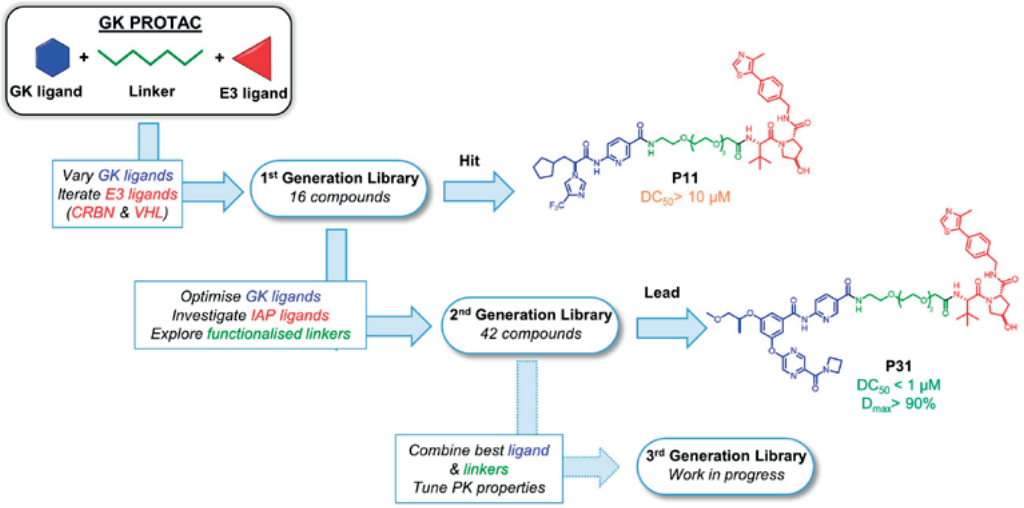
Non-Alcoholic Fatty Liver Disease (NAFLD) is now prevalent in around 25% of the global population and is the most common cause of chronic liver disease. Despite its wide prevalence and well-defined phenotype, there are still no approved pharmacological therapies for the treatment of NAFLD.[1] The complex pathogenesis of NAFLD is yet to be fully elucidated, but it is associated with decreased insulin sensitivity and raised hepatic glucose output resulting from elevated glucokinase (GK) activity.[2] There are no selective inhibitors of GK reported to date, so the project strategy involves the incorporation of known allosteric activators of GK (GKAs) as ligands for GK in PROTACs. This is the first such example of activators being used for targeted protein degradation by means of PROTAC technology, essentially reversing the native pharmacology of the parent ligand.




Several iterations of novel GK PROTACs were synthesised by modular assembly of known GKAs with flexible linkers and ligands targeting the E3 ligases, Cereblon (CRBN) and von Hippel-Lindau (VHL). So far, two generations of GK PROTACs have been synthesised and current work is focused on the synthesis of a 3rd generation library which involves the use of rigidified linkers and alternative ligands for VHL in an effort to improve potency and address pharmacokinetic challenges. These proof-of-concept tools were subject to biological evaluation in hepatic and pancreatic cell-lines to investigate their effect on GK activity and abundance. Orthogonal, activity-based methods have been used to screen libraries of compounds and follow-up characterisation has taken place on the most promising compounds.

**P31** is our current best tool compound which has undergone vigorous evaluation in a variety of enzymatic and cell-based assays in order to investigate its effect on GK activity and protein levels. We have since shown **P31** to be a potent and selective degrader of GK, with a $DC_{50} < 1\mu M$ and $D_{max} > 90\%$ . Washout studies show that P31-induced GK degradation is sustained over multiple days and is reversible. **P31** also shows significant, dose-dependent reduction in glucose phosphorylation rates whereas the epimer which does not recruit VHL, cis-P31, did not show any reduction in either GK activity or protein levels. Additional mechanistic studies are ongoing to further validate **P31** as a probe that would be a useful tool for the entire metabolic therapeutic community as its ability to reduce GK activity levels is currently unprecedented.

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# TAILORED DRUG ACCESS TO THE CNS: AN INTERDISCIPLINARY STUDY EXPLORING SOLUTIONS FOR IMPROVED TREATMENT OF BRAIN DISEASES

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The limited efficacy in traversing brain barriers constitutes a critical impediment to the future development of neurotherapeutics. Drug delivery strategies only target the blood-brain barrier (BBB), the blood-cerebrospinal fluid barrier (BCSFB) has been largely overlooked, despite its potential to access deep cerebral regions through the flow of cerebrospinal fluid (CSF)[1]. Notably, certain neurotropic viruses, including the Mumps virus (MuV), have evolved the capacity to breach the BCSFB, either as unbound viral particles or by exploiting immune cells[2]. This extraordinary ability of MuV is attributed to the small hydrophobic (SH) protein, hypothesized to confer its brain-penetrating ability. The SH protein interacts with GPR125, an adhesion G-protein coupled receptor prominently expressed in the choroid plexus, a gateway utilized by MuV for brain entry via receptor-mediated endocytosis. Motivated by this mechanism, we developed a peptide-drug conjugate (PDC) composed of the extracellular N-terminal fragment (2-11) of the MuV SH protein and a glucagon-like peptide-1 receptor (GLP-1R) peptide agonist, namely Exendin-4 (Ex4). To ascertain the binding interaction between the PDC and GPR125 *in vitro* investigations we conducted, while also assessing its *in vivo* capacity to deliver Ex4 to the brain.

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# DISCOVERY AND DEVELOPMENT OF JNK3 INHIBITORS AS THERAPEUTICS FOR ALZHEIMER'S DISEASE

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JNK (c-Jun N-terminal Kinase) is a member of the MAPK (Mitogen-Activated Protein Kinase) family and plays a critical role in intracellular signal transductions. Overexpression of JNK has been implicated in neuronal cell necrosis and apoptosis, making it a promising target for neurodegenerative diseases such as Alzheimer's disease (AD).

Building on previous structure-activity relationship (SAR) studies and docking analysis, we chose to modify the core scaffold to 1,4,5,6-tetrahydrocyclopenta[d]imidazole. Biochemical kinase assays demonstrated that the resulting derivatives exhibited significantly higher potency as JNK3 inhibitors and showed excellent isoform selectivity compared to previously synthesized inhibitors. Notably, several derivatives demonstrated subnanomolar range of IC50 values for JNK3 (0.716, 0.564, 0.379, 0.779 nM), surpassing the potency of any known JNK3 inhibitors. Moreover, these JNK3 inhibitors effectively protected neuronal cells against amyloid beta-induced apoptosis. Docking studies indicated that the tetrahydrocyclopenta[d]imidazole scaffold retained optimal interactions. Furthermore, BBB-PAMPA assay and ADME prediction revealed favorable pharmacokinetic profiles for the derivatives. Collectively, our findings suggest that 1,4,5,6-tetrahydrocyclopenta[d]imidazole could serve as a promising scaffold for the development of potent and selective JNK3 inhibitors as potential therapeutics for AD.

# HARMICINES AS ANTICANCER HITS

**Maja Beus, Ivana Perković, Goran Poje, Marina Marinović, Kristina Pavić, Zrinka Rajić**

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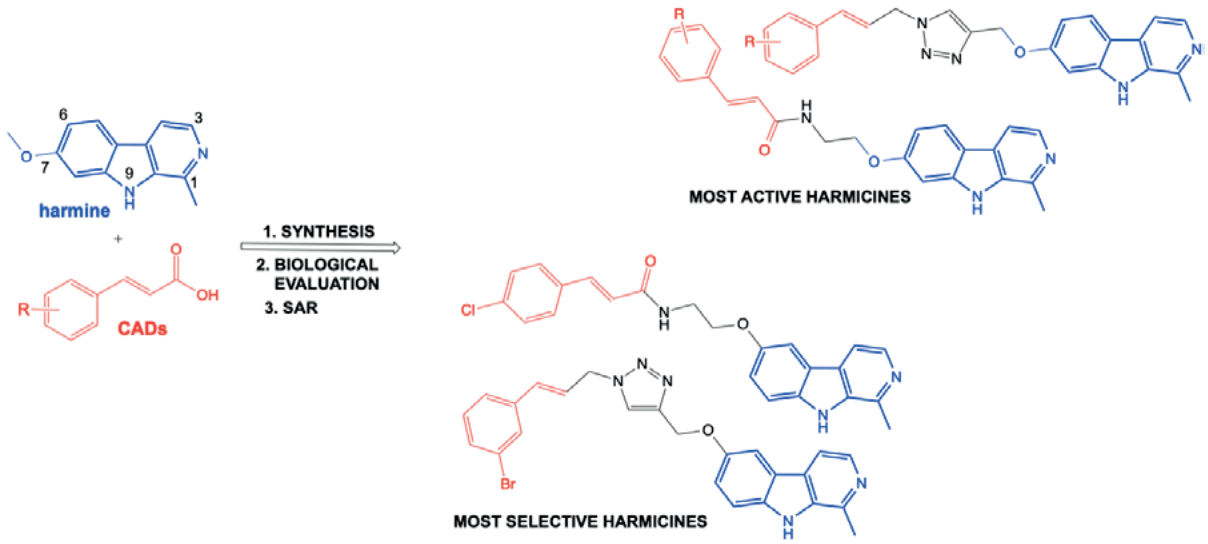
As cancer remains one of the major health burdens worldwide, novel agents, due to the development of resistance, are needed. Harmicines represent hybrid compounds that combine two agents with anticancer properties, β-carboline alkaloid harmine and a cinnamic acid derivative (CAD). Previously we have prepared a library of 80 compounds, which differ in 1) the type of the linker between two moieties (a triazole ring or an amide bond), 2) the position of the substitution on the harmine's β-carboline core (C-1, C-3, O-6, O-7 or N-9), and 3) type of CAD (1-3). Here we disclose their antiproliferative activities in vitro against four human cancer (MCF-7, HCT116, SW620, and HepG2) and one human non-cancer cell line (HEK293T).
The results have shown that the most sensitive cell lines were MCF-7 and HCT116. Structure-activity relationship analysis revealed that both amide- and triazole-type harmicines, prepared at O-7 of the β-carboline core, exhibit strong, but unselective, antiproliferative activities (IC50 in the single-digit micromolar range). The most selective activities were exerted by amide- and triazole-type harmicines substituted at O-6, namely p- chloro- and m-bromocinnamic acid derivatives (selectivity index = 10.6 and 13.3, respectively). We further examined the intracellular distribution of the selected harmicines in MCF-7 cells, based on their fluorescence properties. The preliminary resulty have shown that harmicines are localized exclusively in the cytoplasm.
In conclusion, the results of this study point to harmicines as powerful anticancer hits and warrant further investigations in that direction.




This work was fully supported by the Croatian Science Foundation under the project number UIP-2017-05-5160 and by the Young researcher's career development project – training of doctoral students of the Croatian Science Foundation founded by the European Union from the European Social Fund.

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# DIKETO ACID HETEROCYCLIC DERIVATIVES AS NEW SARS-CoV-2 NSP13 INHIBITORS BLOCKING VIRAL REPLICATION

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The diffusion of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) caused a pandemic with unprecedent socioeconomical impact, enlightening the need of new antiviral agents able to block viral replication. The development of vaccines is essential in the containment of the diffusion of the virus, and an incredible joint effort led to a global vaccination campaign in about 1 year after the virus outbreak. However, vaccines may be less or no effective against emerging variants of SARS-CoV-2 and, also, it is still unknown how long this vaccine-induced immunity will last. Therefore, the development of antiviral drugs against SARS-CoV-2 is of pivotal importance.

The SARS-CoV-2 non-structural protein 13 (nsp13) has been identified as a target for antiviral drugs thanks to its critical role in viral replication and to its high sequence conservation within the coronavirus family.[1] Nsp13 targets the natural nucleotides and deoxynucleotides as substrates when performing its adenosine triphosphatase (ATPase) activity, utilizing the energy of nucleotide triphosphate hydrolysis to catalyze the unwinding of double-stranded DNA or RNA in a 5′ to 3′ direction.[2]

Although the roles of nsp13 in the viral lifecycle, there is a paucity of information about small molecules compounds reported in literature endowed with nsp13-inhibitory activity. Aryl diketo acids (DKAs) have been previously described as inhibitors of nsp13 of SARS-CoV-1.[3] Basing on these literature data and thanks to our longstanding expertise in the design and synthesis of DKA derivatives, we carried out a semi-random screening on our in-house library of DKA compounds, identifying a promising hit compound as micromolar nsp13 inhibitor. We synthesized a set of indolyl DKA derivatives as structurally correlated with the identified hit, obtaining new dual SARS-CoV-2 nsp13 ATPase and helicase inhibitors, also capable of inhibiting viral replication. Mode-of-action studies revealed ATP-non-competitive kinetics of inhibition, not affected by substrate-displacement effect, suggesting an allosteric binding. The data coming from the biological assays will be shown and discussed.

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# SYNTHESIS OF SUGAR BASED DI-VALENT CLICK DERIVATIVES CONJUGATED WITH SERINOL

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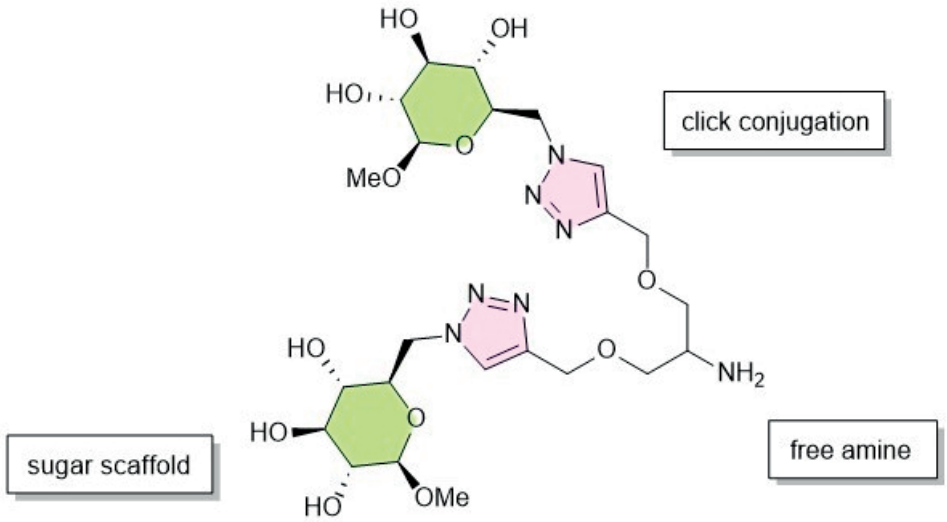
The discovery of promiscuous carbohydrate processing enzymes has enabled chemists to access unnatural glycoconjugates with regio- and stereo selectivity.[1-3] Oligosaccharides, both as free endogenous molecules and as components of glycoconjugates play important role in numerous biological processes including, cell adhesion, cell-cell recognition and modulation of signal transduction pathways.[4] As a consequence, the development of multivalent carbohydrates as effectors of biological processes has been a topic of interest and carbohydrates involved in the receptor binding are generally linked with a linker.[5,6] In the present work, di-valent sugar click derivatives have been synthesized by conjugating methyl-α-D-2,3,4-triacetoxy-6-deoxy-6-azidoglucopyranoside to N-protected serinol-alkyne via copper-catalyzed alkyne-azide click reaction (CuAAC). These newly synthesized compounds are suitable to be employed as building blocks for more complex multivalent molecules by introducing florescent moieties at amine part for enzyme inhibition probing and various biological activities.

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# NOVEL AZASPIRO COMPOUNDS FOR THE TREATMENT OF HUAMAN AFRICAN TRYPANOSOMIASIS. SYNTHESIS, BIOLOGICAL EVALUATION AND DOCKING STUDIES

**Marco Rizzo (1,4), Valeria Francesconi (1), Lorenzo Tagliazucchi (2), Anabela Cordeiro-da-Silva (3), Rebecca Wade (4), MariaPaola Costi (2), Michele Tonelli (1)**

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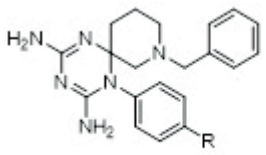
Trypanosomiasis is a threatening neglected tropical disease (NTDs), which is endemic in several countries like South and Central America and Africa. Several progresses have been achieved with the introducing of fexinidazole and the association between eflornithine-nifourtimox. However, their use is hindered by their side effects and their high toxicity. Moreover, the drug resistance issues, and the existence of animal reservoirs make the development of new safer and more efficient drugs a compelling need.

A valid strategy to treat the Human African Trypanosomiasis (HAT), is represented by the inhibition of the two major enzymes involved in the folate pathways. Trypanosomatids are auxotrophic for folates and pterins that are crucial cofactors for the biosynthesis of nucleic acids and proteins, so it`s reasonable to think that the inhibition of their folate-dependent enzymes, namely dihydrofolate reductase (DHFR-Ts) and pteridine reductase 1 (PTR-1) of *Trypanosoma brucei* (*Tb*), may represent a seccessfull strategy for the treatment of HAT. Cycloguanil (CYC) is a well known DHFR inhibitor, which also showed to act as PTR1 inhibitor[1]. The binding mode analysis of CYC to *Tb*DHFR-Ts and *Tb*PTR1 active site, lead to us to design and synthesize two novel series of compounds that maintain the amino 1,6-dihydrotriazine moiety of CYC[2].
Azaspiro-2,4-diamino-1,6-dihydrotriazine (A) and (B), we replace the CYC C6 moiety with a bulky group in order to increase the lipophilicity. The compounds have undergone evaluation of their on-target activity (TbPTR1 and TbDHFR-Ts), human DHFR inhibition to ascertain their selectivity for the protozoan enzymes, cytotoxicity and antiparasitic effect. These compounds are also under computational studies at the Heidelberg Institute for Theoretical Studies where we are docking them and through molecular dynamics studies we expected to better understand how these compounds are able to dampen the activity of the folate enzymes and hopefully will lead us through a rational design of new safer and more effective antiparasitic compounds.



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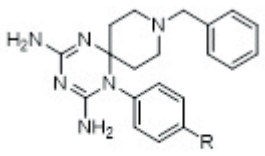

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# SYNTHESIS OF ANNULATED AZINOPYROL HETEROCYCLIC FRAMEWORKS AS PURINE ANALOGS

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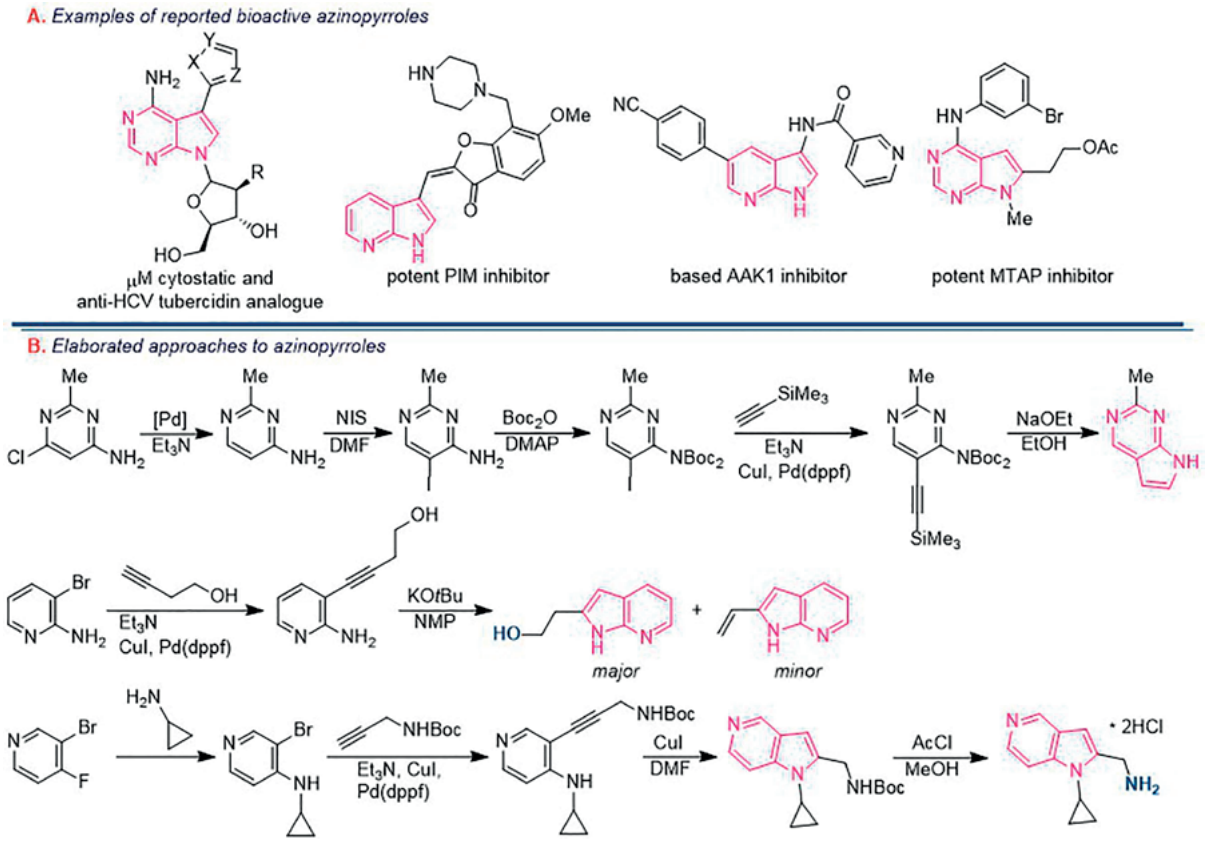
Purine core is a venerable motif in medicinal chemistry, which was employed in construction of numerous drug molecules. Owing to purine nucleotides being bricks of genetic information keepers DNA and RNA most of them are antiviral and anticancer agents interfering with normal replication and processing of these macromolecules. In this regard, diversification of purine core came to the spotlight of scientific community aiming at identifying more effective and selective agents. Approaches to modifying the core have been evolving in two directions: its functionalization and changing heteroatom pattern within purine framework. The second one opened the door to investigation of novel compounds as purine isosteres and revealed compounds with kinase inhibiting, antibacterial, antidiabetic activity etc. (Scheme, **A**) Herein we present results of our synthetic investigations directed to elaborating approaches to one of such isosteric groups, namely compounds containing pyrrole moiety annulated with different azine cores (Scheme, **B**). The strategy to achieve the target derivatives was based on two-steps process – (1) Sonogashira coupling of *ortho*-halogenamines of different aza-heterocycles with terminal alkynes and (2) heterocyclization of the products in the presence of a strong base or organometallic catalysts. The pathway is convenient as it tolerates many functional groups and allows manipulating the structure of the final product *via* the use of easy-to-obtain (often commercially available) starting compounds. Thus, started azines 2-methyl-4-amino-6-chloropyrimidine, 2-amino-3-bromopyridine, 3-bromo-4-fluoropyridine after 2 to 5 stages ended up as pyrrolo[2,3-d]pyrimidine, pyrrolo[2,3-b]pyridine and pyrrolo[3,2-c]pyridine derivatives correspondingly.



# DESIGN AND SYNTHESIS OF NOVEL PYRROLO-PYRIMIDINE BASED DDR1/2 INHIBITORS FOR INHALED ADMINISTRATION

**Roberta Mazzucato, Nicolò Iotti, Eleonora Ghidini, Andrea Rizzi, Serena Bertolini, Fabio Bignami, Alessandro Fioni, Valentina Mileo, Annalisa Murgo, Fabio Vaccaro, Allisson Freire Bento, Anna Maria Capelli, Laura Carzaniga**

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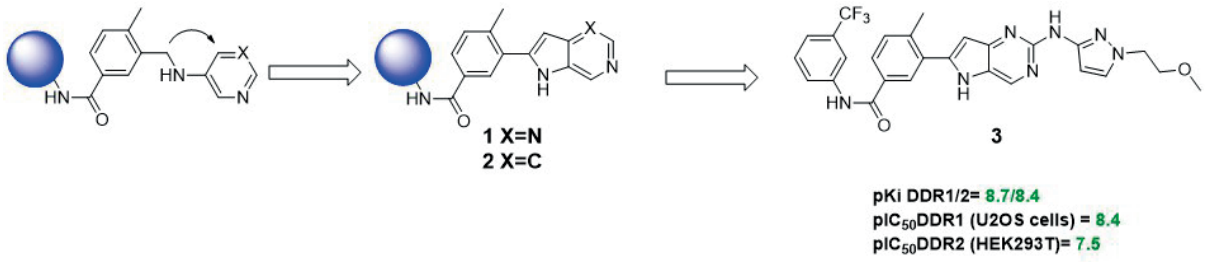
Idiopathic Pulmonary Fibrosis (IPF) is a serious chronic disease with a poor prognosis and high mortality, characterized by an irreversible pulmonary function decline caused by lung tissue fibrosis[1]. Currently, pharmacological treatments can only slow disease progression while showing significant side effects[2,3]. Discoidin domain receptors (DDRs) are transmembrane receptor tyrosine kinases involved in pathways that contribute to fibrosis progression[4]. Preclinical evidence highlighted a possible link between DDR1/2 activity and pulmonary fibrosis[5,6] and inhibition of DDR1/2 could represent a valid strategy for the treatment of IPF and other fibrotic disorders. In this communication, we report our efforts to discover a new class of DDR1/2 inhibitors suitable for inhalation, an unprecedented route of administration for this target. We designed a novel series of compounds characterized by 5:6 bicyclic fused heterocycles, through a ring closure approach that led to conformationally constrained analogues. Following an in-depth SAR exploration, hits **1** and **2** showing high affinity for DDR1/2 were identified. Optimization of their ADME properties for inhaled delivery resulted in the identification of compound **3** showing high cellular potency and an *in vivo* pharmacokinetic profile appropriate for future development as inhaled therapeutic. (Figure 1)

**Figure 1**

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# CUBANE AS A 3D SCAFFOLD FOR FRAGMENT LIBRARY CONSTRUCTION

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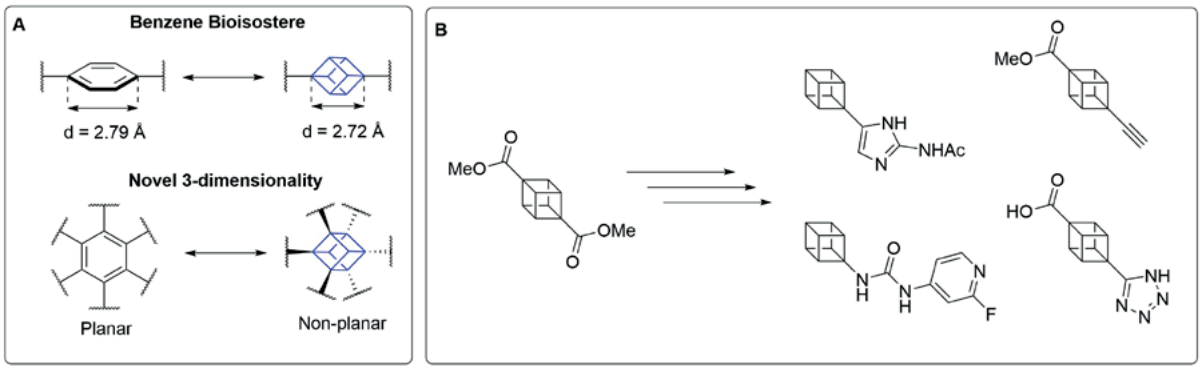
Much innovation in drug discovery has been built on improved synthetic manipulation of an increasing number of hydrocarbon scaffolds that has provided a large source of structurally-diverse molecules with unique chemical and physical properties.[1] Furthermore, a recent push in pharmaceutical sciences to escape from the "flatland" of planar aromatic molecules has assisted the privileged structure status of caged hydrocarbons for their novel three-dimensionality.[2] One such hydrocarbon, cubane, has gained much contemporary research interest due to its remarkable stability, low toxicity and its validation as a bioisostere of benzene.[3] More importantly, the potential to functionalise cubane at each carbon atom provides an opportunity to generate complex three-dimensional molecules with unique biological activities.[4]

Fragment-based drug discovery methodology allows the identification of low molecular weight ligands that can be elaborated into potent, drug-like molecules.[5] Compared to high-throughput screening, even modestly-sized compound libraries can be used to develop leads provided that broad structural and chemical space is represented. To accelerate the incorporation of the cubane scaffold into the drug discovery pipeline, a library of mono-/poly-substituted fragment-like compounds bearing the cubane moiety is to be constructed. The syntheses of several novel cubane fragments will be described.




(A) Geometric comparison of cubane and benzene highlighting bioisosteric relationship and three-dimensionality. (B) Condensed scheme for the synthesis of several novel cubane fragments

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# TARGETING THE CELL-ADHESION MOLECULE PSGL-1 WITH A SMALL MOLECULE INHIBITOR

**Ciyana James (1), Varsha Kanabar-Raivadera (2), Clive P Page (2), Lorraine Martin (1), Gerd K Wagner (1)**

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P-selectin glycoprotein ligand-1 (PSGL-1) is a major selectin ligand expressed on most peripheral T cells, B cells, neutrophils, monocytes, and platelets [1]. It plays a major part in tethering blood cells to endothelial selectins, facilitating transmigration of leukocytes and platelets into inflamed tissue and driving chronic inflammatory processes. PSGL-1 is upregulated on leukocytes from patients with chronic obstructive pulmonary disease (COPD) [2]. Pharmacological reduction of elevated PSGL-1 levels may therefore be a promising new strategy for intervention with PSGL-1-mediated cell adhesion in COPD and other chronic inflammatory airway diseases.

We have previously shown that treatment of primary human peripheral blood mononuclear cells (hPBMCs) with the pro-inflammatory cytokine IL-1b leads to elevated PSGL-1 levels in vitro [3]. Herein, we report that these elevated PSGL-1 levels can be reduced to basal levels with a drug-like small molecule (Fig. 1).

The molecular target(s) and mode of action of this inhibitor are currently unknown. To enable target identification studies, we have developed an optimised synthetic route for this inhibitor using well-established chemistries (e.g., Suzuki-Miyaura cross-coupling). The target molecule was obtained in 12% total yield over 6 synthetic steps via this route, which can also be readily adapted for the development of photoaffinity probes.

To identify potential molecular targets involved in the inhibitor-mediated downregulation of PSGL-1, we used 5 computational target and activity prediction servers (RF QSAR, Swiss target prediction, SEA Search Server, SuperPRED and LigTMap) [4-8]. These algorithms compare the structure and binding profiles of a query inhibitor with that of the natural ligand of potential protein targets and generate a structure and binding similarity score. Ranking and comparison of potential targets from all 5 servers identified a number of common candidate targets, including several kinases (e.g., NEK2, MAPK 8, and MAPK 14). Experiments to investigate these target hypotheses are currently ongoing, and preliminary results will also be reported.

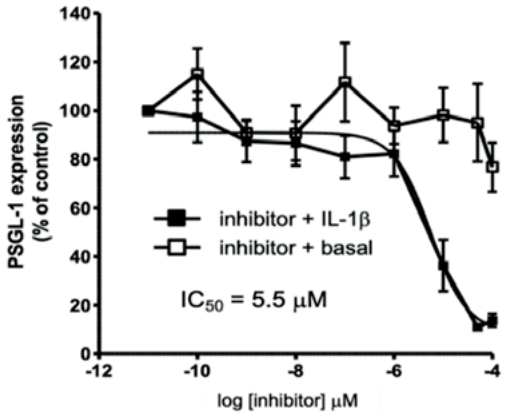


**Fig. 1:** Incubation with inhibitor leads to reduction of induced cell-surface PSGL-1 levels on hPBMCs treated with IL-1β. The inhibitor has no significant effect on constitutive cell surface PSGL-1 levels on untreated hPBMCs.

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# SYNTHESIS, IN SILICO ADME/T ANALYSIS AND BIOLOGICAL ACTIVITY OF NEW HYDRAZIDE ANDROSTANE DERIVATIVES

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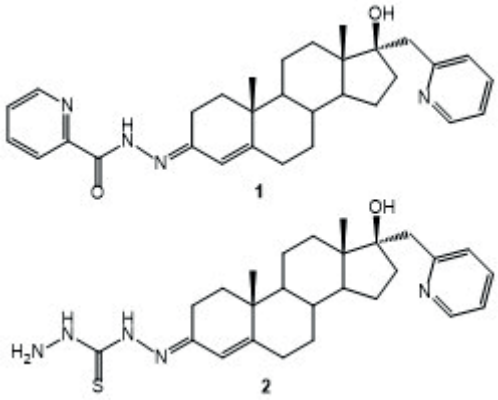
Hydrazides are hydrazine derivatives where one hydrogen atom is substituted by an acyl group. Besides having rich biological and pharmacological properties, hydrazides are also important synthetic precursors for various biologically active nitrogen-containing heterocyclic compounds [1-4]. In addition to high biological potency, they are also used as herbicides and in the paint industry [2, 3]. On the other hand, steroid compounds are generally known to exhibit a wide range of biological activities, primarily antitumor. Analogues of steroid hormones are powerful inhibitors of steroidogenic enzymes and steroid receptor modulators, which makes them attractive candidates for the treatment of hormone-dependent malignancies. They are also considered to inhibit the activity of certain aldo-keto reductases involved in cancer drug resistance. At the same time, they are less toxic compared to many commercially available drugs and highly bioavailable, since they can penetrate the cell membrane.

Given the very small number of papers on the synthesis of steroid derivatives with a hydrazide moiety, our goal was to synthesize new acyl hydrazides in the 17α-(pyridine-2-yl)methylandrostane series, which were tested *in silico* for their pharmacokinetic properties. Thus, here we present the synthesis of one steroid compound with hydrazide (**1**) and one with a hybrid hydrazine-hydrazide group (**2**) at the C-3 position of the steroid nucleus (Fig. 1).




Physicochemical properties were predicted using the SwissADME web tool, where parameters for both compounds were within the optimal range suggested for drug-like molecules. A toxicity profile, determined using the ProTox-II web server, indicates possible immunotoxicity, but no significant toxicity toward healthy cells. These preliminary *in silico* analyses show that both synthesized compounds are good candidates for further

*in vitro* tests. These compounds were also evaluated *in vitro* for affinity to the ligand-binding domains of selected steroid receptors using a fluorescent assay in yeast and potential to inhibit certain enzymes, targets for endocrine therapy of hormone-dependent diseases.

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# A NEW POTENT REAGENT FOR COMBATING BACTERIAL BIOFILM INFECTIONS

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Chronic infections caused by microbial biofilms are predicted to rise to unmanageable levels by the World Health Organization (WHO), United Nations (UN) and Center for Disease Control and Prevention (CDC) if the appropriate actions are not taken. The recalcitrance of microbial biofilms to antimicrobials and to the immune system is a major cause of persistence and clinical recurrence of these infections [1]. Poor antibiotic efficacy against chronic infections has broad and deep health impacts, as well as severe social and economic implications [2], [3]. In biofilms, the bacteria are located in densely packed, slow growing microcolonies concealed in a self-produced matrix of biopolymers. The extracellular matrix protects biofilm bacteria from the actions of antibiotics and effector cells of the immune system. In this life-mode, the bacteria attain the highest levels of protection from present assortment of antibiotics and our immune system [2], [3].

Infections involving bacterial biofilms is estimated to cover about 60–70% of hospital infections [4]. WHO and other agencies has evaluated Pseudomonas aeruginosa and five other strains to be a top global threat [5] and potentially even the next global pandemic. These biofilm infections cause problematic infections of the lungs, urinary tract, and surgical wounds of hospitalized patients often referred to as hospital acquired infections (HAI). HAI patients are often catheterized or intubated, and Catheter Associated Urinary Tract Infections (CAUTI) is one of the most common HAIs worldwide.

Evidence indicates that a high level of c-di-GMP in bacteria correlates with a higher amount of biofilm formation, while low intracellular levels of c-di-GMP lead to increased motility and biofilm dispersal. Diguanylate cyclase (DGC) enzymes catalyse the formation of c-di-GMP, whereas phosphodiesterase (PDE) enzymes catalyse c-di-GMP degradation [6]. We identified 4-arylazo-3,5-diamino-1H-pyrazoles as a novel group of such antibiofilm agents that stimulate the activity of the PDE BifA present in Pseudomonas aeruginosa and induce dispersal of formed biofilm, additionally inhibit formation of new biofilm [7]. A thorough structure-activity-study (SAR) containing more than 60 compounds revealed a very specific binding site and a very potent antibiofilm compound 4-(2-(2-fluorophenyl)hydrazineylidene)-5-imino-4,5-dihydro-1H-pyrazol-3-amine [8].

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# PHOTOCATALYZED MINISCI REACTION AS A KEY STEP TO ACCESS NEW N-HETEROCYCLES

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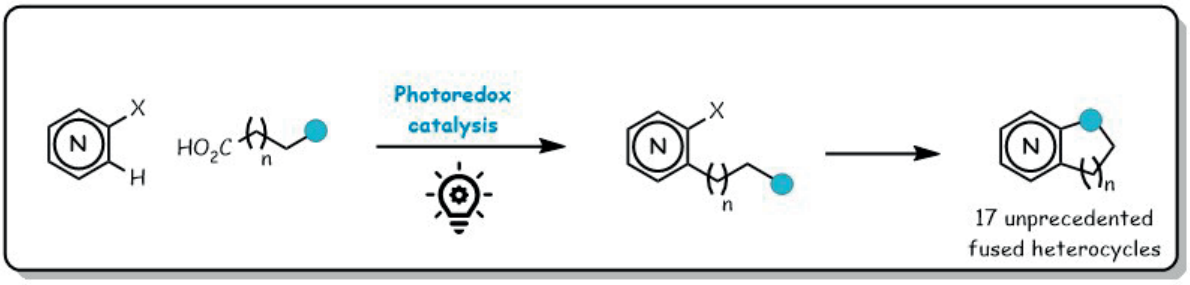
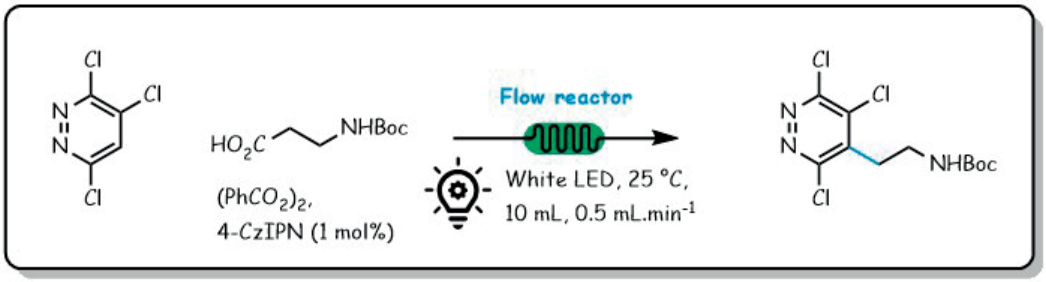
In pharmaceutical research, access to original heterocyclic scaffolds remains a major challenge. Indeed, they constitute molecular structures with unique chemical and biophysical properties. They are also innovative molecular frameworks on which various chemical functionalities can be grafted and are therefore pivotal structures for drugs discovery.[1] Specifically, partially unsaturated bicyclic scaffolds would be of great value for escaping from the traditional and over-explored chemical “flat land”.

The Minisci reaction, a nucleophilic radical substitution, is a strategic reaction manifold to functionalize electron deficient heterocycles, widely used in medicinal chemistry programs.[2] The seminal reactions conditions require harsh conditions to generate radicals, leading to moderate yields and limited scopes. Photoredox chemistry is an interesting alternative to easily generate radicals under mild conditions.




Based on these considerations, we developed a photoredox Minisci / Cyclization sequence that allowed the synthesis of uncharted new heterocyclic entities. A wide scope (> 15) of *N*-containing bicyclic structures, new potential scaffolds for drug discovery programs, was obtained by simple variation of reaction partners.




The transfer to flow chemistry was investigated. The reaction between 3,4,6-trichloropyridazine and N-Boc-β-amino acid was developed. After optimization and a slight modification of the reaction parameters, the continuous photoredox Minisci was achieved in good yield and enhanced productivity compared to the batch mode.

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# AN ALTERNATIVE APPROACH IN ANTIMALARIAL DRUG DESIGN BY INHIBITING MEMBRANE-BOUND PYROPHOSPHATASES

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Many pathogenic protozoan parasites have a negative impact on human health around the world. One common feature of some human pathogens, including *Plasmodium* species (malaria), is large homodimeric integral membrane proteins called membrane-bound pyrophosphatases (mPPases).[1] These enzymes couple the hydrolysis of pyrophosphate to pumping of $H^+/Na^+$ ions and consequently generate an electrochemical potential across the acidocalcisomal membrane. Despite the fact that mPPases play an essential role for many pathogenic protozoan parasites, they do not exist in humans, thereby making them promising drug targets.

Our aim is to develop new mPPase inhibitors capable of disrupting this key ion gradient of pathogenic protozoan parasites to decrease their viability. So far, the therapeutic utility has been limited and only few non-phosphorus inhibitors of mPPases inhibitors have been reported.[2,3] However, through screening efforts of *Thermotoga maritima* PPase we found novel organic inhibitors. Here, we present the exploration of fragment hits and the discovery of small mPPase inhibitors with low micromolar inhibitory activities in our *T. maritima* test system.[4–6] In addition, some of these inhibitors retained their activity against *Plasmodium falciparum* mPPase in membranes.

**Keywords:** drug discovery, inhibitor, membrane-bound pyrophosphatase, protozoan diseases, *Plasmodium falciparum*, *Thermotoga maritima*

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# DEVELOPMENT OF TOOLS FOR THE TARGETED DEGRADATION OF GPCRs

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G protein-coupled receptors (GPCRs) are frequently targeted in the clinic by small molecule antagonists to inhibit specific signalling processes in disease. While this approach has been very successful, newer pharmacological modalities may target GPCRs more effectively, potentially generating improved therapeutics for the treatment of many diseases. In recent years, methods have been developed to exploit the ubiquitin-proteasome system to artificially degrade target proteins. While targeted protein degraders have potential as novel therapeutics and as biological tools for discovery research, targeted degradation of GPCR has remained challenging.[1]

We have investigated the degradation of different GPCRs using the $FKBP12^{F36V}$ dTAG fusion system.[2] Additional studies were performed on the $\beta_2$-adrenoceptor ($\beta_2$AR), as suppressing its signalling action leads to more favourable outcomes in triple negative breast cancer.[3] Our dTAG fusion experiments demonstrated more effective degradation when targeting the von Hippel-Lindau enzyme compared to cereblon. To determine whether the un-tagged $\beta_2$AR could also be degraded, we designed genetically encoded nanobody-based degraders and PROteolysis TArgeting Chimeras (PROTACs). These experiments validate that the $\beta_2$AR is amenable to targeted protein degradation technology. Ultimately, these studies are the first steps towards developing targeted degradation tools and PROTACs for the $\beta_2$AR and other GPCRs. Our studies will help pave the way for the development of novel GPCR degrader tools.

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# PHOTOSWITCHING HDAC INHIBITORS FOR THE SPATIOTEMPORAL TARGETING OF CANCER CELLS WITH LIGHT

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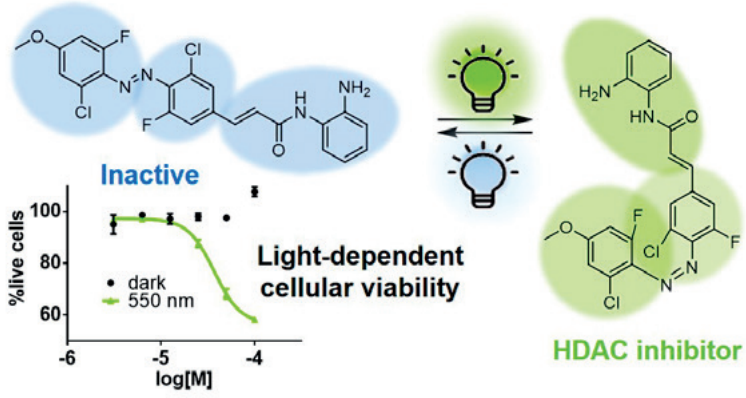
Cancer treatment still represents a major challenge, with continuous increase in incidence, morbidity and relapse. It is difficult to identify drugs that can fully distinguish between cancer and healthy cells, and many chemotherapy agents are limited by their narrow therapeutic window. Thus, novel therapeutic approaches that direct drugs to the tumour area are needed. In this context, photopharmacology emerges as a promising solution.

Photopharmacology uses light to modulate the biological activity of molecules with high precision,[1,2] and it is based on two main designs: photoswitching and caged. An ideal photoswitching drug is completely inactive in the dark, and it is only activated after illumination with a particular light wavelength in a reversible manner. In the field of oncology, we envisage that such drug could be administered in the dark, and be activated with light irradiated only in the tumour area, hence achieving the desired anticancer effect without undesired side effects in other tissues.

Histone deacetylase inhibitors (HDACis) are an example of anticancer drugs that can effectively eliminate cancer cells, but which often lack sufficient selectivity. To achieve the necessary selectivity for their clinical applicability, herein we aimed to develop light-activatable HDACis.[3] Firstly, we prepared a library of photoswitching HDACis to gain insight on SAR of both their photochemical properties and enzyme activity, and identified compounds with over 50-fold differences between dark and light conditions. Secondly, we designed optimised inhibitors that can be activated under visible light, which is more tissue permeable and less harmful than UV light. Remarkably, in cellular assays, some of these molecules showed activity only upon illumination, but not in the dark.

We will present the optimisation process to these molecules, including their design and photocharacterisation to determine the optimal switching conditions and half-time, as well as their light-regulated activity in enzymatic and cellular assays. These constitute amongst the very few examples of reversible light-activatable molecules with anticancer activity that can be modulated with visible light.

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# EFFICIENT SYNTHESIS AND BIOLOGICAL EVALUATION OF NOVEL CHALCONE-BASED COMPOUNDS

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Privileged structures have become a useful tool for the discovery of novel Natural Product (NP) inspired drug-like small molecules [1]. Among NPs, chalcones are considered excellent versatile molecular scaffolds, and both synthetic and natural have shown a wide variety of bioactivities among which the antitumoral against a large number of human cancer cell lines (leukemia, melanoma…) stands out [2]. Particularly, our main aim is to develop synthetic methodologies using rational and efficient processes to prepare collections of novel compounds containing chalcone-based scaffolds with high biological relevancy through direct, simple, and readily scalable reactions [3]. Thereby, the construction of the general a,b-unsaturated system of the chalcones through the well-known Claisen-Schmidt reaction is complemented by the addition of pharmacologically relevant substructures using interesting links (triazoles, amides, ethers...) on both sides of the central core. Design, synthesis and biological evaluation of a collection of valuable compounds is described in this communication.

**Acknowledgements**

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# NEW FIDAXOMICIN ANTIBIOTICS: COMBINING METABOLIC ENGINEERING AND SEMISYNTHESIS

**Erik Jung (1), Maja Hunter (2), Jordan Costafrolaz (3), Yves Benoît Mattenberger (3), Silvia Dittmann (4), Tizian Griesser (5), Michael Meuli (5), Alexander Major (1), Daniel Schäfle (5), Andrea Dorst (1), David Dailler (1), Peter Sander (5,6), Susanne Sievers (4), Patrick Viollier (3), Rolf Müller (2), Karl Gademann (1)**

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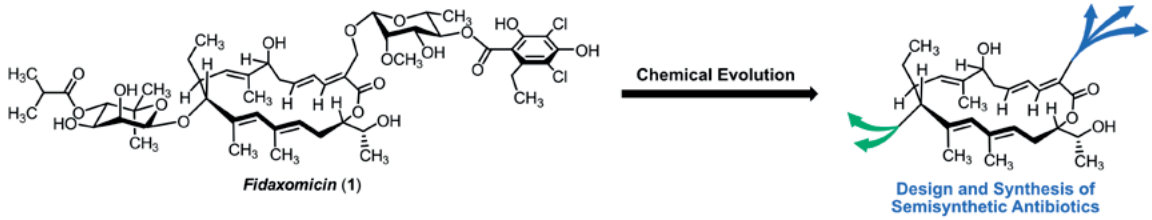
Fidaxomicin (**1**, tiacumicin B, lipiarmycin A3)[1,2] constitutes a macrocyclic antibiotic which demonstrates potent activity against various Gram-positive bacteria through inhibition of RNA-polymerase (RNAP).[3,4] Fidaxomicin is the standard of care to treat *Clostridioides difficile* infections and it also features *in vitro* activity against resistant strains of *Mycobacterium tuberculosis*, yet its unfavourable PK/PD profile prevents its application as a systemic antibiotic.

Structural evolution of a parent natural product is the primary source of new antibiotics. Even now, 50 years after the discovery of **1**, only a few derivates have been described to date. Selective chemical modification of this complex scaffold holds the key to create new generations of Fidaxomicin antibiotics. Our goal is to expand the use of **1** into new treatment areas[4–6], thus we have developed a platform that allows for the selective replacement of each of the moieties decorating the macrocycle. With this poster, we will present our synthetic strategy, the computationally-guided design of new derivatives, and explore their biological activities against various relevant pathogens.




***Fidaxomicin* (1)**

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# FUNCTIONALIZED AND HYPERMODIFIED NUCLEIC ACIDS FOR TARGETING PROTEINS

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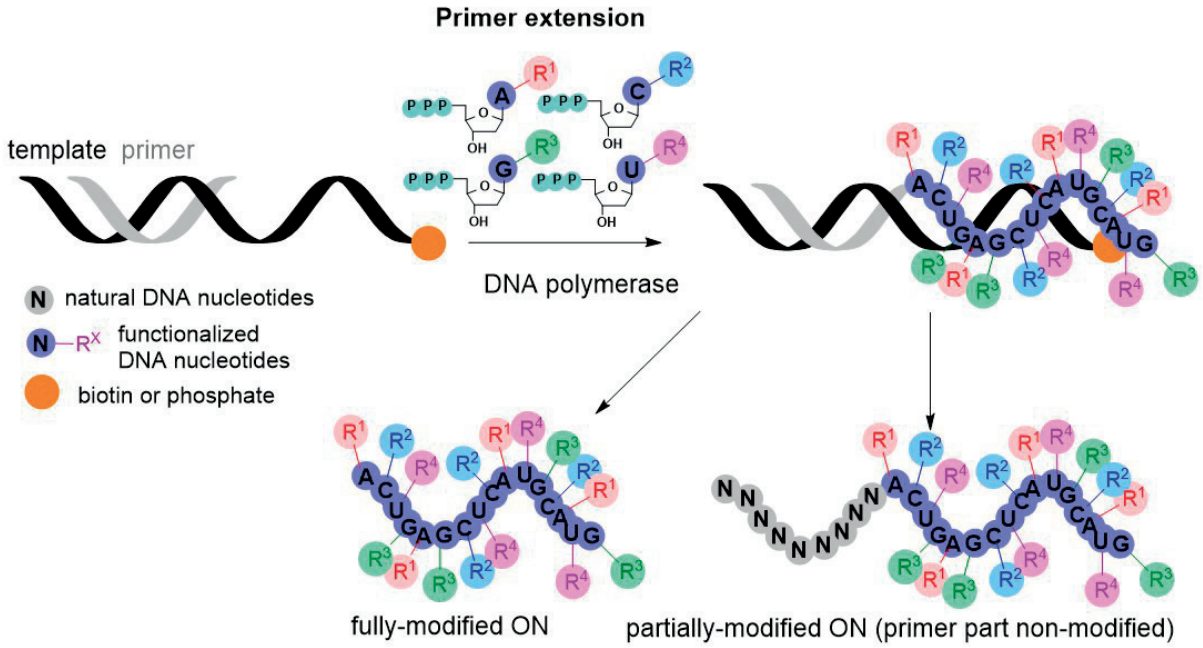
We developed enzymatic approaches to the synthesis of different types of base-modified DNA or RNA starting from modified nucleoside triphosphates. Diverse DNA or RNA polymerases were used for the synthesis of DNA or RNA bearing either one or several modified nucleotides. Moreover, combinations of four modified dNTPs were used for polymerase synthesis of hypermodified DNA containing all four bases bearing different modifications.




We have applied the methodology to the synthesis of modified nucleic acids and oligonucleotide probes for bioanalysis and imaging (through environment-sensitive fluorophores), bioconjugations, as well as targeting proteins through covalent cross-linking (reactive oligonucleotide probes) or through specific interactions (development of base-modified DNA aptamers).

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# STAPLED-PEPTIDE PROTACs SYNTHESIZED BY HYPERVALENT IODINE REAGENTS

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Protein–protein interactions (PPIs) are deemed undruggable due to the lack of well-defined binding pockets on corresponding proteins. Peptides can target undruggable proteins by mimicking PPIs. Indeed, development of peptide-based inhibitors of PPIs is one of the major topics in current medicinal chemistry. PROTAC (proteolysis targeting chimera) technology is an emerging modality to degrade pathological proteins.[1] Most of the PROTACs are synthesized by linking two small-molecule ligands: a POI (protein of interest) ligand and an E3 ligand. However, the development of small-molecule ligands for undruggable proteins is in itself challenging. Thus, the availability of POI ligands is hampering the development of PROTACs for undruggable proteins.

We developed E3 ligand-loaded hypervalent iodine staples based on our previous report,[2] which readily transformed peptides into stapled-peptide PROTACs for degradation of undruggable proteins. Peptide stapling is known to stabilize α-helices and improve inappropriate properties for in vivo efficacy of peptides, namely low membrane permeability and low proteolytic stability. These staples modify peptides to have degradation activity as well as good physicochemical properties targeting intracellular environment at the same time. Thus, this tool would pave the way for rapid drugging of currently untouched proteins, even if small-molecule ligands are not available. We first chose to target the steroid receptor coactivator-1 (SRC-1) which interacts with transcription factors, with a reported SRC-1 degrader as a benchmark.[3]


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# DIAGONALLY PROTECTED 1-METHYLCYCLOBUTANE-1,3-DIAMINES IN ABROCITINIB ANALOGUES SYNTHESIS

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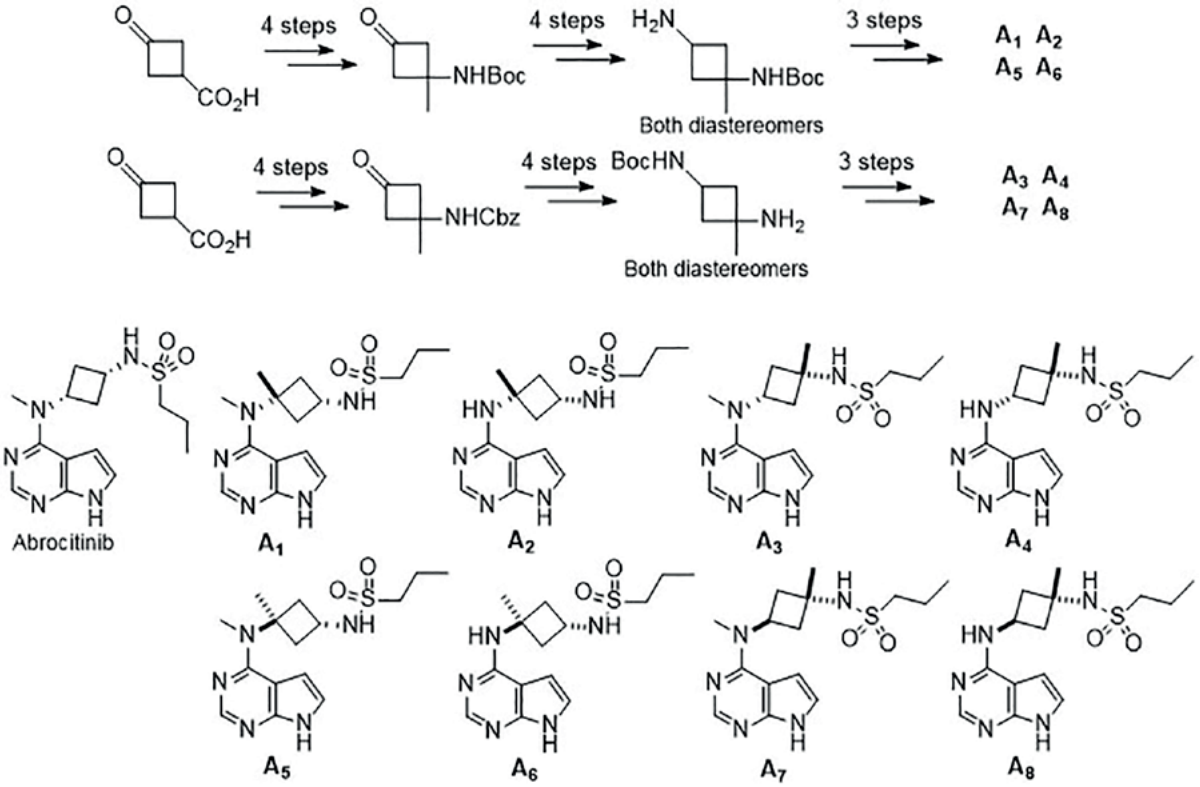
Introduction of a methyl group to biologically active molecules tends to change their properties drastically[1]. The effect is known as “magic methyl”. Therefore, methylated building blocks are required for drug discovery. Hereby, we present syntheses of 1-methylcyclobutane-1,3-diamines. Due to the presence of a cyclobutane moiety, the molecules are conformationally limited. Diagonal protection of the amino groups enables further chemoselective modifications. The substances were obtained as single diastereomers; relative configuration of the products was confirmed by NOE experiments. The reaction sequence requires common starting materials and consists of easily performable steps. The procedure was scaled up to yield 30 g of the desired amines in a single run.

To test the synthesized amines for modification of bioactive compounds, we obtained methylated Abrocitinib analogues and compared their physicochemical and biochemical properties.

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# THE SYGNATURE CHARMeD PROTAC PLATFORM

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*United Kingdom*

Conventional single-compound synthesis and analysis is time-consuming and poses challenges for selection of degrader components, predictive SAR and appropriate ADME properties for the assembled bifunctional compounds. Here, we demonstrate an integrated platform that incorporates high-throughput combinatorial chemistry, live-cell kinetic degrader screening and assessment of in vitro DMPK properties. Alongside this, we have in place computational methods for ternary complex prediction and linker selection. Altogether, our approach facilitates rapid screening and identification of bifunctional lead compounds.

# SYNTHESIS AND ANTIPROLIFERATIVE ACTIVITY OF NOVEL C-5 CLEISTANOLATE ANALOGUE

**Jelena Kesić (1), Ivana Kovačević (1), Mirjana Popsavin (1), Marko V. Rodić (1), Vesna Kojić (2), Velimir Popsavin (1,3)**

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Natural cleistanolate was isolated from the methanol extract of leaves of *Cleistochlamys kirkii*, Annonacae.[1] Our first total synthesis of natural cleistanolate,[2] and the results of a preliminary antitumour assay, encouraged us to synthesize new analogues of the natural product. Herein, we report the synthesis of novel cleistanolate analogue **1** starting from D-ribose (Figure 1) that was also used for the synthesis of the proposed structure of natural cleistanolate **2**.[2] Antitumour activity of compound **1** will be presented. The influence of the arrangement of functional groups as well as stereochemistry on antitumour activity will be discussed in detail.

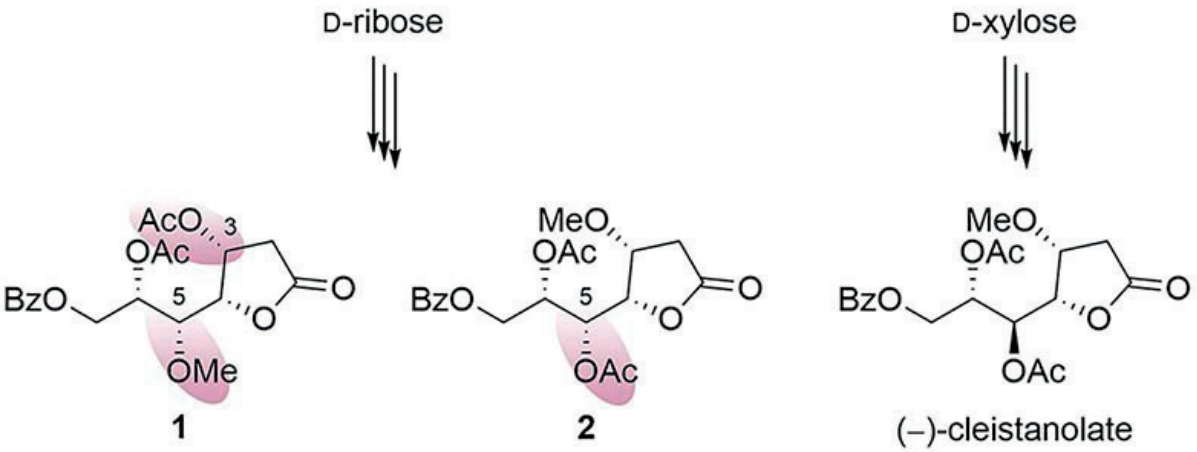


*Figure 1.* Structure of novel 3-*O*-acetyl-5-*O*-methyl analogue **1**, as well as C-5 epimer of cleistanolate **2** and natural product

*Acknowledgment*: The work was supported by a grant from Ministry of Education, Science and Technological Development (Grant No. 451-03-9/2021-14/200125, 451-03-68/2022-14/200125, 451-03-47/2023-01/200125), and (in part) by a research project from the Serbian Academy of Sciences and Arts (Grant No. F-130).

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# IDENTIFICATION OF HIT COMPOUNDS FOR DENGUE VIRUS INHIBITORS THROUGH LIBRARY SCREENING

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Dengue virus (DENV), a member of the flavivirus family, displays significant genetic diversity, leading to its classification into four distinct serotypes. These serotypes have a worldwide distribution and are linked to a wide range of clinical manifestations, from mild fever to the potentially life-threatening dengue hemorrhagic fever (DHF).[1] Importantly, the risk of severe disease increases when an individual is exposed to a different serotype after a primary infection. This heightened severity during secondary infections can be attributed to the presence of non-neutralizing or sub-neutralizing, cross-reactive antibodies generated from a previous heterotypic DENV infection. These antibodies can bind to the infecting DENV particles, but fail to effectively block or eliminate the infection. Instead, their interaction facilitates enhanced uptake of the virus by immune cells and promotes viral replication within the host, ultimately exacerbating the disease symptoms. Given the clinical significance of dengue and the risk associated with secondary infections, there is an urgent need for the development of a novel, small-molecule antiviral agent that can effectively target all four DENV serotypes.

In a recent development, the research group led by Neyts introduced acyl-indole and acyl-indoline derived compounds as potential anti-dengue viral agents.[2-3] These compounds have demonstrated significant antiviral activity against different serotypes of the dengue virus in both *in vitro* and *in vivo* studies. Particularly, JNJ-1802, a compound incorporating the acyl-indole moiety, is currently undergoing phase II clinical trials. Building upon these findings, we screened over 500 compounds and identified 30 primary hit compounds with diverse chemical structures. Some of these compounds have shown slight inhibition of DENV-2 replication. Currently, we are focusing on the development of anti-dengue viral agents by optimizing these primary hit compounds.

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# DESIGN AND SYNTHESIS OF TRUNCATED ADENOSINE DERIVATIVES AS $A_3$ ADENOSINE RECEPTOR ANTAGONISTS

**Minjae Kim, Siddhi D. Naik, Dnyandev B. Jarhad, Lak Shin Jeong**

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We conducted a study from Thio-Cl-IB-MECA, a potent and selective agonist for the human $A_3$ adenosine receptor (AR) with a $K_i$ value of 0.38 nM. Through molecular modeling, we determined that the hydrogen atom in the uronamide group plays a crucial role as a hydrogen bond donor, facilitating the ligand's induced fit into the $A_3$AR. Consequently, we observed selective and species-independent antagonism at the $A_3$AR when we removed the 4'-uronamide group, leading to the loss of the hydrogen bonding donor ability. Our objective was to investigate the steric effects by modifying the 4' position of the truncated 4'-thionucleoside with various small to bulky α and β alkyl substituents while ensuring the absence of a hydrogen bond donor, thereby preserving the antagonistic properties. To synthesize the desired nucleosides, we employed stereoselective organometallic addition and Vorbrüggen condensation as key steps, starting from 2,3-*O*-isopropylidene-L-erythrofuranose. Our structure-activity relationship (SAR) study yielded the following results: the $A_3$AR binding affinity decreased in the following order: β-methyl > dimethyl > α-methyl. We observed that only small substituents, such as H or Me, were tolerated in the antagonism binding pocket of the $A_3$AR. We will provide detailed information regarding the design, synthesis, and binding affinity to ARs in our presentation.

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# SYNTHESIS AND BIOLOGICAL EVALUATION OF 2-PHENYLBENZIMIDAZOLE DERIVATIVES AS NOVEL ANTI-INFLAMMATORY AGENTS

**Min Ji Kim (1), Min Ju Gwon (1), Hye Min Choi (1), Jayeon Lee (1), Chang Sang Moon (1), Kyung-Tae Lee (2), Jae Yeol Lee (1)**

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Endogenous prostaglandin $E_2$ ($PGE_2$) plays an important role in maintaining the homeostasis conditions. However, the over-expression of $PGE_2$ in response to various inflammatory stimuli is an important target of anti-inflammatory drugs. This study aims to develop novel anti-inflammatory drugs through selective inhibition of mPGES-1. In previous studies we reported that 2-phenylbenzimidazole derivatives showed significant inhibitory activity on $PGE_2$ production in A549 cells. Among them, **MPO-0221** showed the strongest reduction of $PGE_2$ levels ($IC_{50}$ = 0.42 μM). Compared to $PGE_2$ assay data, however, **MPO-0221** showed weak inhibitory activity against human mPGES-1 enzyme. To solve this problem, we decided to optimize the structure of **MPO-0221**. For this goal, we designed and synthesized a new series of 2-phenylbenzimidazole derivatives by introducing various functional groups into the scaffold. The newly synthesized compounds were evaluated for the inhibition of $PGE_2$ formation in IL-1β-stimulated A549 cells and exhibited the wide range of inhibitory activity. Especially, **MPO-0257** inhibited the production of $PGE_2$ in IL-1β-stimulated A549 cells with $IC_{50}$ value of 0.19 μM via the selective inhibition of mPGES-1 ($IC_{50}$ = 3.54 μM).

# CONCISE ENANTIOSELECTIVE TOTAL SYNTHESIS OF SALINOSPORAMIDES

**Sanghee Kim, Soojun Park**

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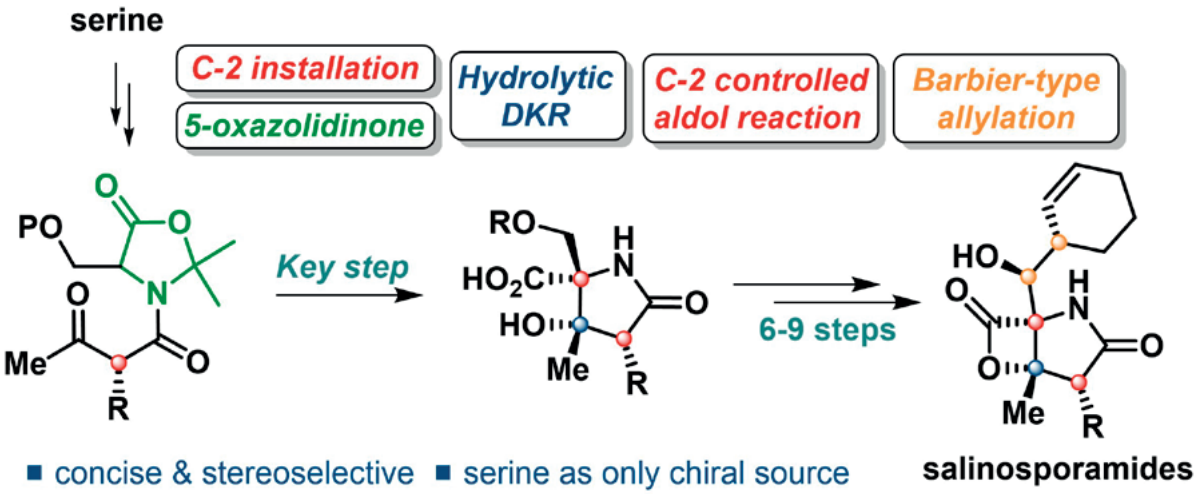
The characteristic structural feature of a family of salinosporamides is a densely functionalized $\gamma$-lactam-$\beta$ -lactone bicyclic core. The representative member, salinosporamide A, is a highly potent irreversible inhibitor of the 20S proteasome and was entered into human clinical trials under the name marizomib.[1] Due to their interesting structures and biomedical properties, salinosporamides have attracted great interest from scientists within the synthetic and medicinal research communities.[2]

With a strategy that allows rapid access to the chiral pyrrolidinone core, the total syntheses of salinosporamide A/B and cinnabaramide A/E/F were completed both concisely and with potential modularity. The key to the success of this synthesis was to explore and exploit the innate properties of the serine-derived 5-oxazolidinone as a stereochemical inducer. We have found that the 5-oxazolidinone moiety acts in a manner similar to that of Evans' 2-oxazolidinone chiral auxiliary and utilized this moiety for selective installation of stereocenters. In addition, we observed an interesting and unexpected hydrolytic dynamic kinetic resolution (DKR) in hydrolyses of 5-oxazolidinone/pyrrolidinone bicyclic aldol products. This substrate-driven hydrolytic DKR of diastereomers is the first example and was utilized in selective preparation of the pyrrolidinone core with excellent efficiency.

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# DISCOVERY OF NOVEL SYNTHETIC TOLL-LIKE RECEPTOR 7 AND 8 DUAL AGONISTS AS VACCINE ADJUVANT

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The advent of modern vaccines has witnessed a significant shift away from utilizing whole, killed, or attenuated pathogens towards subunit components. Although this approach has resulted in diminished immunogenicity, there is a critical demand for vaccine adjuvants that can potentiate the immune response to purified antigens. Adjuvants offer a multitude of benefits, including dose sparing, enhanced vaccine efficacy in immunocompromised individuals, and the potential to confer protection against highly mutable pathogens by broadening the immune response. Among the promising adjuvant candidates are Toll-like receptor (TLR) agonists, renowned for their capability to bridge the innate and adaptive immune responses. TLRs represent a prominent family of transmembrane receptors predominantly expressed by innate immune cells. Each TLR exhibits a leucine-rich repeat (LRR) segment responsible for recognizing pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs). Additionally, a Toll/interleukin-1 receptor (TIR) domain facilitates downstream signal transduction, triggering an inflammatory response. Consequently, TLRs serve as excellent targets for adjuvants, capable of providing a vital "danger" signal to instigate an effective immune response that bestows enduring protection.

Within our research program, we have devoted extensive efforts to unraveling the intricate structure-activity relationships governing small molecule agonists that specifically target TLR7 and TLR8. Our primary objective is to identify potent vaccine adjuvants that not only elicit robust immune responses but also minimize any potential for reactogenicity. Notably, we observed higher antibody levels when administering a combination of TLR7/8 agonist as an adjuvant alongside SARS-CoV-2 spike protein antigen, compared to administering the antigen alone. This breakthrough discovery holds great promise for advancing the field of adjuvant development and improving the effectiveness of vaccines against a wide array of infectious diseases.

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# UNLOCKING NEW THERAPEUTIC OPPORTUNITIES: COVALENT INHIBITION OF KRasG13C FOR PRECISION CANCER TREATMENT

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The small GTPase KRas acts as a molecular switch in cellular signaling by cycling between the inactive GDP-bound and active GTP-bound states. In 25% of all human cancers, this mechanism is dysregulated by oncogenic Ras mutations that shift the equilibrium towards the active form, thus making KRas an attractive drug target for precision medicine.[1)] In 2013, the Shokat lab[2)] achieved a tremendous breakthrough by covalently targeting a previously undiscovered allosteric pocket (*switch II pocket*) of KRasG12C,which finally led to the development and approval of sotorasib (AMG510) and adagrasib (MRTX849), which have revolutionized the treatment of Ras-dependent lung cancer.[3-5)] Since then, several approaches to utilize this pocket for addressing other G12 point mutations, including G12D and G12S, have been described.[6-7)]

Based on these breakthrough approaches in targeting KRas variants, we aim to address the oncogenic mutant KRasG13C [8)], which until today remains unexplored for therapeutic intervention. Here, we report for the first time, the structure-based design and synthesis of novel allosteric small molecule modulators that covalently bind to KRasG13C. The resulting molecules have been tested using a set of biochemical methods and will be further evaluated with regard to their cellular efficacy. Additionally, crystallization experiments are underway that will give valuable insights into inhibitor binding and thus guide the chemical optimization of this novel class of covalent modulators.

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# 3-$CF_3$/$C_2F_5$-SUBSTITUTED PROLINE ANALOGS: SYNTHESIS AND THE PROTEOLYTIC STABILITY OF THEIR AMIDE DERIVATIVES

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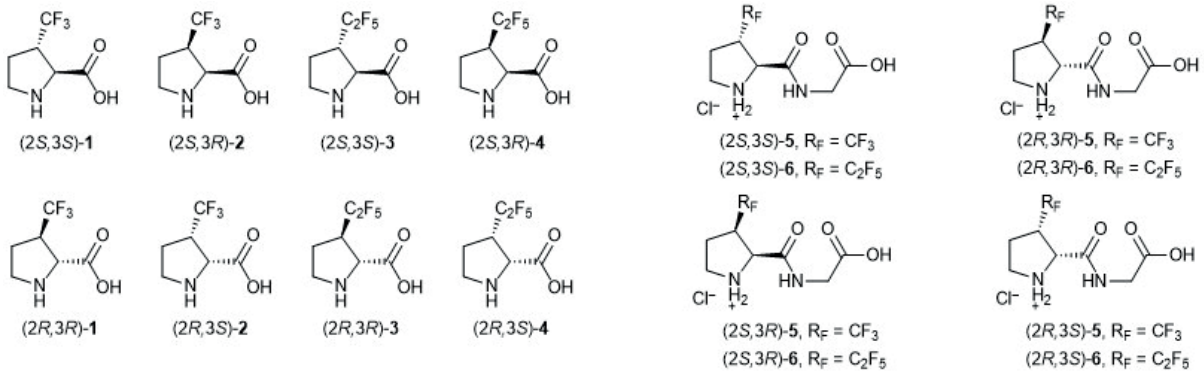
Fluorinated proline analogs became important and valuable tools in peptide studies, protein engineering and medicinal chemistry [1,2]. Fluorinated motifs provide unique modulation capabilities and affect proline's acidity/basicity, lipophilicity, conformation, and trans/cis rotameric preferences of its amide derivatives. Therefore, medicinal chemists often apply fluorinated proline derivatives to improve the physicochemical properties, selectivity, and potency of novel pharmaceuticals.

Recently our group has reported the synthesis of pure cis-/trans- 3-$CF_3$-and 3 $C_2F_5$-Proline **1-4** [3]. As a continuation of the work, we optimized the reaction sequence and synthesized all the enantiomers of compounds **1-4**. In addition, we studied the impact of the fluorine substituent on the hydrolytic stability of model dipeptides5-6 prepared from the corresponding $CF_3$- and $C_2F_5$-substituted prolines [4]. Particularly, *cis*-isomeric derivatives (2*S*,3*R*)-**5,6** demonstrated remarkable proteolytic stability in comparison with *trans* isomers (2*S*,3*S*)-**5,6**.

The particularities of the synthetic approach and proteolysis data will be demonstrated in detail during the presentation.

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# DISCOVERY OF N-SUBSTITUTED-5H-DIBENZO[a,d][7]ANNULEN-5-AMINES AS POTENT N-METHYL-D-ASPARTATE RECEPTOR ANTAGONISTS FOR THE TREATMENT OF NEUROLOGICAL DISORDERS

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*N*-Methyl-d-aspartate (NMDA) receptors are ionotropic glutamate receptors widely distributed in the CNS and are responsible for synaptic plasticity and mediating learning and memory functions. Overactivation of NMDA receptors leads to neuroinflammation which plays a crucial role in neurological disorders such as Alzheimer´s disease, Parkinson´s disease, stroke and many others. Thus, use of NMDA receptor antagonists hold a potential in treatment of those conditions. However, NMDA receptors antagonists are often connected with negative side effects such as hyperlocomotion, psychosis and cognitive disruption. Thus development of novel, potent NMDA receptor antagonists with better safety profile is in high importance for the treatment of neurological diseases. In our study, we designed, synthesized, and biologically evaluated 29 novel *5*H-dibenzo[*a,d*][7]annulen-5-amines analogues. The in silico prediction suggested good oral availability together with potential to permeate through the blood-brain barrier (BBB). An initial assessment of the biological profile included determination of the NMDA receptor antagonism at the GluN1/GluN2A and GluN1/GluN2B subunits, along with cytotoxicity profile in the CHO-K1 cell line. Those results suggested novel compounds as potent antagonists of NMDA receptor with good cytotoxicity profile. Behavioral evaluation of selected derivatives showed no signs of elevated locomotion compared to typical NMDA antagonist MK-801 suggesting no side effects of lead compounds.

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# AUTOMATED SYNTHESIS OF PROTEIN DEGRADERS FOR DRUG DISCOVERY

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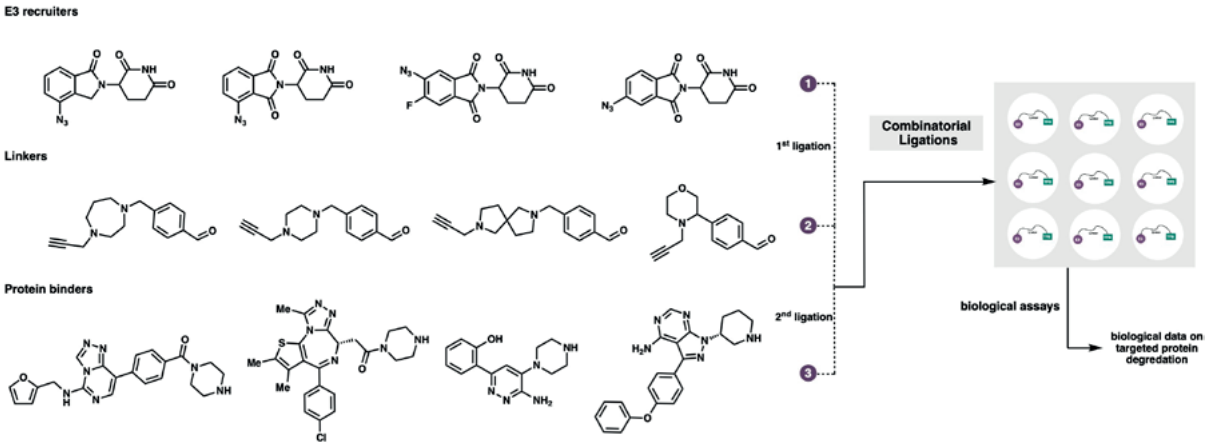
As part of ongoing efforts to automate the preparation of PROTACs, we are employing new technologies that make synthesis safer, more reliable and more predictable. In this respect, we have developed an automated synthesis platform technology for the assembly of libraries of potential PROTACs, which utilises a three-component coupling of a protein binder, a linker and an E3 recruiter. This three component coupling-based approach can be effected using a number of different complementary chemical reactions and enables the generation of multiple candidates in a combinatorial fashion.




In this poster, we report on the design and synthesis of novel, appropriately functionalised linkers, which can be combined with E3 recruiters and protein binders using click chemistry and reductive amination reactions. Furthermore, this library assembly can be effected in an automated, 96-well plate format, enabling the rapid and reliable preparation of large numbers of PROTACs.

# DEVELOPMENT OF SMALL MOLECULE CHEMICAL PROBES TO INVESTIGATE FABP5 FUNCTION

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Fatty-acid binding proteins (FABPs) are involved in the transport and metabolism of hydrophobic molecules such as fatty acids and hormones. It has become apparent that besides their primary role as chaperones, FABPs are also involved in complex signalling pathways. Epidermal FABP, also known as FABP5, has been linked to a range of diseases including cancer and metabolic diseases.[1, 2] Although the specific processes that involve FABP5 are not fully understood, a common feature of these diseases is that they involve aberrant lipid utilisation.To better understand these mechanisms, potent and selective FABP5 chemical probes are needed.

Our approach aims to combine knowledge from literature and fragment-based screening (FBS) to develop high affinity and selective chemical probes for FABP5. A fragment screening cascade by ligand-detect and protein-detect NMR has identified 26 fragment hits. Structural information of these fragments along with literature data enables us to identify opportunities for fragment linking/growing. Structural differences between FABP5 and FABP4 are also being exploited with the aim to generate selectivity over FABP4, the most closely related FABP isoform to FABP5. Compounds that are synthesised in this project are tested for their ability to bind to FABP5 and FABP4 in a range of different biophysical binding assays, including NMR, ITC and SPR. In parallel, a thiophenylamide scaffold described in the literature has been investigated. Compounds from the thiophenylamide series have been identified that have high affinity with good physicochemical properties, but are not selective for FABP5.[3] Efforts are currently focused on elaboration of the thiophenylamide scaffold using the rich source of fragment binding information to achieve selectivity over FABP4. Alongside this, a structure-based design approach is being followed for the development of FABP5 binding fragments. The ultimate goal is to combine these two approaches towards the design of potent and selective FABP5 ligands.

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# NOVEL ANTITUBERCULAR AGENTS BASED ON 5H-PYRROLO[3,2-d]PYRIMIDINES

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Tuberculosis (TB) is one of the top 10 causes of death worldwide from a single infectious agent (*Mycobacterium tuberculosis*-Mtb). The World Health Organization (WHO) estimated 10.6 million new cases and 1.4 million deaths from TB in 2021.[1] Some strains of mycobacteria causing TB show numerous resistances to first-line drugs (isoniazid /INH/ and rifampicin) and to second-line drugs (fluoroquinolones, amikacin, bedaquilin, etc.). The development of new anti-TB drugs with new mechanism of action is necessary to improve TB therapy and to fight against resistant TB as well.

In our previous study we identified purine-based compound K2032 with good anti-TB activity with minimum inhibitory concentration $MIC_{99}$ = 1 μM against $H_{37}Rv$ strain (for comparison, $MIC_{99}$ (INH) = 0.5 μM). The whole-genome-sequencing of resistant strains revealed that this compound is noncovalent inhibitor of DprE1. In this follow-up study, we focused on new class of antitubercular agents 5*H*-pyrrolo[3,2-*d*]pyrimidines, by the application of scaffold hopping strategy. During the study we elucidated the structure-activity relationships (SAR) and determined cardiotoxicity, cytotoxicity, aqueous solubility and antibacterial activity of the most active compounds.

The results of the study highlighted two compounds, namely K2653 and K2654. These derivatives showed high anti-TB activity not only against the drug-sensitive $H_{37}Rv$ strain but also against extremely resistant strains (K2653 and K2654: $MIC_{99}$ = ($H_{37}Rv$, XDR-TB) = 0.06 μM). Moreover, these two hits also showed high activity against G+ bacteria, especially against methicillin-resistant *Staphyloccus aureus* ($MIC_{99}$ = (MRSA *S. aureus*) = 2 μM, MBC = (MRSA *S. aureus*) = 10 μM).

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# HUMAN AGO2 INHIBITOR SCREENING ASSAY

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Argonaute 2 (Ago2) controls protein expression by binding short RNA sequences (such as miRNAs) and forming the base for the RNA-induced silencing complex (RISC) and RNA-induced transcriptional activation complex (RITA). Preventing the formation of these complexes would offer new possibilities in the treatment of diseases where protein or miRNA expression is disturbed, such as cancer. Only a few micromolar inhibitors of Ago2 have been published in the literature (1), and in this project we would like to find more potent inhibitors, for which we need an in vitro assay. Our assay is based on a previously published methodology based on fluorescence polarization (1). Simply put, the fluorescently labeled RNA displays high polarization values when it is unbound, and binding to the protein reduces this value. We have set up and optimized local production of the human Ago2 protein, optimized the assay by experimenting with different RNA sequences, fluorescence polarization wavelengths, incubation times and validated the assay using known Ago2 inhibitors. We also conducted a small-scale virtual screening campaign of the Finnish Molecular Medicine database (FIMM) for Ago2 inhibitors. As a conclusion, we have set up a relatively cost-effective in vitro screening assay for human Ago2 inhibitors.

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# SELECTION CRITERIA FOR CAPPING AGENTS FOR DNA-ENCODED LIBRARY TECHNOLOGIES (DELT)

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DNA-encoded library technologies allow selection of the desired bioactive molecules from $10^7$-$10^{10}$ candidates, while HTS screening campaigns are usually limited to $10^6$-$10^7$ compounds[1]. The other advantage is the run time for a DEL experiment is largely independent of a library time. Although enabling the greater size of combinatorial libraries and faster analysis of druglike compound collections, DELT have strict requirements for capping agents.

Some of the limitations (molecular weight, logP, number of hydrogen bond donors and acceptors) stem from Lipinski's rules. While the abovementioned requirements are applicable for both DEL and traditional building block libraries, there are also some special limitations for DEL capping agents, e. g. compatibility with DNA, ability to react in aqueous, non-acidic medium, at temperatures below 90°C with high conversion and selectivity. More information about properties, requirements, and diversity of capping agents that are currently available is needed for efficient construction of DNA-encoded libraries. Currently, the landscape of the market of the available molecules in the world stock is determined by the demands of HTS, not DELT. Hereby, we will discuss broad spectrum of applicability criteria for the available pool of building blocks, in terms of "DELT incompatibility" and "likely DELT compatibility", using E-molecules, Chemspace and Enamine databases as a case study.

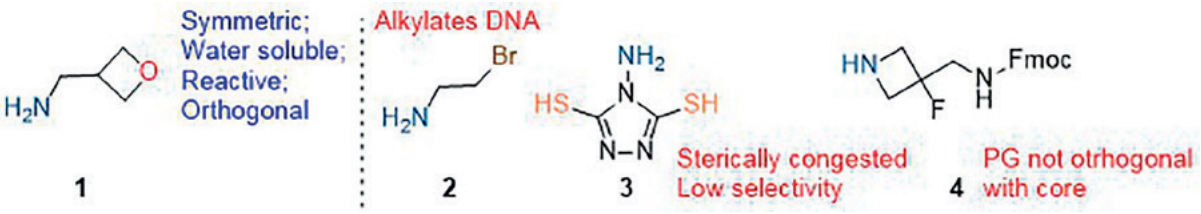


**Figure 1.** Examples of amines – capping agents candidates that are "likely DELT compatible" (compound **1**), and "DELT incompatible" (compounds **2**-**4**).

In the current work, we will discuss our in-house chemoinformatical filters that allow extracting "likely DELT compatible" building blocks from the general pool, using criteria that account molecular weight, substituents compatibility, $Fsp^3$, possible reactivity, water solubility, and chirality. We will disclose the statistical data on the content of the "likely DELT compatible" molecules comparing to the overall array of stock building blocks in the given dataset and discuss the classification of the selected compounds.

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# SEARCH FOR CORE POLYFUNCTIONAL BUILDING BLOCKS FOR DNA-ENCODED LIBRARY TECHNOLOGIES (DELT) IN CHEMICAL DATABASES

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DNA-encoded library technologies enable investigation of druglike molecules on an unprecedented scale while trillions of molecules are examined in a single project. As the compounds are tested as a mixture, an average price of DEL is much lower in comparison to HTS screening library. Despite great advantages of DELs, cores (i. e. bifunctional and trifunctional molecules) need to meet strict requirements, e. g. compatibility with DNA, orthogonality of functional groups, appropriate reactivity in aqueous non-acidic media at temperatures below than 90°C. Therefore, an appropriate collection of commercially available bifunctional and trifunctional building blocks is demanded. An analysis of the open source databases representing the building blocks market shows that bifunctional and especially trifunctional molecules are much fewer in number than monofunctional ones.[1]

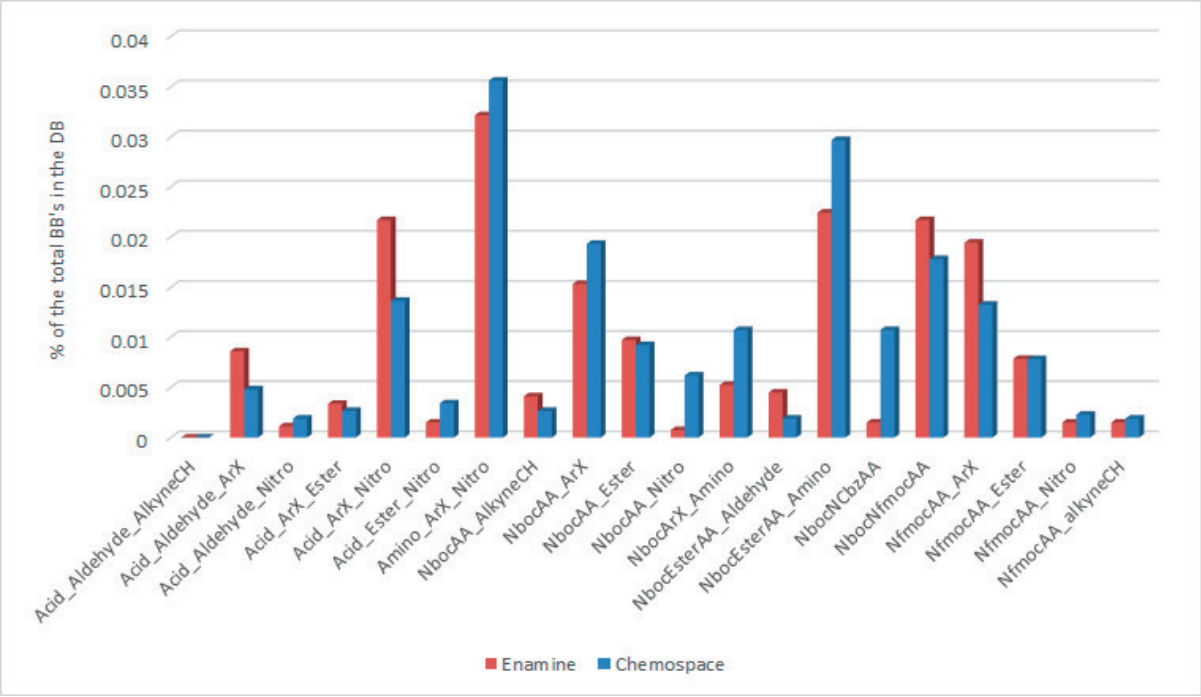


**Figure 1.**Percent of trifunctional DEL-compatible building blocks related to the total count of stock compounds, Enamine collection (red) and Chemspace database (blue).

We analyzed E-molecules, Molport, Chemspace and Enamine databases and found that the show similar pattern of distribution between capping agents (monofunctional building blocks) and the cores (di- and trifunctional). Using our in-house filters on Enamine database, we managed to extract a pool of 13347 bifunctional blocks and classify them in 25 subcategories, and 371 trifunctional cores representing 18 subcategories. The in depth statistical analysis of the current state of the core building blocks market within the given dataset was performed. The optimal search and selection algorithms for polyfunctional DELT-compatible molecules were found.

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# A NEW DRUG FOR TREATING CANINE COGNITIVE DYSFUNCTION

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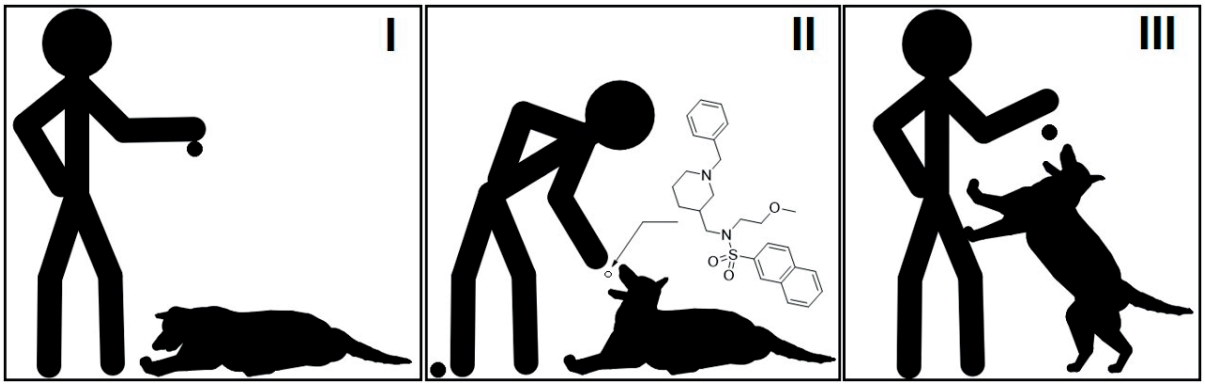
Canine cognitive dysfunction (CCD) is an incurable neurodegenerative disease which effects 60% of older dogs and its most prominent sign is cholinergic hypofunction[1]. As long as this disease cannot be prevented or cured, symptomatic treatment is critical. The only FDA approved drug for treating symptoms CCD is selegiline. This monoamine B inhibitor has adverse effects which make tolerability a significant limitation[2]. However, this problem can be overcome by inhibiting a different enzyme, butyrylcholinesterase (BChE).

We used virtual screening to discover our hit compound, a novel piperidine-based nanomolar selective BChE inhibitor[3]. Using ligand-based and structure-based drug design for hit-to-lead optimization, we produced and evaluated a huge number of various derivatives[4]. Our most promising compound is the sulfonamide derivative which improved memory, cognitive functions and learning abilities of dogs suffering from CCD with no adverse effects. All owners of treated dogs reported a drastic improvement in the quality of life and dog-owner interaction.[5] Our selective BChE inhibitor is a new drug for treating CCD and is a drug candidate for treating other forms of dementia, like Alzheimer's disease in humans.

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# A WAY TO BROAD SPECTRUM ANTIVIRAL AGENTS: SMALL MOLECULES BASED ON PIPERAZINE CORE AS POTENTIAL FLAVIVIRIDAE NS3 PROTEASE INHIBITORS

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*Flaviviridae* virus family is subdivided into four genera namely, *Flavivirus, Hepacivirus, Pegivirus* and *Pestivirus* [1,2]. The incidence of Flavivirus infection has grown significantly, with approximately 400 million people infected annually [3] and widely distributed as consequence of globalization and climate changes, causing public health problems worldwide [4,5]. Currently, there are no effective drugs available to treat most of these infections. All genera of the *Flaviviridae* family show similarities in the organization of the viral genome, which is characterized by a single-stranded positive-sense RNA molecule. The whole viral genome is translated into a viral polyprotein, which is further processed by both host and viral proteases into 9 to 12 mature proteins, consisting of structural and nonstructural (NS) proteins [1,6]. The NS proteins participate in the replication of the RNA genome, virion assembly and interaction with innate host immune response [7]. As a result, these viral proteins represent relevant targets for the development of novel antiviral therapies. Among these, the NS32B proteases play an important role in the virus life cycle, making them attractive targets for antiviral drug discovery [8].Taking advantage of our previous research focused on the development of effective antiviral agents based on piperazine backbone [9], we have recently described two piperazine-derived compounds as promising and non-cytotoxic broad spectrum anti Flavivirus agents [10].The compounds were designed using a privileged structure-based approach, and their antiviral properties were determined by a live virus cell based phenotypic assay against ZIKV and DENV [11], leading to the identification of these promising lead compounds. Based on these important results, this study focuses on the design and synthesis of a small library of molecules acting as potential NS3 protease inhibitors. Our approach involves an optimization process aimed at preserving the molecular complexity of these compounds, which includes three aromatic/aliphatic rings linked by amide and urea/sulfonamide functions. Due to the relevance of the piperazine ring in biological activity of these compounds [12], we decided to retain it as the central core.The focus of this research is to improve the antiviral activity against both viruses and simultaneously obtaining non-cytotoxicity products associated with these lead compounds.

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# Gem-DIFLUOROMETHYLENE DECORATED CYCLIC BUILDING BLOCKS - IMPACT ON PHYSICOCHEMICAL PROPERTIES

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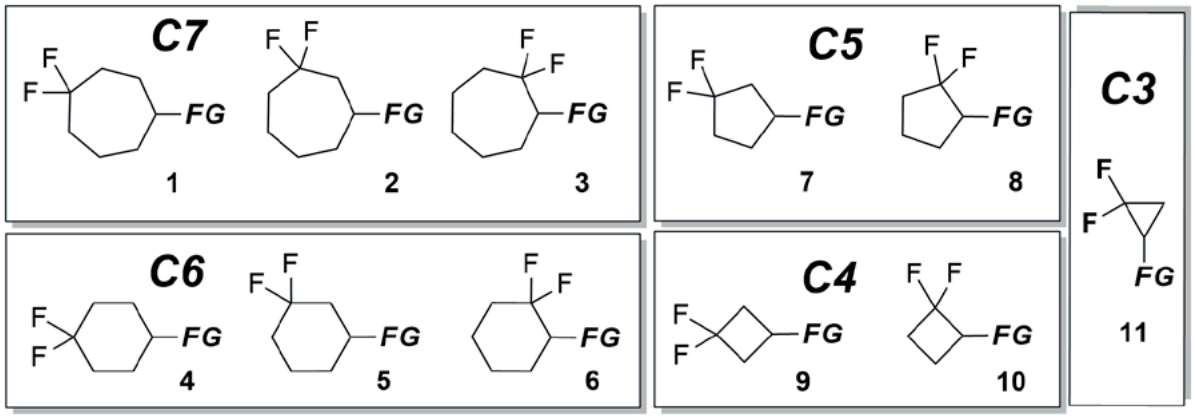
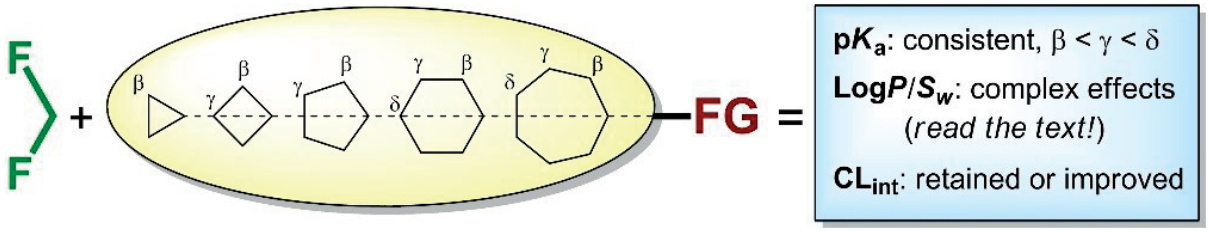
Fluorinated cycloalkane building blocks are useful structural motifs that become increasingly important in various areas, and most of all in drug discovery and agrochemistry. Recently, we elaborated chemical routes to the compounds types **1-11**, which can be promising for further industrial scale-up. These investigations, as well as pioneering lab-scale approaches to the C7 **1-3** types compounds and cyclobutanes, type **10** building blocks, will be discussed in the report.




FG = COOH, $NH_2$, OH, Br, $B(OAlk)_2$ etc.

Also, the effect of *gem*-diflurination on acidity/basicity ($pK_a$), lipophilicity (LogP), aqueous solubility ($S_w$), and metabolic stability (intrinsic clearance, $CL_{int}$) of functionalized C3–C7-cycloalkanes is established and compared to those of non-fluorinated and acyclic counterparts. All these investigations will be discussed in the presentation.

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# TAG-FREE ENCODING OF COMPOUND COLLECTIONS BY HIGH EFFICIENCY ISOTOPE LABELLING

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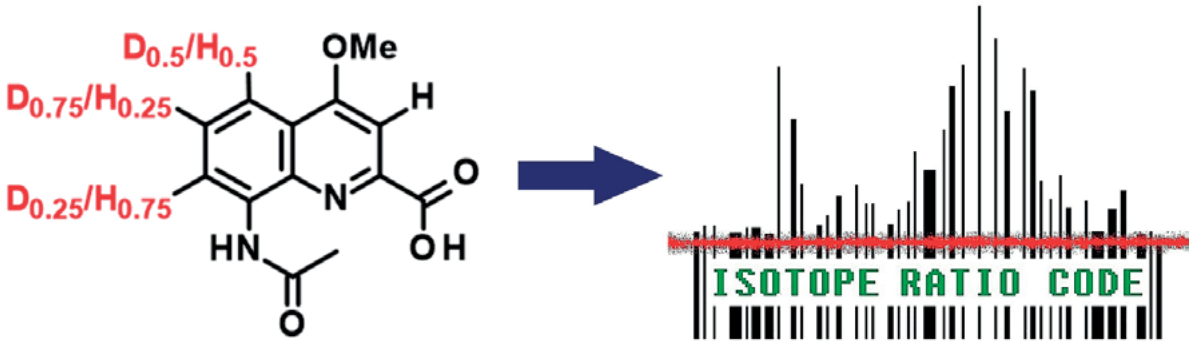
The efficient and reliable screening of large compound collections against a biological target is an evergreen topic in drug discovery. Over the last decades the scientific community developed multiple approaches to address this need ranging from the high throughput screening of single molecules to the testing of compound mixtures where each compound carries a unique chemical tag (i.e. DNA encoded libraries). In an ideal case we should be able to combine the advantage of both approaches: screen large compound collections in a single experiment, where each compound would be distinguishable from the others without bearing any chemical tag. Since the molecular interaction of a given protein and a small molecule is typically independent of their isotopic composition, isotope labelling could help to achieve this dream. Although the concept of using isotopes for compound coding has been around for several decades[1] there were several unsolved problems that blocked its practical use.

We set out to establish the necessary tools to enable the use of isotope-encoded compound collections in drug discovery. The major challenges we addressed in the process include:

- establishing of efficient synthetic methods for the highly efficient and selective introduction of less abundant isotopes ($^{2}H$, $^{13}C$, $^{15}N$) into organic molecules
- defining an isotope distribution based coding method that has the power to uniquely encode large compound collections
- validating the code reading technique that enables the unambiguous identification of any compounds




The presentation describes our achievements in the above areas that led to the concept of isotope ratio encoding.[2] It also discusses the on-going implementation of this method to identify hits for biological targets of interest, and potential applications beyond medicinal chemistry and chemical biology.

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# THE DISCOVERY OF NOVEL ABC PROTEIN MODULATORS WITH IMPLICATION TO ALZHEIMER´S DISEASE THERAPY

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Alzheimer's disease (AD) is a progressive neurodegenerative disorder causing neuronal death and severe cognitive impairment due to pathological accumulation of macromolecular Aβ and tau in the brain. The treatment of AD is only symptomatic, with limited proof-of-concept for disease-modifying agents. The peptide chains forming the Aβ protein aggregate have been found to be substrates of adenosine triphosphate-(ATP)-binding cassette (ABC) transporters. Activators of these membrane efflux proteins capable of binding and/or translocation of Aβ may pose a breakthrough in the treatment of AD. Although the knowledge about ABC transporter activators is still limited, the few molecules that were reported contain substructural motifs of multitarget (pan-)ABC transporter inhibitors. The starting point for the development of innovative activators to boost Aβ clearance from the brain is to explore and potentially exploit the recently proposed multitarget binding site of pan-ABC transporter inhibitors, which represents a promising strategy to develop new drug candidates. Molecular relationships between functional bioactivities and physicochemical properties of small-molecules are essential to understand these processes. This contribution is an analysis of recently reported unique multitarget dataset including the correlation between multitarget bioactivity and physicochemical properties. Six novel pan-ABC transporter inhibitors were evaluated bearing sub-structural motifs of ABC transporter activators, which emphasizes the importance of the multitarget binding site for the targeted development of novel AD diagnostics and therapeutics.

Study was supported by the EEA and Norway Grants 2014-2021 and state budget of the Czech Republic within the TARIMAD project sponsored by TACR Kappa programme (Iceland, Lichenstein, Norway and Czech Republic; No. TO01000078), and the EU Joint Programme – Neurodegenerative Disease Research (JPND) project PETABC (MSMT No. 8F21002)

# ELECTROPHILIC [4+1]-CYCLIZATION OF PICOLINES – EFFICIENT AVENUE TOWARDS 6-AZAINDOLE AND 6-AZABENZOFURANES

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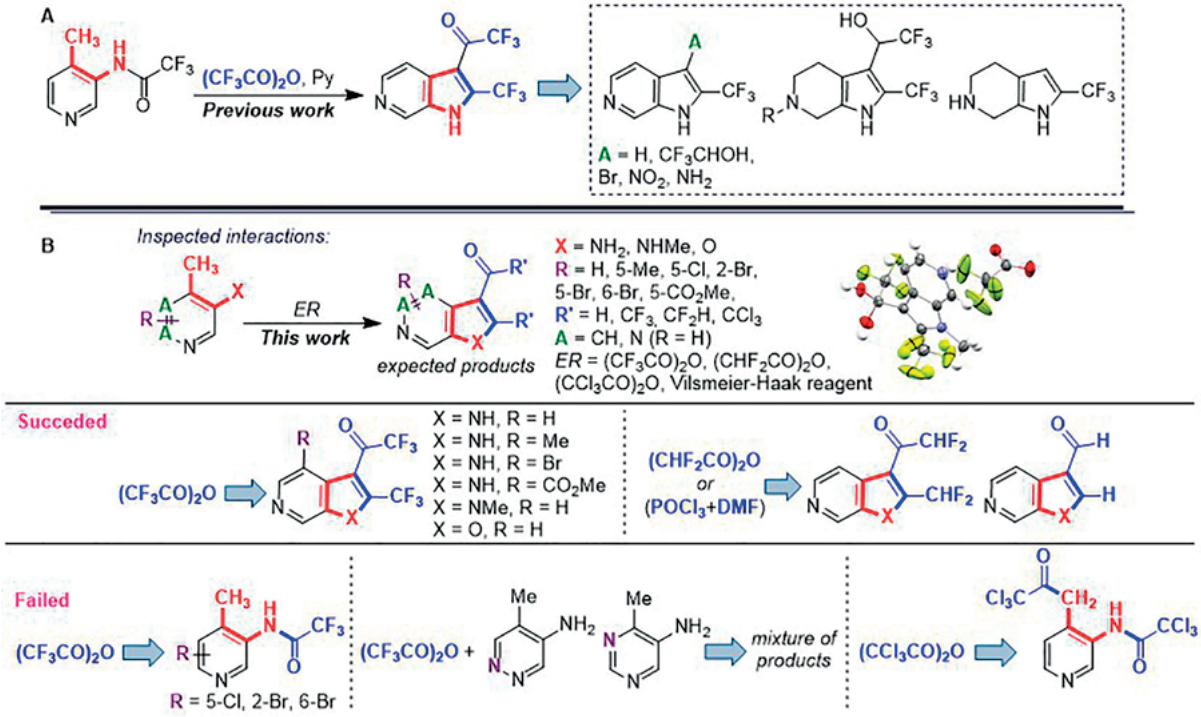
Formal substitution of benzene part in some condensed heterocyclic scaffolds with its aza-analogue has become an effective strategy to modify and widen a range of biological activities for known synthetic and natural compounds. Thus, switch from coumarin to 7-azacoumarin core is a justified and well-reported approach in medicinal chemistry of heterocyclic compounds. In our previous work, we have communicated a scalable and efficient synthesis of the 6-aza-counterpart of reputable indole scaffold (Scheme, **A**).[1] In its turn, 6-azaindole has already gain a great value in medicinal chemistry. Although this system has not been found in nature, its benzoannulated analogue β-carboline is a part of about 800 natural products. The reported method relied on interaction of 3-trifluoroacetylaminoamino-4-methylpyridine and trifluoroacetic anhydride (TFAA) providing 2-trifluoromethyl-3-trifluoroacetyl-6-azaindole. Ways to several derivatives of the azaindole have been disclosed as well. In the project reported herein, we expanded the scope of the elaborated approach with reference to both the aminopicoline and the electrophilic reagent (Scheme, **B**) and also modified the procedure to a one-pot option starting from the corresponding free NH(OH)-derivative. The investigations allowed us to establish some regularities and limitations of the interaction. Thus, 4-methylpyridines bearing in β-position $NH_2$, OH or NHMe group and a substituent in another β-position gave corresponding fused pyrrolo-/furano- derivatives in preparative yields. On the other hand, in the case of pyridines bearing α-substituents or strong σ-acceptors in the β-position (Cl) and reacting with the same reagent the isolated product was only corresponding N-acylated derivatives. As for other electrophilic components, while difluoroacetic anhydride and Vilsmeier-Haack reagent gave results similar to trifluoroacetic anhydride, trichloroacetic anhydride afforded the double $NH_2$/Me acylated product without expected cyclization.The obtained results provide understanding of the cyclization mechanism and enable to predict the substrates' activity.

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# SYNTHESIS AND EVALUATION OF BUTEIN DERIVATIVES FOR IN VITRO AND IN VIVO INFLAMMATORY RESPONSE SUPPRESSION IN LYMPHEDEMA

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Lymphedema occurs when an abnormality or injury in the lymphatic system blocks adequate lymph drainage from the arm or leg. Lymphedema is classified into congenital lymphedema and acquired lymphedema. Acquired lymphedema is often caused by cancer treatments, such as surgery, radiation, and chemotherapy, or after physical trauma or infections. The manifestations of lymphedema include swelling, reduced functionality of the limbs, and subsequent secondary infections, lead to psychological distress and detrimental effects on social and work life. Currently, there is no therapeutic agent that can inhibit lymphedema progression or alleviate its symptoms. Complete decongestive therapy (CDT), a physiotherapeutic approach, is commonly accepted as the primary treatment for lymphedema as it aims to decrease fluid accumulation.

Although many efforts have been underway to find new targets for systemically controlling lymphedema, its molecular mechanisms are not clearly defined. It is known that the accumulation of interstitial fluid leads to inflammation, fat deposition, and fibrosis. Many reports have indicated that immune responses related to inflammatory cell infiltration play a critical role in the pathophysiology of lymphedema. Upregulation of inflammatory genes have been observed in both murine models and patientswith lymphedema. Based on previous studies, inflammatory responses were necessary for lymphedema formation during adipose tissue inflammation in obese mouse models. Swapna et al. also revealed that the proinflammatory responses caused by macrophage infiltration can be significantly affected in response to lymphedema. Patients using CDT showed reduced expression of tumor necrosis factor(TNF)-a and other pro-inflammatory mediators. These data suggest that anti-inflammatory agents can be potential targets for alleviating lymphedema progression.

Several anti-inflammatory compounds have been tried in experimental lymphedema models and have shown promising effects. Ketoprofen, a commonly used nonsteroidal anti-inflammatory drug (NSAID), was subcutaneously applied to a murine tail and it ameliorated swelling and other pathologic symptoms. Tacrolimus, an anti-T-cell agent used for chronic cutaneous inflammation and fibrosis, was successful in preventing and improving lymphedema in the above-mentioned lymphedema model. Although ketoprofen and tacrolimus are currently being assessed as novel clinical lymphedema therapies, their potential toxicities may limit their long-term use.

In our investigation into finding therapeutic candidates, we previously established a new lymphedema mouse model that mimics lymphedema in cancer patients by removing a superficial inguinal lymph node, a popliteal lymph node, a deep inguinal lymph node, and the femoral lymphatic vessel. Using the model, we found that Rhus verniciflua Stokes (RVS) ameliorated hind leg edema. RVS extract contains several anti-inflammatory polyphenols, including sulfuretin and compound **1**. Compound **1** is more potent than other polyphenols, e.g., sulfuretin, in suppressing lipopolysaccharide (LPS)-induced nitrite and prostaglandin $E_2$ production in macrophages. In addition to its anti-inflammatory activity, compound **1** can inhibit adipogenesis and fibrosis. These properties prompted us to explore the systemic effect of compound **1** on lymphedema. At the same time, we attempted to optimize the chemical structure of compound **1** in order to improve in vitro and in vivo efficacy through structure-activity relationship (SAR) analysis. The goal was to produce a prodrug of compound **1** to enhance the potency, solubility, permeability, and pharmacokinetic parameters, including half-life and pharmaceutically active levels in the blood.

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# NEW N-(1-HYDROXY-1,3-DIHYDROBENZO[C][1,2]OXABOROL-6-YL)(HETERO) ARYL-2-CARBOXAMIDES AS POTENTIAL COVALENT INHIBITORS OF MYCOBACTERIAL LEUCYL-tRNA SYNTHETASE

**Marta Kucerova (1), Petr Slechta (1), Adam Anthony Needle (1), Ondrej Jandourek (2), Pavla Paterova (3), Klara Konecna (2), Pavel Barta (4), Martin Dolezal (1)**

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With the aim to combat tuberculosis (TB) and find novel anti-TB drugs[1], a series of N-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-6-yl)(hetero)aryl-2-carboxamides was prepared via the acylation of 6-aminobenzo[c][1,2]oxaborol-1(3H)-ol with various activated (hetero) aryl carboxylic acids[2]. These novel compounds have been tested in vitro against a wide panel of clinically important fungi and bacteria, including mycobacteria. Some of the compounds inhibited the growth of mycobacteria in the range of micromolar concentrations. Their activity was retained also against multidrug-resistant clinical isolates. The toxicity of the compounds against the HepG2 cell line was low for most of the compounds. Their selectivity against mycobacteria was proved in screening tests against representatives of G+, G- bacterial and fungal strains. Some structure-activity relationships have been derived. A molecular docking study confirmed a selectivity toward the potential target leucyl-tRNA synthetase without an impact on the human enzyme. The presented compounds can become promising leads in future antimycobacterial research.

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# BEYOND UAMC-3203: EXPLORING THE CHEMICAL SPACE TO DEVELOP THE NEXT-GENERATION OF FERROPTOSIS INHIBITORS

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Ferroptosis is non-apoptotic programmed form of cell death triggered by iron-dependent lipid peroxidation.[1] The term “ferroptosis” was introduced by Dixon *et al.*[2] in 2012. Since that time, ferroptosis has been linked to the pathophysiological processes of many diseases, including neurological disorders, acute kidney injury or ischemia/reperfusion which gives tremendous therapeutic potential for inhibitors.[3-6]

As ferroptosis is characterized by lipid peroxidation, the main strategy to block this phenomenon is to trap the radicals initiating the oxidative process inside cell membrane. UAMC-3203, used as one of key ferroptosis inhibitor in the field, was developed by our group as an improved version of Fer-1 showing impressive *in vivo* result and favorable (yet perfectible) pharmacokinetic properties.[7,8]

Recently we designed, synthesized, and evaluated a whole new generation of ferroptosis inhibitors derivated from UAMC-3203. In this poster, we will present our latest pharmacomodulations in the scaffold and the new tools implemented in our group to select the potential best new hit.

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# ADDRESSING FLUOROQUINOLONE RESISTANCE: DISCOVERY OF BACTERIAL DNA GYRASE AND TOPOISOMERASE IV INHIBITORS WITH A UNIQUE BINDING MODE

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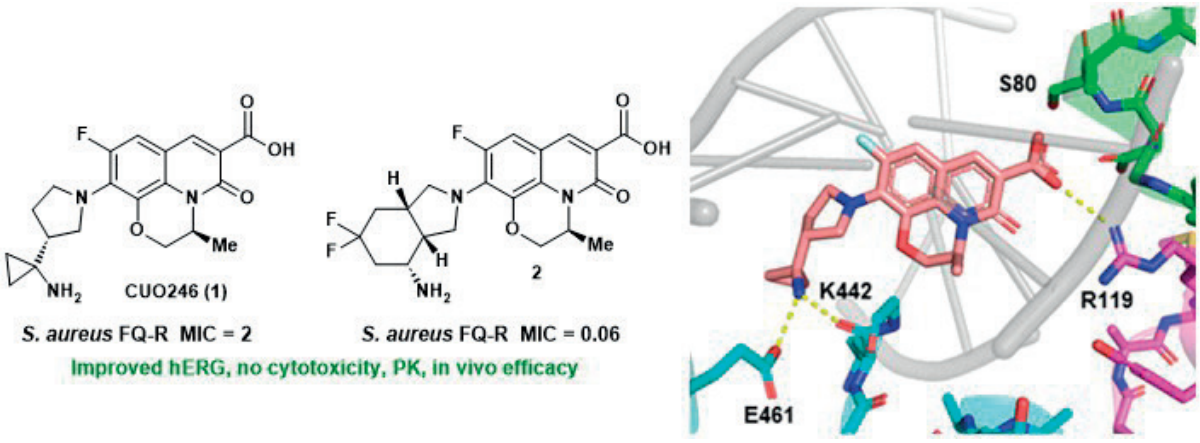


DNA gyrase and topoisomerase IV are structurally and functionally related targets of enormous importance for antibiotic chemotherapy. Both enzymes are critical for bacterial cell viability, and one of the most widely used inhibitor, the fluoroquinolone ciprofloxacin, still accounts for over 20 million prescriptions annually. As with all classes of antibiotics, resistance to quinolones has emerged and continues to develop, threatening their long term utility for treatment of a broad range of infections caused by Gram-positive and Gram-negative bacteria. Resistance to quinolone antibiotics is mostly driven by mutations to residues in the binding pocket that prevents formation of a crucial water-metal ion bridge interaction, thus rendering quinolones ineffective.

We developed a series of novel DNA gyrase and topoisomerase IV inhibitors with a unique binding mode that demonstrate potent activity and efficacy against fluoroquinolone-resistant Gram-positive bacteria. The potency of those compounds do not rely on the water-metal ion bridge that characterizes the binding of quinolone antibiotics. The resulting compounds show excellent overall profile including good antibacterial activity, low hERG inhibition and good ADME properties. The favorable PK profile of our lead compound translated into remarkable *in vivo* efficacy that was superior to comparator compound with greater *in vitro* potency (e.g. moxifloxacin). CUO246 **(1)** and analogs starts to cover gram-negative pathogens, including fluoroquinolone-resistant isolates, and early efficacy data show promises toward targeting those difficult-to-kill strains.

# TARGETING THE R-SPINE: NOVEL TYPE I½ p38α MAP KINASE INHIBITORS WITH OPTIMIZED TARGET RESIDENCE TIME: APPLICATION TO TREAT COLORECTAL CANCER

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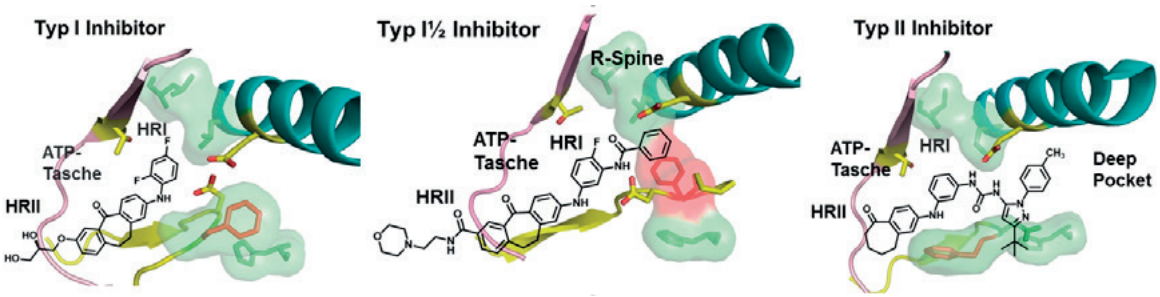
p38 MAP Kinase inhibitors are widely investigated for a plethora of inflammatory diseases including RA and COPD. Latest results however may open an avenue for cancer and CNS-diseases as well. For such indications however, Inhibitors with very particular properties are necessary.




We recently reported Skepinone-L as a Type I p38α MAP kinase inhibitor with high potency and excellent selectivity *in vitro* and *in vivo*.[1] However, as a Type I inhibitor it acts entirely ATP competitive and shows just a moderate residence time. Thus, the scope was to develop a new class of advanced compounds maintaining the structural binding features of Skepinone-L scaffold like inducing a glycine flip at the hinge region and occupying both hydrophobic regions I and II. Extending this scaffold with suitable residues resulted in an interference with the kinase's R-Spine. By optimizing this interaction, we could significantly prolong the target residence time up to 4.000 s, along with an excellent selectivity-score of 0.006 and an outstanding subnanomolar potency. This new binding mode was validated by cocrystallization, showing all binding interactions typifying Type I½ binding.[2,3]. Long term MD-simulation underlines the decisive Role of Water and Protein Dynamics in Residence Time of p38a MAP Kinase Inhibitors [4].

Based on the concept of Type I½ Inhibitors with improved TRT, the drug development project *ImproveCRC* (funded by the Landesstiftung Baden-Württemberg) [5] led to a preclinical candidate to treat colorectal cancer, which underwent extensive in vitro and in vivo characterization. An alternative synthetic access from milligram to gramm to kilogram scale was developed as well. [6]

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# DOCKAI: EFFICIENT EXPLORATION OF ULTRA-LARGE CHEMICAL SPACES USING ACTIVE LEARNING

**Maxime Laugeois (1), Brice Hoffmann (1), Paul Join-Lambert (1), Anna Kriukova (1), Maud Jusot (1), Ennys Gheyouche (1), Stéphanie Labouille (1), Juan Sanz Garcia (1), Christopher Housseman (1), Nicolas Brosse (1), Clarisse Descamps (1), Guillaume Plum (1), Nicolas Drizard (1), Cédric Thao (1), Hamza Tajmouati (1), Brian Atwood (2), Nicolas Do Huu (1), Quentin Perron (1), Yann Gaston-Mathé (1)**

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Hit discovery, the process of identifying small molecules with the potential to become drug candidates, is often the first and most critical step in the process of a drug discovery campaign upon target identification and characterization. This is often achieved by virtually screening (via protein-ligand docking) large chemical libraries (~106) in order to narrow down a vast pool of compounds to a more manageable set of potential hits that can be further evaluated, and this has led to some success over the last two decades [1]. With the advent of make-on-demand ultra large chemical libraries [2] the possible chemical search space has increased by orders of magnitude (~109), and the deployment of traditional virtual screening methods has become prohibitively long and costly [3]. To address this we have developed a proprietary solution called DockAI which is a fast, low cost, and highly effective AI-based method for virtual screening of ultra large scale databases of virtual compounds. It is based on an active learning approach, which allows us to identify most promising compounds for further evaluation by docking a small fraction (typically de novo synthesizable (virtual) molecules based on available building blocks and chemistry that are not constrained by any database, thus allowing exploration of a previously unexplored chemical space and discovering novel and highly active compounds that were previously unforeseen. DockAI's machine learning and active learning models outperformed Schrodinger's comparable approach on a dataset of Dopamine receptor (D4) compounds (n~140 million), by retrieving ~75% of the hit molecules after docking only 1 million compounds, demonstrating the efficiency of our method. DockAI has been successfully implemented to real-world projects with a biotech company and a not-for-profit foundation, with other projects under way. In its current technical implementation, DockAI is deployed on AWS enabling docking up to 105 molecules in parallel, providing results of our screening campaigns within 24 hours. Drug discovery campaigns are venturing into novel modalities, drugging more challenging targets, and rapid, cost-effective and technically efficient active-learning based docking tools like DockAI are expected to become a part of the solution.

# HIGH-THROUGHPUT OPTIMIZATION AND DISCOVERY OF COVALENT LIGANDS

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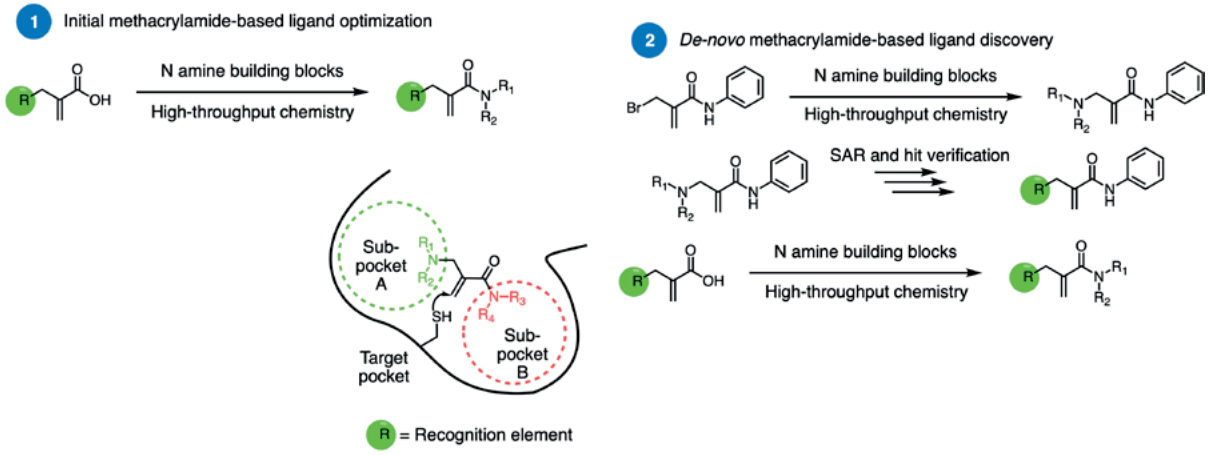
Interest in covalent probes is increasing due to their advantages, such as increased potency, enhanced selectivity, and longer duration of action.[1,2] However, their discovery remains challenging. Recently, members of our group have characterized the alpha-substituted methacrylamide as a new type of electrophile for covalent protein targeting.[3,4] This warhead can target two sub-pockets around a cysteine residue, yet this capability was overlooked. In this work, we rapidly optimized a SARS-CoV-2 inhibitor containing a methacrylamide warhead. For this purpose, we used nanomole-scale high-throughput chemistry and a direct-to-biology screening approach to rapidly synthesize and screen two methacrylamide-based libraries. We identified ten hits by screening 771 compounds directly from the crude mixtures, which we verified in a dose-response experiment. The top hit improved potency over the previous inhibitor almost two-fold, even though it was assayed as a crude compound. Therefore, we expect it to improve inhibition even more in its pure version.

Then, we used the knowledge we gained to find a completely new methacrylamide-based ligand for Keap1 in a 3-step process. We started by screening a general methacrylamide library, and after finding a more potent analog of our best hit, we made a protein-specific library. By screening this last 771-membered protein-specific library, we identified five hits. Although these hits didn't improve the potency of the intermediate ligand, they have properties that predict their reactivity to be lower than the reactivity of the intermediate ligand.[5] Therefore, their specificity is potentially better. Overall, we demonstrated that high-throughput chemistry can be used to optimize existing methacrylamide-based ligands and find new ones quickly and efficiently.

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# DEVELOPMENT OF NOVEL MITOCHONDRIA-TARGETING PHOTOSENSITIZERS THAT INDUCE PYROPTOSIS FOR CANCER CELL ABLATION

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Mitochondria have been identified as a crucial source of energy for cancer development and progression. In this study, we report JH14+ as a promising mitochondria-targeting photosensitizer for photodynamic cancer therapy. Real-time confocal fluorescence images of cancer cells showed that JH14+ was selectively localized in the mitochondria. The cancer cell death was triggered by mitochondrial reactive oxygen species caused by JH14+, leading to pyroptosis via gasdermin-mediated pore formation on the cellular membrane. Our findings suggest that the synthesized JH14+ could be employed in photodynamic therapy as a potential photosensitizer with significant anticancer activity.

# AZULENES IN MEDICINAL CHEMISTRY - THE POTENTIAL OF AN OVERLOOKED BICYCLE

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Azulene is a bicyclic non-benzenoid aromatic hydrocarbon. It is a structural isomer of naphthalene, but it can be considered to be an isostere of indole, as both azulene and indole consist of an electron-rich five-membered ring fused to a larger ring. Reports of azulene derivatives in medicinal chemistry applications are scarce,[1] unlike those concerning naphthalene and indole.[2] Antiulcer drugs egualen sodium and sodium gualenate are the only azulene-based drugs that have reached the market so far.[3] In addition, the reported bioactivities of azulene derivatives include anticancer[4] and antimicrobial[5] effects, as well as orexin receptor[6,7] and dopamine D4 activation[8], among others.

We recently explored the general potential of azulene as a scaffold in medicinal chemistry by determining the physicochemical and *in vitro* parameters relevant for drug discovery for a series of azulene derivatives.[9] Additionally, we synthesized a scaffold hopping series of corresponding azulene, indole and naphthalene derivatives and compared their polarity, permeability, solubility, and metabolic stability to reveal differences between these analogous bicyclic scaffolds. Our results demonstrate that azulene scaffold has no general liabilities due to which its use in medicinal chemistry should be avoided, and thus it has potential applicability as a ring structure in drug molecules.

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# ANTICANCER DRUG DESIGN AND DISCOVERY: 4-THIAZOLIDINONE/THIAZOLE DERIVATIVES APPLICATIONS

**Roman Lesyk (1), Serhii Holota (1), Andrii Lozynskyi (1), Anna Bielawska (2), Krzysztof Bielawski (2)**

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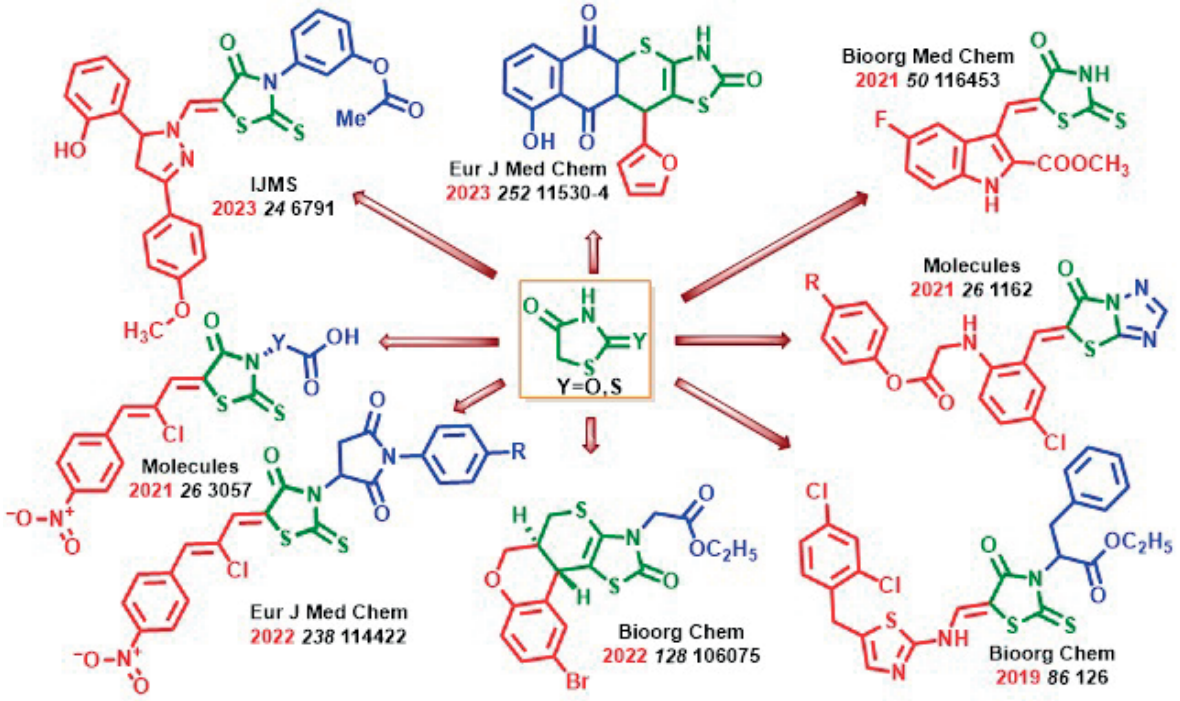
Thiazolidinone/thiazole derivatives are a well-known class of patented lead-compounds and drugs. In modern medical chemistry, the thiazolidinone core is a powerful biophore for the "drug like" molecules design. The study of the 4-thiazolidinones/thiazoles pharmacological profiles allowed to establish anticancer activity as one of the most promising for these heterocycles. Along with this is undeniable evidence of such derivatives affinity to biotargets involved in the main metabolic pathways of tumor cells growth (TNF-α-TNFRc-1, JSP-1, antiapoptotic complex Bcl-XL-BH3, etc [1].

At the present synthetic strategy of our team is based on chemical modification of the 4-thiazolidinone/thiazole cycle using various types of reaction (Knoevenagel condensation, [2+3]-, [2+4] and [3+3]-cycloadditions, Michael additions, domino and tandem reactions, etc.). As a result, we obtained the in home library contained chemically diverse thiopyrano[2,3-*d*]thiazoles, thiazolidinone-pyrazolines and thiazolidinone-benzothiazole hybrid molecules, isothiocoumarin-3-carboxylic acids, thiazolo[4,5-*b*]pyridines, etc.




Based on the antitumor activity screening of more than 2,000 synthesized compounds, 260 hit compounds were identified [2,3]. The in-depth study of the most interesting compounds was reasoned, which allowed coming to the following statements: a) Leukemia (CCRF-CEM, HL-60(TB), RPMI8226, SR, K-562, MOLT-4, Jurkat), Melanoma (LOX IMVI, UACC-62), CNS cancer (U251), non-small cell lung cancer (HOP-92), renal cancer (UO-31, 786-O), gastric cancer (AGS), colon cancer (HCT-116), as well as breast cancer (MDA-MB-231) cell lines, were the most sensitive; b) the apoptosis-dependent mechanism of antitumor activity was confirmed; c) experimentally confirmed anticancer properties of hit-compounds via PPARγ receptors modulation, inhibition of tubulin polymerization and topoisomerase II.

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# SELECTIVE DELIVERY OF DRUGS TO CNS FOR TREATMENT OF NEUROLOGICAL DISEASES

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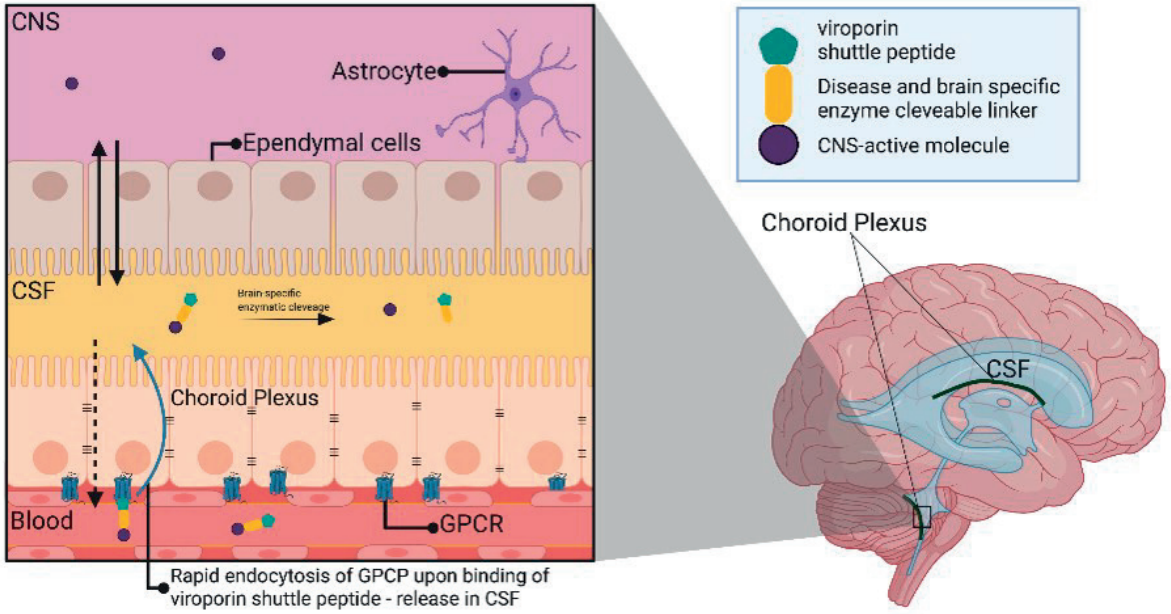
Neurological diseases that affect the brain are devastating and poorly treated diseases[1]. Not only is drug delivery to the brain limited by the presence of the blood-brain barriers (BBBs), which only allows the passage of crucial important brain functioning nutrients[2], but it is also affected by many side-effects as a result of low selectivity. The purpose of this study is to develop novel drug conjugates for improved delivery of drugs to the brain. The drug conjugate consists of three parts: a delivery unit, a drug and a brain selective linker to facilitate the delivery of drugs into the brain where the main focus of this study is on the brain-selective linker (Fig. 1). One linker strategy investigates acetylcholinesterase (AChE)-cleavable linkers. AChE is an enzyme overexpressed in the brain and it is involved in the termination of impulse transmission by rapid hydrolysis of the neurotransmitter acetylcholine in the CNS[3]. Another linker strategy investigates reactive oxygen species (ROS) cleavable linkers. The brain exhibit a robust prodruction of ROS as well as too high levels of ROS is associated with neurodegerative diseases[4]. Therefore, both a AChE- and a ROS-cleavable linkers would be highly brain-specific. This study will conduct Structure-Activity relationship (SAR) studies of a library of dye-conjugated AChE- and ROS-cleavable building blocks to explore the cleavage efficiency, selectivity and plasma stability aiming to identify the most optimal cleavable linkers for selective release of drugs in the brain.

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# RECENT ADVANCES IN HETEROCYCLIC SYNTHESIS IN ASTRAZENECA ONCOLOGY CHEMISTRY

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The ability to rapidly synthesise and derivatise novel building blocks is essential to generating new chemical equity in a pharmaceutical research program. Using modern techniques such as photoredox chemistry and high-throughput experimentation, we have developed a number of useful transformations and syntheses of novel heterocycles as part of our ongoing Oncology research programmes. These include tetrahydroisoquinolines via Pictet-Spengler and other cyclisations, triazolopiperazines, substituted and bridged pyrrolidines. Also, we describe the introduction of diverse amines to lenalidomide-like scaffolds via Buchwald–Hartwig cross couplings, generating a range of PROTAC drug candidates.

# THE SYNTHESIS OF FLUORESCENT CYCLIC PEPTIDES VIA GOLD(I)-CATALYZED MACROCYCLIZATION

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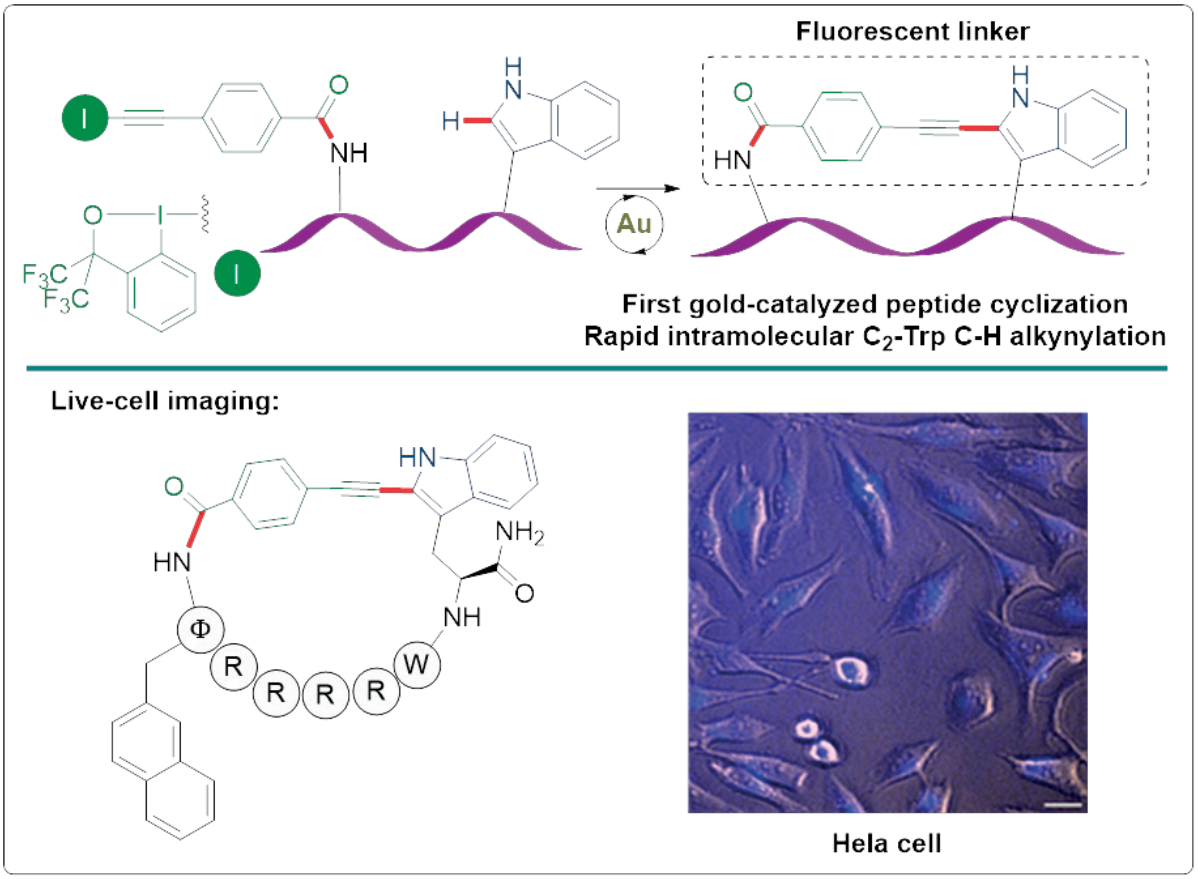
Rapid and efficient cyclization methods, forming novel 3D macrocycles, are still urgently needed. Herein, we presented here the first gold(I)-catalyzed peptide macrocyclization of peptide-EBXs (ethynylbenziodoxolones). This reaction was carried out in the presence of protecting group free peptide sequences enabled by simple and commercial gold catalyst (AuCl·$Me_2S$). The method displayed rapid reaction rate (within 10 min), wide functionality tolerance (26 unprotected peptides were tested) and good yield. This unique highly conjugated cyclic peptide linker, formed through alkynylation, can be directly applied to cell imaging without further attachment of fluorophores.

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# A COMBINATORIAL APPROACH TOWARDS SELECTIVE INHIBITORS OF OXYTOCINASE

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Oxytocinases are a subfamily of M1 aminopeptidases formed by three enzymes called Endoplasmic Reticulum Aminopeptidases 1 and 2 (ERAP1 and ERAP2) and Insulin Regulated Aminopeptidase (IRAP). They contain a Zn atom in their active site and cleave the N-terminal amide bond of peptide substrates. These three enzymes play a critical role in the process of antigenic peptides generation and the overall control of adaptative immune response. Chemical tools, like inhibitors or probes, specific for these enzymes could lead to a better understanding of the molecular mechanisms behind autoimmune diseases and at the same time create new inhibitors that could be used in the future for the treatment of autoimmune diseases or even improve cancer immunotherapy.[1]

Potent inhibitors have already been synthesized against ERAPs and IRAP. However, only small progress has been made regarding their selectivity which is attributed to the large structural similarities of their active sites that hinders the design and synthesis of selective inhibitors.[2] One of the most potent inhibitors against these enzymes, DG013A, is a phosphinic pseudopeptide which mimics the structure of a peptide but contains a phosphinic acid and a methylene group in lieu of the carbonyl and the NH of the amide bond, respectively. Phosphinic inhibitors allow the competitive inhibition of the enzyme by mimicking the substrate and occupying the binding pocket.[3] This strategy has been successfully applied to various medicinally relevant Zn-metalloproteases, obtaining potent and selective inhibitors.[4,5]

In the present work, a combinatorial approach involving solid phase synthesis of phosphinic pseudopeptide libraries has been developed. Each library carries a common phosphinic psedotetrapeptidic core but with different side chains that interact with the primed and unprimed cavities in the binding pocket of the three target enzymes. In vitro enzymatic evaluation of the libraries revealed important structure-activity relationships concerning ligand preferences of the aminopeptidases and at the same time shed light on selectivity patterns that can contribute to the design of selective inhibitors.

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# DISCOVERY OF LEISHMANIA DRUGGABLE SERINE PROTEASES BY IN-HOUSE FLUOROPHOSPHONATE-BASED PROBES

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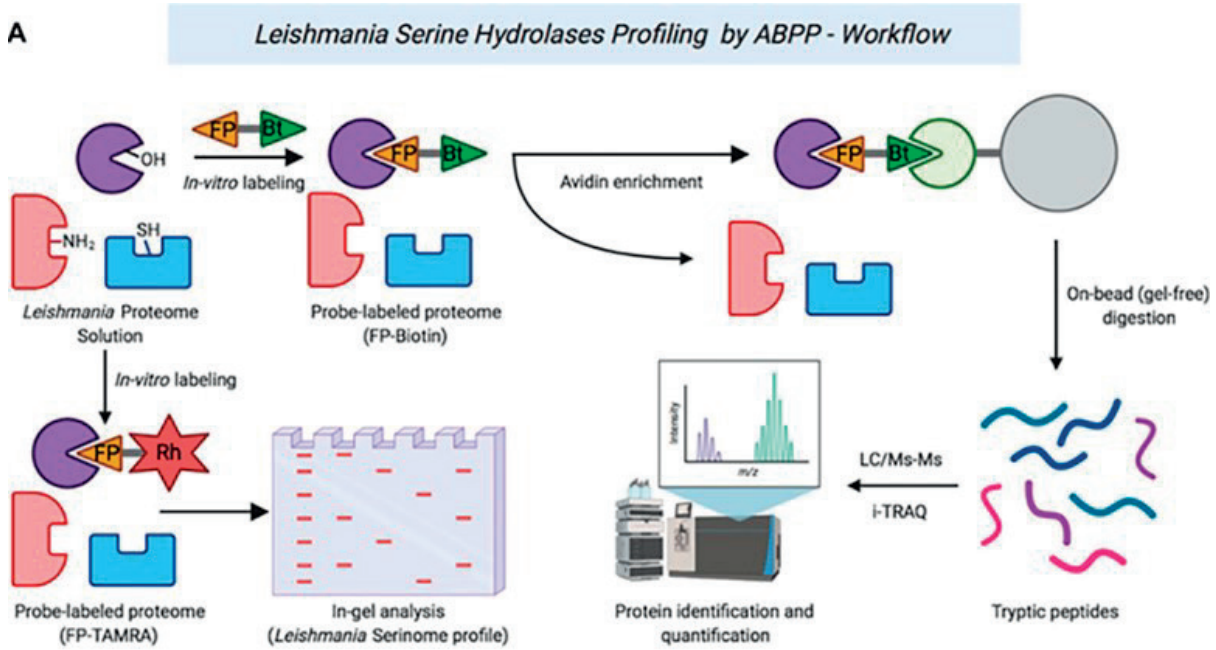
Leishmaniasis is caused by parasitic protozoa of the genus *Leishmania* and current treatments are limited by factors such as difficult administration, high cost, poor efficacy, toxicity, and growing resistance. There is an urgent need for new agents with new mechanisms of action to treat these diseases. Although extensively studied in other organisms, serine proteases (SPs) have not been widely explored as antileishmanial drug targets. This study reports, for the first time, an activity-based protein profiling (ABPP) strategy to investigate new therapeutic targets within the SPs of *Leishmania* parasites (Figure A). A library of 15 synthetic in-house active-site directed fluorophosphonate probes (chemically diverse and and clickable) were used to detect and identify active *Leishmania* serine hydrolases (SHs) in native conditions. Significant differences in SHs expression levels were observed throughout the *Leishmania* life cycle and between different *Leishmania* species. Using Tandem Mass Tag (TMT)-labelling-based quantitative proteomic mass spectrometry, 10 targetable SPs in *Leishmania mexicana* were identified. Druggability was ascertained by selective inhibition using commercial serine protease inhibitors, such as chymostatin, lactacystin, dabigatran, camostat, benzamidine, and ZPP, which represent templates for future anti-leishmanial drug discovery programs. The ABPP method complements existing genetic methods for target identification and validation in *Leishmania*, and the details and results of this study will be presented.

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# QUINOLINONYL DERIVATIVES AS DUAL INHIBITORS OF THE HIV-1 INTEGRASE CATALYTIC SITE AND INTEGRASE-RNA INTERACTIONS

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Over the past three decades, the outcome of HIV infection has been revolutionized by considerable progress in the therapeutic options available, as a result of the introduction of effective multi-pills regimens (ART). Although ART is purposely devised to overcome the high viral genetic variability and the subsequent emergence of resistance, multi-drug resistant strains can still be detected in some patients and long-term drug toxicities still represent an unresolved concern in HIV management.[1]

HIV integrase (IN) is vital for viral replication and it is an important therapeutic target. In this regard, integrase strand transfer inhibitors (INSTIs) which bind to the active site of the viral enzyme, have proven to be highly effective, becoming a potent first-line therapy to treat infected patients. However, despite the effectiveness of INSTIs as therapeutic options and the high barriers with the second-generation FDA-approved INSTIs, the pharmacological therapy selects for drug resistance and mutations responsible for multiple INSTIs resistance have been described in clinical practice,[2] underscoring the need for the development of novel and more effective antiretroviral compounds. The development of small molecule protein-protein interaction inhibitors is a new attractive strategy for discovering anti-HIV agents. In this field of research, allosteric IN inhibitors (ALLINIs), are a promising new class of antiretroviral agents. These inhibitors act differently in respect to INSTIs, in fact, they alter the functional IN multimerization. Recently, it was unraveled that aberrant IN multimerization underlies the inhibition of IN-vRNA interactions by ALLINIs.[3] In doing so, ALLINIs indirectly disrupt the IN-vRNA binding leading, as a result, to the formation of defective viral particles with greatly reduced infective potential with mis-localization of the vRNA outside the viral capsid.[4] While the indirect disruption of IN-vRNA binding (caused by the impairment of functional IN multimerization) has been described with the treatment of virus-producing cells with ALLINIs, the direct disruption of this binding (without affecting IN multimerization properties) by small molecules has not been reported so far. We describe a series of compounds identified as inhibitors of the IN-vRNA binding *via* a direct mechanism. In particular, we deepened the mechanism of action of some compounds previously described by us as INSTIs.[5] Indeed, we speculated that these quinolinonyl derivatives, being endowed with two DKA chains, could also act as protein-nucleic acid interaction inhibitors. To verify our hypothesis, we decided to test a set of derivatives and their analogues endowed with a variable "base-like" functional group. We assessed the capability of our derivatives of inhibiting at low micromolar concentrations both the IN 3'-processing (3'-P) and strand transfer (ST) reactions in a LEDGF/p75 independent assay. In addition, we performed *in vitro* binding assays, and we found that our quinolinonyl derivatives are able to disrupt the IN-vRNA interaction, that is vital for a correct generation of a functional infective virion. The data coming from the biological assays will be shown and discussed.

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# ALKYNONES AS VERSATILE TOOLS TOWARDS SELECTIVE BIOCONJUGATION

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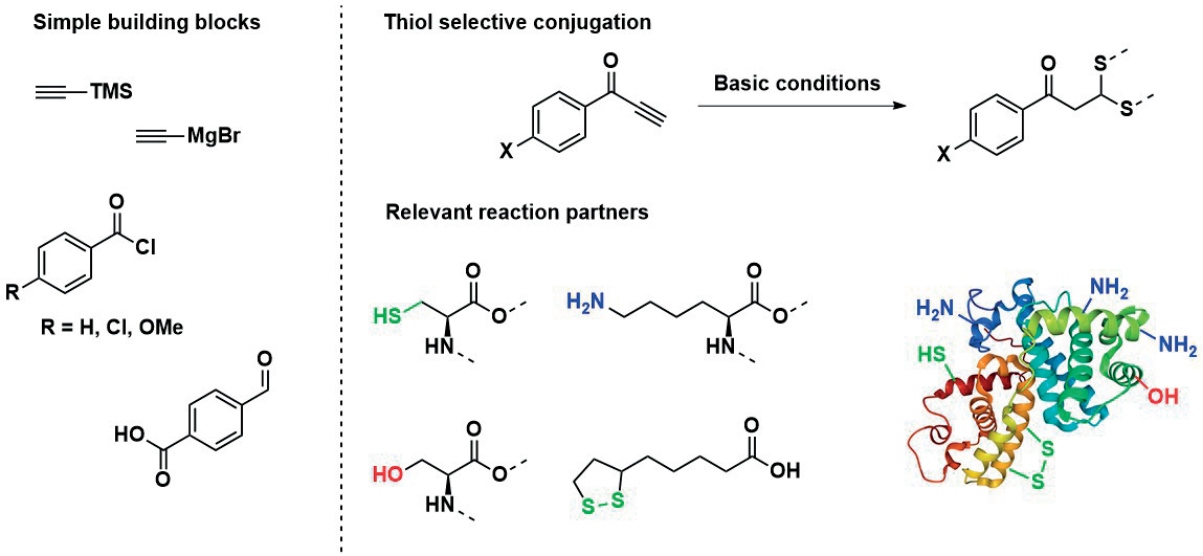
**Sulphur-based chemistry** have attracted chemists in various fields and has become a hot topic over the years. Thanks to the inherent reactivity profile of sulphur containing molecules, applications can range from biological/medicinal context to a more polymer material orientation.[1,2,3] In proteins, the thiol groups in **cysteine residues** can be used for further conjugation with **Michael acceptors** such as maleimides, vinyl sulfones etc.[1] Herein, the conjugation of thiols to maleimides has obtained most of the attention as a result of its ease in handling, click-like character and versatile reactivity profile. However, maleimides suffer from the lack of hydrolytic stability.[4] Therefore, various strategies to circumvent this problem are required.[5,6] We present the use of **alkynones** as a **dual reactive moiety** towards cysteine as a new platform for bioconjugation. From previous research, we proved the dynamic nature of the formation thioacetals by changing the electronic character.[7] The knowledge obtained in this preliminary study resulted in a profound knowledge on the behaviour of **thioacetals and/or alkynones** which opened the field of **cysteine selective bioconjugation.**

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# FOSTERING DRUG DISCOVERY THROUGH THE DELEOPMENT OF HIGH-PERFORMANCE INNOVATIVE PROBES

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Among the remarkable advantages, fluorescent ligands demonstrated to be suitable for different type of experiments such as fluorescence microscopy, high contest screening, FRET, HTRF and BRET in both pre-transfected and living cells[1]. However, fluorescent ligand employment has not yet been adopted widely due to the challenging development process and the consequent lack of optimal fluorescent probes available for the majority of the targets of interest. In Celtarys Research we have developed a rational and versatile synthetic strategy enabling to rapidly identify optimal fluorescent probes to tag diverse molecular targets. The relevant role of GPCRs in drug discovery[2] led us to start applying our technology in this field, developing fluorescent probes for different receptor families and applications. Here we report case studies of two of the probes developed so far: CELT-327, an $A_{2B}/A_3$ adenosine receptor fluorescent antagonist and CELT-419, a $D_3$ fluorescent antagonist. CELT-327 allowed semi-quantitative receptor mapping in living cells and validated the specific expression of $A_{2B}$AR in colorectal cancer (CRC) cell lines. As well, this probe was effective at monitoring real-time $A_{2B}$AR receptor labeling using live-imaging modalities, and displayed high efficiency when used to label complex 3D cellular systems such as CRC tumor spheroids.[3] On the other hand, CELT-419 was employed in the development of dopamine D3 receptor-ligand binding assays with fluorescence polarization and quantitative live cell epifluorescence microscopy. The fluorescence anisotropy assay using 384-well plates achieved Z' value of 0.71, which is suitable for high-throughput screening of ligand binding. The assay can also be used to determine the kinetics of both the fluorescent ligand and reference unlabelled ligands. CELT-419 was also used with live HEK293-D3R cells in epifluorescence microscopy imaging for deep-learning- based ligand binding quantification.[4] These examples highlight the wide range of applications which can be explored using Celtarys technology in fluorescent probes development.

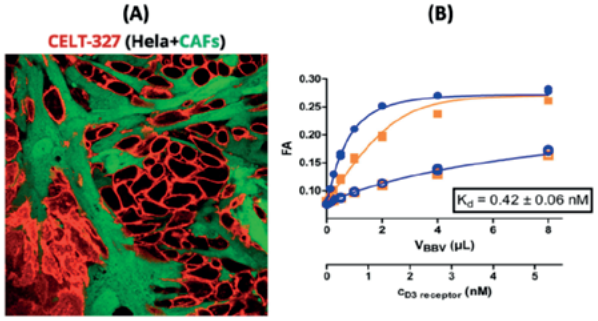


Figure 1: A) HeLa cells were co-cultured with fibroblasts from the stroma around tumors (CAFs) previously labelled in green with a cell-tracker. The figure shows in red the plasma membrane staining revealed by CELT-327, specifically in HeLa cells and excluding fibroblasts; B) Binding curves of CELT-419 binding to D3 receptors in BBVs. FA of 3 nM (orange squares) or 0.5 nM (blue circles) CELT-419 were measured after 2 h incubation with different amounts of D3 receptor displaying BBVs. Non-specific binding (NS, open symbols) was determined in the presence and total binding (T, filled symbols) in absence of 50 µM Spiperone

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# DEEPER INSIGHT INTO THE LANDSCAPE OF THE ACTIVITY OF STYRYLQUINAZOLINE DERIVATIVES AS TYROSINE KINASE INHIBITORS

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Developments in medicinal chemistry and rational drug design underpin modern treatments. Currently, much research in this field focuses on the mechanisms of tumourigenesis, identifying a particular role for molecules involved in cell cycle progression and signal transduction pathways. An important role in this regard is played by tyrosine kinases, which, due to their overactivity and critical role in intracellular signal transduction, are attractive drug targets for the treatment of certain types of cancer.

Among tyrosine kinase inhibitors, quinazoline-based compounds represent a large and well-known group of multi-target agents. We have previously investigated a series of quinolines [1, 2] and 4-aminostyrylquinazoline derivatives, which are structurally related to CP-31398 (reactivator of p53). These compounds exhibited good anticancer activity as multi-targeted kinase inhibitors (e.g. ABL or SRC kinases) [3]. Recently, we have investigated their analogues - a series of 2-[(*E*)-2-phenylethenyl]quinazolin-4-yl benzenesulfonates. Interestingly, we found that these analogues acted as competitive ATP inhibitors depending on the pattern of their substituents. In addition, anticancer activity of tosylates corresponded with a strong cell cycle arrest in GBM and mitotic inhibition similar or higher than that of paclitaxel [4]. Beside this, our work has focused on the biological characterisation of a group of sulphamoyl derivatives (IDH1 R132H inhibitors) for which allosteric binding to the ABL kinase has been demonstrated, further indicating for the first time the common features and geometry of the allosteric site in ABL and IDH1 R132H [5]. Finally, based on these results, in our current study we focused on the sulfanyl group. Conversion of a C-SO2R group into a sulphide is a strategy to increase biochemical stability while maintaining good bioavailability. Thus, we designed several thio-analogues of styrylquinazoline derivatives with respect to previously described benzodioxole- and phenyl- substituted compounds. Our results showed high inhibition potential against non-receptor tyrosine kinases for several compounds. Molecular docking studies showed differential binding to the DFG conformational states of ABL kinase for two derivatives. The compounds showed sub-micromolar activity against leukaemia. Finally, in-depth cellular studies revealed the full landscape of the mechanism of action of the most active compounds, focused on cell cycle inhibition, apoptosis and autophagy induction.

In conclusion, these results may provide new insights into the behaviour and mechanism of action of styrylquinazoline derivatives at the cellular level. Thus, these data may be useful to develop new multi-target inhibitors characterised by a desirable mode of binding to kinases that makes them effective in anticancer therapy.

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# DISCOVERY AND DEVELOPMENT OF GPR84 ANTAGONISTS

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GPR84 is a pro-inflammatory G protein-coupled receptor that is activated by medium chain free fatty acids.[1] It is mainly expressed in peripheral immune cells and in microglia in the central nervous system.[2] GPR84 functions as an amplifier of inflammatory mediators, as its activation induces chemotaxis and release of pro-inflammatory cytokines and chemokines.[3-5] Moreover, GPR84 has been shown to contribute towards the pathogenesis of fibrosis,[6] reflux esophagitis,[7] neuropathic pain,[8] acute myeloid leukemia,[9] and inflammatory bowel disease.[10] Therefore, blockade of the receptor function could be a viable therapeutic approach for the treatment of inflammatory and fibrotic diseases. Herein, we describe the conversion of a GPR84 agonist to antagonists and inverse agonists, and structure-activity relationship explorations, leading to the discovery of highly potent compounds.

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# TAMING THE IMIDAZOLE CORE: MILD ENTRY TO ENANTIOPURE 6,7-DIHYDROBENZIMIDAZOLES VIA ORGANOCATALYTIC [4+2] CYCLOADDITIONS

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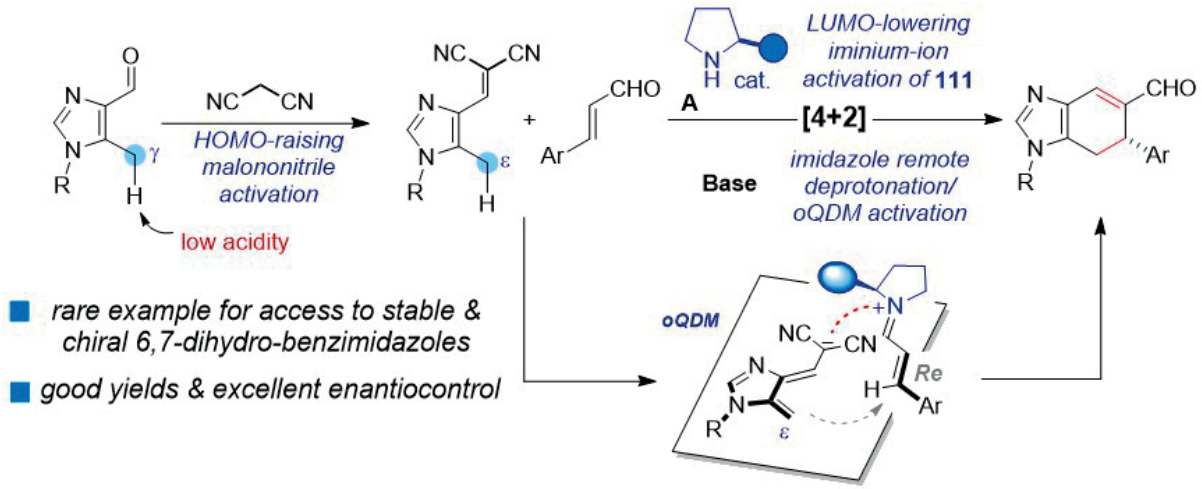
Benzimidazole derivatives are privileged nitrogen-containing heterocyclic scaffolds with high potential in drug discovery, due to their isostructural pharmacophore of naturally occurring bioactive molecules. Synthetic methodologies for accessing three-dimensional and chiral dihydrobenzimidazoles remain still elusive, the majority of methods pertaining to the domain of bidimensional and achiral aromatic benzimidazole relatives.[1] Documented herein is an unprecedented example of organocatalytic eliminative [4+2] formal cycloaddition reaction between novel imidazole-based alkylidene malononitriles **1**, functioning as γ-activatable *o* -quinodimethane (*o*QDM) diene precursors, and diverse enals **2** functioning as organocatalytically activatable dienophiles.[2] A series of *N*-protected dihydrobenzimidazoles of type **3** was efficiently obtained in one step with excellent enantioselectivities, which could be further elaborated into valuable imidazole-containing products without losing the chiral integrity. Different challenging issues had to be faced in this endeavour, including the non-facile, remote γ-deprotonation/temporary dearomatization[3] of the highly stable 5-methyl-imidazole-4-carbaldehyde precursors, and cohabitation of the basic/nucleophilic imidazole ring with the amine organocatalyst. Finely merging the traceless-malononitrile activation strategy[4] with the chiral iminium ion LUMO-lowering activation modality[5] turned out to be a good option, successfully consigning the designed products upon careful optimization of the experimental conditions.

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# ORGANIC CYCLIC CARBONATES OBTAINED BY PALLADIUM-CATALYZED INSERTIONS OF $CO_2$ INTO PROPARGYL ALCOHOLS

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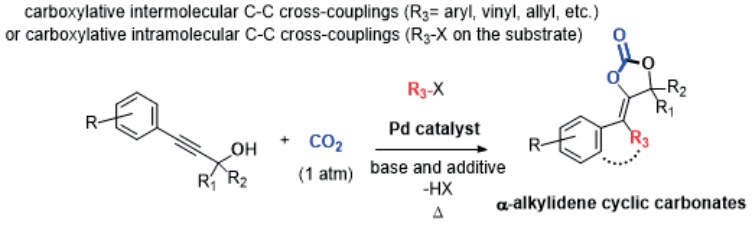
Carbon dioxide ($CO_2$) gas is considered as an abundant and renewable C1 synthon for the preparation of highly valued chemicals.[1] Catalytic $CO_2$ capture by propargylic substrates -alcohols or amines- affords α-alkylidene cyclic carbonates or carbamates, respectively.[2] Combination of $CO_2$ capture with C-C cross-coupling reactions may give direct access to complex products that otherwise require multistep syntheses.[3] Such transformations are particularly desirable because they follow the principles of green chemistry for atom and step economy. We are accordingly presenting, herein, Pd-catalyzed carboxylative intermolecular or intramolecular C-C cross-coupling reactions on various propargylic alcohol substrates. Our experiments are supported by calculations based on density functional theory (DFT) method which show that these reactions are exergonic because of product stabilization through the formation of additional C-C bonds, thus overcoming the thermodynamic and kinetic inertness of $CO_2$ even under atmospheric pressure. Currently, our efforts are focused on the elucidation of the reaction mechanism.

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# TERPENOIDS AS ALLOSTERIC MODULATORS OF HUMAN 5-LIPOXYGENASE.

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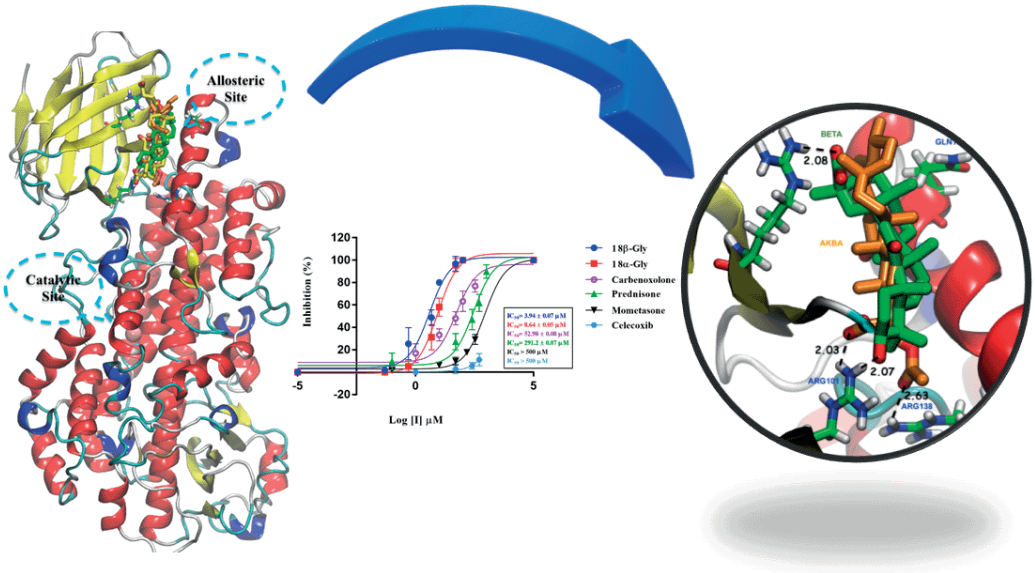
To investigate novel mechanisms of human 5-lypoxigenase (5-hLOX) inhibition, natural terpenes with structural similarities to acetoxy boswellic acid (AKBA), were examined using a 5-hLOX enzymatic bioassay to assess potential anti-inflammatory activity.[1] This enzyme synthesizes lipid messengers such as leukotrienes implicated in inflammation and other clinical disorders. This investigation focused on the allosteric 5-hLOX inhibition elicited by sesquiterpenes acting at the recently identified 5-hLOX allosteric site, revealed following enzyme crystallization. Present research combined a 5-hLOX inhibition biochemical assay, with *in silico* modelling, to determine whether natural boswellic acid analogs mimicked AKBA enzyme inhibition.[2,3] 5-hLOX potency determinations plus binding energy calculations of these chemicals to the reported allosteric enzyme site together with molecular dynamics simulations allowed ranking the inhibitory potency of these compounds as 5-hLOX inhibitors. In addition, the identification of the putative amino acids associated with the ligand-enzyme interaction were discovered. We now describe that α and β glycyrrhetinic acids, carbenoxolone, and to a minor extent prednisolone, inhibited 5-hLOX activity with low micromolar potency, values which correlate significantly with the calculated *in silico* allosteric site binding energy. In contrast, other steroidal or non-steroidal anti-inflammatory agents exhibited inhibitory potencies larger than 500 μM. Ethnobotanical medical reports support that sesquiterpenes examined have potential anti-inflammatory activity, but their pharmacodynamics remain elusive. We propose that AKBA analogs, acting on the 5-hLOX allosteric enzyme pocket, highlight a novel anti-inflammatory strategy of therapeutical relevance; these chemicals may impact the future development of novel anti-inflammatory medicines.

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# AMIDE-FUNCTIONALIZED COUMARIN COTREATMENT PROTECTS MICE FROM BRAIN AND LUNG INJURIES DURING EXPERIMENTAL SEVERE MALARIA

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Cerebral malaria is the most severe neurological condition of *Plasmodium falciparum* infection. With over 575,000 cases every year, children in sub-Saharan Africa are the most affected, presenting severe neurological and cognitive dysfunctions. An *in vitro* experimental screening of the antimalarial profile a family of phenolic compounds with previous neuroprotective activity was carried out. An amide-functionalized coumarin stood out among all the compounds, being subsequently tested *in vivo*. *Plasmodium berghei* ANKA (PbA) infection in mice resembles severe malaria in humans, reproducing injuries including cerebral malaria and acute respiratory distress syndrome.[1] PbA-infected mice treated with the new molecule alone or in combination with chloroquine increased survival rates, and protected cognitive abilities and lung function, compared with untreated mice. The combined treatment reduced brain and lung inflammation, the production and accumulation of microglia and immune cells producing the inflammatory cytokines TNF and IFNγ, and upregulating the production of the anti-inflammatory cytokine IL-10 by innate and adaptive immune cells. Overall, the combined treatment promotes immunomodulatory, neuroprotective, and lung function preserving effects, being a strong therapeutic candidate for severe malaria.

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# EVALUATION OF PHYSICOCHEMICAL FEATURES AND BIOLOGICAL ACTIVITY OF PROPARGYLAMINE-CONTAINING COMPOUNDS FOR PARKINSON'S DISEASE

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Parkinson's disease is one of the main causes of morbidity worldwide, associated with a deterioration in motor, mental, and functional skills. Monoamine oxidase B (MAO-B) has largely been considered a druggable target. Its ability to catalyze the degradation of biogenic amines has traditionally made its inhibition a viable therapeutic strategy to treat neurodegenerative disorders in which the balance between neurotransmitters is impaired. The two main reference compounds for MAO-B inhibition are rasagiline and selegiline. These FDA-approved drugs are used in the treatment of Parkinson's disease, and they both bear a propargylamine moiety in their structure. Our group has recently studied the importance of this moiety in the development of drugs targeting neurodegeneration.[1] We have also reported promising preliminary findings by introducing this moiety to the coumarin scaffold (Figure 1), whose versatility has largely been exploited for the design of central nervous system (CNS) targeting agents.[2] This communication aims to report the trends observed in our group for MAO inhibition of coumarin-rasagiline hybrid compounds, provide insights into the synthetic versatility of this scaffold, the evaluation of the physicochemical features, the MAO-B inhibitory activity, and the prediction of the binding modes of these ligands using molecular docking and molecular dynamics.

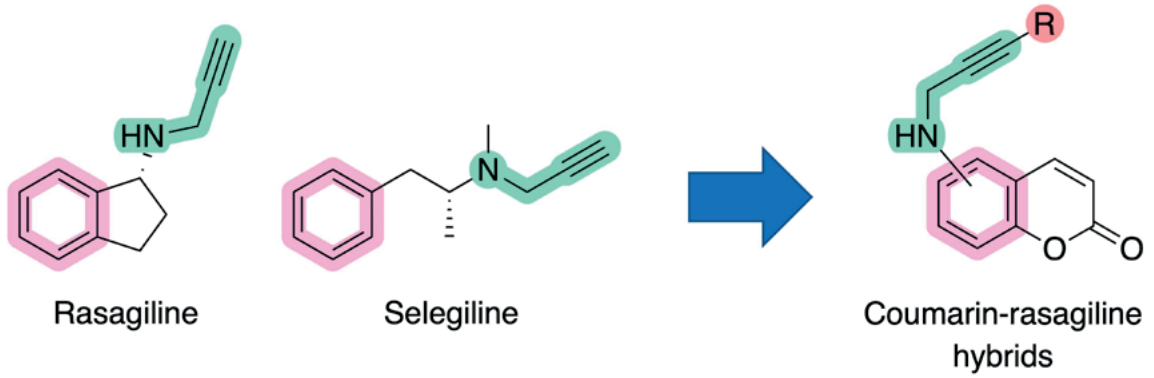


**Figure 1.** Design of coumarin-based MAO inhibitors inspired by rasagiline and selegiline.

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# CARBAMATES AS POTENTIAL NEW DRUGS IN THE TREATMENT OF ALZHEIMER`S DISEASE

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Alzheimer's disease (AD) is a multifactorial neurodegenerative disease characterized by the decline of cognitive functions. Although, the multifactorial nature of AD points to the existence of a number of possible targets, the existing treatment of AD is directed at the restoration of cognitive functions of patients by increasing the concentration of the neurotransmitter acetylcholine (ACh) by inhibiting the action of the enzymes responsible for its hydrolysis, acetylcholinesterase (AChE), primarly and butyrylcholinesterase (BChE). Considering the role of BChE in the development of AD and the symptom progression, selective inhibition of BChE over AChE can represent a promising pathway in treating advanced stages of AD.

The carbamate group is a important structural motif in drugs currently or previously in use for the treatment of AD, which act as cholinesterases inhibitors and displayed significant positive effects on cognitive symptoms. In our study, we synthesized carbamates modelled after the known potent and selective BChE inhibitor and determined their inhibitory potential toward both cholinesterases and inhibition selectivity. The ability of carbamates to cross the blood–brain barrier by passive transport, their cytotoxic profile, their antioxidant capacity and their ability to chelate biometals were also evaluated. According to our results, we could point to two carbamates as a promising compound for the development of more effective drugs for the treatment of adavnced stages of AD .

*Supported by the Croatian Science Foundation (Grant No. IP-2020-02-9343 (PI: A. Bosak))*

# DISCOVERY AND PRECLINICAL DEVELOPMENT OF SELECTIVE NKCC1 INHIBITORS FOR THE TREATMENT OF NEURODEVELOPMENTAL DISORDERS WITH DEFECTIVE NKCC1/KCC2 RATIO

**Luce Micaela Mattio (1,), Gabriella Vitali Forconesi (1), Michele Ruggeri (1), Marco Borgogno (2), Annalisa Savardi (2), Andrea Malizia (2), Laura Cancedda (2,3), Marco De Vivo (1,2)**

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A number of clinical and pre-clinical studies have demonstrated that the intracellular Cl homeostasis is impaired and Cl transporter cellular expression is dysregulated in several neurodevelopmental disorders, including Autism Spectrum Disorders (ASD) and Down Syndrome (DS). Interestingly, NKCC1 (Sodium Potassium Chloride cotransporter) was found to be upregulated in the DS and ASD brain, causing $Cl^-$ imbalance. The inhibition of NKCC1 by the FDA-approved diuretic bumetanide reverts cognitive deficits in DS mouse models (Ts65Dn mice) as well other core symptoms in ASD mouse models (VPA mouse model). However, bumetanide has a strong diuretic effect due to its inhibition of the kidney $Cl^-$ transporter NKCC2, which prevents it from becoming a sustainable therapy for brain disorders. Therefore, starting from bumetanide's structure, we applied a ligand-based computational strategy to identify new molecular entities able to selectively inhibit NKCC1. Extensive synthetic efforts and structure-activity analyses of the selected hit compounds improved the *in vitro* potency, efficacy, and drug-likeness of the initial chemical scaffold. As a result, we identified lead compound ARN23746, which displayed optimal drug-like properties and was able to restore the physiological [$Cl^-$] *in vitro* .When administered *in vivo*, ARN23746 was able to recover social and repetitive behaviors in ASD and DS mouse models, without showing any sign of toxicity or diuretic effects. For these reasons ARN23746 was extensively characterized in preclinical studies, showing an overall excellent metabolism profile, and good brain penetration and pharmacokinetics. Taken together, these results support ARN23746 as a solid candidate for clinical trial-enabling studies. Moreover, new selective chemical classes are currently in the discovery and optimization phases to further enrich our portfolio of novel and selective NKCC1 drug-like inhibitors, towards unprecedented sustainable therapeutics in ASD, DS and many other pathologies characterized by defective $Cl^-$ homeostasis.

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# FROM QUANTUM MECHANICS TO METABOLIC PATHWAYS

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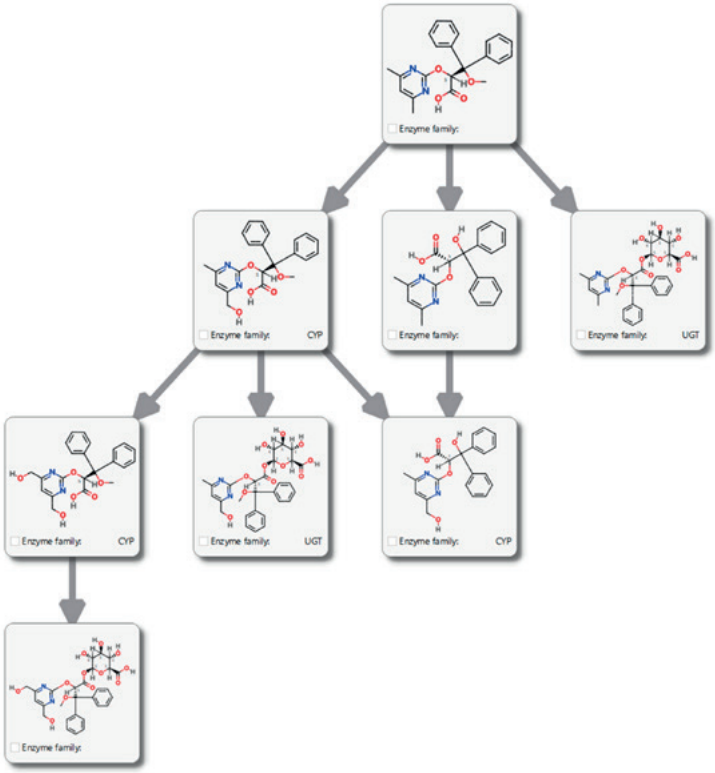
Unexpected metabolism can lead to the failure of many late-stage drug candidates or even the withdrawal of approved drugs. Therefore, during early research, it is important to predict the sites of metabolism and metabolites of potential drug-like molecules.

Historically, predictive models have targeted metabolism by human Cytochrome P450 (CYP) isoforms due to their well-documented importance in phase I metabolism.[1] Here, we will present methods to predict isoform-specific metabolism for a broad range of enzymes involved in phase I and phase II metabolism, including aldehyde oxidases (AOs)[2], flavin-containing monooxygenases (FMOs)[2,3], sulfotransferases (SULTs)[4] and UDP-glucuronosyltransferases (UGTs)[2,3] alongside CYPs. These models are based on a consistent framework, combining mechanistic quantum-mechanical simulations with machine learning, and are rigorously validated with experimental data. The resulting models predict if a potential site of metabolism on a compound is likely to be metabolised by the specified enzyme

We will demonstrate how these site-of-metabolism models can be combined with models that predict which enzymes and isoforms[5] are likely to metabolise a compound. Applying models iteratively to a parent compound and its metabolites enables the prediction of metabolic pathways and the resulting metabolites observed in vivo. We validate these pathway predictions by comparison with experimentally observed metabolite profiles.

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# LIGHTS, CAPTURE, EXTRACTION! A PHOTOAFFINITY PROBE FOR ZINC METALLOPROTEIN PROFILING IN LIVE CELLS

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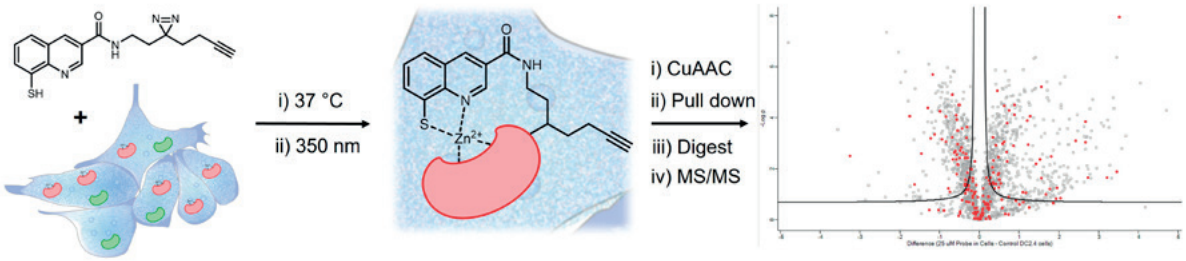
Zinc imparts vital structural characteristics and catalytic function to a wide variety of cellular proteins.[1,2] These zinc-dependent metalloproteins are exciting targets for investigation towards the development of novel therapeutics. However, their covalent capture for protein profiling studies can be highly challenging.[3] Here, we describe the design and preparation of a novel, photoactivatable affinity-based probe bearing an 8-mercaptoquinoline motif, a privileged ligand able to engage several zinc metalloproteins.[4,5] We report the synthesis of the probe and downstream proteomic analysis of metalloproteins labelled in a competitive and UV-dependent manner. Furthermore, we report the successful translation of this photoaffinity probe to labelling proteins in live DC2.4 cells. This work represents an important contribution to the library of cell-permeable probes with inducible reactivity for profiling a range of therapeutically significant biomolecules.[6]

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# FIRST-IN-CLASS TRPV6 INHIBITORS FOR THE TREATMENT OF PROSTATE CANCER

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TRPV6, a calcium channel, is an oncochannel that is overexpressed in epithelial cancers, including prostate cancer.

We have developed a novel series of first-in-class small molecule TRPV6 inhibitors for treatment of advanced prostate cancer. Our preclinical candidate QED-203 has been shown to inhibit calcium influx at low nM potency in TRPV6 cellular assays (FLIPR, electrophysiology), and has low nM potency in a TRPV6-driven NFAT assay (NFAT luciferase reporter). Inhibition of TRPV6 with our TRPV6 inhibitors or by siRNA knockdown causes changes in genes related to NFAT and WNT signalling, ER stress and the cell cycle (RNAseq and qPCR analysis), and causes cell cycle arrest, decreased proliferation and apoptosis in prostate cancer cells (demonstrated via imaging and FACS analysis).

Importantly, QED-203 demonstrates potency in enzalutamide (AR therapy standard of care (SOC)) resistant cell lines (*in vitro* proliferation inhibition) and has superior potency over enzalutamide and darolutamide SOC drugs in prostate cancer cell lines with AR mutations or the ARV7 variant.

QED-203 has high bioavailability with a pharmacokinetic profile amenable to once-a-day oral dosing. QED-203 is well tolerated in rodents and has *in vivo* efficacy (tumour growth and PSA inhibition) in a castrated mouse model of prostate cancer.

We have also shown QED-203 target engagement in rodents by demonstrating a change in calcium levels in the urine, and we have also observed changes in genes consistent with a TRPV6-specific mode of action in xenograft tumours (demonstrated via qPCR).

QED-203 targets a novel, non-hormonal mechanism in prostate cancer, and could be used to treat prostate cancer patients who have developed resistance to AR therapies, where there is a large unmet need.

# NOVEL MACROCYCLIC NLRP3 INHIBITORS

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Innate immunity is the first and immediate line of defense against invading endogenous or exogenous pathogens. Here, the NOD-like receptor family pyrin domain containing 3 (NLRP3) acts as a sensor that detects a broad range of danger signals [1]. Upon its activation, pro-caspase-1 is cleaved to form mature caspase-1, leading to subsequent release of IL-1 ß and Gasdermin N-terminal fragments, and ultimately to inflammation and pyroptosis [2].

Aberrant activation of NLRP3 due to *e.g.* persistent tissue damage, misfolded proteins or crystal deposits has been linked to multiple chronic inflammatory disorders such as cryopyrin-associated period syndrome (CAPS), Parkinson's disease, gout and numerous others [3]. Hence, there has been an increasing interest in NLRP3 inhibitors as therapeutics. A first generation of NLRP3 inhibitors bearing a sulfonylurea core such as MCC950 (developed by Pfizer) were discovered by phenotypic screening, however its mode of action was only elucidated later [4]. Based on MCC950 second-generation inhibitors were developed, aiming to overcome some liabilities such as moderate potency and drug induced liver injury (DILI) [5]. During the optimization of these (second-generation) inhibitors, conformational studies lead to the identification of novel macrocycles [6]. Here we report the discovery and optimization of this class of NLRP3 inhibitors.

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# LOOKING FOR AN IDEAL HTS SCREENING COLLECTION

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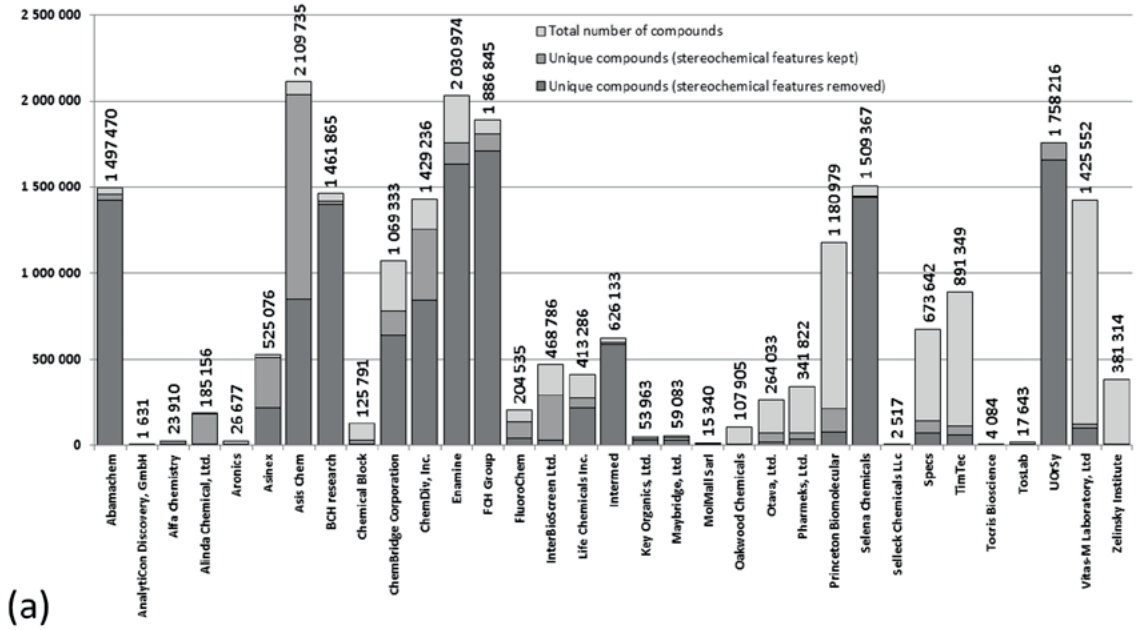
The number of new drugs approved per $1 billion spent has been halving every 9 years since 1950[1]. An average R&D spending per new pharmaceutical product surpassed $2,7 billion[2] in 2017. In order to increase economic efficiency of the process, we aimed to create an 'ideal' (in terms of diversity and druglikeness) screening collection of compounds. Initially, we searched for a one million compound set (50K scaffolds represented by minimum 20 examples each). Using ZINC database, we analyzed[3] an array of **16,902,208** unique structures (including stereoisomers) from 33 commercial suppliers. We mapped the purchasable molecules in chemical space and determined the parameters of the set (diversity, distribution accordingly to molecular weight, logP, Fsp$^3$, number of heavy atoms, rotatable bonds, aromatic rings, hydrogen bond donors and acceptors etc.). Evolution of these values during the last decade was analyzed. It was found that the average parameters of the commercially available collections shifted in more 'druglike' way (i.e. lower MW and logP).

Accordingly to our analysis, it is currently impossible to buy an 'ideal' one million compound set. However, an 'ideal' 500K set can be purchased from only six suppliers with 350K set available from just three suppliers. It looks like large companies have been looking for their ideal sets for HTS too.



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# PREPARATION OF CHOLESTEROL DERIVATIVES OF MANNOSYLATED DESMURAMYL PEPTIDE

**Danijela Mihelec (1), Željka Car (1), Rosana Ribić (2), Vesna Petrović Peroković (1)**

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Immunostimulators, known as adjuvants, are substances that are added to vaccines to enhance the specific immune reaction to a specific antigen. Muramyl dipeptide (MDP, *N*-acetylmuramyl-L-alanyl-D-*iso*glutamine) and its analogue without the hydrophilic *N*-acetylmuramyl unit, desmuramyl dipeptide (DMP, L-Ala-D-*iso*Gln) are the well-known adjuvants. Structure – activity relationship studies have shown that L-Ala-D-*iso*Gln pharmacophore is essential for immunostimulatory properties [1]. In order to increase the adjuvant activity of DMP we recently reported the preparation of mannosylated derivatives of DMPs, primarily modified by liphophilic subunits such as adamantan-1-yl, adamantan-1-ylethyl and $C_{12}$ alkyl chain, each connected to DMP *via* triazole ring. Their adjuvant activities were tested *in vivo* [2]. The results showed that the introduction of triazolyl liphophilic subunits in the DMP pharmacophore as well as its mannosylation improved its adjuvant activity.

In this work we described the synthesis of mannosylated DMPs with cholesteryl (**I**) and cholesteryl-triazole (**II**) moiety linked to α-position of D-*iso*Gln through an amide bond (Fig. 1). Peptide precursor, protected dipeptide L-Ala-D-Glu, was synthesized first and then successfully modified by an amidation reaction on α-position of D-*iso*Gln with previously prepared cholesterol amine and cholesterol-triazole amine. Benzyl protected mannose subunit was linked via glycolyl linker to the N-terminal amino group of alanine. The target molecules **I** and **II**, prepared by the protecting groups removal in the last step of the synthesis, will be tested for their adjuvant activity.

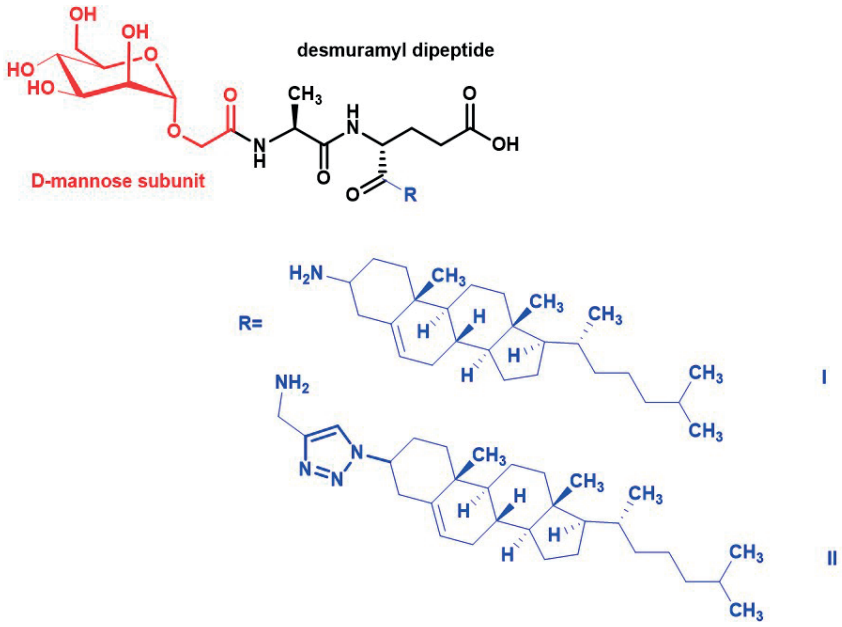


**Figure 1.** Cholesterol derivatives of mannosylated desmuramyl peptide

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# SYNTHESIS AND BIOLOGICAL INVESTIGATION OF 6-ALKYL-2-PHENYL-2H-PYRAZOLO[4,3-c]PYRIDINES

**Vaida Milisiunaite (1,2), Eva Řezníčková (3), Asta Žukauskaitė (4), Eglė Arbačiauskienė (2), Vladimír Kryštof (3), Algirdas Šačkus (1,2)**

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Pyrazoles and their derivatives represent a class of important nitrogen-containing heterocyclic compounds that cover a broad range of natural products and synthetic compounds with innumerable chemical, biological, agrochemical, and pharmacological properties. Derivatives with pyrazolopyrimidine scaffold possess various pharmacological properties including but not limited to antiproliferative, anticancer, antitubercular, sedative, antibacterial, anti-fungal, cyclin-dependent kinase (CDK) inhibitory activities. In the view of the diverse pharmacological profile of condensed pyrazoles, design and synthesis of such compounds has been a subject of many research studies.

We have previously developed an efficient approach for the synthesis of variously substituted 2*H*-pyrazolo[4,3-*c*]pyridines, employing Sonogashira cross-coupling and a subsequent substituent-tolerant annulation reaction in the presence of ammonia. Thus obtained 2*H*-pyrazolo[4,3-*c*]pyridines, varying by the substituents at the 2-, 4-, and 6-positions, were evaluated for their cytotoxicity against selected cancer cell lines. The most active compounds exhibited anticancer activity *in vitro* through arresting cell cycle in mitosis and induction of apoptosis.[1]

Therefore, in this study, we further examined the applicability of our synthetic approach and prepared a library of various 6-alkyl-2-phenyl-2*H*-pyrazolo[4,3-*c*]pyridines which were then assessed for their biological activity.

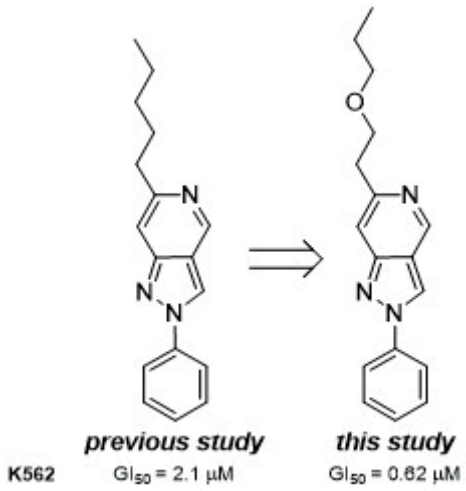


**Figure 1.** *In vitro* cytotoxicity of previously and newly synthesized lead compounds

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# DEVELOPMENT OF A NEW METHODOLOGY TO ACCESS RACEMIC AND ENANTIOENRICHED TRIFLUOROMETHOXYLATED SULFOXIDES

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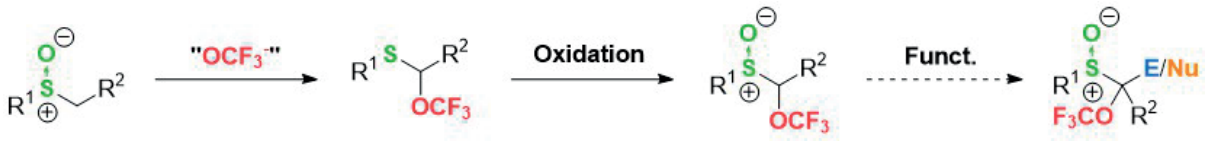
The introduction of fluorinated groups on molecular targets has emerged as an important area of research in organic chemistry, with applications in material science, medicinal chemistry, and agrochemicals. The substitution of a proton by a fluorinated group can lead to enhanced chemical stability, modified lipophilicity, altered pharmacokinetics and dynamics, and an overall improved biological activity.[1] The trifluoromethoxy moiety in particular can drastically increase the lipophilicity of a molecule relatively to a fluorine atom or a trifluoromethyl group (Hansch lipophilicity parameter $\pi(OCF_3) = 1.04$, $\pi(CF_3) = 0.88$, $\pi(F) = 0.14$).[2] Sulfoxides were substrates of choice for the study of the introduction of $OCF_3$ group on stereogenic centers.

Sulfoxides are easy-to-handle versatile chiral intermediates, which can be readily converted to various functional groups. They are known to have relatively high inversion energy barriers compared to many other types of organic molecules, making them attractive targets for this trifluoromethoxylation study.[3]

To obtain chiral compounds bearing the $OCF_3$ unit, a new method was investigated for the trifluoromethoxylation of sulfoxides *via* a Pummerer-type reaction followed by an oxidation. The synthesis of starting substrates is straightforward, in one or two steps, with low costs and excellent yields. Two different trifluoromethoxylating agents were used: trifluoromethyl *p*-toluenesulfonate (TFMS) is a bench-made air and room temperature-stable oil and 2,4-dinitro-trifluoromethoxybenzene (DNTFB) is an affordable commercially available liquid. Both show great efficiency for the release of the $CF_3O^-$ anion.[4,5,6]

The synthesis of the trifluoromethoxylated sulfoxides as well as mechanistic insights into the reaction pathway will be presented and their reactivity will be discussed.

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# DISCOVERY OF A SELECTIVE GPR55 BIASED MODULATOR

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G protein coupled receptors (GPCRs) are the largest family of membrane receptors in eukaryotes. The pharmacological manipulation of GPCRs is a well-validated approach for human therapeutics in numerous pathologies. GPR55 is an orphan class A GPCR that recognizes a subset of cannabinoid ligands, suggesting its potential relationship with the endocannabinoid system. GPR55 has been found to be implicated in inflammatory pain, neuropathic pain, metabolic disorder, bone and neuronal development, and cancer, indicating the promising potential of its ligands. However, while much information exists regarding signaling mechanisms and biological effects of the cannabinoid receptors, the pharmacology of GPR55 remains disputed. In the absence of selective and potent ligands for GPR55 and receptor structural data, we focused on developing and evaluating a novel functionally selective GPR55 ligand. This compound was designed based on the structure of ML184, a GPR55 agonist. Pharmacological characterization using β-arrestin recruitment and SRE luciferase reporter assays in CHO cells overexpressing h-GPR55 evidenced its bias profile at GPR55. Its GPR55 selectivity in a panel of over 40 GPCRs, including CB1 and CB2, and its lack of cytotoxicity demonstrate its interest for further preclinical development.

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# ELUCIDATION OF THE ABSOLUTE STRUCTURE OF THE PUTISOLVINS

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Putisolvins are cyclic lipopeptides produced by several *Pseudomonas putida* strains via non-ribosomal peptide synthesis. These compounds were initially discovered in Bloemberg's group in 2004 and were reported to reduce surface tension and potentially inhibit biofilm formation.[1,2] Additionally, three more lipopeptides, Putisolvins III, IV, and V, were isolated from *Pseudomonas koreensis* in Höfte's group in 2020.[3] The group of Eberl later discovered two similar lipopeptides being expressed by *Pseudomonas putida* IsoF, and demonstrated the ability of putisolvins to trigger the asocial motility of the bacteria cells.[4] All putisolvins consist of 12 amino acids, a fatty acid, and a macrocycle with 4 amino acids linked via a Ser-ester bond. However, the absolute configuration of these lipopeptides remained unknown.

In this study, we aim to elucidate the structure of several cyclic lipopeptides of the putisolvin family.[5] The study addresses several structural issues, including the sequence of amino acids in the lipopeptides and their absolute configurations, the secondary structure of the lipopeptides, and the tertiary structure of potential aggregates. Various approaches were utilized, such as chemical and enzymatic degradation, UHPLC-MS on chiral stationary phases, 2D NMR spectroscopy, H/D exchange, CD spectroscopy, computational studies (DP4+ and molecular dynamics simulations), and the comparison to the synthesized lipopeptides.

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# IMINE- THIAZOLE HYBRIDS AS POTENTIAL DUAL INHIBITORS OF COX-2 AND 5-hLOX. SYNTHESIS AND BIOLOGICAL EVALUATION

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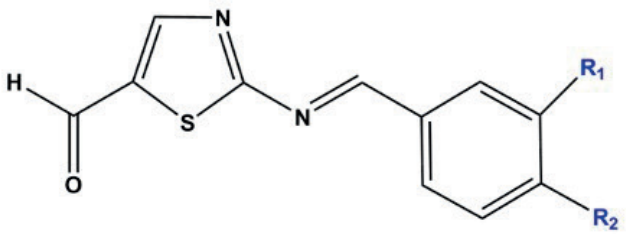
The inflammation arises as a biological response of the organism to a harmful stimulus, either due to damage to cells, an external agent in the organism, or an injury. [1] These inflammatory processes are linked to developing diseases like diabetes, hypertension, arthritis, asthma, and even diseases such as cancer and Alzheimer's. Prostaglandins (PG) and leukotrienes (LT) are mediators of the inflammatory response, which are synthesized by the action of the enzymes cyclooxygenases (COX) and 5-lipoxygenase (5-LOX) from their substrate arachidonic acid (AA). The use of selective COX-2 inhibitors has been shown to redirect AA metabolism toward the upregulation of the 5-LOX enzyme, increasing the release of LT. [1] To avoid this adverse effect, the interesting proposal to develop dual inhibitors of 5-LOX and COX-2 arises, as is the case of curcumin and darbufelone, potent dual inhibitors. [2]

In this sense, continuing with the search for 5-hLOX/COX-2 inhibitors, a family of new imine-thiazole hybrids ( **IT**) was synthesized and evaluated as potential dual inhibitors (Figure 1). Docking studies were performed between the **IT** ligands and the 5-hLOX and COX-2 enzymes. Our results suggest an attractive affinity with binding energies between -3.6 and -4.6 kcal/mol for 5-hLOX and -3.9 and -5.4 kcal/mol for COX-2. These interesting results motivate us to continue our study and evaluate *in vitro* the potential dual inhibitory activity of these imine-thiazole hybrids.




IT-1: $R_1$=$R_2$=H
IT-2: $R_1$=$R_2$=OH
IT-3: $R_1$=$OCH_3$; $R_2$=OH
IT-4: $R_1$=H; $R_2$=OH
IT-5: $R_1$=H; $R_2$=$OCH_3$

| Ligand | Binding energy (kcal/mol) 5-hLOX | | Binding energy (kcal/mol) COX-2 |
|---|---|---|---|
| | Active site | Allosteric site | Active site |
| IT-1 | - 4.49 | - 4.10 | - 4.46 |
| IT-2 | - 3.84 | - 3.80 | - 5.42 |
| IT-3 | - 4.20 | - 3.61 | - 3.93 |
| IT-4 | - 3.58 | - 3.33 | - 4.62 |
| IT-5 | - 4.55 | - 3.68 | - 4.23 |

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# MONOCHARGED PYRIDINIUM OXIMES IDENTIFIED IN SILICO ARE EFFICIENT REACTIVATORS OF PHOSPHYLATED HUMAN CHOLINESTERASES

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The cholinesterase reactivators (so called “oximes”) are used as causal antidotes in case of organophosphorus intoxications. The effectiveness of the reactivator is strongly dependent on the formation of active nucleophile, the oximate anion. Its formation can be supported by optimizing the physical-chemical properties (i.e. p$K_a$) of the oxime and the decreased p$K_a$ should lead to enhanced reactivation of phosphylated cholinesterases [1].

In the recent work, we have used *in silico* techniques to identify further molecular scaffolds of oxime nucleophiles that could modify oxime properties and enhance OP reactivation. The original ligands were obtained from Zinc database [2] and selected on the basis of particular physical-chemical properties. The oxime moiety was attached to selected molecular scaffolds and the molecular docking with OP-inhibited cholinesterases was performed. Over 250 modified oxime nucleophiles were selected with possibly beneficial binding mode. After manual inspection, over 15 modified oxime nucleophiles were chosen for chemical synthesis. The stability and efficiency of oxime anion formation (p$K_a$) was determined for all prepared molecules. Some modified nucleophiles were proved to be *in vitro* effective and excellent for reactivation of nerve agent surrogates, i.e., NEMP, NIMP, NEDPA or paraoxon-inhibited cholinesterases [3].

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# DECARBOXYLATIVE-CARBONYLATIVE NICKEL-CATALYZED CROSS-COUPLING FOR THE EFFICIENT ISOTOPIC LABELING OF ARYL-ALKYL KETONES

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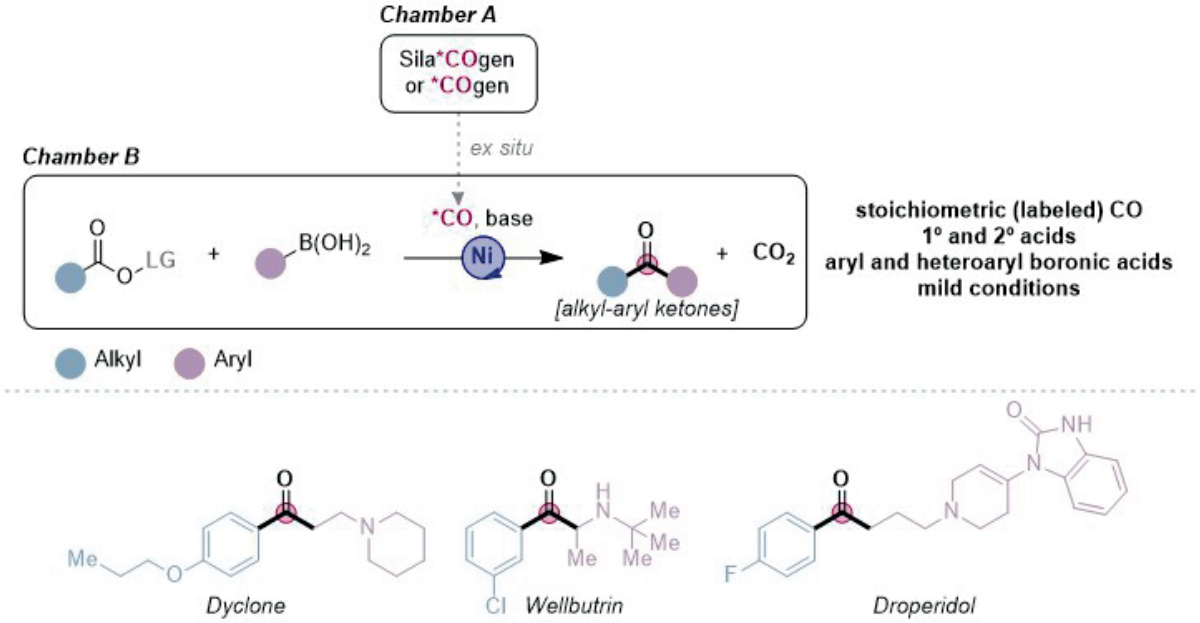
Isotopologues enriched with a radioactive isotope, such as tritium or carbon-14, provide access to essential ADME data through *in vivo* studies, both in animals and humans. The radioactive labeling of drug candidates can often be challenging. On the one hand, the radiolabel must be located at a chemically and biologically stable position of the molecule in order to track the fate of the drug candidate. While many drug metabolism pathways are known, predicting a metabolically stable position of a new drug candidate remains difficult and often results in the synthesis of molecules with different labeling patterns. Therefore, it is desirable to incorporate the labels at a late or even last stage of the synthesis, which can save time and money. On the other hand, there is only a limited and generally highly expensive pool of labeled starting materials that can be used as a source for radiolabeling. A powerful tool for incorporating a carbon label is $^{13/14}$C-isotopically labeled carbon monoxide, itself generated from a CO-releasing substrate.

Due to the frequent presence of aryl-alkyl ketones in drug structures, a general late-stage carbonylation methodology, which allows for the incorporation of a carbon isotope, has been identified as a powerful transformation. Herein, an efficient methodology for the nickel-catalyzed carbonylative cross-coupling of alkyl carboxylic acids with aryl boronic acids to obtain aryl-alkyl ketones and their isotopologues is described. The developed method uses stoichiometric amounts of (labeled) CO released from SilaCOgen or COgen in combination with a common nickel catalyst and ligand. In this manner, a wide range of aryl-alkyl ketones bearing various functionalities was successfully synthesized under mild conditions. Moreover, this methodology was expanded to the synthesis of pharmacologically relevant compounds and their $^{13/14}$C-enriched isotopologues. Finally, the reaction mechanism was experimentally investigated and supported by DFT calculations.



# SYNTHESIS, BIOLOGICAL AND IN-SILICO EVALUATION OF NOVEL PYRAZOLES AND PYRAZOLINES DERIVED OF BENZENESULFONAMIDE AND CATECHOL AS DUAL INHIBITORS OF CYCLOOXYGENASE-2 AND 5-LIPOXYGENASE

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Chronic inflammation caused by the overexpression of cyclooxygenase-2 (COX-2) and 5-lipoxygenase (5-LOX) enzymes has become an important health problem due to its direct relationship in the development of different types of cancer, autoimmune and neurodegenerative diseases. Currently, several inhibitors have been designed with the aim of interfering with the metabolism of the substrate of both enzymes, arachidonic acid (AA).[1] Thus, **celecoxib** has stood out as the main COX-2 inhibitor drug, while **NDGA**, zileuton (the only commercial drug), ZD-2138 and AKBA are classified according to the mechanism of deactivation of the 5-LOX enzyme into redox, iron chelating, non-redox and allosteric inhibitors, respectively. In addition, inhibitors such as **curcumin**, have been able to interfere with the metabolization of the AA substrate by both inflammatory pathways.[2] In this sense, the use of molecular hybrids in the design of dual inhibitors has achieved great popularity, because these systems combine together with molecular scaffolds (pyrazole, pyrazolines, triazole, among others) structural characteristics or pharmacophore fragments of known inhibitors of these enzymes, obtaining $IC_{50}$ values that are in the micromolar range and, that present a higher selectivity index towards the COX-2 isoform in relation to COX-1 (enzyme in charge of vital physiological processes in our body).[3,4]

Considering the background, we propose the synthesis, *in-silico* (docking and molecular dynamics) and *in-vitro* evaluation of novel molecular hybrids functionalized through pyrazoline (**1a-b**) and pyrazole (**2a-b**) scaffolds with the pharmacophoric fragments benzenesulfonamide, catechol and vanillin of the inhibitors **celecoxib** (COX-2), **NDGA** (5-LOX) and **curcumin** (dual inhibitor) respectively. Preliminary molecular docking results have evidenced the favorable binding affinity of the proposed systems at the allosteric site of 5-LOX and the active site of COX-2 enzyme.

[1]

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# CUT AND PASTE: A NOVEL CYSTEINE REBRIDGING METHOD

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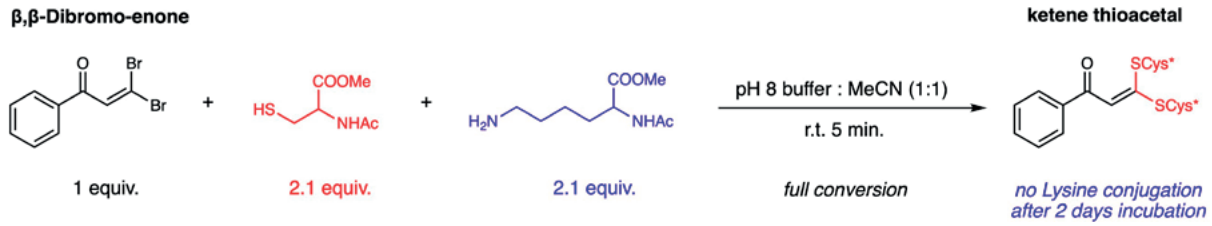
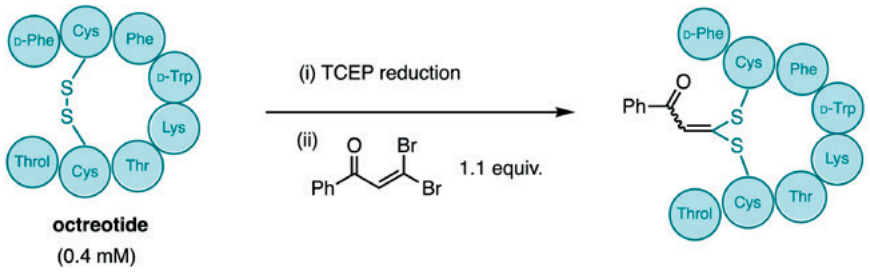
Synthetic alteration of the native structure of biomolecules such as **peptides and proteins** has shown to be of great utility in the field of biotechnology and medicine. Fluorescent labeling of proteins allows elucidation of cellular pathways[1] while conjugation of cytotoxic drugs to antibodies gives rise to receptor-specific anticancer bullets.[2] Despite the various applications, developing a reliable, fast, and chemoselective bioconjugation strategy is still highly challenging. The amino acid residue of choice for **site-selective bioconjugation** remains cysteine due to its low natural abundance. However, most cysteine residues in proteins are in the **disulfide bridge** state, rendering them unavailable for conjugation. Here, we report a novel strategy for modifying bridged cysteine-containing biomolecules using geminal substitutable Michael acceptors.




**Dibromo-enones** in particular were observed to undergo selective addition by two cysteine moieties, even in the presence of other amino acids. The reaction rapidly delivers the hydrolytically stable thioacetals in aqueous media at room temperature without the need for catalysis or UV irradiation. An example of the potential of this method is the disulfide rebridging of the cyclic peptide octreotide in a **one-pot reduction/conjugation** fashion delivering the labeled peptide with full conversion in less than an hour.

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# 1,4-BENZOQUINONES AS POTENT AGENTS FOR ALZHEIMER'S DISEASE TREATMENT

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According to the World Health Organization (WHO), currently, more than 55 million people live with dementia caused by Alzheimer's disease (AD) worldwide, and in the EU and associated countries, in 2018 more than 9.8 million people lived with dementia. It is estimated that in 2050 these numbers will progressively rise by 10 million new cases every year. AD is a complex neurological disorder, with multiple aetiology such as deposition of amyloid-ß peptides, depletion of the neurotransmitter acetylcholine (ACh) dyshomeostasis of biometal cations, accumulation of hyperphosphorylated τ-protein and oxidative stress. Currently approved treatments are based on reversible inhibition of acetylcholinesterase (AChE), and increasing the level of the neurotransmitter ACh in the brain. Quinones are a ubiquitous class of natural and synthetic organic compounds and exhibit a broad spectrum of biological activities. Recently it was shown that both natural and synthetic compounds bearing quinone scaffold possess promising activity at the low micromolar range as inhibitors of the key targets in cholinergic, β-amyloid and tau hypothesis.[1] In the continuation of our investigation of synthetic quinones[2] and new anti-AD drugs,[3] we have evaluated synthetic derivatives of 1,4-benzoquinones for their potential as symptomatic AD agents. In our study, we prepare a series of derivatives of 2-*tert* -butyl-1,4-benzoquinone (TBQ) designed as biomimetic of natural product avarone to evaluate their potency to inhibit both AChE and BChE. Compounds were prepared starting from TBQ or avarone *via* Michael reaction using corresponding S- or N-nucleophiles. It was shown that obtained derivatives are promising structural scaffolds for the design of novel anti-AD agents, with promising potential to act in the central nervous system due to their unique structure, high inhibitory potential toward both cholinesterases, potential to cross the blood-brain barrier and low toxicity. It was found that structures of introduced substituents and regioisomerism as well, influence inhibitory activity and the mode of the mechanism of inhibition. Based on these results, and the evaluation of the ability of tested compounds to chelate biometal ions $Cu^{2+}$, $Zn^{2+}$ and $Fe^{2+}$, and docking models of interactions with AChE peripheral (PAS) and catalytic anionic site (CAS) potency of tested compounds to act as anti-AD agents will be discussed.

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# ACCELERATING PROTAC DISCOVERY

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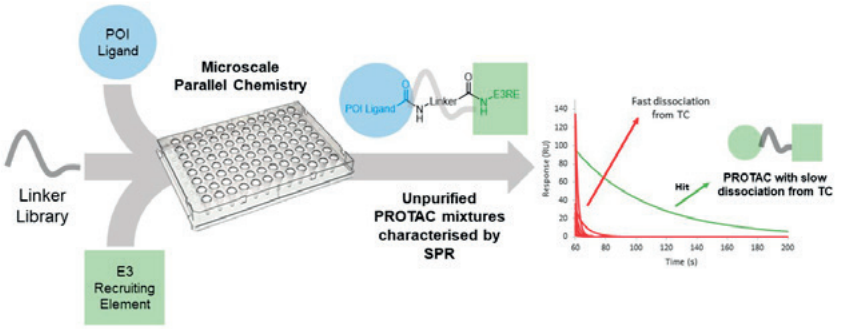
Proteolysis targeting chimeras (PROTACs) are small molecules that hijack the ubiquitin proteosome system in order to label proteins of interest (POI) for degradation. PROTACs are comprised of a POI binder and an E3 ligase recruiting element (E3RE) that are covalently tethered by a linker. Recent studies have highlighted the importance of the linker's role to afford stable and long-lived ternary complexes. Long-lived complexes appear to be necessary for efficient ubiquitination and degradation.[1, 2] Therefore, efficient strategies are required to identify optimal linkers that generate a stable ternary complex. To address this, we have designed a diverse library of linkers utilising Tanimoto similarity and pharmacophore diversity metrics, whilst simultaneously enriching for linkers that are found in active PROTACs.[3] From this analysis we selected a 43-membered linker library. The library was used to synthesise potential PROTACs in parallel and at microscale (2.5 µmol).[4] The products from this parallel synthesis will be screened by surface plasmon resonance (SPR) with minimum work-up and purification. We hypothesis that this will enable dissociating ternary complexes (TC) to be identified via "off-rate screening".[5] In this work, the linker library was used to synthesise potential PROTACs with amide coupling chemistry featuring different POI targeting ligands. Two POIs were selected; the well-characterised bromodomain Brd4$^{BD2}$, as well as fatty-acid binding protein 4.

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# DISCOVERY OF DPP8 INHIBITORS USING IN SILICO FRAGMENT MAPPING METHOD

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Dipeptidyl peptidase 8 (DPP8) is a intracellular serine protease belonging to the peptidase S9B family, which cleaves off N-terminal dipeptides from specific substrates (preferentially postproline). Recently, DPP8 is unveiled to be associated with pathophysiological roles in immune response and cancer biology. While other DPP family member, such as DPP4, is extensively characterized in molecular terms as a validated therapeutic target of type II diabetes, DPP8 inhibitors are yet to be clinically applicable. In this study, we aimed to identify novel DPP8 inhibitors by multistep virtual screening based on our in silico fragment mapping method.
The screening procedure makes the most of a database named as the Canonical Subsite-Fragment DataBase (CSFDB) and the knowledge-based fragment mapping program, Fsubsite. The CSFDB consists of various pairs of subsite-fragments derived from crystal structures of known protein-ligand complexes. Fsubsite searches the surface of a target protein for similar topographies to subsites stored in the CSFDB. When a local topography similar to the subsite is found on the target protein, Fsubsite places the corresponding fragment with its matching subsite. In this study, the mapping procedure identified fragments with novel modes of interaction at the ligand binding site of DPP8. Next, we constructed two three-dimensional (3D) pharmacophore models and retrieved several candidate compounds from a commercial database. This selection was achieved using a multistep virtual screening protocol, combining 3D pharmacophore-based searches, docking calculations, and chemical structure clustering. Assay for detecting DPP8 inhibition is being performed.

# A NEW SERIES OF TETRAZOLE-HYDRAZONE HYBRIDS ENDOWED WITH ANTICANDIDAL ACTIVITY

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Hydrazones occupy a prominent place in the development of novel drugs for the treatment of several diseases, particularly bacterial and fungal infections due to their unique structural features such as carrying hydrogen bond donor and acceptor groups allowing these ligands to interact with key residues of crucial biological targets.[1] Besides, tetrazole, the bioisoster of carboxylic acid, is one of the most important five-membered heterocycles and its derivatives have been reported to show diverse biological activities including antifungal activity.[2]

In an attempt to identify potent anticandidal agents, herein new tetrazole-hydrazone hybrids were synthesized efficiently. All compounds were examined for their anticandidal activities using a broth microdilution assay and minimum inhibitory concentration (MIC) was determined for each compound. Compounds **1** and **3** (Fig. 1) were found to be as effective as tioconazole (MIC= 50 µM) on *Candida krusei* (ATCC® 6258™) and *Candida parapsilosis* (ATCC® 22019™) with a MIC value of 50 µM. MTT assay was performed to evaluate their cytotoxic effects on NIH/3T3 mouse embryonic fibroblast cells. These compounds did not show cytotoxicity on NIH/3T3 cells at the tested concentrations indicating that the anticandidal effects of both compounds were selective. The pharmacokinetic profiles of compounds **1** and **3** were predicted by means of QikProp, a predictive Absorption, Distribution, Metabolism and Excretion (ADME) module within the Maestro suite produced by Schrödinger. According to Lipinski's rule of five and Jorgensen's rule of three, both compounds were found to possess favorable drug-likeness and oral bioavailability. *In vitro* and *in silico* studies pointed out the potential of compounds **1** and **3** as promising candidates for the treatment of candidiasis.

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# SYNTHESIS OF STERYL AND PHYTOL PHENOLIPIDS AND THEIR ANTIOXIDANT EVALUATION IN LIPOSOMES

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Phenolic compounds are potent antioxidants that may play an important role both in biological systems and in formulated lipid dispersions. However, their efficiency is often limited by their solubility or bioavailability. Lipophilization of phenolic acids (phenolipids) with high radical scavenging properties, such as caffeic or ferulic acid, may increase their solubility in lipophilic matrices and their penetration throw lipid bilayers, conferring better antioxidant protection in food and biological systems [1, 2]. Recently, attention has been given to some natural phenolipids such as steryl ferulates and caffeates. The overall concentration of these compounds in some cereals may be ten times fold higher than the one of total tocopherols, being very important antioxidants in cereals [3]. These compounds have also shown cholesterol-lowering properties as well as anti-inflammatory and antitumor activity.

Although the esterification reaction has been widely studied, few reports concern the esterification of phenolic acids. These compounds, in particular the catecholic ones, have a particular chemistry and stability that prevents the direct use of the more common esterification strategies. Therefore, previous protection of the aromatic hydroxyl groups is usually required in order use reaction conditions with better esterification yields. However, this strategy is time and material consuming and the overall yield is not great because the need of more reactional steps and purifications.

In this work, the esterification reaction conditions were study in order to obtain phenolipids in good yields by the direct esterification of phenolic acids (caffeic, dihydrocaffeic, 3,4-dihydroxyphenyl ethanoic and protocatechuic acids) with cholesterol or phytol. By changing temperature, catalyst type, acidity and solvent type, we were able to improve yields. Optimized reaction conditions were not the same for all phenolic acids and alcohols being in all cases very depend on the used solvent. The best conditions for phytol phenolipids were obtained mainly with enzymatic catalysis with yields up to 99%. In contrast, cholesterol phenolipids were only efficiently obtained by chemical catalyzes with yields around 60%. Preliminary antioxidant evaluation of these phenolipids in soybean PC liposomes showed that these compounds have a much higher protecting efficiency against the AAPH-induced oxidative stress than the corresponding phenolic acid.

**Acknowledgments**

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# DESIGN OF DIVERSE BIOSENSORS FOR VISUALIZING CYTOSKELETON MODULATIONS AND DYNAMICS

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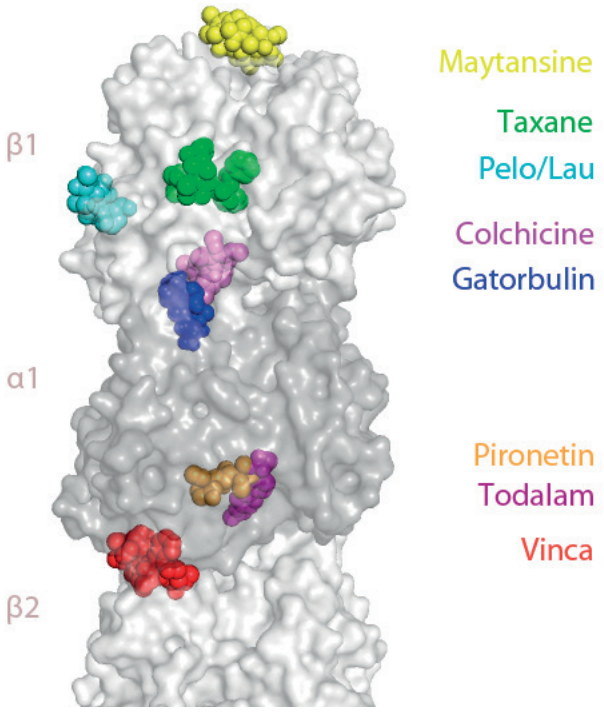
Microtubules (MTs) are key cytoskeletal elements involved in track formation for intracellular transport, structural support and spindle formation for chromosome segregation. They are composed of α- and β- tubulin dimers that enable the MTs to undergo successive cycles of growth and shrinkage to perform its function. [1] MT targeting agents are useful chemical tools and drugs, being 16 microtubule binding agents (MTAs) approved for clinical use. To better understand of the features triggered in pharmacological treatment there is a need of new tools able to assess the function of MTs in the presence of drugs. This information would enable the design of new drugs with stronger activity and less side effects.

Here we have synthesized fluorescent derivatives of MTAs specifically recognizing structural features of MTs as visualization tools. For that purpose, we have used a semi-synthesis strategy in which the tubulin-binding derivatives are chemically modified to incorporate functional groups that allow the linkage with fluorophores. Particularly, we have approached the synthesis using amine-carboxylic acid coupling reactions and click-type reactions (1-3 dipolar cycloaddition).

Finally, our recently developed kinesin and dynein probes enable to assess intracellular trafficking in the presence of MTAs. [2]

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# DUAL GAT-3 AND BUCHE INHIBITORS TARGETING COGNITIVE, BEHAVIORAL AND PSYCHOLOGICAL SYMPTOMS OF ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is an incurable neurodegenerative disorder affecting around 35 million people worldwide. Due to complex and still not fully explored etiopathogenesis initiating changes in the brain long time before the first symptoms, AD is a particularly difficult area to search for new therapies. The most characteristic symptoms of the disease, which include memory and cognitive disorders, are accompanied by the behavioural and psychological symptoms of dementia (BPSD) such as agitation, aggression, apathy, depressive mood, anxiety, psychosis and reduced sociability[1].

The memory-related symptoms of AD are caused by disturbances in neurotransmission systems mainly cholinergic but also GABAergic. Observed impairment of cholinergic neurotransmission may be compensated by inhibition of enzymes hydrolysing acetylcholine: acetylcholinesterase (AChE) and/or butyrylcholinesterase (BuChE)[2]. The observed overexpression of γ-aminobutyric acid transporter subtype 3 (GAT-3) on the astrocytes and microglial cells of the hippocampus in animal models of AD may be overcome by the inhibition of GAT-3[3]. Therefore, we undertook research on new multifunctional ligands targeting BuChE and GAT-3, assuming that simultaneous modulation of these two neurotransmitter systems may be more effective in improving memory deficits in AD.

We performed a biological screening towards GAT-3 of our in-house library of BuChE inhibitors that led us to the selection of a "hit" compound[4] that we optimized in terms of biological activity and drug-likeness. As a result, we discovered compound JT-3, a potent inhibitor of BuChE and GAT-3 with favourable ADME-tox properties observed *in vivo* in mice. In further pharmacodynamic studies JT-3 showed procognitive activity in memory tests: passive avoidance task, Morris water maze and Barnes maze and activities in forced swim test, four-plate test and elevated plus maze test indicating potential in the treatment of BPSD including anxiety and depression.

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# PYRENE-ARMED CALIX[4]ARENE DERIVATIVES AS HIGHLY SENSITIVE FLUORESCENT REPORTERS

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Due to their chemical versatility and application possibilities, calixarenes have been a subject of continuous interest in the scientific community over the last four decades.[1] Their capability for efficient entrapment and coordination of different metal cations makes them extremely interesting systems in terms of analytical applications in environmental and biological areas.[2] The structural upgrade of the parent calixarene cavity with specially designed coordination arms provides additional development of new biomimetic systems able for the specific guest molecule recognition/binding.[3] For instance, the covalently linked pyrene functional group is especially interesting due to its unique spectroscopic properties. The ability to form non-covalent interactions with DNA/RNA revealed pyrene's extreme spectroscopic potential as a sensitive fluorescent probe widely used to study different micro-heterogeneous systems. For these reasons, our research interests in this field were directed to the synthesis of calixarene binders by grafting pyrene fluorophores to the calix[4]arene basket. A simple peptide coupling between 4-(1-pyrenyl)butyric acid, and an amine linked to a calix[4]arene rim builds bis-functionalized derivatives **1** and **2** designed as highly sensitive to the microenvironment and thus able to report the binding event. Several spectroscopic methods were combined to study the interactions of new compounds with cations, mononucleotides, and DNA/RNA, and to get insight into the mode of interaction. Their excimer and exciplex emission properties were used as fluorescent chemosensing processes to investigate affinity and selectivity upon binding to certain targets. The results finely delineate the high sensitivity of studied systems to the presence of ligands, more precisely quenching of the emission upon binding to the mono/polynucleotide (Fig. 1) or upon coordination of certain metal cations. Molecular dynamics simulations of **1** and **2** and their $Zn^{2+}$-complexes suggest the most likely exists conformations of studied calixarene-based fluorescent reporters depending on the presence or absence of the metal cation close to the ionophoric cavity of calixarene.

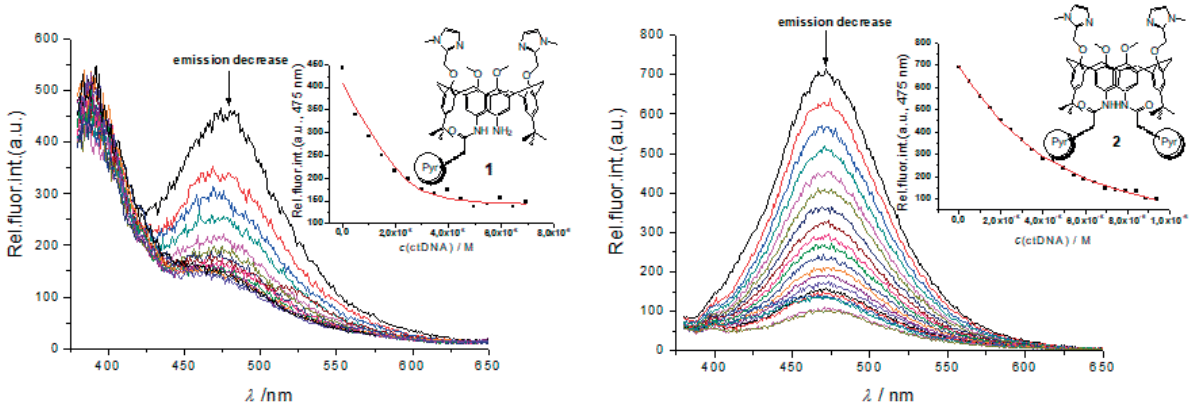


**Figure 1.** Changes in the fluorescence spectra of: a) **1** and b) **2** upon titration with ctDNA at $\lambda_{exc}$= 350 nm. Insets: Dependence of **1** and **2** emission intensity at 475 nm on c(ctDNA).

The authors gratefully acknowledge financial support from Croatian Science Foundation (Grants IP-2020-02-3786 and IP-2018-01-5475).

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# LIPID INTERACTIONS WITH DIFFERENT CATIONIC PEPTIDES: TOWARDS THE UNDERSTANDING OF MEMBRANE DISRUPTING ACTIVITY

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The emerging problem of antibiotic-resistant bacteria has caused the need for alternative strategies to combat bacterial infections. One of the promising avenues for the development of new antibiotics is the use of membrane active peptides. These peptides are able to disrupt or penetrate the cellular membrane by interacting with the lipid bilayer, without targeting a specific receptor [1]. The cationic amino acids arginine (Arg or R) or lysine (Lys or K) provide the affinity for anionic prokaryotic membranes, while hydrophobic amino acids such as phenylalanine (Phe or F) perturb lipid packing, and both are important for ensuring cell lysis [1,2]. Despite the equal net charge of Arg and Lys, they seem to have different affinity towards membranes, with Arg being more prevalent in naturally occurring membrane active peptides [3]. Research has already confirmed different interactions of Arg guanidinium group and Lys amine group [4], but the exact mechanism behind those effects is yet unconfirmed. Here, a combined theoretical and experimental study is presented on the interaction of cationic peptides RRRRRFF and KKKKKFF with zwitterionic 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC), anionic 2-dipalmitoyl-*sn*-glycero-3-phosphatidylserine (DPPS), and mixed membranes. The peptides were prepared by solid phase synthesis, and their interaction with lipid bilayers was characterized by calorimetric and spectroscopic techniques. Molecular dynamics was employed to evaluate peptide binding and membrane disrupting effects. The preference of membranes for Arg-rich peptides over Lys-rich ones was noted. The results of the study will help illuminate the preferred structure and mechanism of action of antimicrobial peptides.

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# MANNOSYLATED FERROCENE DERIVATIVES OF DESMURAMYL PEPTIDE: SYNTHESIS AND ADJUVANT ACTIVITY

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Derivatives of muramyl dipeptide (MDP), a well-known peptidoglycan fragment, have been extensively studied as potential adjuvants for human and animal vaccines.TheL-Ala-D-*iso*Gln pharmacophore is essential for immunostimulatory properties. Therefore, numerous MDP analogues without the *N*-acetylmuramyl moiety, called desmuramyl peptides (DMP), have been prepared. The introduction of lipophilic substituents was found to contribute to adjuvant activity. We have shown that the binding of lipophilic substituents (adamantyl, dodecyl, and adamantylethyl) through triazole motif is beneficial for adjuvant activity, as well as additional mannosylation of DMP derivatives.1,2

In this work, we have described the synthesis of novel class of DMP derivatives in which lipophilic ferrocene unit was introduced in DMP at D-*iso*Gln side chain via ester or amide bond, and additionaly to the *N*-terminus of DMP. The ferrocene moiety is connected to DMP through alkyl spacers of different lengths. Mannose is attached to the N-terminus of the ferrocene derivatives of DMP via a glycolyl spacer. Additionally, with the aim of examining the influence of the position of the ferrocene subunit on the immune response, mannosylated DMP derivatives with the ferrocene subunit attached to the a-position of D-*iso*Gln were prepared. All compounds were screened for their *in vitro* adjuvant activity on HEK-Blue NOD2 cells.

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# EFFICIENT CELL-BASED DEL SCREENING

**Rico G. Petersen, Allan B. Christensen, Andrzej Taranta, Carlos Azevedo, Charlotte Andersen, Daniel Madsen, Frank A. Sløk, Leif K. Larsen, Lars K. Petersen, Ole Kristensen, Peter Blakskjær, Pratibha Dhumale, Nils J. V. Hansen**

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Screening of DNA-encoded libraries (DELs) has gained momentum as an important hit identification technique over the last two decades. In contrast to high throughput-screening, DNA barcoding of molecules in conjunction with PCR-amplification and next-generation sequencing allows for a deeper and more cost-effective screening of chemical space (multi-million library members).

In a common DEL screen, a highly purified protein of interest (POI) is immobilized on a solid matrix and used to interrogate a DEL in an aqueous solution containing buffer and salts. This non-physiological screening environment can give rise to artefacts and high attrition rates. Furthermore, many proteins are not amenable to common DEL screens as they cannot be successfully purified. Considering this, it is highly desirable to develop and improve the DEL screening technology. Recently, we published a new *cellular binder trap enrichment* technology (cBTE) for screening DELs inside living cells.[1]

Two fundamental challenges of screening DELs inside living cells are to get the DEL into the cell and to enable discrimination between DEL binding to POI or endogenous cell proteins. The challenge of getting inside the cell was overcome by using *Xenopus laevis* oocytes which are large enough (~1 mm in diameter) to allow direct injection of the DEL. The challenge of discriminating DEL binding was overcome by labelling the POI with DNA *in vivo* utilizing the affinity of a small bait molecule for a prey protein. The POI was expressed as a fusion with the prey and the DNA was conjugated to the bait molecule and injected together with the DEL. Because the DEL is injected into the cell and the screen performed directly in the cell, the technology is not dependent either on a special method for getting DNA across cell membrane or on protein stability and purification *in-vitro*. An additional advantage of the technology is that it allows for screening of multiple targets simultaneously.

The poster will focus on further applications of the cBTE technology, and two case studies will be presented. In the first study, the purification of a DNA conjugate of a notoriously difficult helicase-like domain of a polymerase was compromised by tetramer formation. Hence, the target could not be subjected to a classic DEL screen. In comparison, the target was screened successfully with cBTE identifying hits in nine different chemical series. For several hits, enzyme activity was confirmed upon off-DNA resynthesis. In the second study, a full-length enzyme and three separate domains of the same enzyme were screened individually. Hits in three different chemical series were identified for one of the separate enzyme domains only, showcasing the strength of the technology.

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# DESIGN AND SYNTHESIS OF BRAIN PERMEABLE ANTI-NAEGLERIA FOWLERI AGENTS TARGETING CYP51 FOR PRIMARY AMOEBIC MENINGOENCEPHALITIS TREATMENT

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The free-living ameboflagellate *Naegleria fowleri* is an opportunistic pathogen causing a fulminating brain infection namely primary amoebic meningoencephalitis (PAM) that can result in death within days, with a worldwide distribution and over 97% fatality rate.[1] Even though PAM is considered rare, with 381 global PAM cases reported by the Center for Disease Control and Prevention,[2] this is likely an underestimate of the true worldwide occurrence of PAM. Currently, there is no standard regimen to treat *N. fowleri* infections in humans and only seven patients have been successfully treated so far using Amphotericin B (AmpB), either alone or in combination with other drugs.[3] However, clinical use of AmpB is limited due to its toxicity, including acute infusion-related reactions and dose-related nephrotoxicity.[4] For these reasons the development of effective and safe drugs for the PAM treatment represents a real unmet medical need. Over the past few years, we validated several steroidogenic enzymes as drug targets. In particular, disruption of sterol 14-demethylase (CYP51) function by sterol biosynthesis inhibitors, induced massive autophagocytosis leading to *N. fowleri* cell death after 24 h of drug exposure.[5] Notably, *in vitro* growth inhibition of *N. fowleri* by CYP51 inhibitors, including antifungal conazole drugs, has been reported in literature and some of them, even though endowed with poor blood-brain barrier (BBB) permeability, have been used in combination therapies with AmpB for the treatment of PAM patients.[6] In this work, we provide evidence that miconazole-like compounds could be considered as drug candidates for the treatment of PAM. We used a combination of the cheminformatics, target-based and phenotypic drug discovery methods to identify a lead scaffold conducive to BBB permeability capable of targeting *N. fowleri* CYP51 (NfCYP51). 124 compounds pre-selected *in silico* were tested against *N. fowleri* trophozoites, allowing to identify nine hits with $EC_{50} \leq 10$ μM. The top hit was identified *via* cross-validation in co-crystallization with the NfCYP51 target that singled out a miconazole-like scaffold having the best drug-target fit. Based on the co-crystal structure, a set of analogs was synthesized and biochemically evaluated, confirming the superiority of the S- over R-configuration and the advantage of ether over ester linkage. The two best acting compounds exhibited improved $EC_{50}$ and $K_D$ compared to hit, both readily distributed into the brain. Brain-to-plasma distribution coefficient of the best acting compound was 1.02±0.12, holding a promise for further optimization into a drug candidate. Synthetic pathways, *in vitro* activities, X-ray crystallography data and pharmacokinetic study will be shown and discussed.

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# CELL PERMEABILITY ASSESSMENT OF DITHIOLANE CONTAINING PEPTIDES THROUGH NANO-CLICK ASSAY

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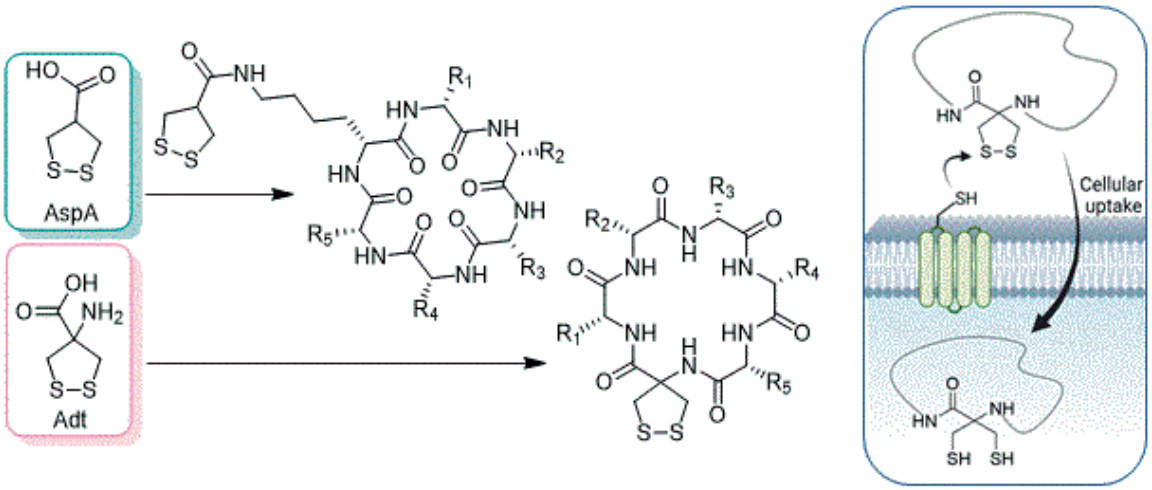
Cell permeability is a relevant characteristic for molecules showing *in vitro* activity to go into later stages of drug development. This is the case with peptides, as their low membrane permeability limits their therapeutic application.

Constrained cyclic disulfides such as Asparagusic acid (AspA) and 4-amino-1,2-dithiolane-4-carboxylic acid (Adt) have proved to exhibit thiol-exchange-mediated cell permeability.[1,2,3] In contrast to positively charged CPPs, the dithiolane ring has a lower risk of presenting issues such as membrane lysis or non-specific interactions. We aim to introduce these moieties into head-to-tail cyclic peptides in order to study the structure-cell permeability relationship. AspA is attached to a lysine residue during SPPS of the linear peptides, while strategies for using Adt in NCL-based SPPS are currently being studied.




In order to assess the cell permeability of these cyclic peptides we have set up the NanoClick assay in our lab. Cell permeable peptide azido-octa-Arginine and cell impermeable azido-ONEG are positive and negative controls. Azido-lysine has been introduced in the sequence of dithiolane containing peptides as a handle for NanoClick assay.[4] Cell permeability of an initial library of dithiolane containing peptides was assessed

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# DESIGN, SYNTHESIS AND EVALUATION OF A BOSUTINIB-INSPIRED MACROCYCLIC ULK1 INHIBITOR

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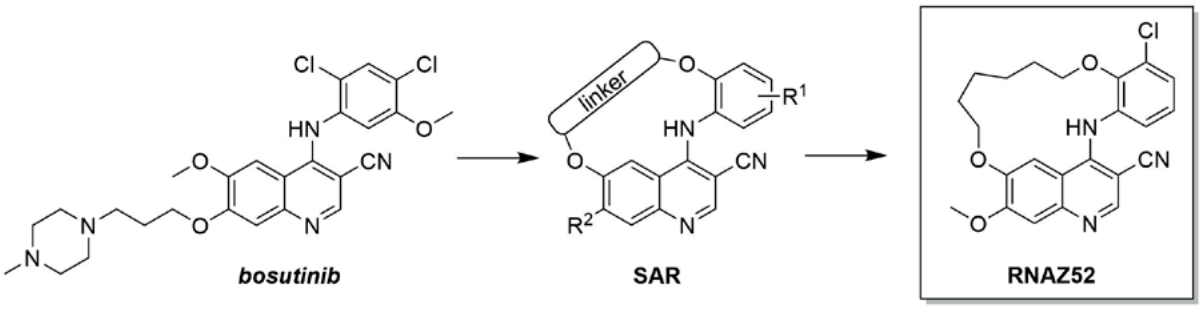


The well-characterized kinase inhibitor *bosutinib (SKI-606)* has desirable binding affinity to many kinases, but is not selective.[1)] Upon binding *bosutinib* adopts its bioactive conformation in a target-dependent manner, as one can observe comparing different co-crystallstructures. Macrocyclization is a drug development tool that can be used to restrict the flexibility of acyclic ligands to limit the number of bioactive conformations.[2)] We initiated a proof-of-concept study on the 4-amino-3-cyano-quinoline core of *bosutinib* and designed and synthesized a series of macrocyclic derivatives of *bosutinib.* Therefore, an efficient synthetic route for derivatization and the ring closing reaction was developed. The newly synthesized compounds were evaluated in an initial selectivity screen against approximately 100 kinases via differential scanning fluorimetry and the selectivity of the macrocyclic compounds were compared to the acyclic lead *bosutinib*. Preliminary results showed that ether functionalization at position 7 of the quinoline ($R^2$) was important for the binding affinity. Similarly, we saw that position 2 of the aniline was a favourable linker attachment point for the macrocyclization. By selecting different linker lengths and alternating the substitution pattern of the phenyl ring, we finally identified a very potent unc-51 like autophagy activating kinase (ULK1) inhibitor in compound RNAZ52, which also showed an excellent selectivity profile. In this structure-activity relationship study, we demonstrated how macrocyclization can be used to modify and optimize the properties of an acyclic kinase inhibitor for other targets.

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# APPROACH TO RATIONAL IDENTIFICATION OF LEAD MOLECULAR GLUE DEGRADERS FOR CASEIN KINASE 1 ALPHA

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There has been a tremendous explosion of interest in the targeted protein degradation (TPD) in drug discovery in recent years. One approach that has been widely exploited is the use of proteolysis targeting chimeras (PROTACs). Here, a heterobifunctional compound with 2 ligands linked by a flexible/rigid spacer has one ligand that binds to an E3 ligase while the other binds to a protein of interest (POI). This action brings the POI in close proximity to the E3 ligase for polyubiquitination and subsequent degradation by cellular proteasome machinery. The limitations of this approach are that it has usually led to the identification of compounds that are outside of the rule of five, have molecular weights >500 and in most cases, are not orally bioavailable. An alternative TPD approach involves the use of molecular glue degraders (MGD). To date there are few examples of cases where a rational approach has been used to identify potent MGDs and most MGDs have been discovered by serendipity or by happenstance. Unlike PROTACs, MGDs generally have molecular weights of

In a recent report[1], a series of MGDs have been designed based on the stabilization of the ternary complex by modelling ligands in the crystal structures.

In this study, we use a structure-based virtual screening approach in conjunction with ternary complex stabilization to identify potent MGDs for our CK1a protein target. CRBN and CK1a have a relatively weak native protein-protein interaction with Kd of about 2 mM. These types of interactions, between the domain of one protein with the sequence motif of the other, are generally weak as they possess only a small buried surface area (BSA). The goal of a MGD is to increase these interactions (if present) and stabilize the resultant ternary complex. Indeed, when lenalidomide binds to CRBN, it increases its affinity for CK1a and the resultant ternary complex has a Kd of about 75 nM. This could also be viewed as lenalidomide binding to the binary complex of CRBN- CK1a and strengthening the interaction within the ternary complex.

Using a 2.5 Å resolution crystal structure of CRBN-lenalidomide-CK1a (PDB: 5FQD), a structure-based virtual screen was performed using lenalidomide scaffold-based Enamine library. The highly scored hits with favorable interactions from the screen were selected for profiling in binding stability studies of the ternary complex using molecular dynamics simulations. Subsequently, the compounds were screened in biological assays for CRBN binding affinity, CRBN-ligand-CK1a ternary complex formation, CK1a polyubiquitination, cellular target engagement (CRBN) and cellular CK1a degradation. As a validation of our assays the known CRBN binders lenalidomide, thalidomide, CC-885 and CC-90009 were all shown to bind to CRBN. Lenalidomide, CC-885 and CC-90009 had the activity of stimulating the formation of CRBN-MGD-CK1a ternary complex, enhancing binding affinity by 2-4 fold in vitro. The compounds were then tested in ubiquitination assay to assess if ubiquitin could be conjugated to CK1a in vitro via E1-E2-E3 ligase system. Ubiquitination was visualized with Western blot, where Lenalidomide, CC-885 and CC-90009 were all shown to enhance ubiquitination of CK1a. In addition, at the cellular level, the compounds were membrane-permeable, capable of binding to intracellular CRBN and promoting the ubiquitination and degradation process of CK1a. The results of these studies will be presented to showcase how our approach of combining in silico screening with biology could help rapidly identify lead MGD for protein targets.

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# PYRIMIDINE-BASED COMPOUNDS AS NEW PROMISING PROTEIN KINASE INHIBITORS FOR CANCER TREATMENT

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Pyrimidine-based scaffold is typical of some of the most promising anticancer agents recently discovered. Indeed, though biologics currently dominate clinical scenarios and seem ready to revolutionize cancer therapies, heterocyclic-based small molecules remain of great prominence continuing to play significant roles in the development of innovative treatments. Small molecules display some favorable pharmacological advantages and oncology drug discovery has significantly benefited from progress in understanding how to target enzymes intimately involved in cancer expression and proliferation with small molecules. Nitrogen-based heterocyclic nuclei have been the core parts of these new entities, representing today the 75% of the approved anticancer drug. In particular, pyrimidines are currently considered privileged scaffolds since they show intrinsically a wide range of biological activities. Many aminopyrimidine derivatives exerted their anticancer activity through inhibiting different types of Protein Kinases (PKs) as they are considered as bioisosteres to purine scaffold from which ATP is formed. Kinase deregulation has emerged as a relevant mechanism by which cancer cells evade normal physiological constraints and kinases inhibitors have become one of the most intensively pursued classes of recent antitumoral drugs.[1,2]

Owing to the significance of pyrimidine derivatives as anticancer agents through kinase inhibition and our longstanding expertise in pyrimidines drug discovery, we designed and synthesized various classes of anilino and bis-anilinopyrimidines. Most of them were found active in *in vitro* HTRF inhibition assays in low nanomolar range against one or more kinases, like EGFR, c-KIT, VEGFR, PDGFR, Akt and AURKA, wild type or mutated and double-mutated isoforms. Some compounds were also crystallized in the active site of some kinases, showing a preference for DFG-in or DFG-out conformation. Subsequently, the antitumor activity of selected compounds was evaluated on three different human cancer types chosen on the basis of their unsatisfactory therapeutic strategies and poor prognosis: glioblastoma multiforme (U-87 MG cells), triple-negative breast cancer (MDA-MB231 cells), colon adenocarcinoma (HT-29 cells), tongue squamous carcinoma (CAL-27 cells) and hypopharyngeal squamous carcinoma (FaDu cells). Various pyrimidines demonstrated to also hinder cell proliferation and cell cycle and to induce apoptosis in all cell lines, without exerting cytotoxic effects at the same concentrations. The data coming from the biological assays will be shown and discussed.

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# EVALUATION OF ANTIPLASMODIAL AND ANTIPROLIFERATIVE ACTIVITIES OF HARMIPRIMS, HARMINE AND PRIMAQUINE HYBRIDS

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Cancer and malaria remain significant global health threats due to high mortality rates and drug resistance challenges. Molecular hybridization strategy is a useful tool for the development of new agents targeting such complex diseases and may overcome some drawbacks of the combined chemotherapy. Harmine (HAR), alkaloid of the β-carboline type, and well-known antimalarials such as primaquine (PQ) possess a wide range of biological properties. HAR has been shown to cause cell cycle arrest or DNA intercalation [1], as well as selective inhibition of *Plasmodium falciparum* heat shock protein 90 [2], making it a potential candidate for the treatment of cancer and malaria. In addition, numerous PQ derivatives act as potent antiproliferative agents against various cancer cell lines [3]. To this end, we investigated the antiproliferative and antiplasmodial activities of harmiprims (HP), harmine–primaquine hybrids of triazole (TT) or ureido type (UT) (Figure 1).

TT HPs **1–5** and **11** were obtained from harmine-based terminal alkynes and PQ-based azide by applying standard Cu(I)-catalyzed azide–alkyne cycloaddition, while UT-HPs **6–10** were prepared from harmine-based amines and PQ-benzotriazolide, as described previously [4]. In the synthesis of TT-HP **3** at position 6 of the β-carboline core, *bis*-derivative **11** coupled to position 5 of the quinoline ring was obtained alongside hybrid **3**. Screening of their antiproliferative activity was performed against four human cancer cell lines: MCF-7, HepG2, SW 620 and HCT 116, and one non-cancer cell line, Hek293T. In general, UT-HPs **6–10** showed more pronounced antiproliferative activity than their TT analogs. The most active HP against all cell lines tested was UT-HP **6,** which was substituted at position 1 of the β-carboline core, whereas all other HPs were only moderately active or inactive. Only TT-HP **5** was selective against MCF-7. Interestingly, *bis*-TT-HP **11** was completely inactive, in contrast to mono derivative **3** which showed moderate activity against all human cell lines tested. To our surprise, **11** displayed significant antiplasmodial activity, while the other HPs were virtually inactive. Its activity against the erythrocytic stage of CQ-sensitive strain of *P. falciparum* (*Pf*3D7) was comparable to that of chloroquine (CQ), whereas it was one order of magnitude more potent against the CQ-resistant strain (*Pf*Dd2). HP **11** also showed strong activity against the liver stages of *P. berghei* and mature gametocytes of *P. falciparum* NF54 and IGP-1 strains, indicating its potential as a transmission blocking agent. As a result, TT-HQ **11** was identified as a valuable hit, that could be used in the development of antimalarial agents targeting different stages of the *P. falciparum* life cycle.

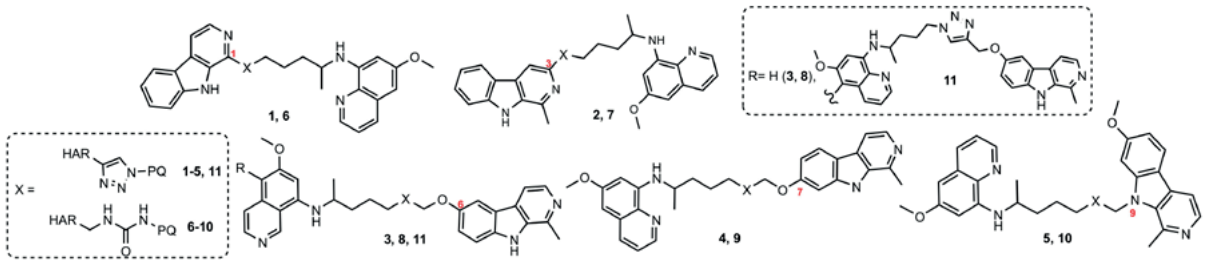


**Fig. 1.** Structures of TT- (**1–5**, **11**) and UT-HPs (**6–10**).

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# NOVEL 1,4-DIHYDROPYRIDINES AS SPECIFIC BINDERS AND ACTIVATORS OF SIRT3 IMPAIR CELL VIABILITY AND CLONOGENICITY, AND DOWNREGULATE HYPOXIA-INDUCED TARGETS IN CANCER CELLS

**Carola Castiello (1), Alessia Raucci (1), Clemens Zwergel (1), Michele Aventaggiato (2), Sabrina Garbo (3), Elisabetta Di Bello (1), Bruno Fassari (4), Beatrice Noce (1), Chiara Lambona (1), Dante Rotili (1), Rossella Fioravanti (1), Marco Tripodi (3), Gianni Colotti (5), Clemens Steegborn (6), Cecilia Battistelli (3), Marco Tafani (2), Sergio Valente (1), Antonello Mai (1)**

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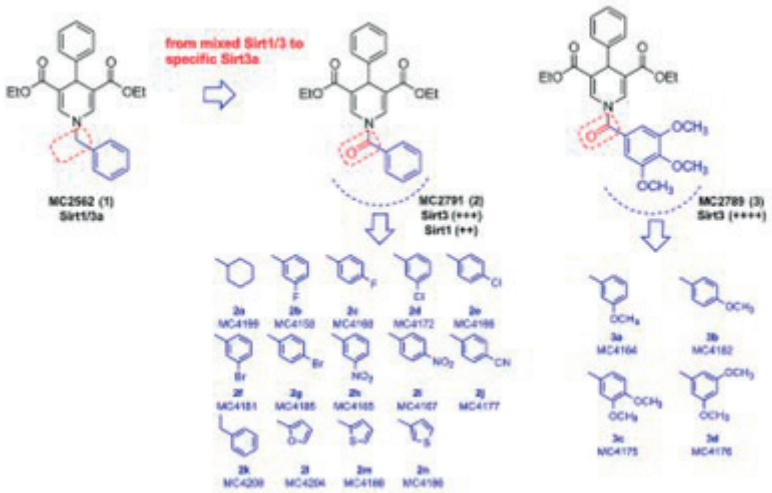
The Sirtuins family is composed of $NAD^+$-dependent lysine deacetylases that, in humans, contains seven members (SIRT1-7) and belongs to the class III histone deacetylases.[1] Among the other isoforms, SIRT3 localizes mainly in the mitochondrial matrix and plays a pivotal role in regulating many cellular processes, including mitochondrial metabolism and protection against oxidative stress by catalyzing reactive oxygen species (ROS) detoxification.[2] Indeed, SIRT3 has been shown to play a central role in several biological pathways regulation, sush as cancers, metabolism and hypoxia-related diseases.[3] It's also known that SIRT3 activation can lead to anticancer effects.[4] For example, it has been shown that SIRT3 can deacetylate and modulate several targets directly or indirectly involved in glycolysis regulation leading to anticancer effects. In addiction, a study proved that SIRT3 overexpression destabilized HIF1-α in hypoxic human breast cancer cells, where the SIRT3 catalytic activity was required for the complete repression of HIF1-α target genes.[5] On these bases, SIRT3 activation would be a fascinating approach for treatment of several metabolic-dependent diseases, especially cancer. Very recently, our research group discovered new 1,4-dihydropyridines, compounds **2** and **3**, able to selectively/specifically activate SIRT3. A novel small series of such compounds has been developed, and among them **3c** displayed the strongest SIRT3 binding and biochemical activation, with a $K_D$ of 29 μM and 387% of enzyme activation, respectively. Differently, compound **3d** was the most effective in enhancing GDH activity and deacetylating K68- and K122-AcMnSOD in triple-negative MDA-MB-231 breast cancer cells. Moreover, **3d** was the most effective also to downregulate hypoxia-induced factors, such as HIF-1α, EPAS-1, and CA-IX, but also epithelial to mesenchymal transition master regulators and extracellular matrix components, such as SNAIL1, ZEB1, SLUG, COL1A2.

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# PROBING THE SIGNALLING AND BIOSYNTHETIC PATHWAY OF VALDIAZEN

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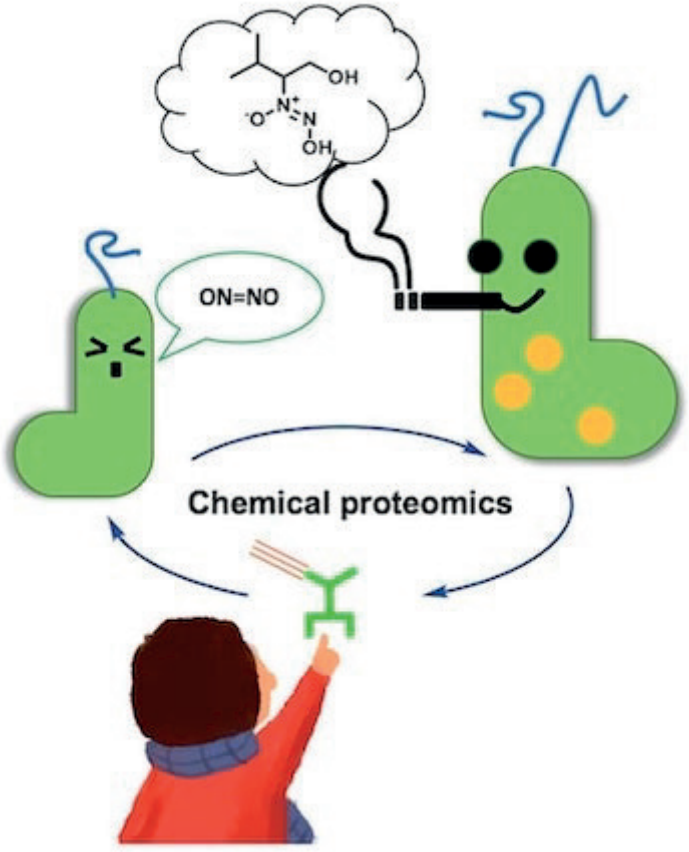
Quorum sensing refers to a special signalling pathway that regulates genes based on the local population density of microbes.[1] Such a cell-density-dependent pathway is often involved in complex phenomena in bacteria such as biofilm formation, virulence factor regulation, and cross-species communication.[1] The recent discovery of valdiazen in *Burkholderia cenocepacia* H111 marked the first example of a diazeniumdiolate-containing quorum-sensing signal.[2] This volatile and heteroatom-packed signal controls the expression of over 100 genes including those involved in the biosynthesis of an antifungal agent, (–)-fragin.[2] Chemical derivatisation and chemoproteomic profiling were attempted to shed the light onto the sophisticated signalling and biosynthetic pathway of valdiazen.

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# PHENYLETHYNYL ANTHRANILIC ACID BASED DIHYDROOROTATE DEHYDROGENASE INHIBITORS FOR THE USE AS ANTIVIRAL AGENTS

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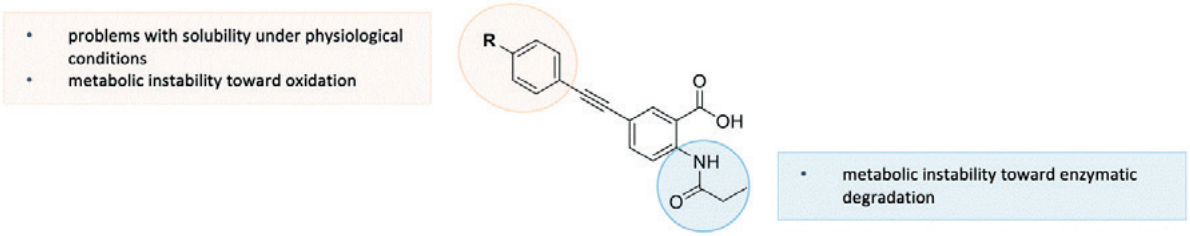
Dihydroorotate dehydrogenase (DHODH) is a mitochondrial enzyme involved in the *de novo* pyrimidine synthesis leading to the formation of uridine monophosphate (UMP). Afterwards, UMP is converted to the other pyrimidine nucleotides needed for the biosynthesis of RNA and DNA. In resting cells, the demand for pyrimidines is sufficiently covered by the *salvage* pathway, whereas fast proliferating cells, like cancer cells or also virus replicating cells, additionally depend on the *de novo* pyrimidine synthesis. The fact that a high supply of pyrimidine nucleotides is required for a fast viral replication renders the enzyme DHODH as a promising target for the development of new antiviral agents. Inhibition of DHODH leads to reduced levels of pyrimidine nucleotides that primarily affect the replication of the viral genome. DHODH inhibitors function as host factor targeted antiviral agents that provide several advantages compared to inhibitors targeting single viral enzymes, such as acting as potential broad-spectrum antivirals which provide a high barrier to resistance.[1,2]

We have developed different series of DHODH inhibitors based on anthranilic acids that showed broad-spectrum antiviral activities against several RNA viruses, including bunyaviruses, flaviviruses and filoviruses.[3] Herein, we describe structural optimizations using an identified lead structure of the phenylethynyl anthranilic acid series, especially with regard to the enzymatic stability and aqueous solubility. The objective of this study is to improve the drug-like properties of the selected compounds without the loss of antiviral efficacy.

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# MACROCYCLIC-BASED COMPOUNDS AS A TREATMENT FOR VISCERAL LEISHMANIASIS

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Visceral Leishmaniasis (VL) is the most severe type of Leishmaniasis[1] and can lead to severe symptoms, e.g. irregular fever, spleen-liver enlargement, and anemia.[2] For VL, >90% of untreated cases are fatal.[2] Leishmaniasis manifests in areas such as Asia, Africa, and South America,[2] but only five treatments are currently available, with varying approval statuses in different regions.

The treatments are associated with severe side effects, high costs, and cold chain dependence. No new drug has been introduced after miltefosine approval in 2002. Even though new classes of derivatives are undergoing clinical development, the risk for attrition is high.[3]


| Compound | $IC_{50}$ (μM) | Solubility |
|---|---|---|
| 1 | 1.32 - 2.01 | MS |
| 2 | 43.07 - 64.00 | S |
| 3 | 2.00 - 3.17 | MS |
| 4 | 7.25 - 20.24 | S |
| Miltefosine | 5.15 - 5.28 | S |

S= soluble; MS = moderately soluble (13% DMSO/water)

Ongoing research at Uppsala University is focused on the design and synthesis of natural product-derived macrocyclic compounds with novel bioactivities.[4,5] Compound **1** (Fig. 1A) was found to be the most potent inhibitor of *L. infantum* and *L. donovani* ($IC_{50}$ values 1.3-2.0 μM). Therefore it was chosen as a hit compound for further optimization to improve potency and reduce lipophilicity to increase solubility and reduce metabolic oxidation. The optimization first involved variations of the structure of the macrocyclic ring (**P1**).

The *para*-linkage of one of the phenyl rings within the macrocyclic ring has been modified into a *meta*-linkage ( **2**) to understand the influence of reduced ring size and altered conformation on the potency and PK characteristics of the compound. In parallel, the effect of ring enlargement has been investigated by incorporation of homo-Tyr instead of Tyr (**3**). The role of the Phe residue (**P2**) is being studied by substitution of the phenyl group with a 2-pyridinyl one (**4**).

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# EASY-TO-PERFORM LARGE SCALE APPROACH TO 1-METHYLADAMANTANE AND ITS DERIVATIVES – SCARCE THOUGH PROMINENT MEDCHEM BUILDING-BLOCKS

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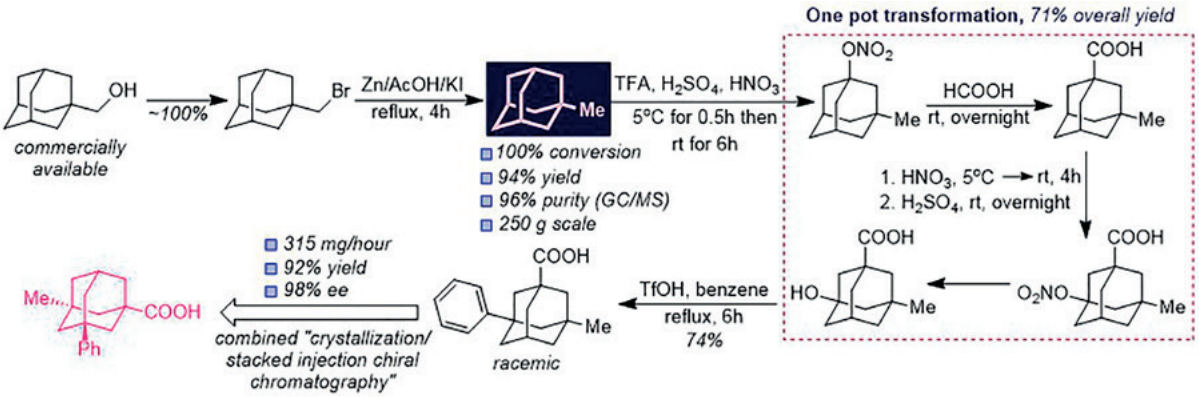
So far, application of a simple 1-mehyladamantane hydrocarbon framework as well as its derivatives in research projects has been restricted because of imperfection of existing preparative methods towards them. These methods feature unsafe conditions, lead to inseparable mixtures, or use expensive starting materials that prevent preparation of the discussed derivatives in a large scale. Simultaneously, low synthetic availability of 1-methyladamantanes leads to their poor representation in medicinal chemistry. The last fact is a really annoying obstacle when consider a recent discovery of an efficient *anti*-Ebola virus agent with a backbone of 1-methyladamantane having nanomolar $EC_{50}$ value, remarkable water solubility and excellent stability in the organism against metabolic enzymes. What is more, 1-methyladamantane is a perfect platform for synthesizing chiral molecules with cage-derived chirality, which might be very beneficial from the biological assessment viewpoint. With a purpose to fill the mentioned gap, the current project was designed to change the status quo for 1-methyladamanthanes and make them easily available for the needs of research community.[1]

For the synthesis of the target 1-methyladamantane, we used a classical approach based on reduction of 1-bromomethyladamantane, which was obtained from relatively cheap and commercially available 1-adamantylmethanol. Methods for the transformation know from the literature turned out to be unsuitable due to low conversion, prolonged reaction times and by-products formation. We found that reduction of the bromide with 4 equiv of Zinc powder in acetic acid in the presence of 20 mol % of KI provides 1-Me-adamantane with 92% yield and 96% purity on a scale of 250 g. The latter was one-pot functionalized with carboxyl function and phenyl group *via* successive "nitroxylation (with $HNO_3$)→carboxylation (with $HCO_2H$)→another nitroxylation" transformations resulting in racemic 3-hydroxy-5-methyladamantane-1-carboxylic acid. The (1S,3R,5R,7S)-isomer of the acid is a direct precursor to above-mentioned anti-Ebola virus agent. Hence, we worked out innovative approach consisting of sequential (1) crystallization and (2) stacked injections chiral chromatography steps enabling multigram isolation of the pure isomer.




In summary, described here achievements pull out 1-Me-adamantane and its derivatives from the shadow and open the door to their wide application in different branches of industry.

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# NOVEL AZASPIRO COMPOUNDS FOR THE TREATMENT OF HUMAN AFRICAN TRYPANOSOMIASIS

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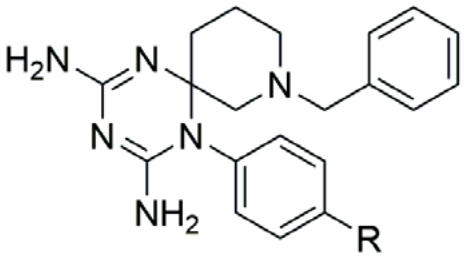
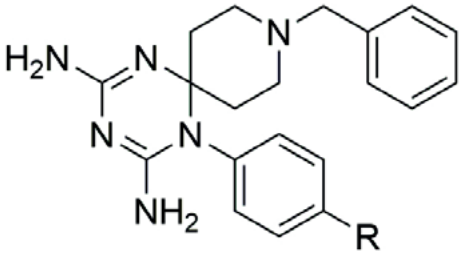
Trypanosomiasis is a threatening neglected tropical disease (NTDs), which is endemic in several countries like South and Central America and Africa. Several progresses have been achieved with the introducing of fexinidazole and the association between eflornithine-nifourtimox. However, their use is hindered by their side effects and their high toxicity. Moreover, the drug resistance issues, and the existence of animal reservoirs make the development of new safer and more efficient drugs a compelling need.

A valid strategy to treat the Human African Trypanosomiasis (HAT), is represented by the inhibition of the two major enzymes involved in the folate pathways. Trypanosomatids are auxotrophic for folates and pterins that are crucial cofactors for the biosynthesis of nucleic acids and proteins, so it`s reasonable to think that the inhibition of their folate-dependent enzymes, namely dihydrofolate reductase (DHFR-Ts) and pteridine reductase 1 (PTR-1) of *Trypanosoma brucei* (*Tb*), may represent a seccessfull strategy for the treatment of HAT. Cycloguanil (CYC) is a well known DHFR inhibitor, which also showed to act as PTR1 inhibitor[1]. The binding mode analysis of CYC to *Tb*DHFR-Ts and *Tb*PTR1 active site, lead to us to design and synthesize two novel series of compounds that maintain the amino 1,6-dihydrotriazine moiety of CYC[2].
Azaspiro-2,4-diamino-1,6-dihydrotriazine (A) and (B), we replace the CYC C6 moiety with a bulky group in order to increase the lipophilicity. The compounds have undergone evaluation of their on-target activity (TbPTR1 and TbDHFR-Ts), human DHFR inhibition to ascertain their selectivity for the protozoan enzymes, cytotoxicity and antiparasitic effect. These compounds are also under computational studies at the Heidelberg Institute for Theoretical Studies where we are docking them and through molecular dynamics studies we expected to better understand how these compounds are able to dampen the activity of the folate enzymes and hopefully will lead us through a rational design of new safer and more effective antiparasitic compounds.




**A**




**B**

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# LEISHMANICIDAL EFFECT OF SELENOCYANATE COMPOUNDS

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Leishmaniasis is a neglected tropical disease (NTD) that is caused by a protozoan parasite from over 20 *Leishmania* species. It is transmitted through the bites of infected female phlebotomine sandflies. According to the symptoms, Leishmaniasis can be visceral, cutaneous or mucocutaneous [1,2].

In this work, 17 selenocyanate derivatives were synthesized and screened against promastigotes of three species of *Leishmania* (*L. major*, *L. amazonensis* and *L. infantum*). Half-maximal effective concentration ($EC_{50}$) and toxicity on murine macrophages were studied. In the preliminary screening against *L. major* promastigotes 6 out of 17 compounds tested showed $EC_{50}$ values lower than 15µM.

In general, promastigotes from *L. infantum* were more sensitive to selenoderivatives than the other species. Compounds **1**, **3** and **16** showed the best toxicity results and good selectivity values, especially Compound **16** which showed a high selectivity value for *L. infantum* (SI >32.57).

These results inspired us to analyze leishmanicidal activity of these compounds against the intracellular amastigotes. The three selected compounds (**1, 3** and **16**) decreased the percentage of infected macrophages after 72h treatment. Compound **16** was the most effective reducing by 90% the infection ratio in *L. infantum*-infected macrophages at the lowest tested concentration (12.5 µM).

Further studies were performed with Compound **16**. This selenoderivative did not produce haemolytic effect on human red blood cells at the studied doses (10-100 µM). Furthermore, gene expression of infected murine macrophages was studied after treatment with Compound **16**. Genes related to cell death, cell cycle and selenoprotein synthesis pathway in amastigotes were altered, while no changes were observed in their murine homologues supporting the specificity of Compound **16** against the parasite.

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# SINGLE STEP SYNTHESIS OF ß- AND γ-AMINO ACID DERIVATIVES BY ELECTROCHEMISTRY

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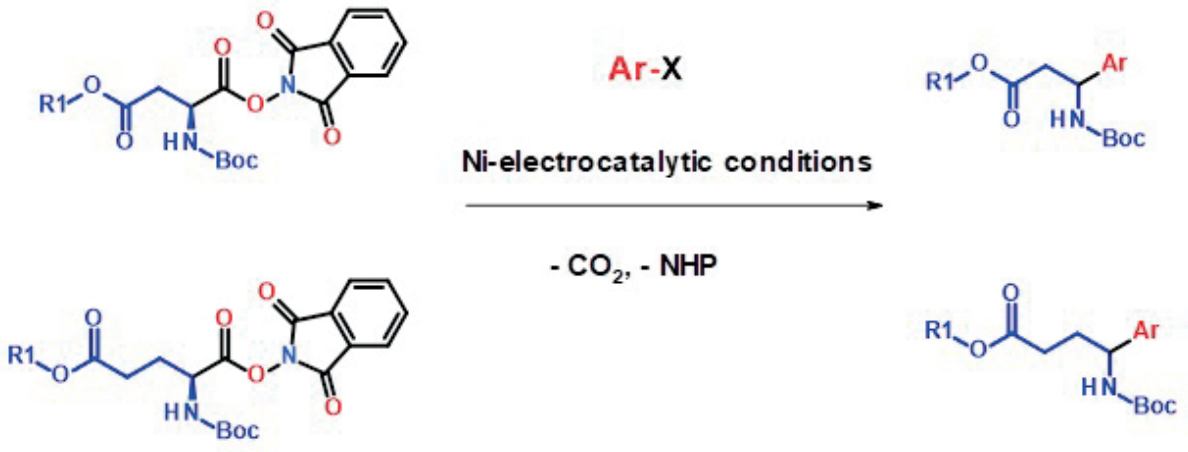
Electrochemistry has received a lot of attention over the recent years as an enabling tool for rapid construction of complex scaffolds and intermediates of pharmaceutical interest by a single step transformation. In addition, electrochemical approaches can offer clear advantages in terms of sustainability and mild reaction conditions.[i] This development was significantly facilitated by the publication of several recent reviews providing a more systematic understanding of electrochemical reaction conditions.[ii] [iii] [iv]

Among the variety of cross coupling reactions, $Csp^2$ – $Csp^3$ couplings are of special interest in medicinal chemistry because they allow the "Escape from Flatland" when searching for drug candidates with better physicochemical properties.[v] In this context, the combination of electrochemistry with nickel catalysis resulted in highly interesting decarboxylative cross-coupling (DCC) protocols of redox-active esters (RAE) and halo(hetero)arenes. [vi] [vii] [viii]

We recognized the potential of these published methodologies to deliver a novel approach to β- and γ- amino acids by starting from the corresponding RAEs of N-Boc-protected aspartic and glutamic acid esters as shown below.




In summary, we have succeeded in providing a systematic set of novel ß- and γ-amino acids by a single step Ni-electrocatalytic $Csp^2$ – $Csp^3$ coupling reaction. We hope that these results will contribute to the popularization of synthetic electrochemistry and motivate our community to utilize electrochemical methodologies more frequently.

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# NEW DIAZAQUINAZOLINE UREA SUBSTITUTED ANALOGUES DESIGNED AND SYNTHESIZED AS POTENT ANTICANCER INHIBITORS

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One of the biggest causes of death in developed nations worldwide during the past few decades has been cancer. Although there have been several attempts to generate effective anticancer drugs, the majority of the successful cytotoxic techniques that have been established result in molecules with no selectivity. Due to its wide range of pharmacological effects, quinazoline, an heterocyclic scaffold from the benzodiazines family, is one of the most used cores in medicinal chemistry. The compounds of synthetic quinazoline have antiviral, anticancer, antibacterial, antifungal, anti-inflammatory, antihypertensive, anti-malarial, and anticonvulsant properties.

The design and synthesis of diazaquinazoline derivatives substituted on C 4 and C 7 are presented here as a result of this. Drugs that are readily available on the market, including sorafenib and erlotinib, were employed as model molecules. Using a seven step process that began with thiourea and 2-chloro-4-nitroaniline, the required analogues were produced in good yields.

The anticancer activity of the new compounds was tested against a panel of 110 proteins (106 kinase and 4 bromodomains) and cancer cell lines. Three of them turned out to be extremely potent, and the majority of them are active against all of the examined cell lines. Four of the novel inhibitors showed highly intriguing findings for the BRAF kinase in the thermal shift assay ($\Delta$Tm = 10.0, 12.0, 12.5 and 22.0 °C). The results for the most cytotoxic compounds are intriguing since they suggest the existence of another target. In order to find the new target, a novel strategy based on proteomic analysis is currently being implemented.

# THERMAL [2+2] SYNTHESIS OF 3-OXOCYCLOBUTYL BORONATES VIA KETENIMINIUM SALTS

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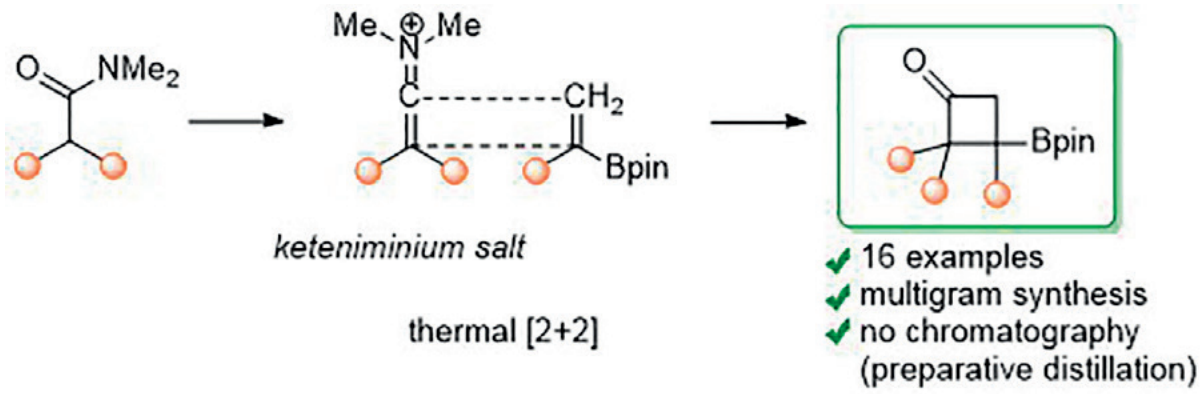
Owning to their small size and defined geometric organization, substituted cyclobutanes have become structures of high demand in contemporary medicinal chemistry. At least ten molecules containing cyclobutane ring have been approved as drugs, and more shall follow.[1] In this work, we developed synthesis of cyclobutene-based substances containing pinacol boronates.[2] The synthesis proceeded *via* formation of keteniminium salts, followed by thermal [2+2] cycloaddition to vinyl- or allyl-pinacol boronates. By using this protocol, we prepared 16 compounds in moderate yield on multigram scale. The purification of target substances was typically performed by distillation, thus bypassing cost- and time-consuming chromatography. Obtained 3-oxocyclobutyl pinacol boronates are compounds of high synthetic value for coupling reactions, and they provide an easy access to other cyclobutane-based structures as demonstrated by few examples.

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# SYNTHESIS OF VERSATILE STELLANE DERIVATIVES IN MULTIGRAM SCALE

**O. K. Smyrnov (1,2), A. E. Pashenko (1,2,3), K. P. Melnykov (1), D. M. Volochnyuk (1,2,3), S. V. Ryabukhin (1,2,3)**

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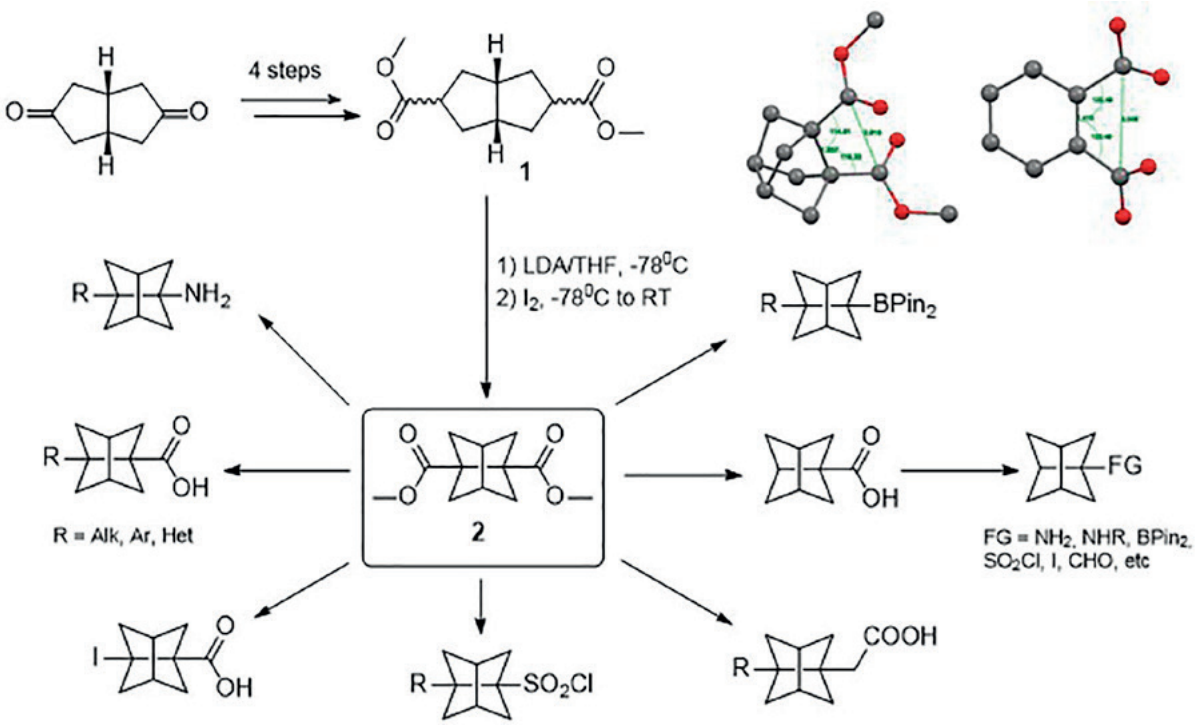
Stellanes have been fascinating scientists with their beauty since 1960s[1]. Nevertheless, their applications for drug discovery were limited by lack of easy, cheap and scalable synthetic approaches. Due to close values of bond lengths and angles in 1,2-disubstituted stellane system, it can be considered as bioisosteric to 1,2-disubstituted benzene ring. Although compounds containing flat aromatic rings are still exteremely popular in medicinal chemistry[2], "escape from Flatland"[3] concept gets more and more applications. Therefore, demand for 3D-shaped benzene bioisosters is constantly rising.

Hereby, we propose a procedure for synthesis of 1,2-stellane dicarbonic acid dimethyl ester **2** in 100 g scale in a single run. An intermediate **1** was obtained as a diastereomeric mixture: *exo,endo*- and *exo,exo*-isomers smoothly afforded the desired product **2**, while *endo,endo*-isomer gave an undesired Claisen cyclization product. The mechanism of transformation from **1** to **2** was modelled *in silico*. More than 30 diverse building blocks were obtained in multigram amounts. All of them were designed to bear a functional group (i.e. $CO_2H$, CHO, $NH_2$, $SO_2Cl$, $BPin_2$) that enables further chemical modification.

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# BIOLOGICAL EVALUTION OF 3-AMINOQUINUCLIDINE QACs WITH LONG ALKYL CHAINS AS NEW POTENT ANTIMICROBIAL AGENTS

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The increasing resistance of bacteria to existing antimicrobial agents is becoming a global health problem, as the spread of various infectious diseases cannot be prevented or controlled. Naturally occurring alkaloids form the structural backbone of many currently available drugs and continue to inspire scientists in drug discovery [1]. One of these is the quinuclidine molecule, more specifically 1-azabicyclo [2.2.2]octane. Its quaternization leads to quaternary ammonium compounds with different biological properties.

In a previous publication, we reported the synthesis and biological activity of 3-amidoquinuclidine as new potent biodegradable QACs [2]. The degradation products yielding 3-aminoquinuclidine QACs with alkyl chains of different lengths (12, 14, and 16 C atoms) were synthesized to further explore the structure-activity relationship. The compounds were tested against a range of Gram-positive and Gram-negative bacteria. QACs with 14 and 16 C atoms showed antimicrobial activity in a concentration range from 12.5 to 100 μM. The candidates were found to be less susceptible to the induction of bacterial resistance than commercially available QACs and were able to suppress bacterial growth by killing the bacteria in a time- and concentration-dependent manner. Similarly, membrane permeability, and cytotoxicity of the compounds were also determined showing that candidates are less toxic toward human cells for which reason they might be considered as new potential antimicrobial agents.

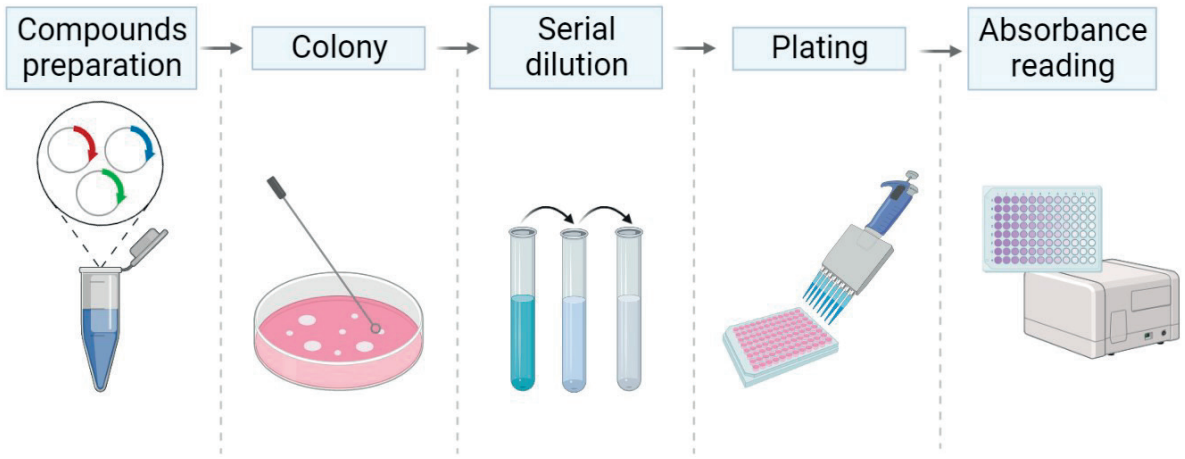


Figure 1: *Step-by-step schematic representation of the microdilution method.*

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# PREPARATION OF NEW [2.1.1]-BICYCLOHEXANES AND THEIR DERIVATIZATIONS TO ACCESS UNCHARTED CHEMICAL SPACE

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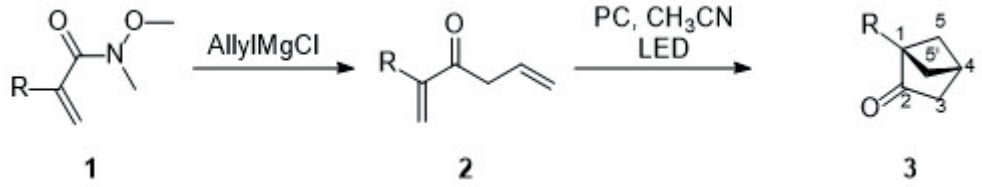
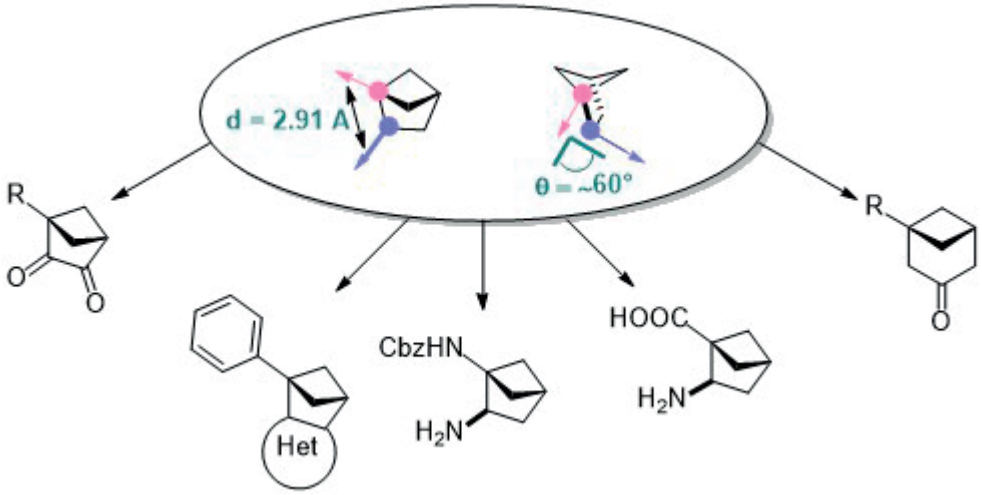
Saturated bicycles are becoming ever more important in the design and development of new pharmaceuticals. In line with these aspirations, SpiroChem continues to expand the collection of isosteres available by exploring new chemical space while following the mantra *escape from flatland.*[1] Surprisingly, the synthesis of 1,2 substituted [2.1.1]-bicyclohexanes is barely described and the incorporation of exit vectors remains scarce. The most common method for the synthesis of [2.1.1]-bicyclohexanes is via a crossed [2+2]-cycloaddition of a 1,5- diene.[2] We developed an innovative and scalable route towards scaffolds such as **3** using Blue or UV light with the help of a photocatalyst (PC).[3]




With **3** hand, the ketone at the position 2 was derivatized to obtain new functional groups, such as amine, aldehyde, carboxylic acid or hydrazone. The R group was also diversified to incorporate exit vectors as carboxylic acid, alcohols or substituted aromatic rings. Furthermore, we used this platform to access ring-expanded products and 1,2,3 substituted [2.1.1] systems.




The new synthesized building blocks open new chemical space and offer a new dihedral angle interesting for medicinal chemists.

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# A WAY TO BROAD SPECTRUM ANTIVIRAL AGENTS: SMALL MOLECULES BASED ON PIPERAZINE CORE AS POTENTIAL FLAVIVIRIDAE NS3 PROTEASE INHIBITORS

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*Flaviviridae* virus family is subdivided into four genera namely, *Flavivirus, Hepacivirus, Pegivirus* and *Pestivirus* [1,2]. The incidence of Flavivirus infection has grown significantly, with approximately 400 million people infected annually [3] and widely distributed as consequence of globalization and climate changes, causing public health problems worldwide [4,5]. Currently, there are no effective drugs available to treat most of these infections. All genera of the *Flaviviridae* family show similarities in the organization of the viral genome, which is characterized by a single-stranded positive-sense RNA molecule. The whole viral genome is translated into a viral polyprotein, which is further processed by both host and viral proteases into 9 to 12 mature proteins, consisting of structural and nonstructural (NS) proteins [1,6]. The NS proteins participate in the replication of the RNA genome, virion assembly and interaction with innate host immune response [7]. As a result, these viral proteins represent relevant targets for the development of novel antiviral therapies. Among these, the NS32B proteases play an important role in the virus life cycle, making them attractive targets for antiviral drug discovery [8].

Taking advantage of our previous research focused on the development of effective antiviral agents based on piperazine backbone [9], we have recently described two piperazine-derived compounds as promising and non-cytotoxic broad spectrum anti Flavivirus agents [10]. The compounds were designed using a privileged structure-based approach, and their antiviral properties were determined by a live virus cell based phenotypic assay against ZIKV and DENV [11], leading to the identification of these promising lead compounds.

Based on these important results, this study focuses on the design and synthesis of a small library of molecules acting as potential NS3 protease inhibitors. Our approach involves an optimization process aimed at preserving the molecular complexity of these compounds, which includes three aromatic/aliphatic rings linked by amide and urea/sulfonamide functions. Due to the relevance of the piperazine ring in biological activity of these compounds [12], we decided to retain it as the central core.

The focus of this research is to improve the antiviral activity against both viruses and simultaneously obtaining non-cytotoxicity products associated with these lead compounds.

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# SENSITIVE QUANTIFICATION OF THE PROTEIN TARGETING CHIMERA (PROTAC) TL 13-112 IN RAT PLASMA USING AN LC-MS/MS WORKFLOW

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## Introduction

Proteolysis targeting chimeras (PROTACs) are endogenous protein degradation tools, capable of removing specific protein targets using a cell's own disposal machinery. PROTACs have evolved as a therapeutic modality, as several candidates have now moved into clinical trials. Sensitive and selective assays for high-confidence detection and quantification of PROTACs are needed to ensure safety and efficacy in the drug development pipeline and because PROTACs have expressed high potency in nanomolar drug concentrations. In this study, low-pg/mL quantification for the PROTAC, TL 13-112, and its inactive control, TL 13-110, was achieved at a lower limit of quantification (LLOQ) of 10 pg/mL using a highflow LC-MS/MS platform.

## Methods

PROTACs were spiked into rat plasma at concentrations ranging from 10 pg/mL to 15000 pg/mL. Following protein precipitation, samples were vortexed and centrifuged at room temperature. The supernatant was transferred to a new Eppendorf tube and dried under nitrogen flow. Dried extracts were reconstituted prior to the analysis.

PROTACs were separated using a Phenomenex Kinetex XB-C18 column (2.1 x 50 mm, 1.7 µm, 100 Å). The LC system was operated at a flow rate of 0.3 mL/min. Analysis was performed on a SCIEX 7500 system in positive mode. Collision energy, source and MS parameters were optimized to achieve sensitive MS/MS quantification.

## Preliminary data

Calibration curves were constructed across concentrations ranging from 10 pg/mL to 15000 pg/mL. Individual concentrations were run in triplicate.

An LLOQ of 10 pg/mL was achieved for TL 13-112 and TL 13-110. No interferences were observed in the matrix blank (rat plasma) for either analyte. Strong linearity was achieved for both analytes with a linear dynamic range (LDR) of 3.2 orders of magnitude.
Analytical performance was evaluated based on the requirement that the accuracy of the calculated mean should be between 80% and 120% at the LLOQ and between 85% and 115% for the higher concentrations. The %CV of the calculated mean of the
concentration should be below 20% at the LLOQ and below 15% for all higher concentrations Accuracy was within ±11% and ±12% of the nominal concentration for TL 13-112 and TL 13-110, respectively. The %CV was Calculated values for accuracy and %CV were within the acceptance criteria at each concentration level.

Overall, a highly sensitive method for the quantification of PROTACs in rat plasma was developed with excellent accuracy and precision at low-pg/mL levels.

## Novel aspect

Development of a highly sensitive method for low-pg/mL quantification of PROTACs in a complex matrix using highflow LC-MS/MS

# IDENTIFICATION OF A DUAL REV-ERBβ AND AUTOPHAGY INHIBITOR WITH IN VIVO ANTICANCER EFFICACY

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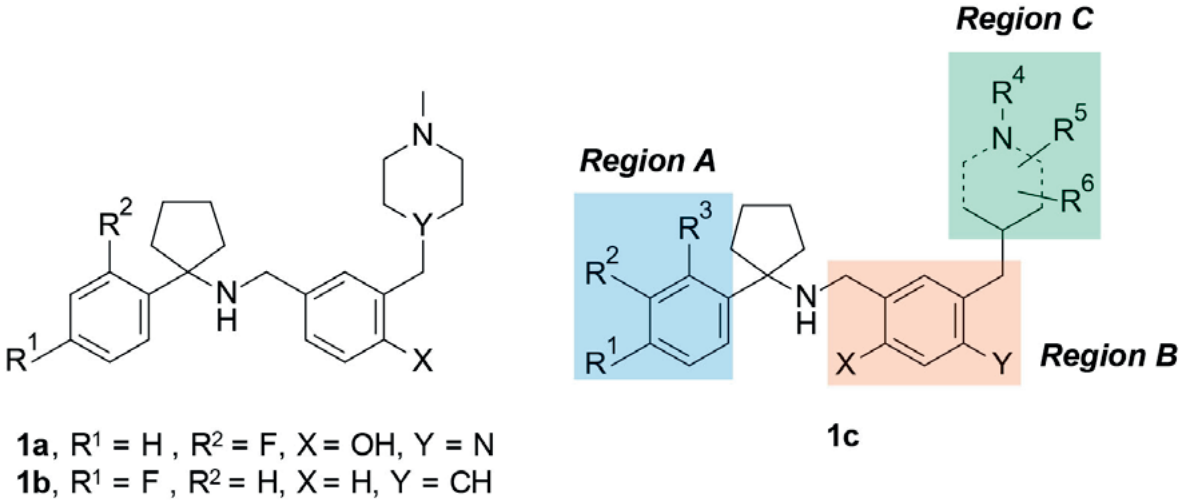
We previously demonstrated that a dual inhibition of circadian nuclear receptor REV-ERBβ and autophagy represents a better anticancer approach than the single inhibition of autophagy.[1,2] The first class of dual REV-ERBβ and autophagy antagonists is exemplified by our proprietary compounds **1a** and **1b** (**Figure 1**).[3, 4] Despite the enhanced potency and improved 'drug-likeness' of **1b** compared to the initial hit **1a**, its moderate metabolic stability limits its use in *in vivo* studies. Herein, we present our lead optimization strategies which involved the chemical exploration of the three main regions of **1c** (*regions A*, *B* and *C*, **Figure 1**). These studies led to the identification of an optimized compound with an improved biological profile, optimal 'drug-like' properties and efficacy in a mouse xenograft model of melanoma as a single anticancer agent.




**1a**, $R^1$ = H , $R^2$ = F, X = OH, Y = N
**1b**, $R^1$ = F , $R^2$ = H, X = H, Y = CH

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# INHIBITING FERROPTOSIS: DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION OF NOVEL RADICAL TRAPPING ANTIOXIDANTS

**Camilla Scarpellini (1), Greta Klejborowska (1), Caroline Lanthier (1), Niccolò Chiaramonte (1), Pieter Van Der Veken (1), Behrouz Hassannia (2,3,4), Tom Vanden Berghe (2,3,4), Koen Augustyns (1)**

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Ferroptosis is a mechanism of regulated cell death mainly characterised by peroxidation of polyunsaturated fatty acyl phospholipids (PUFA-PLs), the availability of redox-active iron and the loss of lipid peroxide repair capacity by the phospholipid hydroperoxidase GPX4.[1] Ferroptosis plays an important regulatory role in the occurrence and development of many diseases, including ischemia-reperfusion injury, kidney injury, cardiac diseases, transplantation and neurodegenerative diseases.[2] Thus, ferroptosis inhibitors may have therapeutic potential in the treatment of these disorders. Different strategies aiming to halt ferroptosis have been identified, including the use of iron chelators, radical trapping antioxidants (RTAs), lipoxygenase inhibitors, deuterated lipids and ACSL4 inhibitors.[3]

In the Medicinal Chemistry group at the University of Antwerp we focused on the development of lipophilic radical trapping antioxidants.[4,5] Over the last years, several generations of ferrostatins have been synthesised, among which molecule UAMC-3203 showed high potency in animal disease models, increased metabolic stability and solubility as well as no toxicity in mice after daily administration over four weeks.[6] Herein, we present the design, synthesis and biological evaluation of a novel series of heterocyclic RTAs which are supposed to show greater BBB permeability.

The molecules have been fully characterised and tested *in vitro* for their pharmacokinetic properties. Moreover, the activity of the novel compounds as RTAs was verified by the FENIX assay (fluorescence-enabled inhibited autoxidation), a recent spectrometric assay developed by prof. Pratt's group and implemented in our laboratory. The assay enables high throughput screening of lipid peroxidation and provides insight into the mechanism of action of our molecules through the quantification of the antioxidants' reactivity with the phospholipid peroxyl radicals.[7]

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# NOVEL AIE-BASED PHOTOSENSITIZERS FOR BIOIMAGING AND PHOTODYNAMIC THERAPY

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Aggregation-induced emission (AIE) or aggregation-induced emission enhancement (AIEE) properties have received considerable attention due to their potential applications in bioimaging and photodynamic therapy (PDT). Herein, we report a series of phthalimide-based AIEgens that can induce significant fluorescence emission in the aggregated and solid states, along with the selective generation of ROS under light irradiation. Different synthetic strategies were employed to construct derivatives to improve AIE characteristics and photo-induced cytotoxicity. We also assessed the capability of the synthesized compounds for live cell imaging to examine the potential of the derivatives as theranostics. Confocal laser scanning microscopy images of cancer cells revealed that the compounds are efficiently localized in lipid droplets, mitochondria, and lysosomes, depending on the proper directing groups. In addition, the AIE-induced stable fluorescence enabled us to monitor the interactions of lipid droplets with other organelles. Our results demonstrate a rational design of novel photosensitizers that could act as potential theranostics for bioimaging and PDT applications.

# NOVEL AZABICYCLIC HETEROCYCLE SERIES AS POTENT ALLOSTERIC SHP2 INHIBITORS

**Alessio Sferrazza, Alina Ciammaichella, Ilaria Rossetti, Danilo Fabbrini, Esther Torrente, Nicola Relitti, Federica Ferrigno, Paola Fezzardi, Costanza Iaccarino, Monica Bisbocci, Antonella Cellucci, Cristina Alli, Martina Nibbio, Vincenzo Pucci, Jerome Amaudrut, Christian Montalbetti, Carlo Toniatti, Alessia Petrocchi**

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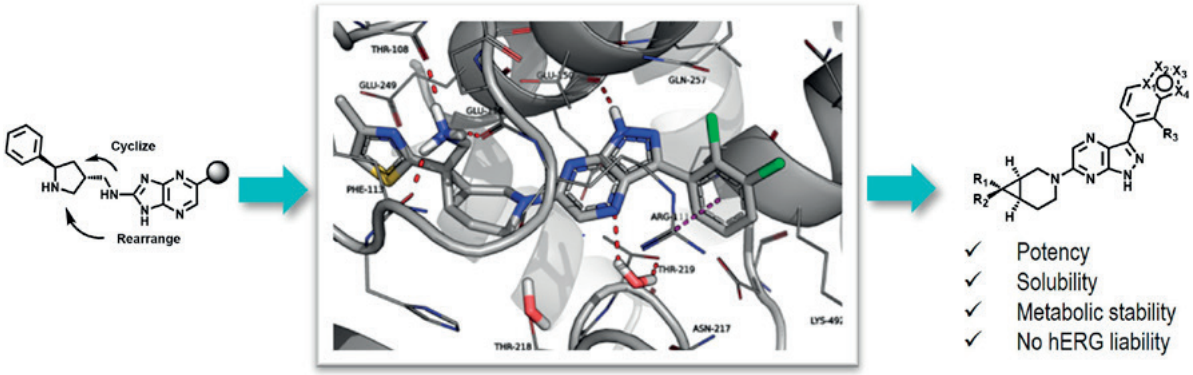
The non-receptor protein tyrosine phosphatase SHP2 (encoded by PTPN11) is a critical component of RAS/MAPK signaling by acting upstream of RAS to promote oncogenic signaling and tumor growth.[1] As part of a drug discovery program[2] aimed at obtaining novel allosteric SHP2 inhibitors, a series of original bicyclic heterocycles was identified.

Extensive preliminary SAR around the novel bicyclic basic moiety (left-hand side) and the heteroaryl portion (right-hand side), followed by a scaffold hopping approach, afforded a highly potent series of SHP2 inhibitors with demonstrated cellular target engagement (pERK inhibition, as downstream marker of MAPK pathway activity) as well as antiproliferative activity against KYSE-520 cancer cell line.

X-Ray resolution of a co-crystal structure of our compounds in SHP2 allowed to identify the interactions of the bicyclic basic moiety forming H-bonds with the carbonyl backbones of three residues (Thr 108, Glu 110 and Phe 113)[3]. Further optimization of the physicochemical properties and the mitigation of *in vitro* off-target liabilities, culminated in the discovery of a unique series of SHP2 inhibitors with a high potential for future developability towards *in vivo* pharmacological studies.

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# FEBUXOSTAT ANALOGS AS AN ANTIBACTERIAL AGENT AGAINST STAPHYLOCOCCUS AUREUS

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The rise of multidrug-resistant bacteria, such as Staphylococcus (S.) aureus, has highlighted global urgency for novel classes of antibiotics.[1,2] Febuxostat (1) is a well-known anti-gout marketed drug, which has been reported as anti-quorum sensing, anti-virulence and anti-biofilm agent against Gram-negative bacterium, Pseudomonas aeruginosa.3 Due to lack of structure-activity relationships (SARs) of febuxostat[3] and considering our recent experience with the development of antibacterial agents against Gram-positive bacteria, S. aureus,[4] we decided to synthesize diverse febuxostat analogs having general structure I (Figure 1). Among 45 synthesized analogs, five analogs having lipophilicity (cLogP) in between 6-7.5 have shown MIC in a range of 5 -20 µM against S. aureus that is almost similar as our recently reported antibacterial agent, etrasimod.4 Follow-up studies (like cytotoxicity, etc.) are currently ongoing. In conclusion, our research results support the utility of febuxostat in the development of antibacterial agents against S. aureus.

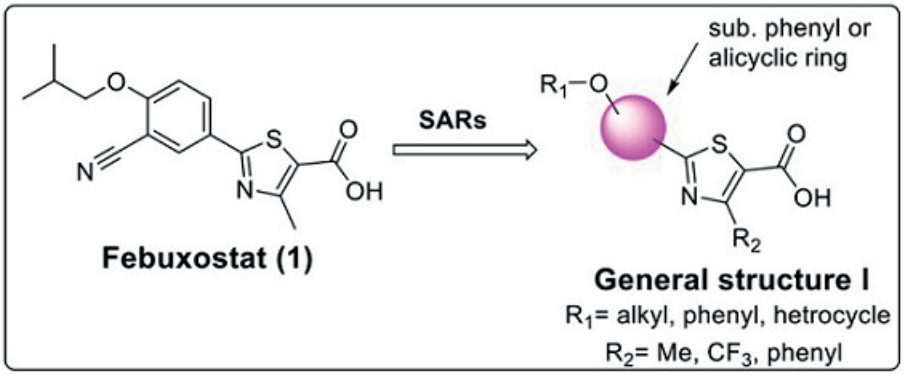


**Fig. 1.** Structure of febuxostat (**1**) and its analogs as General structure **I**

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# NEW HISTONE DEACETYLASE INHIBITORS FOR THE TREATMENT OF GLIOBLASTOMA MULTIFORME

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Glioblastoma multiforme (GBM) is an aggressive brain tumor with no cure, higher recurrence rates and with median survival of 15 months after diagnosis. GBM accounts for 45% of malignant brain [1]. GBM has an incidence of 3.21 for each 100.000 persons. In general, the incidence rate is higher in Caucasian, specifically in persons that lives in industrial areas [1]. The standard therapy includes surgical resection, radiotherapy, and chemotherapy with temozolamide (TMZ). Most of the patients relapse within six months after concomitant therapy [2]. Therefore, novel therapeutic options are necessary.

It has been observed that epigenetic modifications play a pivotal role in GBM development. In this sense, histone deacetylases (HDACs) are promissory therapeutic target due to their pleiotropic effects. HDACs remove acetyl groups from lysine on histone and non-histone substrates [3]. The use of HDAC inhibitors for GBM treatment have been explored, compounds such as vorinostat and valproic acid showed cytotoxic effects on GBM cells. Particularly, the inhibition of HDAC1, 2, 6 and 8 induced cell cycle arrest, apoptosis, and a detriment in proliferation in GBM [4-5].

Based on the above, we synthesized new hydroxamic acids as HDAC-6 inhibitors that showed in vitro antiproliferative activity in the U87-MG cell line. These hydroxamic acid derivatives were synthetized through and amide coupling, and Suzuki reaction or Heck reaction, on solid phase. Our synthetical approach afford compounds with purity higher than 95% (Figure 1).

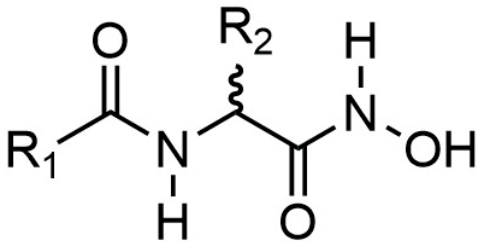


Figure 1. General structure of the hydroxamic acid derivatives synthesized.

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# STEROIDAL POSITIVE ALLOSTERIC MODULATORS OF N-METHYL-D-ASPARTATE RECEPTORS WITH IMPROVED METABOLIC STABILITY AND SOLUBILITY

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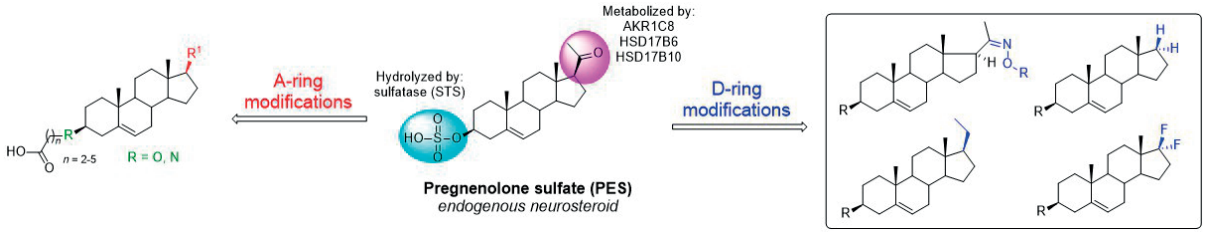
The *N*-Methyl-*D*-aspartate receptors (NMDARs) are a major class of excitatory neurotransmitter receptors in the central nervous system. They form glutamate-gated ion channels that are highly permeable to calcium and mediate activity-dependent synaptic plasticity. NMDAR dysfunction is implicated in multiple brain disorders, including stroke, various forms of neurodegeneration, chronic pain and schizophrenia. NMDARs are activated by agonists - glutamate and glycine, and the activity is modulated by allosteric modulators including endogenous neurosteroids.

Pregnenolone sulfate (PES, 20-oxo-pregn-5-ene-3β-yl sulfate) is an abundantly occurring neurosteroid synthesized *de novo* in the central nervous system that potentiates responses of NMDARs, but wider use is hindered its metabolic liability. Based on our SAR studies we designed structural modifications of analogues to improve metabolic stability and also improve their solubility and permeability profiles. We prepared a series of synthetic steroids with changed functional groups in steroidal A and D-rings. We will present structures of new compounds and their synthesis, ability to modulate NMDARs, evaluation of their chemical, pharmacological and biophysical properties.

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# NEW HYBRID MATERIALS COATED WITH PROTEINS AS POTENTIAL CARRIERS OF PHOTOSENSITIVE DRUGS

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Photodynamic Therapy (PDT) is a clinical method of treating cancer. It uses the action of light and a photosensitizer, which leads to the generation of reactive oxygen species that destroy cancer cells and tissues[1]. Even though this therapy has been widely known for a long time, it has some limitations. They are mainly related to the nature of photosensitizers - the hydrophobic core, causing its excessive aggregation in biological systems. The second problem is the excessive accumulation of the drug in healthy cells and the problem of removing it from the body. Magnetic nanoparticles may be a potential solution to this problem. Ease of modifying their structure (allows to increase their biocompatibility), assimilation by cancer cells (it is built of iron), easy ways of binding photosensitive drugs, and the phenomenon of hyperthermia generated by magnetic nanoparticles - can improve the operation of Photodynamic Therapy and increase its efficiency[2].

The presented research aimed to synthesize magnetic nanoparticles coated with modified chitosan functionalized with hemoglobin and a photosensitive drug. The obtained new material was characterized in terms of structure and surface morphology (ATR-FTIR, SEM, and XRD analyses). The thermal stability of the materials was investigated by thermogravimetric analysis. The size of the nanoparticles was measured using a dynamic light scattering (DLS) measurement. Quantum efficiencies of singlet oxygen generation by the obtained material were also determined, which is crucial in Photodynamic Therapy.

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# NOVEL CALCIUM-SENSING RECEPTOR ALLOSTERIC MODULATORS FOR THE TREATMENT OF ENDOCRINE AND RESPIRATORY DISORDERS

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The calcium-sensing receptor (CaSR) is a class C GPCR that is essential for life. The CaSR is widely expressed in humans and is particularly abundant in the parathyroid glands and kidneys. In these organs, the CaSR responds to elevated calcium ($Ca^{2+}$) by inhibiting parathyroid (PTH) secretion (consequently inhibiting calcium reabsorption from the kidneys, intestine, and bone) and promoting renal excretion of calcium and other salts. Given its central role in calcium homeostasis and PTH secretion and more recently asthma and pulmonary arterial hypertension pathology, the CaSR is an important drug target.

Currently, three CaSR positive allosteric modulators (PAMs) are available on the market for the treatment of *secondary hyperparathyroidism* (SHPT). However, their uses are limited as all three PAMs tend to cause hypocalcaemia. Most of the current negative allosteric modulators (NAMs) were developed with the intention of treating osteoporosis. Due to the weak cooperativity and failure to show clinical efficacy, no NAMs are approved for therapeutic use. Therefore, novel PAMs and NAMs need to be developed to improve the clinical outcomes of CaSR-related diseases.

Most of the current CaSR PAMs and NAMs are targeting the *seven-transmembrane* (7TM) domain (~250 residues), and only etelcalcetide (one of the approved PAMs) acts on the *extracellular domain* (ECD) of the CaSR (~600 residues). In addition, X-ray crystallography[1] and cryo-EM structures[2] confirmed that L-aromatic amino acids (L-aa) and TNCA (an L-tryptophan derivative) bind to CaSR ECD. I*n vitro* data support that L-aa potentiate CaSR activity in the presence of $Ca^{2+}$, thereby indicating the ECD is a feasible drug target to modulate CaSR function.

Herein, we report a fragment-based drug design study to explore novel CaSR allosteric modulators targeting the CaSR ECD, including a primary fragment library screening, hit validation, and SAR study by off-rate screening and parallel synthesis.

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# NEW ANTIMICROBIAL ADJUVANTS TO INTERACT WITH DIFFERENT RESISTANCE MECHANISMS

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Antimicrobial resistance (AMR) is currently one of the greatest global threats. Rising levels of AMR will hinder progress towards many of the Sustainable Development Goals (SDGs). Given the inter-relatedness of human and animal health, food production and safety and the environment, tackling AMR effectively will require concerted action across all sectors, applying the so-called One Health approach.

This threat is epitomized by the spread of antimicrobial-resistant ESKAPE pathogens. Among Gram-negative bacteria, the outer membrane is essential to regulate the influx and efflux of nutrients and toxic compounds. One strategy to address the influx involves bacterial iron transport pathways to deliver antibiotics by using siderophores, often called the “Trojan horse” approach [1]. Other is the concept of inhibiting efflux pump activity, not only because efflux underpins many other mechanisms of resistance but also because many efflux pumps are also required for virulence and biofilm formation [2]. Therefore, we hypothesized that several mechanisms of resistance could be addressed by linking antimicrobials and/or their adjuvants to siderophores.

In this work, we describe the conjugation of natural siderophores or their synthetic mimetics with known antimicrobial adjuvants to obtain new polyfunctional molecules with therapeutical and environmental applications. Following the synthesis of several siderophore mimetics, known antimicrobial adjuvants were coupled with different linkers, and the obtained compounds were subsequently coupled with the siderophore mimetics. Future work includes the evaluation of their antimicrobial potential against a panel of human and fish pathogenic microorganisms addressing different virulent mechanisms with the “One Health” approach.

**Acknowledgments**

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# NOVEL CLASSES OF SMALL-MOLECULE TOLL-LIKE RECEPTORS 7 AND 8 MODULATORS: DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION

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Toll-like receptors (TLRs) are pattern recognition receptors that play a key role in the innate immune system and also serve as a bridge between innate and adaptive immunity by promoting adaptive immune responses.[1] TLR7 and TLR8 are located on endosomal membranes, where they recognize viral single-stranded RNAs as well as synthetic small molecules such as imidazoquinolines (e.g., imiquimod and resiquimod).[2] Endosomal TLRs have been reported to be involved in various autoimmune, inflammatory, and malignant diseases,[2] highlighting the need for the development of novel synthetic small-molecule TLR7 and TLR8 modulators.

Various in silico-based approaches (e.g., ligand- and structure-based virtual screenings, molecular modeling) and subsequent experimental validation led to the identification of novel chemotypes of TLR7 and TLR8 modulators.[3,4] The development and optimization of straightforward synthetic approaches for chromeno[3,4-*d*]imidazol-4(1*H*)-one, 2-(trifluoromethyl)quinazoline-4-amine and 6-(trifluoromethyl)isoxazolo[5,4-*d*]pyrimidin-4(5*H*)-one scaffolds enabled new classes of potent selective TLR7 agonists with $EC_{50}$ values in the low micromolar range.[3] A rapid, efficient, and regioselective method for the preparation of C4-substituted pyrimidines from commercial 2,4-dichloropyrimidines and boronic acids was developed[5] and used for the synthesis of novel TLR8 modulators based on the 6-(trifluoromethyl)pyrimidin-2-amine scaffold, which selectively inhibited TLR8 at low micromolar concentrations and exhibited low cytotoxicity.[4] Further optimization using a novel method to study the structure-activity relationship through the analysis of 3D pharmacophores (i.e., dynophores) led to novel TLR8 modulators with improved inhibitory and physicochemical properties. To test the ability of our TLR7/8 modulators to modify cytokine production, which is imperative for an effective immune response, cytokine secretion was also measured in peripheral blood mononuclear and dendritic cells. The most potent compounds induced secretion of inflammatory cytokines, indicating that they have the potential to be developed into small molecule-based immunomodulators.

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# NEW INSIGHTS INTO QUATERNARY AMMONIUM COMPOUNDS: FROM STRUCTURE-ACTIVITY OPTIMIZATION TO ANTIBACTERIAL MODE OF ACTION

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Quaternary ammonium compounds (QACs) are among the most potent antimicrobial agents known to man. While global consumption of QACs peaked during the recent pandemic, scientists warn of a potential increase in drug-resistant bacteria. Indeed, literature data indicate the upsurge of drug-resistant bacteria, most of which simultaneously carry multiple resistance genes to various antimicrobial agents, including QACs. Therefore, our research focuses on elucidating bacterial resistance mechanisms and developing new effective QACs, which is extremely important in this field.

Guided by these considerations and inspired by the inexhaustible source of diverse chemical structures in nature, we endeavored to develop new naturally derived QACs based on different heterocyclic precursors (aromatic and non-aromatic). We found that the potent antibacterial activity strongly depends on the type of substituent for quaternization (aryl or alkyl) and on the presence of the polar functional groups, but less on the precursor itself. QACs with alkyl substituents with alkyl chains of 14 to 16 C-atoms resulted in the most effective derivatives, which exhibited MIC and MBIC values in the low μM range for selected bacteria. As suggested by atomic force microscopy and flow cytometry, the new compounds have a membranolytic mode of action and exhibit high selectivity toward bacterial over mammalian cells (Fig. 1). Physicochemical characterization showed that derivatives with longer alkyl chains spontaneously form micelles and that their stability also depends on the number of carbon atoms in the chain, although these values show no obvious correlation with bioactivity. In contrast, the presence of polar groups on the QACs resulted either in complete abolition of bioactivity or in derivatives with low activity despite long alkyl chains, most likely due to premature expression of the Qac efflux pumps responsible for bacterial resistance. To synthesize biodegradable and environmentally friendly QAC, we prepared new derivatives functionalized with labile amide groups that were found to be effective and susceptible to enzymatic degradation.

In conclusion, our results suggest that alkyl chains are an important part of the QACs structure, with antimicrobial activity directly correlating with the number of carbon atoms in the chain. Moreover, the introduction of amide functionalization leads to an environmentally friendly variant of QAC that could serve as a substrate for degradation by proteases.

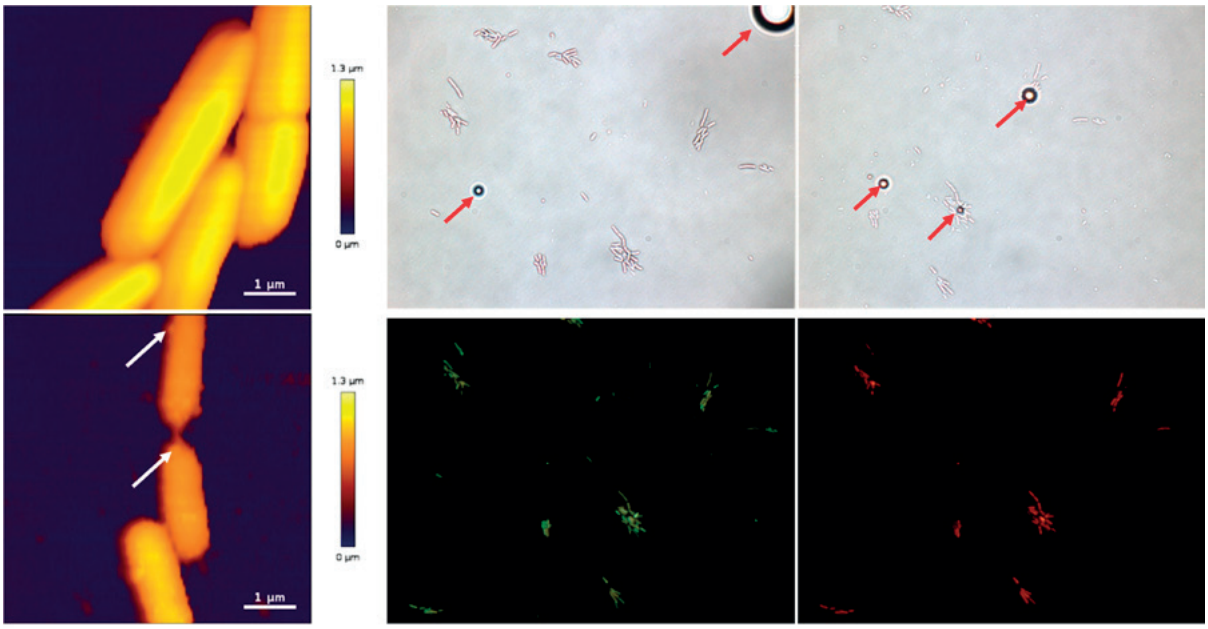


**Figure 1.** Atomic force and optical microscopy data of untreated and treated bacterial cells.

# CHEMICAL BLOCKAGE OF THE MITOCHONDRIAL RHOMBOID PROTEASE PARL BY NOVEL KETOAMIDE INHIBITORS REVEALS ITS ROLE IN PINK1 / PARKIN-DEPENDENT MITOPHAGY

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The mitochondrial rhomboid protease PARL regulates mitophagy by balancing intramembrane proteolysis of PINK1 and PGAM5. It has been implicated in the pathogenesis of Parkinson's disease, but its investigation as a possible therapeutic target is challenging in this context, because genetic deficiency of PARL results in compensatory mechanisms. To elucidate this problem, we undertook a hitherto unavailable chemical biology strategy. We developed potent PARL-targeting ketoamide inhibitors and investigated the effects of acute PARL suppression on the processing status of PINK1 intermediates and on Parkin activation. This approach revealed that PARL inhibition leads to a robust activation of the PINK1/Parkin pathway without major secondary effects on mitochondrial properties, which demonstrates that pharmacological blockage of PARL to boost PINK1/Parkin-dependent mitophagy is a feasible approach to examine novel therapeutic strategies for Parkinson's disease. We are currently developing approaches for efficient mapping of sequence preferences of rhomboid proteases, and we explore the chemical space of the tail substituent to elevate the potency and selectivity of ketoamide inhibitors of rhomboid proteases further.

# SYNTHETIC STUDIES TOWARD DAROBACTIN A

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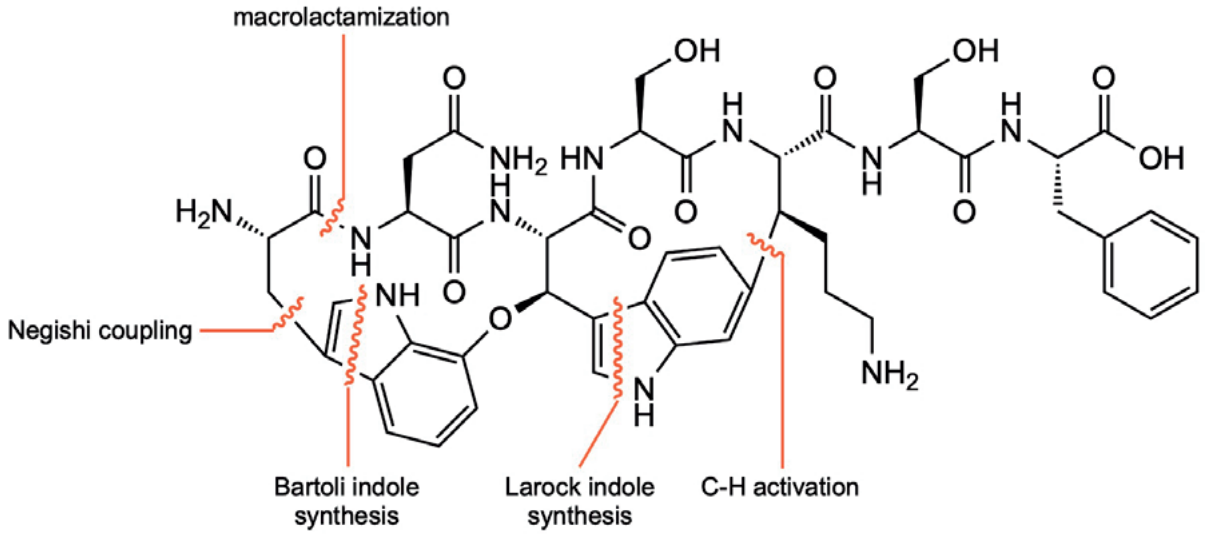
The discovery and development of new antibiotics targeting multidrug resistant bacteria is of utmost importance in the context of global health.[1] In 2019 the group of Kim Lewis isolated darobactin A from *Photorhabdus* symbionts, a compound which showed high potency against Gram-negative strains.[2] darobactin A targets the BamA complex located on the outer membrane of Gram-negative bacteria, an interaction which is dominated by hydrogen bonding between BamA and the peptide backbone of darobactin A.[3] This is enabled by a rigid β-strand conformation of the compound stemming from its unique bismacrocyclic structure featuring an ether bridge and an aromatic-aliphatic carbon-carbon bond which form post-translationally. Herein we describe our efforts toward the total synthesis of darobactin A starting from D-Garner's aldehyde and L-lysine. An initial strategy featured introduction of the crucial ether bond via $S_NAr$ followed by construction of the neighboring indole via Bartoli indole synthesis. The aforementioned carbon-carbon bond was installed by C–H activation. While the eastern macrocycle was successfully closed by an intramolecular Larock indole reaction the desired macrolactamization of the western ring remained futile. In the light of two published syntheses of the same molecule[4,5] – both featuring a second Larock cyclization – the strategy was revised. Combining our precursors and their insights we set out to complete the formal synthesis of darobactin A.




Darobactin A

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# HIERARCHICAL IN SILICO PIPELINE FOR IDENTIFYING ACTIVE COMPOUNDS AND/OR PROTEIN TARGETS

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Publicly available databases with well-curated information on chemical structures and bioactivities such as ChEMBL and PubChem allow researchers to access and analyze large amounts of data, which is beneficial for new drug development. Natural products characterized by great scaffold diversity and structural complexity, have been a source and inspiration for the development of many drugs currently in use. The study of natural products remains an important area of research in drug discovery.[1] In 2021, databases of collected publicly available information on natural products appeared in one place with Coconut Online and later with the Lotus Initiative.[2,3]

We have developed a hierarchical pipeline for high-throughput virtual screening to identify the biological activities of a specific group of compounds (untargeted screening) or to find hit molecules for specific target proteins (targeted screening). The pipeline is based on extensive physicochemical and ADMET characterization plus comprehensive screening involved 1,945 recommended activities including pharmacological effects, mechanisms of action, toxic and adverse effects, antitargets, metabolic effects, gene expression regulation, and transporter-related effects predicted by the PASS (Prediction of Activity Spectrum for Substances) program.[4] We established a workflow for filtering based on the width of the bioactivity spectrum generated by PASS, which we consider to be indicative of compound specificity and selectivity, that is further verified by molecular docking. The pipeline will be presented based on its application to natural molecules.

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# PLATINUM(II) COMPLEX WITH CYTOTOXICITY CONTROLLED WITH LIGHT

**Marta Stolarek (1,2), Mateusz Brela (1), Kamil Kamiński (1), Maria Nowakowska (1), Krzysztof Szczubiałka (1)**

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Cisplatin and other platinum-based drugs are the mainstay of anticancer therapy. It is estimated that every second oncological patient is treated with cisplatin or its analog [1]. The action mechanism of cisplatin is based on its interaction with genomic or mitochondrial DNA with the formation of a cyclic adduct [2] and consequent inhibition of the transcription and translation, ultimately leading to cell death. In addition, cisplatin induces oxidative stress through the formation of reactive oxygen species (ROS). Unfortunately, this drug also reacts with glutathione (GSH), the cytoskeleton, RNA, and proteins of normal cells. These interactions are responsible for the serious adverse effects of platinum-based drugs [3]. These sequelae could be avoided or at least limited if cisplatin cytotoxicity in cancer and normal cells could be more spatiotemporally selective.

Such possibilities are offered by photopharmacological approach which we applied to develop a new, photoswitchable analog of cisplatin (cis-$PtCl_2(PS1)_2$). Cytotoxicity of our platinum(II) complex, unlike that of cisplatin, can be controlled - increased or decreased - by irradiation with light which induces its reversible photoisomerization This was achieved by replacing two chlorine atoms in the cisplatin molecule with two molecules of a photoswitch (PS1), which can undergo reversible *trans-cis* photoisomerization on exposure to the visible light. The photoisomerization entails a significant change in the geometry of the molecule, as confirmed by our *in silico* studies, and, as a result, a change in its physicochemical properties and biological activity. The PS1 used here has favorable photochemical properties. The most important ones from the point of view of potential practical applications include: 1) the possibility to achieve the photoisomerizations in both directions ( *trans-cis* on irradiation with light at 400 nm and *cis-trans* on exposure to light at 530 nm), avoiding the necessity of using the harmful UV light, 2) both photostationary states composed of almost pure photoisomers, 3) high thermal stability of the cis isomer of PS under physiological conditions (half-life of that isomer is almost 9 days at physiological temperature), and 4) resistance of both isomers to photodegradation (at least up to 5 cycles).

Our in vitro studies on tumor cell lines (B16-F10 melanoma, A2780 ovarian cancer, and A549 lung cancer cells) have shown that cis-$PtCl_2(PS1)_2$ containing a photoswitch in the trans conformation (i.e., cis-$PtCl_2$(trans-$PS)_2$) exhibits significantly greater cytotoxicity than the molecule in which PS1 is in the cis conformation (i.e., cis-$PtCl_2$(cis-$PS)_2$).These results suggest that it is possible to increase or decrease the toxicity of the complex by irradiating it with a green (530 nm) or blue (400 nm) light, respectively. That may limit the adverse effects associated with the high toxicity of cisplatin to healthy/normal tissues. Moreover, the tests of the complex cytotoxicity in normal and cancer cell lines of the same organ (NMuMG murine mammary gland and 4T1 murine breast cancer, respectively) showed that trans photoisomer of the photocomplex is more toxic for tumor cells than for normal cells. It can also help to avoid damage the healthy cells during treatment. The complexes of this type can be particularly useful in the treatment of skin malignancies where direct irradiation is possible but it also can be considered for tumors developed in gastrointestinal tract, respiratory system, uterus, bile ducts, sinuses or bladder when the procedure can be carried out endoscopically.

**Key words:** cisplatin, arylazopyrazole, photoswitch, photopharmacology, anticancer

***Acknowledgments***

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# DESIGN AND SYNTHESIS OF 1′-MODIFIED CARBANUCLEOSIDE DERIVATIVES AS ANTIVIRAL AGENTS

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Modified nucleoside analogues are among the most promising class of small molecule-based antiviral and anticancer therapeutics that have been extensively applied in clinical setting. Among them, there is growing interest in 1'-modifications, such as remdesivir, which was introduced as the first treatment for the treatment of respiratory syndrome coronavirus 2 (SARS-COV-2). Here in we designed andsynthesized 1'-modified cabanucleoside with an efficient synthetic protocol that enables the construction of 1′-functionalized carbocyclic N-nucleosides in a stereoselective manner. Also, we carried out structure activity relationship study with the purines and pyrimidines. The screening of final compounds for their antiviral activity against a variety of +RNA viruses including SARS-CoV-2 is ongoing. Design, synthesis and antiviral data of 1′-modified nucleosides will be presented in detail.

# PHENOXYTACRINE DERIVATIVES AS DUAL CHOLINESTERASE INHIBITORS AND N-METHYL-D-ASPARTATE RECEPTOR ANTAGONISTS

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*N*-methyl-D-aspartate receptor (NMDARs) are a subclass of glutamate receptors, which play an essential role in excitatory neurotransmission, but their excessive overactivation by glutamate leads to excitotoxicity. NMDARs are hence a valid pharmacological target for the treatment of neurodegenerative disorders; however, novel drugs targeting NMDARs are often associated with specific psychotic side effects and abuse potential. Motivated by currently available treatment against neurodegenerative diseases involving the inhibitors of acetylcholinesterase (AChE) and NMDARs, administered also in combination, we developed a 30 dually-acting compounds based on 7-phenoxytacrine (7-PhO-THA) derivative. All compounds were tested for their anti-cholinesterase activities, cytotoxicity, ability to cross the blood-brain barrier as well as their impacts on NMDAR antagonism. In addition, we inspected selected compounds (**K1958, K1959**) and evaluated their neuropsychopharmacological properties.

Study was supported by the EEA and Norway Grants 2014–2021 and state budget of the Czech Republic within the TARIMAD project sponsored by TACR Kappa programme (Iceland, Liechtenstein, Norway, and Czech Republic; No. TO01000078), and the EU Joint Programme - Neurodegenerative Disease Research (JPND) project PETABC (MSMT No. 8F21002).

# DISCOVERY OF THE ACTIVATORS OF NRF1 PATHWAY AFFECTING PROTEASOME ACTIVITY AND PREVENTING PROTEING AGGREGATION

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Neurodegenerative disorders (NDs) are one of the most devastating diseases of our time for which there is no cure. A hallmark event in neurodegeneration is the misfolding, aggregation, and accumulation of abnormal proteins, causing proteotoxic stress that leads to pathological stages. This phenomenon occurs in parallel with the decline in proteasome activity. Due to the complicated structure of the 26S proteasome, its biogenesis must be strictly regulated at the levels of transcription, translation, and molecular assembly.

NRF1 (encoded by the *NFE2L1* gene) is a transcription factor that upregulates the expression of proteasome subunits in a concerted manner, especially during stress conditions. Under normal condition NRF1 is degraded by the proteasome. However, when cell proteostasis is disturbed NRF1 is cleaved by the DDI2 protease and as a processed transcription factor, it switches on the expression of proteasome genes and other rescue factors. Therefore, activation of the NRF1 pathway could represent a new approach to delay the onset of neurodegenerative disorders and other disorders with disturbed proteostasis.

Here, we present a series of compounds that are able to induce NRF1-dependent proteasome synthesis both in cell lines and in *C. elegans* model strains. These compounds increase proteasome activity, decrease the number and the size of protein aggregates and importantly, they do not cause cellular stress. Overall, preventing of protein aggregation by increasing the capacity of protein degradation machineries by using our compounds represents a promising novel therapeutic strategy for neurodegenerative diseases and proteinopathies in general.

# INVESTIGATION OF CLOSTRINDOLIN-BASED 3-PHENYLINDOLE DERIVATIVES AS POTENTIAL ANTITUBERCULAR AGENTS

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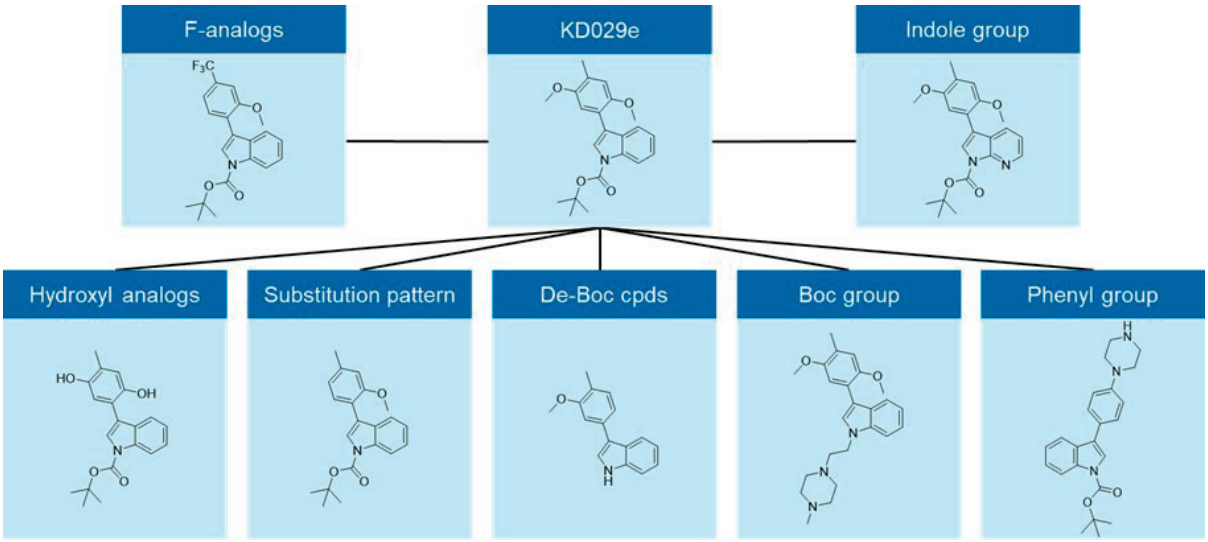
The growing prevalence of multi-drug resistant and extensively drug-resistant tuberculosis cases highlights an urgent need for novel antitubercular compounds with unique modes of action.[1] Natural products (NPs) have a proven track record in inspiring the development of novel therapeutics targeting vital cellular processes, such as RNA synthesis and protein metabolism.[2] Clostrindolin, a literature-reported NP, represents a starting point for further investigations displaying activity against *Mycobacterium vaccae* without detectable cytotoxicity.[3]

In our study, we accomplished hit nomination and hit expansion of a NP-based series with a promising activity against *Mycobacterium tuberculosis* (*Mtb*) starting from clostrindolin. After identification of hit compound **KD029e** (minimum inhibitory concentration (MIC) of 4 µg/mL *Mtb* H37Ra) (Figure 1) an early profiling was performed revealing solubility and metabolic stability as key alerts of this series. We synthesized over 40 compounds to explore the structure–activity relationships (SAR) of **KD029e**. As a result, the kinetic solubility was successfully increased, however a concurrent decrease in potency against *Mtb* was observed. Therefore, future optimization efforts are needed to investigate the potential of this compound class in addressing the challenges of TB treatment.

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# DISCOVERY OF ALLOSTERIC MODULATORS OF A2A RECEPTORS USING MOLECULAR MODELING

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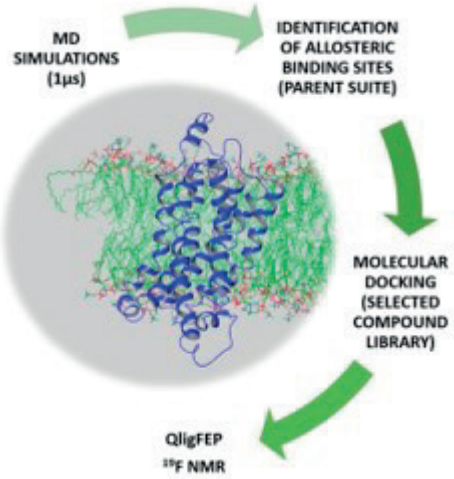
Adenosine receptors (AR) belong to the class A of family of G-protein coupled receptors (GPCR). AR are comprised of four members, named $A_1$, $A_{2A}$, $A_{2B}$, and $A_3$ receptors, found widely distributed in almost all human body tissues and organs. Although they represent pharmacologically very interesting targets, due to their low selectivity and wide spectrum of side effects, they currently have very few applications in clinical practice. On the other hand, positive and negative allosteric modulators have much greater selectivity and their side effect profile is much milder [1]. The difficulty in the development of such ligands is represent the small number of known allosteric modulators of AR, as well as the poor knowledge of allosteric binding sites within these proteins.The adenosine $A_{2A}$ receptor represents a target in cancer immunotherapy, thus a receptor of interest for new drug development[2]. Despite the fact that there is a very good structural coverage of this receptor in both active and inactive forms with different chemotypes (more than 70 structures), only a few allosteric binding sites are known, of which only the $Na^+$ ion binding site is well defined [3]. Currently, only a few molecules that allosterically regulate the work of this receptor are known, while none of the known allosteric modulators are specific for the $A_{2A}$ receptor.

In this work we present combination of computational and experimental methods [4] for determining allosteric binding sites an potential allosteric ligands on the $A_{2A}$ receptor. After molecular dynamics (MD) simulations of selected $A_{2A}$ receptor structures, potential allosteric sites were located using the PARENT program [4] to analyze the obtained MD trajectories. The located allosteric pockets were then compared to known allosteric sites on other GPCR receptors, including the adenosine $A_1$ receptor and the muscarinic $M_2$ receptor [5]. Fragments from different libraries were then docked into the Maestro Schrodinger suite within possible allosteric sites. Selected fragments were examined using $^{19}F$ NMR spectroscopy[6] to locate those that have an impact on the equilibrium of the proportion of active and inactive conformations of the adenosine $A_{2A}$ receptor. These results represent the basis for the design of specific allosteric ligands of adenosine $A_{2A}$ receptors.

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# DEVELOPMENT OF P2X$_7$ RECEPTOR ANTAGONISTS FOR THE TREATMENT OF NEURODEGENERATIVE DISEASES

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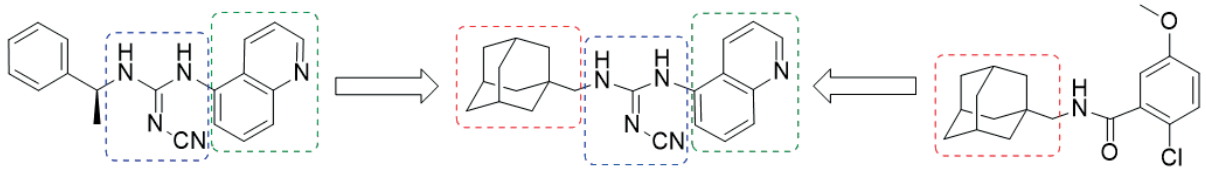
The P2X$_7$ receptor (P2X$_7$R) is an ATP-mediated ligand-gated ion channel upregulated on activated immune cells (*i.e.,* microglia) within the central nervous system (CNS).[1] Activation of the P2X$_7$R triggers a myriad of downstream signaling events that results in the release of interleukin-1β, cell death and proliferation. This is seen in pathological signalling states consistent with neurodegenerative disorders; Alzheimer's Disease and Parkinson's Disease.[2,3]

High-throughput screening campaigns have facilitated the discovery of a multitude of potent P2X$_7$R antagonists.[4,5] However, there is no clinically approved P2X$_7$R antagonist currently on the market, with CNS permeability a common issue throughout the drug discovery process. Previous work developed a hybrid pharmacophore model that displayed potent inhibition at the P2X$_7$R which has since been refined following several structure-activity relationship studies. These studies identified a highly potent adamantly cyanoguanidine framework, exemplified by lead compound **1** (IC$_{50}$ = 18 ± 2.0 nM).[6]

The current study aims to probe interactions between novel ligands and the P2X$_7$R binding site to improve the pharmacophore framework of **1**. This will be achieved by bioisosteric replacement of the cyanoguanidine linker to explore the influence of electrostatic distribution and positioning of hydrogen bond donors (HBD) and hydrogen bond acceptors (HBA). Furthermore, the project aims to reduce the lipophilicity and increase potency of the lead compound to afford optimal *in vitro* activity.

This research has seen the synthesis and initial *in vitro* evaluation of a small compound library. While improved potency was not achieved, a critical HBD necessary for nanomolar potency was identified within the linker framework. Ongoing *in vitro* evaluation of these P2X$_7$R antagonists will influence the direction of future research in this field. Ultimately, this knowledge may lead to the development of a CNS-permeable ligand that reduces IL-1β secretion and restores microglial phagocytosis. This will be the first innovation to combat neuronal degeneration for the treatment of Alzheimer's and Parkinson's Disease.



**2**
Competitive Antagonist
logP = 4.4
IC$_{50}$ = 93 ± 2.0 nM$^a$
T$_{1/2}$ = 0.58 h$^b$

**1**
Slowly Reversible NAM
logP = 4.7
IC$_{50}$ = 18 ± 2.0 nM$^a$

**3**
Reversible NAM
logP = 4.1
IC$_{50}$ = 11 ± 3.1 nM$^a$
T$_{1/2}$ = 0.16 h$^a$

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# REFINING THE DRUG DISCOVERY PARADIGM FOR SCHISTOSOMIASIS

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Schistosomiasis is a major Neglected Tropical Disease (NTD) caused by parasitic worms of the genus *Schistosoma*. An estimated 779 million people are at risk of infection globally and approximately 290,000 disease-related deaths occur annually. [1] Schistosomiasis is among the highest of the WHO's disability-adjusted life years (DALYs) NTDs with 3 million DALYs.[2] Currently there is only one drug, praziquantel, available to treat schistosomiasis, no drugs in clinical trials and an underdeveloped and poorly defined pathway for new treatments. There is an urgent need to discover new treatments due to significant shortcomings with praziquantel, as well as concerns around resistance.[3] Our work focuses on developing the drug discovery pathway for schistosomiasis and using this to progress our current portfolio of hit and lead compounds towards a late lead.

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# DEVELOPMENT OF AN ITERATIVE PIPELINE FOR THE DISCOVERY OF SELECTIVE MULTITARGET TYROSINE KINASE INHIBITORS

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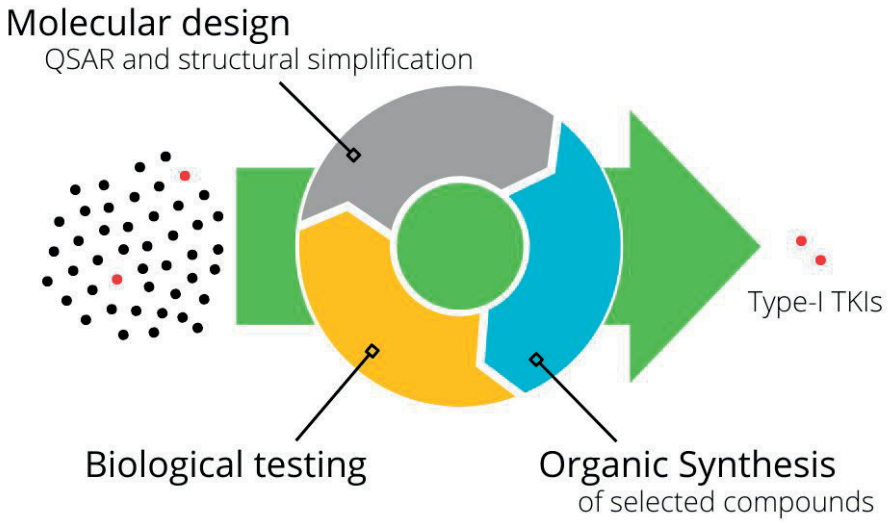
Treating pancreatic ductal adenocarcinoma (PDAC) has always been a challenge as it requires the simultaneous inhibition of several key proteins. Specifically, tyrosine kinase receptors have been found to have an important role in both tumor progression and drug resistance development, making them an interesting target when dealing with the disease [1].

We have developed an iterative pipeline for the discovery of new selective multitarget tyrosine kinase inhibitors. Diversity-oriented combinatorial library selection was coupled with crossed QSAR prediction models for the purpose of identifying the most promising dual inhibitors against fibroblast growth factor receptor 2 (FGFR2) and insulin-like growth factor 1 receptor (IGF1R). Moreover, in contrast to the traditional medicinal chemistry scheme, we included an additional step to reduce molecular complexity, allowing the selection of synthetically feasible compounds and speeding discovery approaches. Following this procedure, we have selected, synthesized and biological tested a total of 18 novel tyrosine kinase inhibitors with potential activity against two key proteins in PDAC; FGFR2 and IGF1R.

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# DEVELOPMENT OF DUAL-FUNCTIONALIZED FLUOROPHORES TO MONITOR ANTIBODY DELIVERY SYSTEMS

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Antibody-based therapeutics[1] and imaging agents[2] are paragons in the present era of precision medicine, yet still pose challenges including off-target cytotoxicity and adverse reactions, due to the sub-optimal pharmacokinetics of the antibody and its tendency to accumulate non-specifically in tumours.[3, 4] These shortcomings result in either low treatment rate of tumours or inaccurate indications of the tumour size, compromising patient survival or an incorrect treatment regimen. Thus, with the imaging capabilities of fluorescence, detailed and accurate representation of the site of interest in real-time is attainable, assisting current imaging techniques.

Here, we report a novel class of click chemistry-dependent switch-on coumarin, naphthalimide, and naphthalene fluorescent probes comprising of two orthogonal chemical handles that offer utility in varied applications, including the ability to monitor click-to-release drug delivery systems based on antibody conjugates and the ability to provide synergistic capabilities to antibody-based tracers, to strengthen current studies in antibody delivery systems.

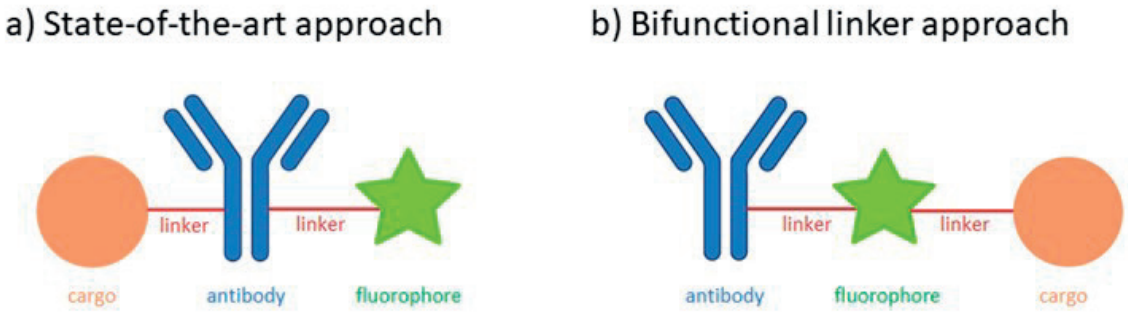


Figure 1. Schematic representation of antibody-conjugates using (a) traditional fluorophores, and (b) dual-functionalised fluorophores.

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# EXPLORATION OF PHENOXAZINE DERIVATIVES FOR THE TREATMENT OF TUBERCULOSIS

**Eric Tran (1), Chen-Yi Cheung (2), Glen P. Carter (3), Yi Sing Gee (1), Nicholas West (4), Amandeep Kaur (1), Gregory Cook (2), Jonathan B. Baell (1), Manuela Jörg (1)**

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Tuberculosis (TB) is currently the second leading cause of death by an infectious disease, only behind COVID-19. The World Health Organisation (WHO) reported a total of 1.6 million people succumbing to the disease, with an estimated 10.6 million people falling ill with TB worldwide in 2021.[1] The causative agent behind this disease is *Mycobacterium tuberculosis* (*Mtb*) and can be fatal if left untreated. While treatments are available for this disease, the emergence of resistant strains to first-line and second-line treatment options make this disease an ongoing public health concern.[2] Therefore, the development of new treatment regimens, repurposing of existing drugs as well as the discovery for new drugs are necessary to combat the disease.[3]

Herein, we report our efforts to explore the structure-activity relationship (SAR) of a phenoxazine-derivative, which was identified as a hit with sub-micromolar activity against a *Mtb* strain through a phenotypic compound screen. The hit had no antibacterial activity when tested against *S. aureus*, *E. faecium*, *E. coli*, *K. pneumoniae*, *A. baumannii* and *P. aeruginosa*. Modifications to different parts of the structure have thus far revealed “steep” SAR. Interestingly, the activity of the hit compound was completely abolished against a mutant strain of TB, while the other analogues retained activity against the resistant *Mtb* strain.

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# IDENTIFYING SMALL MOLECULE MODULATORS OF IR-IGF1R HYBRID FORMATION

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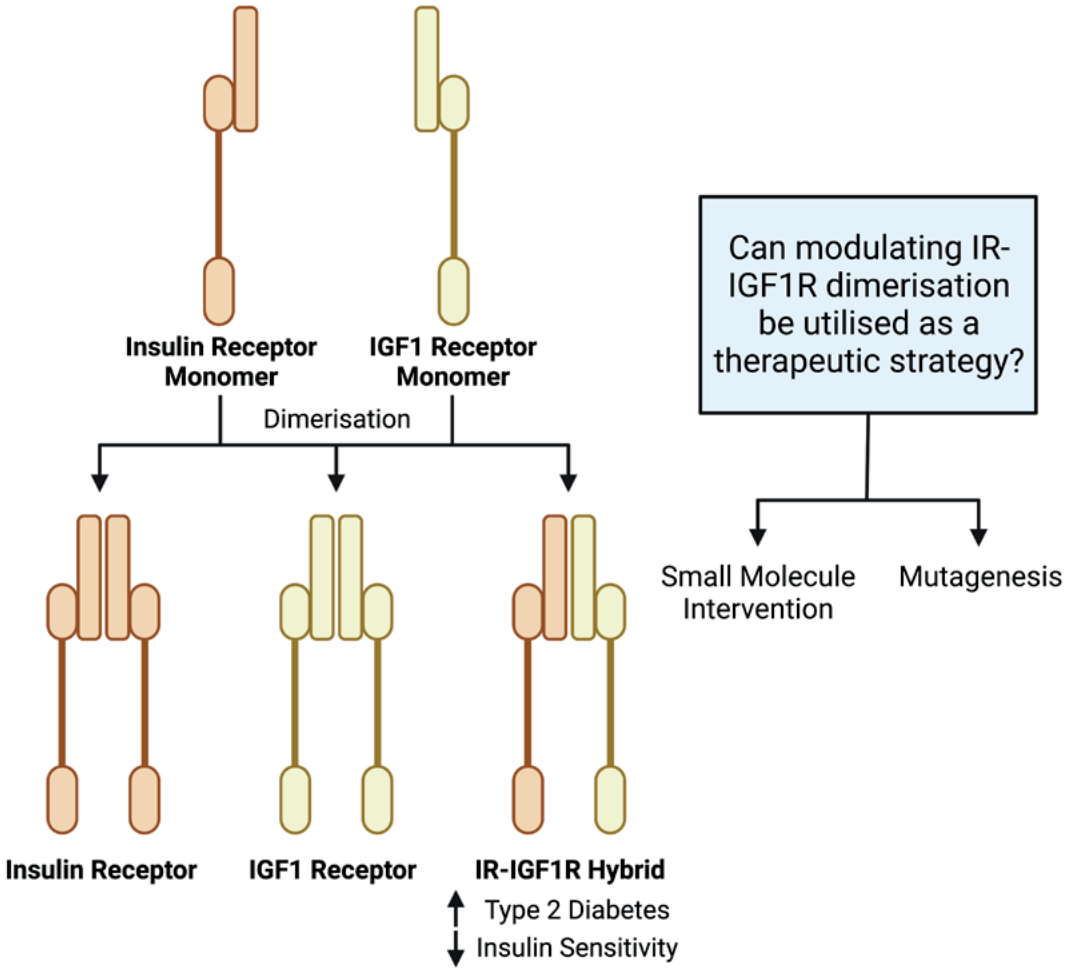


Type 2 diabetes is characterised by the disruption of insulin and insulin-like growth factor (IGF) signalling. The key hubs of these signalling cascades - the insulin receptor (IR) and insulin-like growth factor 1 receptor (IGF1R) – are known to form functional IR-IGF1R hybrid receptors which are insulin resistant. However, the mechanisms underpinning IR-IGF1R hybrid formation are poorly understood. Understanding the factors affecting hybrid formation could form the basis of a novel therapeutic strategy to treat type 2 diabetes. Therefore, we have evaluated the impact of mutations on hybrid formation, identifying hotspot residues at the IR: IGF1R interface and establishing that the affinity of the hybrid complex can be modulated at the protein level. We have leveraged this to guide the identification of small molecule IR-IGF1R hybrid modulators. Using a homology model of the hybrid receptor, a virtual high-throughput screen was directed against epitopes identified by mutagenesis. Compounds prioritised in screening were subsequently evaluated for their ability to modulate hybrid formation in a bioluminescence resonance energy transfer (BRET) assay, with hits corroborated by western blotting. This screening cascade led to the identification of a small molecule that promoted hybrid formation in HEK293 cells at 100 μM when evaluated by BRET. Western blotting confirmed this molecule was similarly able to promote IR-IGF1R hybrid formation in HUVEC cells at 100 μM. These findings emphasise the possibility of modulating IR-IGF1R hybrid formation as a strategy to treat type 2 diabetes.

# THERMALLY TRIGGERED SITE-SELECTIVE TYROSINE BIOCONJUGATION

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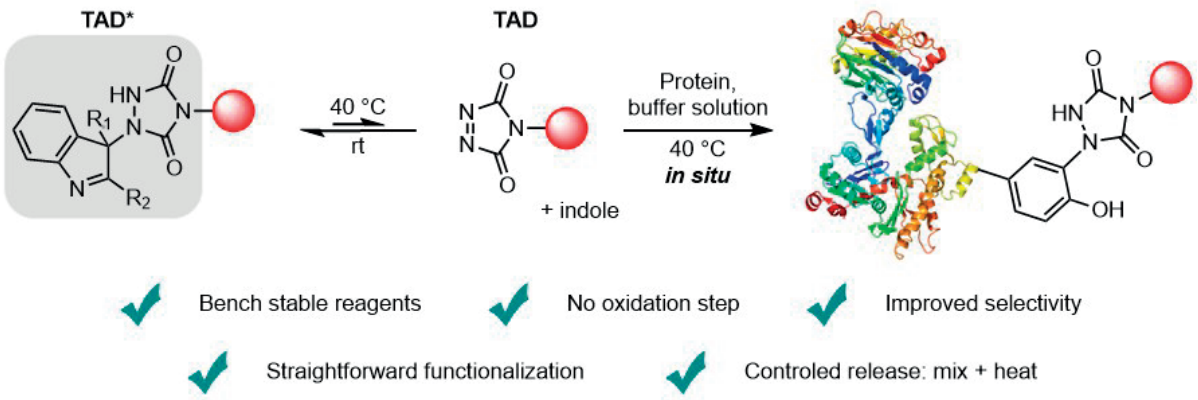


Protein conjugation has become a widely investigated research field as it is essential in a wide range of applications including imaging, protein function elucidation and targeted drug delivery[1]. Despite the increased importance for reliable methods towards synthetically modified proteins, it remains an ongoing challenge to develop strategies that can achieve high chemo- and site-selectivity. Recently, **triazolinedione (TAD)** mediated tyrosine conjugation emerged as one of the fastest and most site specific bioconjugation techniques up to date[2]. Despite operational ease and reliability, TADs are very unstable by itself in aqueous medium and recently revealed off-target tryptophan modification[3]. Also, incompatibility of the biological payload with the corresponding urazole oxidation step limits their functionalization.

Here we introduce an *in situ* bioconjugation strategy involving **protected TAD moieties (TAD*)** which are bench-stable at room temperature, but deblock upon heating in aqueous medium, allowing controlled release and site-selective protein modification. In contrast to TAD, it was possible to synthesize a broad range of functional TAD*s exhibiting an improved shelf-life and solvent stability, via a straightforward one-pot procedure. Also, competition experiments showed an improved selectivity towards tyrosine at physiological pH, without any degradation products. Finally, site-selective modification of several proteins was achieved, demonstrating the generic potential of TAD*-mediated bioconjugation strategy.

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# IDENTIFICATION OF PLASTOQUINONE ANALOGS TO BE EFFECTIVE IN BREAST CANCER TREATMENT

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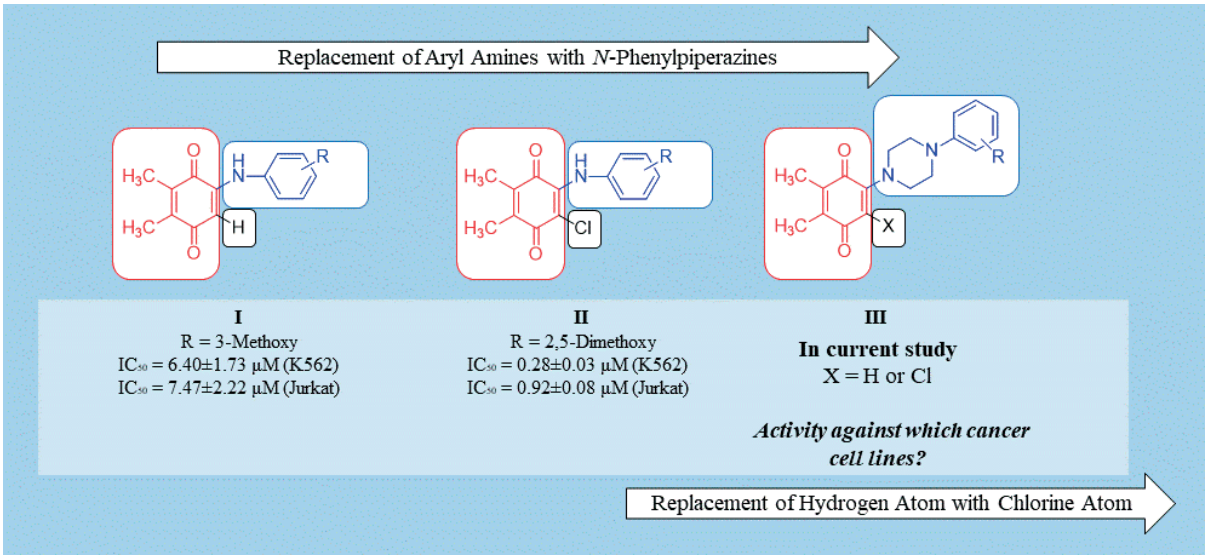
As a part of our research program,[1,2] aiming at the discovery of lead molecules based on quinone chemistry, we recently reported the potent Plastoquinone analogs (**PQ1-12**)[3] have been obtained to understand their antiproliferative profile which contains a piperazine fragment as a key structural element and dimethyl-1,4-benzoquinone as a core moiety. In addition to remarkable $IC_{50}$ values of those molecules selected by the National Cancer Institute (NCI) of Bethesda based on the NCI Developmental Therapeutics Program and tested against the panel of 60 cancer cell lines, the cytotoxicity of the selected PQ analogs (**PQ8**, **PQ9**, **PQ11**, and **PQ12**) was determined using four breast cancer cell lines (MCF7, UACC-2087, MDA-MB-231, and MDA-MB-435) compared to normal cell line (HaCaT). Reactive oxygen species generation, changes in cell proliferation, cell migration, and apoptosis induction were investigated for the selected PQ analog on MCF7 and UACC-2087 cell lines. We could understand from the results, **PQ11** displayed the most promising anticancer activity against MCF7 cell line through increased oxidative stress, apoptosis, and suppression of cell proliferation.

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# DEVELOPMENT OF A METHODOLOGY FOR TRACELESS AFFINITY LABELLING OF BRUTON'S TYROSINE KINASE IN LIVE CELLS

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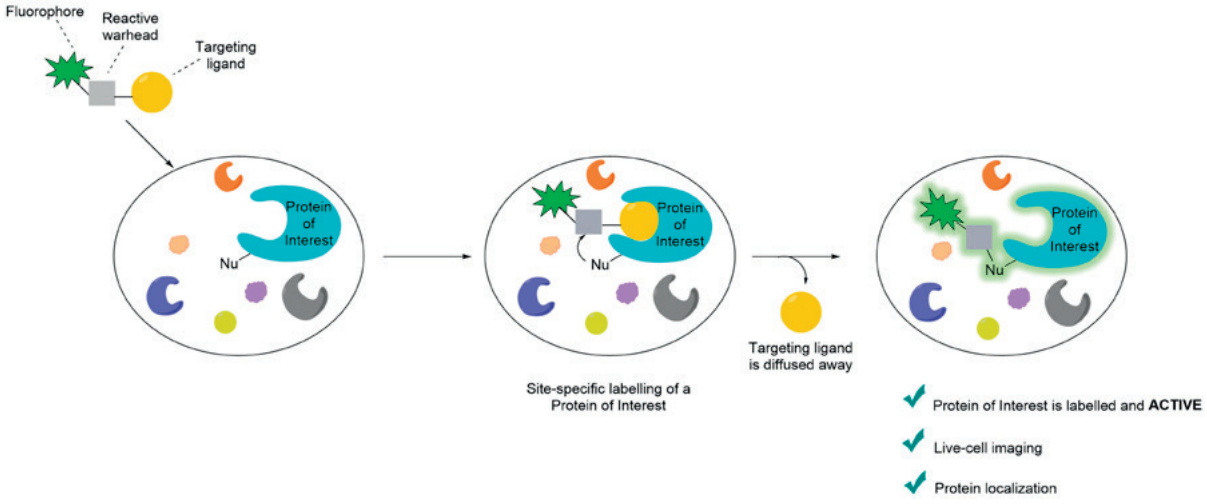
Traceless affinity labelling of a protein of interest is a powerful tool to study protein function, conformation, and cellular signalling pathways without the need for genetic engineering methods. In this technique, a targeting ligand that recognizes and binds to the active site of the protein, is coupled with a probe, such as a fluorophore, using a reactive electrophilic group known as a warhead. The warhead will facilitate the covalent attachment of the probe to the enzyme by reacting with a nucleophilic non-catalytic residue in the binding pocket. As a result, the ligand that binds to the active site is released, and can diffuse away from the enzyme, allowing the kinase to remain active after the labelling. The incorporation of a fluorescent probe enables imaging and localization of the target protein[1,2]. One particular enzyme of interest is Bruton's Tyrosine Kinase (BTK), which belongs to the Tec family and plays a crucial role in B cell proliferation and activation pathways. Due to its important role in B cells, BTK has emerged as an important target in autoimmune disorders and malignancies. BTK has a non-catalytic cysteine residue (Cys481) in the vicinity of the ATP-active pocket, which has been exploited in the development of covalent BTK inhibitors[3]. Herein, we report the synthesis and evaluation of a series of BTK-labelling probes that enable the chemical modification of BTK *in vitro* while preserving the enzymatic activity. The probes have been evaluated for their labelling efficacy in live cells. Preliminary data prove that the probes can enter the cells without perturbing the cellular activity. This method can be applicable to other kinases as well, considering that over 200 kinases are estimated to have an accessible nucleophilic residue (Cys, Lys, or Ser) that can be targeted by ATP-competitive covalent kinase inhibitors. Thus, this approach holds great potential for further elucidating the role of kinases in cell signalling pathways.

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# DISCOVERY OF POTENT TETRAZOLE FREE FATTY ACID RECEPTOR 2 ANTAGONISTS

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The free fatty acid receptor 2 (FFA2/GPR43) is a G protein-coupled receptor broadly expressed in various tissues, including the intestines[1], pancreas[2], immune cells[3], adipose tissue, and ganglia.[4] It is activated by short-chain free fatty acids such as acetate, propionate and butyrate that are produced by gut microbiota upon fermentation of dietary fiber.[5, 6] The receptor is linked to inflammatory and metabolic conditions such as type 2 diabetes[7], arthritis[8] and asthma[9], and represents a promising target for their treatment. Although the potential benefits of either agonists or antagonists is still debated, the only clinical candidate was the FFA2 antagonist GLPG0974 that failed to meet efficacy endpoints for ulcerative colitis.[10] There is still an unmet need for high-quality tool compounds and starting points for drug development.

Here, we report on an SAR investigation of the known FFA2 antagonist CATPB. The bioisosteric replacement of the carboxylic acid head group with a tetrazole moiety led to significantly increased potency. An improved synthetic route to access enantiopure compounds was also developed. The most potent compounds showed favorable physicochemical properties with the preferred antagonist TUG-2304 presenting an $IC_{50}$ = 4 nM and low lipophilicity (log $D_{7.4}$ = 0.84), resulting in a high LLE of 7.5. The compound also showed full inhibitory activity in propionate-mediated respiratory burst and human neutrophil migration assays in a dose-dependent fashion, as well as favorably PK properties in mice and a bioavailability of 44%. Thus, TUG-2304 represents the most potent and arguably the first high-potency FFA2 antagonist and is expected to be a valuable tool in the further studies of the physiological role of FFA2.

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# SYNTHESIS OF UNSYMMETRICAL SQUARAMIDES AS POTENTIAL BIOLOGICAL TARGETS AND DETAILED CONFORMATIONAL ANALYSIS

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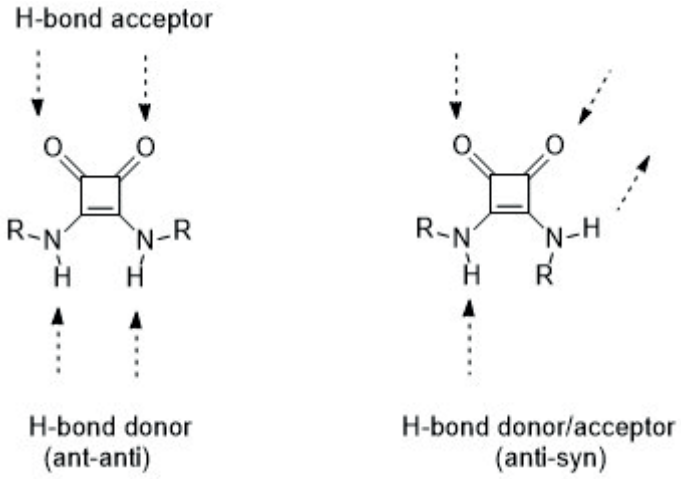
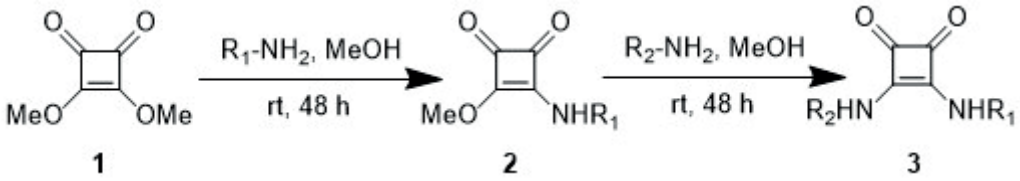
Squaramides[1], a family of conformationally rigid cyclobutene ring derivatives, are rapidly gaining research interest across diverse areas of the chemical and biological sciences. In fact, squaramides display a unique ability to partake in strong bidirectional hydrogen bonding, rendering them useful candidates for supra- and sub-molecular studies at the interface of material sciences and may act as mimetics of several functional groups.




To enrich the possibility of bioisosterically replace urea-containing compounds with squaramides, we synthesized several *mon*o- and *di*- substituted analogs. Their conformational preferences, which affect their behavior were studied in detail.

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# SYNTHESIS OF MOLECULAR PROBES FOR THE DETECTION OF PATHOGENS AND REACTIVE OXYGEN SPECIES

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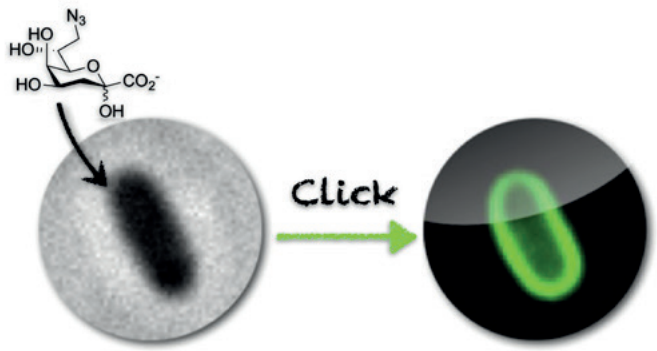
The development and application of synthetic tools to explore biological processes, as well as the use of biological systems as a source of inspiration for the design of molecular devices, are the main focus of our research.

This communication will present a selection of some of our most recent work in this area, which includes the development of new labeling strategies for the detection and identification of live bacteria[1-6] such as *Legionella pneumophila*, a serious pathogen responsible for Legionnaires' disease,[2] as well as the design and evaluation of new, fast-reactive hydrogen peroxide-sensitive borinate triggers, for the elaboration of probes for the detection of reactive oxygen species.[7-8]

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# IN VIVO EVALUATION OF SOLUBLE EPOXIDE HYDROLASE INHIBITORS IN MURINE MODELS OF ALLODYNIA, CHEMOTHERAPY-INDUCED NEUROPATHIC PAIN AND VISCERAL PAIN

**Santiago Vázquez (1), Sandra Codony (1), Beatrice Jora (1), José M. Entrena (2), Miriam Santos-Caballero (3), Andreea L. Turcu (1), Carla Calvó-Tusell (4), Rafael González-Cano (3), Christophe Morisseau (5), Antón L. Martínez (6), Clara Bartra (7), Ruben Corpas (7), Coral Sanfeliu (7), Belén Pérez (8), Bruce D. Hammock (5), Concepción Pérez (9), María Isabel Rodríguez-Franco (9), Christian Griñán-Ferré (10), Mercè Pallàs (10), Sílvia Osuna (4,11), Ferran Feixas (4), M. Isabel Loza (6), José M. Brea (6), Enrique J. Cobos (3)**

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Soluble epoxide hydrolase inhibitors (sEHI) are a new class of non-opioid analgesics, with a representative compound, EC5026, currently in clinical trials for the management of neuropathic pain [1].

Our group has recently designed, synthesized and pharmacologically evaluated novel series of potent benzohomoadamantane-based sEHI [2]. Herein, we report further medicinal chemistry around the abovementioned polycyclic scaffold to improve the potency and, particularly, the DMPK properties of previous hits. After an extensive *in vitro* screening cascade, molecular modeling, and *in vivo* pharmacokinetics studies, three candidates were selected for *in vivo* studies. Two compounds evaluated in a murine model of capsaicin-induced allodynia displayed potent anti-allodynic effect in a dose-dependent manner. Next, the most potent compound was evaluated in the cyclophosphamide-induced murine model of cystitis, a well-established model of visceral pain, presenting robust analgesic efficacy [3]. Finally, considering that chemotherapy-induced neuropathic pain (CINP), a severe side effect of several anticancer agents, is a largely unmet medical need [4], our third candidate was evaluated in a murine model of paclitaxel-induced neuropathic pain. CINP was performed by a daily injection of paclitaxel via i.p. (2 mg/kg), for 5 consecutive days. Mice developed neuropathic mechanical allodynia, which peaked on day 10 after the first paclitaxel administration –time when the acute effects of sEHI were tested. Subcutaneous administration of this candidate (2.5-5 mg/kg) completely reversed in a dose dependent manner the sensory hypersensitivity. Additionally, administration of the sEHI (5 mg/kg, s.c.) 30 min before each paclitaxel injection completely prevented the development of neuropathic allodynia. Collectively, these results suggest interstitial cystitis/pain bladder syndrome and CINP as possible new indications for sEHI.

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# SYNTHESIS OF (OLIGO)ARABINOSIDES TO STUDY NEW DRUG TARGETS FOR TUBERCULOSIS TREATMENT

**Cristiano A. Conceicao, Vanessa T. Almeida, Federico Issoglio, Margarida Archer, Maria Rita Ventura**

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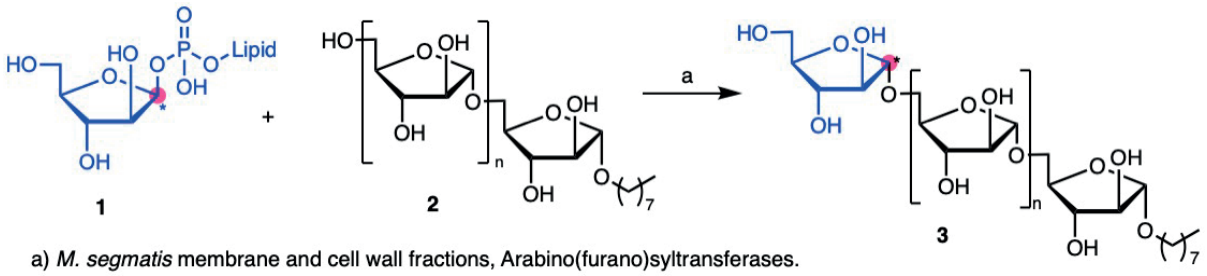
Tuberculosis (TB), caused by *Mycobacterium tuberculosis*, remains one of the primary causes of human death worldwide. The survival and pathogenicity of mycobacteria depends on the integrity of the cell wall, which contains two main polysaccharides, arabinogalactan (AG) and lipoarabinomannan (LAM). D-arabinofuranose (D-Araf) is present in these polysaccharides but not found in mammals, thus compounds that inhibit the enzymes essential for the building of these polysaccharides are potential antimycobacterial drugs.[1] Arabinofuranosyltransferases (AraT) use decaprenylphosphoryl-D-arabinofuranose (DPA) to donate an arabinofuranose residue to a saccharide acceptor and are essential for *M. tuberculosis* growth.[2]

In this work, a multidisciplinary approach was used for the development of novel and efficient enzymatic assays for the characterisation of AraTs. Several linear and branched (oligo)arabinofuranoside acceptors were synthesised and their binding affinity with AraT was screened using differential scanning fluorimetry (nanoDSF) to select the best synthetic glycosyl acceptors. The total synthesis of [1]-$^{13}$C-labelled DPA analogues **1** (Fig. 1) was optimised achieving an overall yield of 38% and an excellent anomeric ratio of 31:1 (beta:alpha). The total syntheses of several linear and branched arabinosyl acceptors for the enzymatic reactions were also efficiently accomplished. In order to study the protein conversions of the synthesised labelled donor with the acceptors a flexible NMR protocol was designed and implemented.

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# STEROIDS WITH UNNATURAL CONFIGURATION

**Mariia Vodolazhenko, Timotej Strmeň, Marina Morozovová, Blanka Klepetářová, Radko Souček, Karel Kudláček, Eva Kudová**

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Among different classes of biologically active compounds in the human body steroids always played one of the most significant roles. They can both participate in our metabolism and be effective drugs with different types of activity: cardioactivity,[1] anticancer, anti-angiogenic, anti-inflammatory, antimicrobial, or neuromuscular blocking agents.[2] Moreover, neurosteroids – endogenous compounds that are synthesized in the brain and for the brain – can be implicated in synaptic plasticity, age-related neurodegenerative diseases, learning and memory function, or disturbances associated with certain neuropsychiatric disorders[3-5] through interaction with ion channels receptors, e.g. ɣ-amino-butyric acid ($GABA_A$) and *N*-methyl-*D*-aspartate receptors (NMDA).[1,2]

Finding new effective steroid-based medicines is a challenging task because most of the studied neurosteroids have several stereocentres that can complicate their synthesis. In this work, we have decided to modify relevant stereocentres to change the overall shape of the skeleton into an atypical shape. As such, a series of new steroids with atypical stereochemistry at positions C-13 and C-14 were synthesized starting from dehydroepiandrosterone. The structures and shapes of skeletons were confirmed by X-ray analysis. Moreover, we have prepared their natural analogues with natural stereochemistry at C-13 and C-14. For both series, variations of the main functional groups were made. Interestingly, our approach allowed us to prepare new compounds with unnatural configurations from a starting material with a natural configuration by multi-step methodologies. For synthesised compounds, their stability, solubility, and permeability were evaluated in comparison with their natural analogues. The results of this study will define new skeletons for the further development of novel neurosteroid drug-like compounds.

*This work was supported by the Czech Science Foundation GACR, No. 23-04922S and Research Project of the Academy of Sciences of the Czech Republic: RVO grant 61388963.*

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# DESIGN, DEVELOPMENT AND EVALUATION OF NOVEL ANTICANCER DRUGS TARGETING PROTEIN KINASE D2 (PKD2)

**Lauren Voets* (1), Olivia Appelmans* (2), Ahmed Shemy* (1), Philippe Gilles (1), Rudra Shankar Kashyap (2), Margaux Billen (1), Johan Van Lint (2), Arnout Voet (1), Wim M. De Borggraeve (1)**

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Protein kinase D (PKD) is a serine/threonine kinase family belonging to the CAMK kinase superfamily. Over the past decade, PKD gained considerable interest after the evidence that dysregulated PKD plays a crucial role in certain cancers including pancreatic, head and neck, and prostate cancer.[1, 2] Protein kinase D consists of three different isoforms, namely PKD1, 2 and 3. PKD2 stands out as an attractive anticancer target due to its pro-oncogenic effect. The PKD1 isoenzyme, on the contrary, counteracts the tumorigenic effects, causing the design of isoform-specific PKD2 inhibitors to offer a new perspective on next-generation cancer therapeutics.[2-4] This work focuses on the usage of computer-aided drug design (CADD) to rationally construct allosteric isoform-specific PKD2 inhibitors. A structure-based virtual screening campaign against a presumed allosteric site of PKD2 resulted in a set of 28 virtual hits. Further *in vitro* validation using ADP-Glo and a radioactive kinase assay against PKD yielded one potent, specific PKD2 allosteric inhibitor with an IC50 below 50 µM. This compound was used as a parent to develop two additional libraries aiming to increase potency and selectivity. Four compounds from the expanded libraries were identified as more potent PKD2 inhibitors with reasonable isoform specificity, unlike the known classic ATP competitive inhibitors. The anti-cancer effects of these compounds were validated *in cellulo* on different cancer cell lines.

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# ANTITUMOR ACTIVITY OF NOVEL 1,2,3-TRIAZOLE CONTAINING QUINOLINE-BENZIMIDAZOLE HYBRIDS

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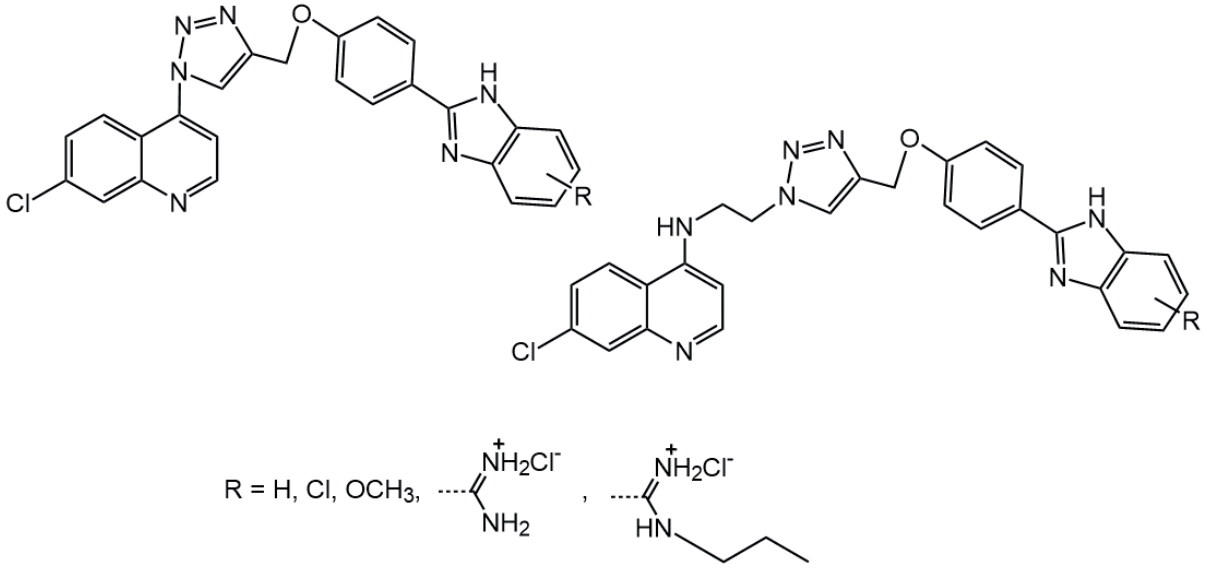
Quinoline, a nitrogen containing heterocycle, is a part of numerous compounds with a broad spectrum of biological activities and 7-chloro-4-aminoquinolines are an important segment of this group. [1] Benzimidazole, again a nitrogen heterocycle, also has significant biological profile [2]. 1,2,3-Triazole, with the emergence of click chemistry, has found its way to a vast number of compounds with significant biological properties. [3] We wish to report on preparation of novel 7-chloro-4-aminoquinoline benzimidazole hybrid compounds with a 1,2,3-triazole linker which were evaluated on one non-tumor and 4 tumor cell lines. Screening revealed that there is a distinct difference between activity of amidine and non-amidine compounds. Amidine derivatives have shown to be more selective but with an overall lower activity.

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# EFFICIENT ONE-STEP APPROACH TO ANNULATED TETRAHYDROFURANS: SUPPORTING THE SEARCH FOR NEW POTENT CCR2 RECEPTOR ANTAGONISTS

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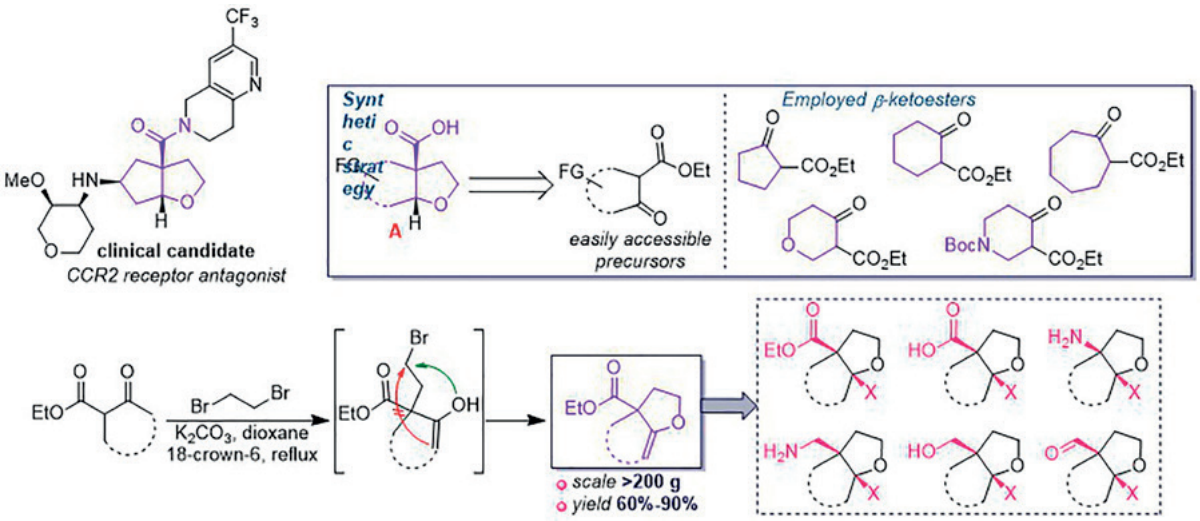
Low molecular conformationally constrained "oxygen-enriched" building blocks are the object of increased interest for medicinal chemistry. A striking confirmation of the statement is recent development of a selective CCR2 receptor antagonist by Janssen. So far, several CCR2 antagonists have entered clinical trials for the treatment of rheumatoid arthritis, multiple sclerosis, neuropathic pain, diabetes mellitus, allergic rhinitis. Owing to a wide activity range of the CCR2 inhibitors there are ongoing efforts on search and optimization of their structure. Back to the Janssen candidate, it was obtained by modifying octahydropentalene structure with oxygen atom. At the same time, possibility of modifying another cyclopentane ring of the scaffold has not been studied. By means of this project, we aim at developing a flexible method for the synthesis of type **A** functionalized compounds, which will apparently support the search for new potent CCR2 receptor antagonists.

Our investigation on annulation of tetrahydrofurane core provided interesting results. Cyclic α-ketoesters react with dibromoethane in a tandem manner first producing C-alkylated products followed by O-alkylation, thus giving the target derivatives in one synthetic step. Expansion and optimization of the procedure led to the multigram scale (>200 g from 1 synthetic run) method for the synthesis of bicyclic fused tetrahydrofurans with various sizes and natures of the adjacent ring. Formed [3+3]-annulated products exhibit highly reactive vinyl alcohol moiety smoothly adding alcohols with formation of corresponding cyclic acetals. Inspection of the reaction scope evidenced that a series of β-ketoesters comprising cyclohexane, cycloheptane, 4-oxopiperidine, and 4-oxotetrahydro-2*H*-pyran moieties can be subjected to the methodology for multigram preparation of the corresponding tetrahydropyrans. The bicyclic compounds can undergo typical functional group interconversions affording functionalized compounds with carboxyl, amine, alcohol, aldehyde functions. Summarizing, the building blocks synthesized will significantly influence the lead optimization approaches *via* increasing the toolbox of synthetic chemists.



# ASYMMETRIC ENE-REDUCTION BY F420-DEPENDENT OXIDOREDUCTASES B (FDOR-B) ENZYMES FROM MYCOBACTERIUM SMEGMATIS

**Suk Woo Kang (1), James Antoney (2), David W. Lupton (3), Colin Scott (4), Colin J. Jackson (5)**

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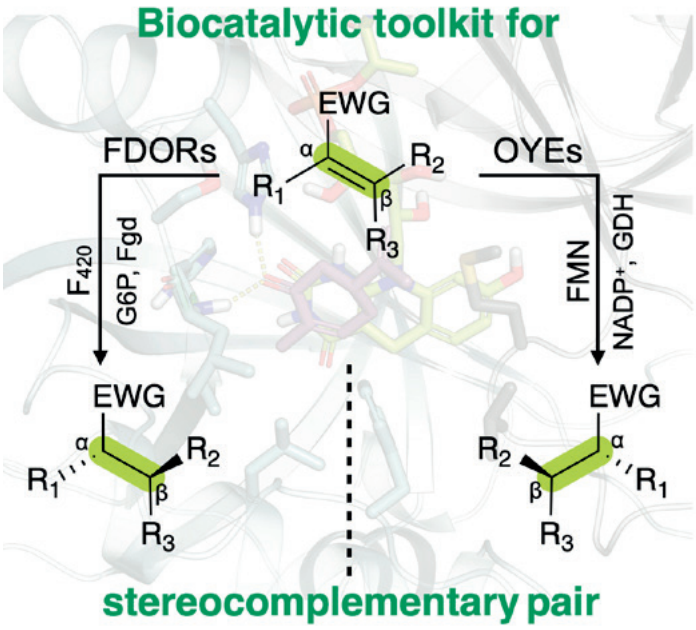
Asymmetric reduction by ene-reductases has received considerable attention in recent decades. While several enzyme families possess ene-reductase activity, the Old Yellow Enzyme (OYE) family has received the most scientific and industrial attention. However, there is a limited substrate range and few stereocomplementary pairs of current ene-reductases, necessitating the development of a complementary class. Flavin/deazaflavin oxidoreductases (FDORs) that utilize the uncommon cofactor $F_{420}$ have recently gained attention as ene-reductases for biocatalysis due to their stereocomplmentarity with OYEs. Although the enzymes of the FDOR-As sub-group have been characterized in this context and reported to catalyse ene-reduction enantioselectively, enzymes from the similarly large, but more diverse, FDOR-B sub-group have not been investigated in this context. In this study, we investigate the activity of 8 FDOR-B enzymes distributed across this sub-group, evaluating their specific activity, kinetic properties, and stereoselectivity against α,β-unsaturated compounds. The stereochemical outcomes of the FDOR-Bs are compared with enzymes of the FDOR-A sub-group and OYE family. Computational modelling and induced-fit docking are used to rationalize the observed catalytic behaviour and proposed a catalytic mechanism.




Our study is the first that investigates asymmetric ene-reductions by FDOR-Bs and explores additional ene-reductase candidates from FDOR family, which will advance our understanding of FDORs as industrial biocatalysts. Due to their presence in a greater variety of bacterial genera than FDOR-A enzymes, FDOR-Bs are expected to offer greater versatility in the search for potent activity against industrially relevant substrates.

# NEXT STOP GLUE CITY - NOVEL MOLECULAR GLUE PLATFORM

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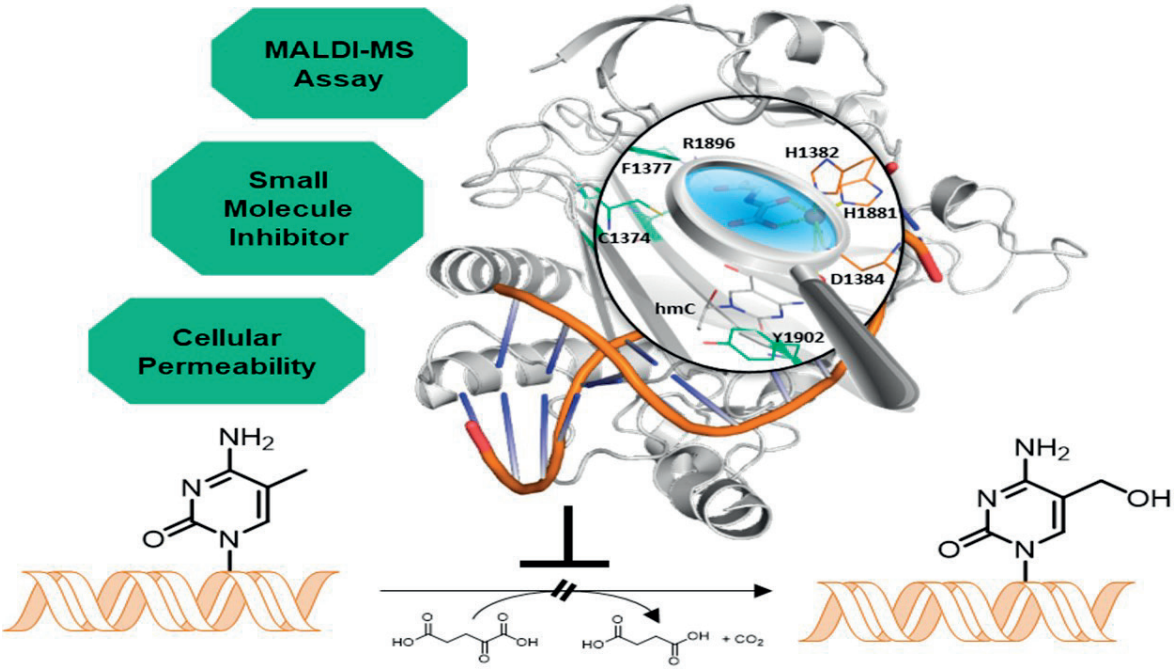
Discovery of molecular glue degraders has historically been serendipitous, and while inroads to their identification have recently been made using cell-based models, biophysical precedent for their discovery is still largely lacking. In this work, we present results on the use of a cell-free HTRF based method relying on the use of purified proteins. As a proof-of-concept, we used the well described inducible interaction between the Cereblon ubiquitin ligase and Casein Kinase 1 alpha. Using this model, we built an assay suitable for compound screening and applied this to screen our fragment library. The results presented here indicate that the method is broadly applicable for identification of molecular glues between any two selected proteins.

# EXPLORING EPIGENETIC REGULATION: UNVEILING THE ROLE OF TET ENZYMES THROUGH THE DEVELOPMENT OF NOVEL SMALL MOLECULE MODULATORS

**Suzanne Willems, Tzu-Chen Lin, Tom Schulz, Lejla Maksumic, Janina Niggenaber, Jörn Weisner, Daniel Summerer, Daniel Rauh**

*Department of Chemistry and Chemical Biology, TU Dortmund University and, Drug Discovery Hub Dortmund (DDHD), Zentrum für Integrierte Wirkstoffforschung (ZIW), Otto-Hahn Str. 4a, 44227 Dortmund, Germany*

Ten-eleven translocation dioxygenases (TETs) were identified a decade ago as a new member of the enzyme class of 2-oxoglutarate-dependent dioxygenases and are central epigenetic regulators of mammalian DNA.[1)] This family of enzymes recognizes DNA sequences containing 5-methylcytosine (5mC), and selectively catalyzes the iterative oxidation to 5-hydroxymethyl-, 5-formyl- and 5-carboxylcytosine using a Fe-active center. This process ultimately results in the active removal of the 5mC epigenetic mark by the base excision repair machinery. Since the discovery of TETs, these enzymes have been shown to be associated with gene mutations that cause various cancers.[2)] The development of cell-permeable, small-molecule TET inhibitors is envisioned to study enzyme function in states of health and disease and to provide the basis for early drug discovery. Using a previously established semi-high-throughput MALDI-MS assay for direct monitoring of substrate turnover, we screened a focused library of quinolines.[3)] Early structure-activity relationship (SAR) studies with derivatives of this scaffold and computational studies focusing on structurally related 2OG-dependent dioxygenases led to the identification and chemical optimization of novel TET2 modulators.

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# SYNTHESIS, INHIBITORY ACTIVITY ON JAKs AND MOLECULAR DOCKING STUDIES OF ROSMARINIC ACID DERIVATIVES

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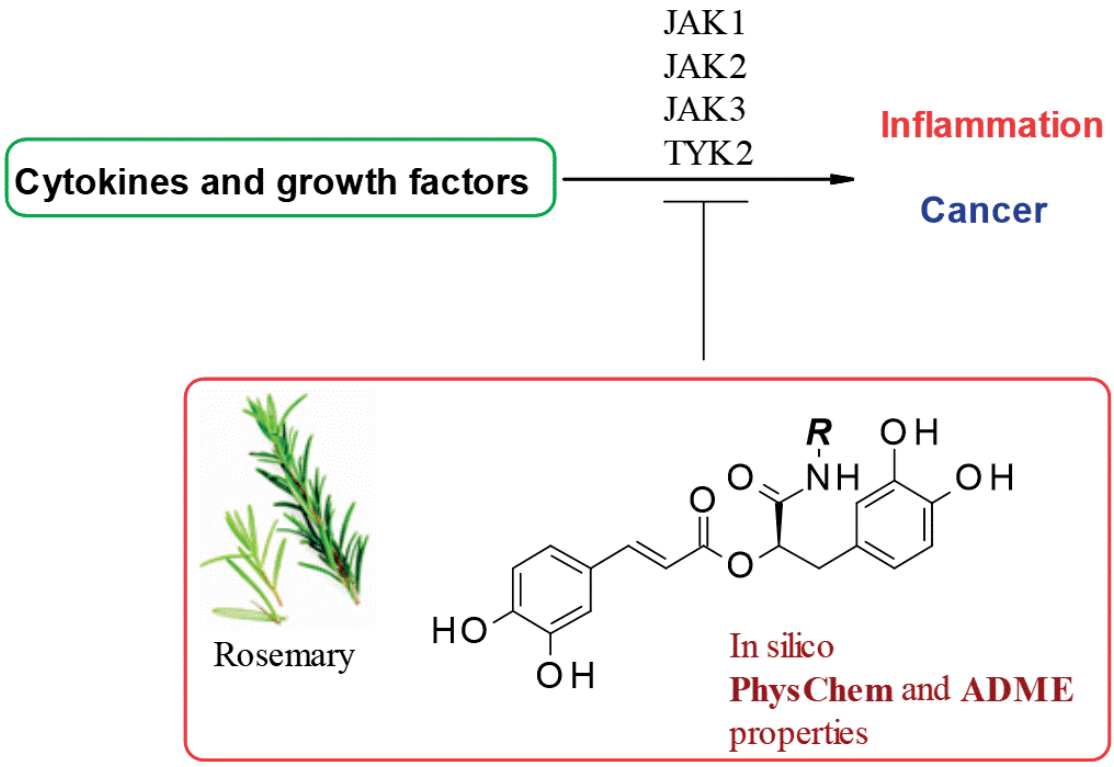


Rosmarinic acid is a natural compound and an ester of caffeic acid (3,4-dihydroxycinnamic acid) and 3,4-dihydroxyphenylacetic acid. In nature, it can be found in a number of plants, and in the highest concentrations, it is found in plants of the family Boraginaceae and Lamiaceae. It posseses diverse biological effects, ranging from anti-inflammatory, anticancer, antioxidant, antimicrobial, antiviral, antidepressant, anti-allergenic to cardio-, hepato- and neuprotective.[1-5] Many rosmarinic acid derivatives are described in literature with the aim to improve biological activity. Clinical JAK inhibitors generally target multiple JAKs at high potency.[6] Specific JAK kinase inibitors may decrease adverse effects, and thus increase safety and efficacy. In this study inhibitory activity of thirty rosmarinic acid amide derivatives on JAK1, JAK2, JAK3, TYR2 was evaluated. Rosmarinic acid derivatives selectively inhibit members of JAK familly at micromolar concentrations. Molecular docking studies have been used to rationalize biological profile of prepared focused library. *In silico* PhysChem and ADME properties are also calculated. In summary, preliminary results indicate potential for further investigation of this class of compounds.

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# SYNTHESIS AND BIOLOGICAL ACTIVITY OF 4''-O-ETHER CLARITHROMYCIN DERIVATIVES

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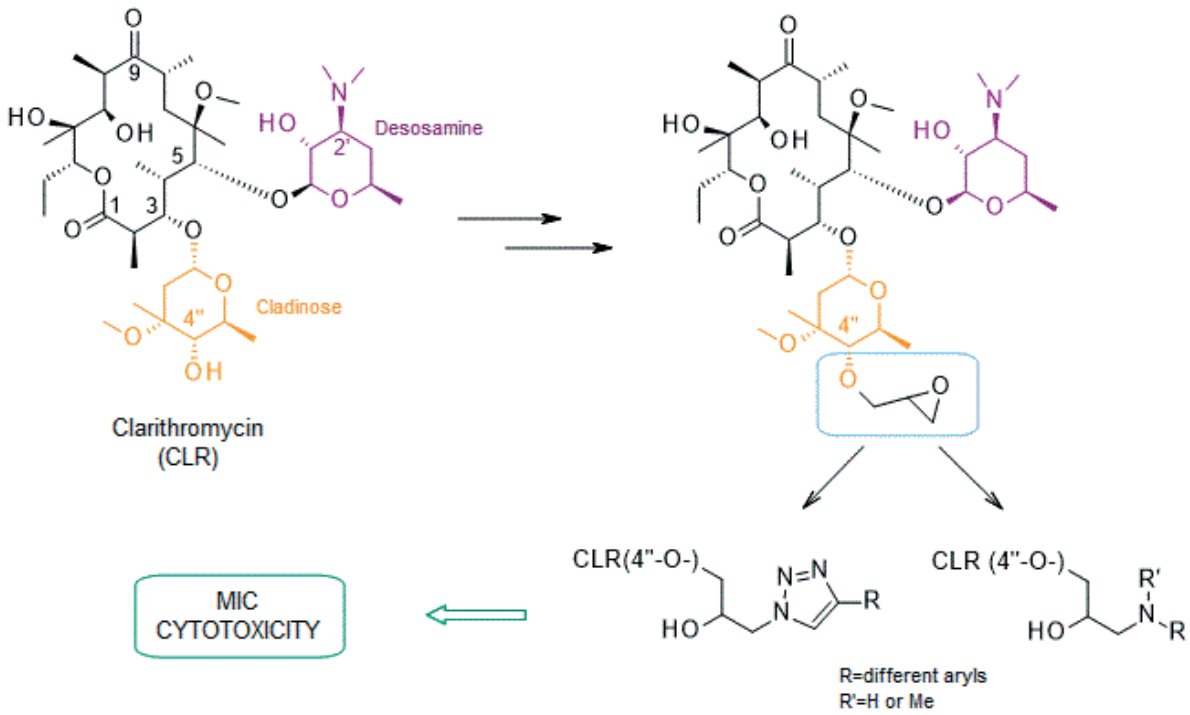


Macrolides are natural or semisynthetic macrocyclic molecules containing aglycone lactone ring (12-16 membered) and one or more deoxy sugars.[1] They are called selective inhibitors of translation, because they interfere with the translation of a specific subset of proteins being synthetized at the nascent peptide exit tunnel inside the bacterial ribosome.[2-3] Macrolides have several binding epitopes to the ribosomal tunnel, desosamine being the one observed in most of the available high-resolution crystallographic structures.[4] Their important characteristics include moderately broad spectrum of antimicrobial activity, an orally effective route of administration and a relatively high therapeutic index.[5] Since one macrolide can manifest different binding sites at ribosomal tunnel, there are difficulties in trying to design macrolide modifications.[4,6]

In this study a series of different 4''-O-ether derivatives were synthetized starting from clarithromycin. Novel molecules were tested against the panel of susceptible and resistant Gram-positive bacteria. Several molecules showed increased inhibition activity against resistant strains of *S. pneumoniae* and *S. pyogenes* when compared to clarithromycin, while only a few have better activity against resistant strains of *S. aureus*. Prepared molecules were tested in a cytotoxicity assay to exclude ones showing activity because of their cytotoxic effect on bacterial cells.

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# RATIONAL CHEMICAL DESIGN OF RX1, A MODULAR 1,2-THIASELENANE REDOX PROBE THAT SELECTIVELY REPORTS ON CELLULAR THIOREDOXIN REDUCTASE ACTIVITY

**Lukas Zeisel (1), Jan G. Felber (1), Karoline C. Scholzen (2), Lena Poczka (1), Dorian Cheff (3), Martin S. Maier (1), Qing Cheng (2), Min Shen (3), Matthew D. Hall (3), Elias S. J. Arnér (2,4), Julia Thorn-Seshold (1), Oliver Thorn-Seshold (1)**

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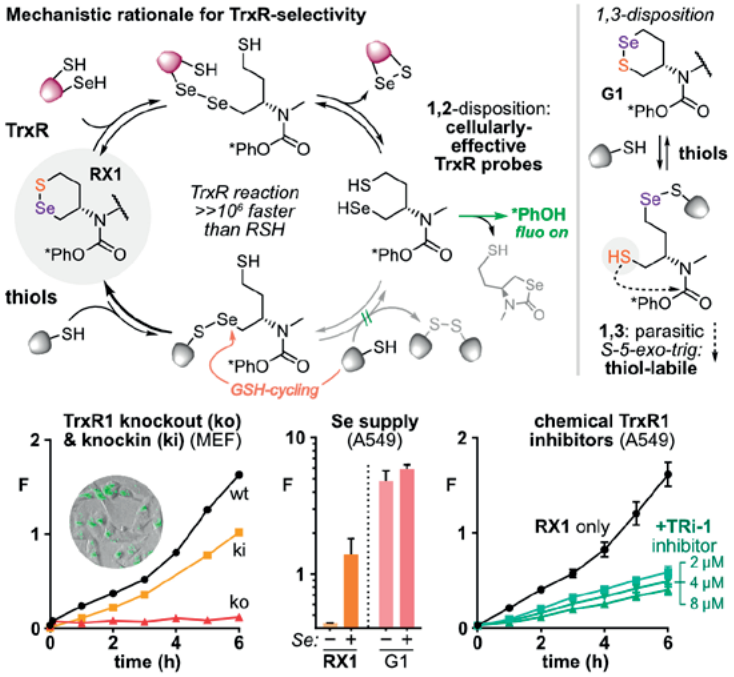


Quantifying the activity of key cellular redox players is crucial for understanding physiological homeostasis and for targeting their perturbed states in pathologies, including cancer and inflammatory diseases.(1) However, cellularly selective probes for oxidoreductase turnover are sorely lacking. We rationally developed the first probes that selectively target the mammalian selenoprotein thioredoxin reductase (TrxR) by using a cyclic selenenylsulfide oriented to harness TrxR's unique selenolthiol chemistry while resisting the cellular monothiol background. Lead probe **RX1** had excellent TrxR1-selective performance in cells, cross-validated through the use of knockout, selenium starvation, knockin, and chemical inhibitors.(2) Its background-free fluorogenicity enabled us to perform the first quantitative high-throughput live-cell screen for TrxR1 inhibitors, which indicated that tempered $S_NAr$ electrophiles may be more selective TrxR drugs than the classical electrophiles used hitherto. The **RX1** design thus sets the stage for *in vivo* imaging of the activity of this key oxidoreductase in health and disease and can also drive TrxR1-inhibitor drug design.

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# DISCOVERY OF THE FIRST FKBP51 PROTACs TO TARGET THE SCAFFOLDING FUNCTION OF FKBP51

**Min Zheng, Michael Walz, Thomas Geiger, Tianqi Mao, Christian Meyners, Felix Hausch**

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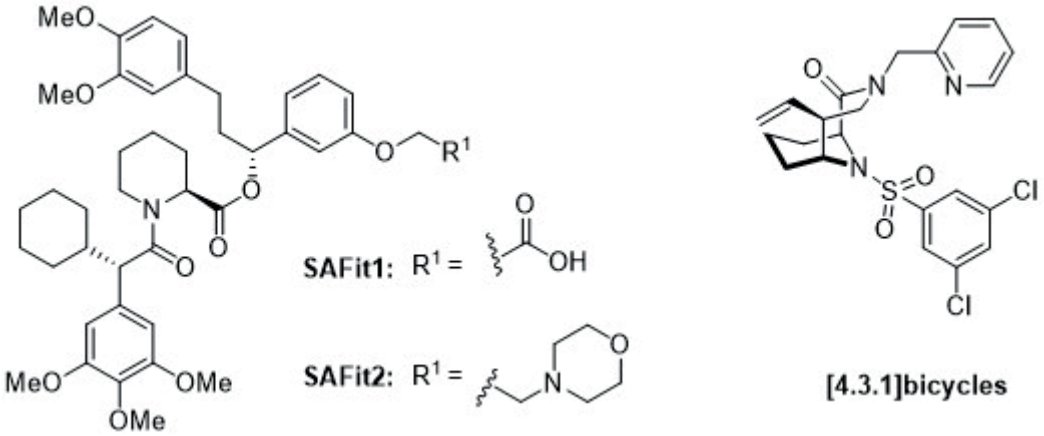
Knockout studies have validated a key role of the FK506-binding protein in animal models of depression, obesity and chronic pain.[1]

Several non-immunosuppressive FKBP51 ligands such as SAFit1, SAFit2 and [4.3.1] bicycles were developed, which potently bind the FK506-binding site of FKBP51. [2-6]

However, biochemical studies showed that some of the scaffolding functions of FKBP51 are unaffected by these FK506 analogs.[1,2]




However, biochemical studies showed that some of the scaffolding functions of FKBP51 are unaffected by these FK506 analogs.[1,2]

We thus set out to develop proteolysis targeting chimeras (PROTACs) based on the available ligands. Over 200 PROTAC candidates (PreTACS) were prepared and analyzed using different exit vectors, E3 ligase ligands and linkers. The small homolog FKBP12 turned out to be a superbly degradable targets (>30 PROTACs with a $DC_{50}$ >20 pM), whereas only 6 PROTACs degraded FKBP51 and only one PROTAC minimally degraded FKBP52. Degradation efficacy of VHL-based PROATCs strongly correlated with positive cooperativity of ternary complex formation. Optimization of the best initial FKBP51 PROTAC led to the potent and selective FKBP51 PROTAC SelDeg51(MWa558), which degraded FKBP51 and could potentially enhance glucocorticoid receptor signaling in HeLa cells.

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# NOVEL ACTIVATORS OF SLACK POTASSIUM CHANNELS FOR THE TREATMENT OF HISTAMINE-INDEPENDENT AND CHRONIC ITCH

**Annika Balzulat (1), Wenxin Felix Zhu (2), Cathrin Flauaus (1), Victor Hernandez-Olmos (3), Jan Heering (3), Mariam Dubiel (4), Amelie Tjaden (2,6), Katharina Metzner (1), Ruirui Lu (1), Robert Lukowski (5), Peter Ruth (5), Stefan Knapp (2,6), Susanne Müller (2,6), Dieter Steinhilber (2), Holger Stark (4), Ewgenij Proschak (2), Achim Schmidtko (1)**

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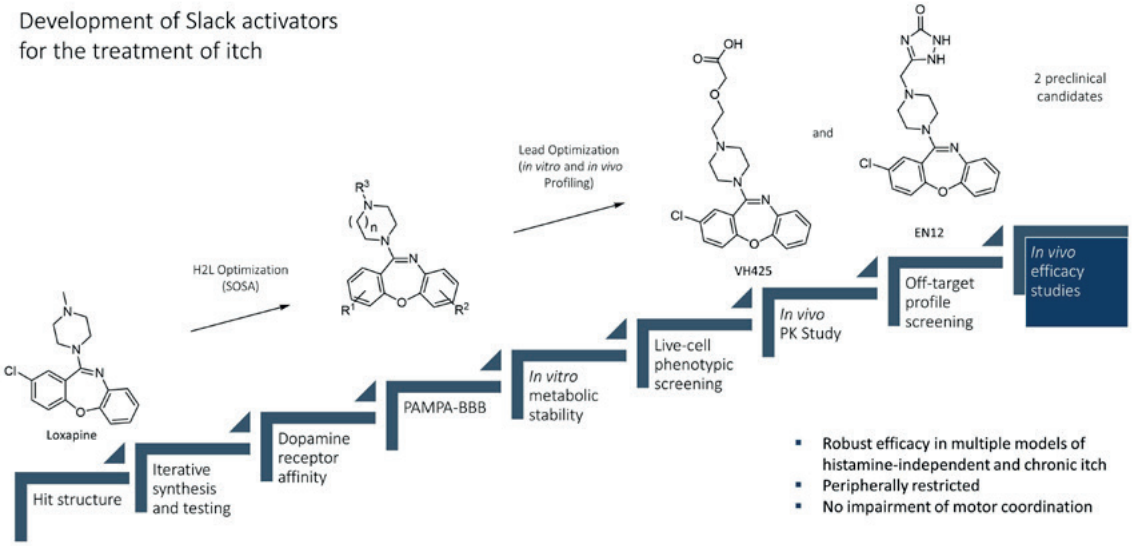


Chronic itch is defined as an unpleasant sensation that evokes a desire to scratch for longer than 6 weeks. In contrast to acute itch, which is an important protective mechanism, chronic itch does not serve a useful function and instead is associated with suffering and a compromised quality of life. It is estimated that around 15% of the general population is affected by chronic itch[1-2] but currently available therapies are often ineffective. Thus there is an unmet need to develop new treatment strategies.[3-5] We hypothesized that pharmacological activation of Slack, a potassium channel highly expressed in itch-sensitive sensory neurons, might hold therapeutic potential for the treatment of itch. We herein present our results on the development of Loxapine-based Slack activators with robust efficacy in several *in vivo* models of histamine-independent and chronic itch, which I) establish Slack as a novel target for the treatment of itch and II) disclose our most potent drug-like lead structures with reduced adverse effects through loss of original dopamine receptor affinity.

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# DISCOVERY OF NEW HUMAN Hv1 PROTON CHANNEL INHIBITORS

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Voltage-gated proton channels (Hv1) are proton-selective, voltage-dependent channels found in various cell types. They are responsible for maintaining intracellular and extracellular pH within physiological values, which is essential for many biological functions such as proliferation, mobility, and apoptosis.[1] Recent studies suggest that Hv1 plays a key role in proton extrusion in tumor cells, and its overexpression has been linked to tumor malignancy in some cases. In an acidic microenvironment, tumor cells can adapt extremely well while immune cell functions are compromised. Currently, there are no known potent and selective inhibitors of Hv1 channels. A selective Hv1 inhibitor would allow us to study how acidity of the tumor microenvironment affects tumor cell functions and tumor growth.[1,2]

The aim of our work was to discover and evaluate a new series of Hv1 inhibitors. We used an open structure of the human Hv1 channel to perform a virtual screen (VS) of an in-house library of compounds.[3,4] The compounds were docked to the binding site of guanidine derivatives at the voltage-sensing domain.[5] A series of molecules were selected and tested by manual patch-clamp technique on CHO and HEK cells expressing hHv1 and other channels. Seven hits were found to block more than 50% of proton currents at 50 μM (Fig. 1a). The results show that compound NZ-58 blocks channels in a dose-dependent manner, that it binds when VSDs are resting or deactivated, and that the binding rate is state-independent. It is likely that binding occurs from the extracellular side. Most hit molecules exhibited low selectivity because they also inhibited voltage-gated sodium and potassium channels. However, compound NZ-13 had lower affinity for the other channels than for Hv1 and the smallest effect on T-cell proliferation (Fig. 1b). Based on these results, a small series of analogues was prepared and evaluated. Overall, we have obtained a solid starting point for the development of new potential Hv1 inhibitors. The selected promising hits will be used for further hit-to-lead optimization to obtain molecules with improved inhibitory potency, better hHv1 channel selectivity, and desired physicochemical properties.

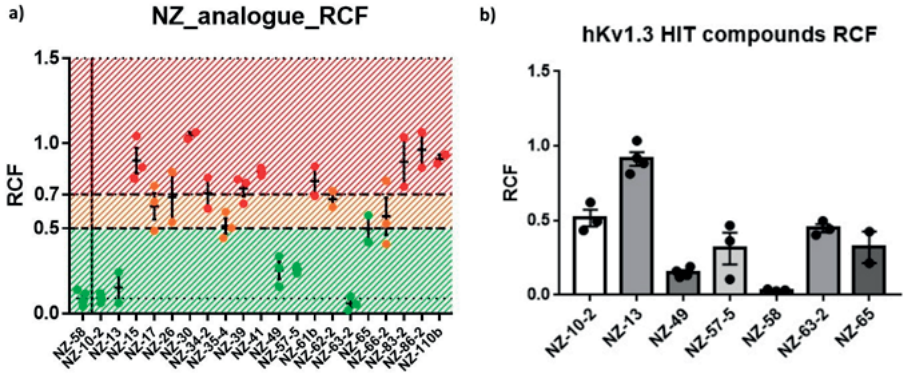


**Figure 1.** Effects of NZ analogues on Hv1 and Kv1.3 currents. a) Remaining current fraction (RCF) for Hv1 measured at +100 mV in the presence of 50 μM of compounds. b) Remaining current fraction (RCF) for Kv1.3 measured in the presence of 50 μM of compounds.

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# RECOGNITION OF DNA STRUCTURES BY A SERIES OF PHENANTHRIDINE DERIVATIVES

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Phenanthridine derivatives are some of the most researched groups of biologically active compounds that efficiently bind to DNA. Over the years, they have become a symbol of the intercalative way of binding to DNA and have been used for decades as the "gold standard" of DNA and RNA fluorescent markers, such as ethidium bromide (1). Phenanthridine derivatives show antifugal, antitumor, antibacterial and cytotoxic activity and when they bound to polynucleotides show an increase in fluorescence, which is why they are interesting as possible therapeutics and fluorescent markers for nucleic acids (2,3). We have synthesized four new phenanthridine derivatives. Our intent was to evaluate the influence of phenanthridine derivatives on recognition of various single-stranded, double-stranded and triple-stranded DNA/RNA structures using several biophysical methods including thermal melting, fluorescence and circular dicroism. MTT assay was applied for *in vitro* study on various tumor cell lines.

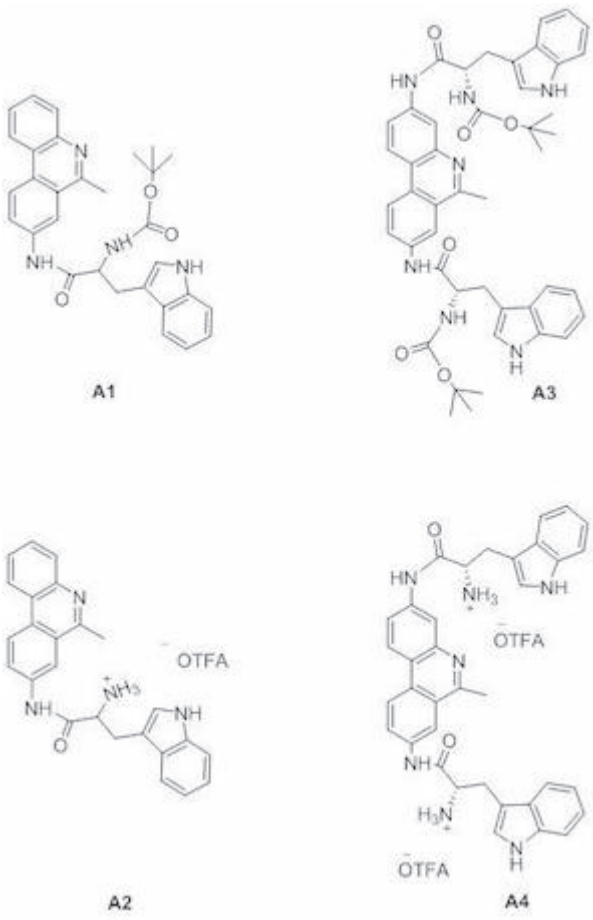


Figure 1. Structures of synthesized phenanthridine derivatives.

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# MESOPOROUS SILICA-BASED NANODEVICE FOR TARGETED CANCER THERAPY

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Cancer remains the major global health issue which continues to impact life expectancy. Many treatment approaches have been developed to address this disease, including surgery, radiotherapy, and chemotherapy [1,2]. Among these, conventional chemotherapy is considered the most effective treatment option for various types of cancer. However, the efficacy of most anticancer agents is hampered by several challenges, including short half-life, low bioavailability, multidrug resistance, non-specific- distribution, and difficulty in crossing physiological barriers. As a result, patients often experience more adverse effects than benefits.

Doxorubicin (DOXO) is a potent anthracycline anticancer drug with a broad spectrum of antineoplastic activity. It is widely used in the treatment of many human cancers, such as breast, ovarian, thyroid, multiple myeloma, and sarcoma [3]. Nevertheless, the use of DOXO in cancer chemotherapy presents serious drawbacks, especially cardiotoxicity, myelosuppression, nephrotoxicity, and the risk of extravasation, which limit its clinical application [4].

In recent years, the rapid advancement of nanotechnology has introduced new ideas and approaches for cancer treatment, particularly in the exploration of novel drug delivery systems. The delivery of anticancer drugs through a nanocarrier is a successful strategy to improve efficacy and safety in cancer therapy. Drug delivery systems (DDSs) offer the benefits of site-specific drug targeting, controlled release, lower dose administration, and reduced side effects. As a result, numerous nanomaterials have gained considerable attention as promising DDSs. Among these, mesoporous silica nanoparticles (MSNs) have emerged as versatile and advanced nanosystems, owing to their advantageous features, including high surface area, large pore volume, tuneable particle size, easy surface functionalization, ability to carry different drugs, good biocompatibility, and low toxicity [5]. Internal surface modifications with responsive functional groups ensure the release of drug under specific physiological conditions, while targeting molecules on the external surface guide MSNs to deliver the drug selectively to the desired tissue [6].

In this study, we present a mesoporous silica-based nanodevice (MSN), named FOL-MSN-DOXO, which incorporates the antineoplastic drug DOXO linked to the MSN pores via a hydrazone bond, and exhibits folic acid (FOL) as a targeting function on the MSN external surface. The nanodevice is designed to release DOXO in response to the acidic tumor microenvironment. In vitro experiments were conducted to assess the efficacy of FOL-MSN-DOXO against folate receptor overexpressing (FR+) cancer cells (HeLa and T47D cells), as well as normal FR-low cells (3T3L1 normal fibroblasts). FOL-MSN-DOXO selectively killed FR+ cancer cells while sparing the FR-low normal cells, whereas free DOXO showed toxicity towards all tested cell lines. Notably, the nanocarrier alone, without the inclusion of the drug, FOL-MSN, exhibited no signs of toxicity, clearly indicating the remarkable biocompatibility and safety of the vehicle itself.

These promising findings highlight the potential of MSN-based technology, making it a valuable and significant contribution to targeted cancer treatment.

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# DEGRADATION OF CYCLIN-DEPENDENT KINASE 9/CYCLIN T1 BY OPTIMIZED MICROTUBULE-ASSOCIATED PROTEIN 1 LIGHT CHAIN 3 BETA RECRUITING COUMARIN ANALOGS

**Yanping Zeng, Jian Xiao, Yuanxin Xu, Fan Wei, Lina Tian, Yinglei Gao, Yi Chen, Youhong Hu**

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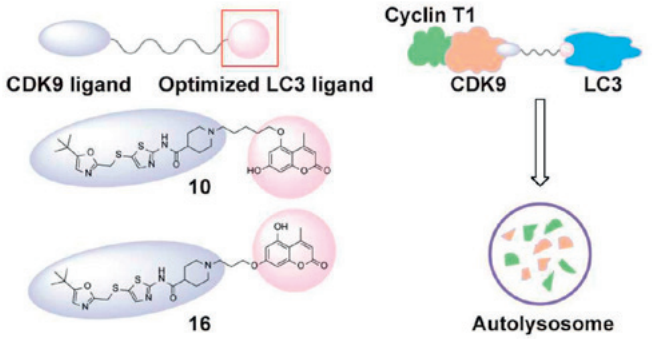
Autophagy is an efficient and attractive protein degradation pathway in addition to the ubiquitin-proteasome system. Herein, systematic optimization of coumarin analogs linked with CDK9 inhibitor SNS-032 is reported that may bind to cyclin-dependent kinase 9 (CDK9) and microtubule-associated protein 1 light chain 3 beta (LC3B) simultaneously, which leads to the selective autophagic degradation of targeted CDK9/cyclin T1 and is different from the PROTAC degrader THAL-SNS-032. Further mechanism studies revealed an autophagy-lysosome pathway, where the degraders formed a ternary complex with CDK9 and LC3B. Furthermore, the degrader **10** showed antitumor efficacy in vivo. Our work optimized a potent LC3B recruiter and demonstrated the feasibility of autophagy-tethering compounds (ATTECs), which could be applied for the degradation of diverse intracellular pathogenic proteins to treat related diseases.




**Modification of coumarin analogs to the selective autophagic degradation of targeted CDK9/cyclin T1 through an autophagy-lysosome pathway**

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# OPTICAL RESOLUTION OF KETOMORPHANS ENABLES THE CONSTRUCTION OF NATURAL PRODUCT-INSPIRED COMPOUND LIBRARIES

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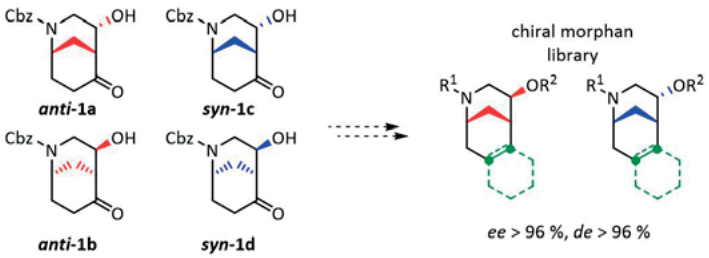
The morphan heterocycle is present in numerous natural products[1] that exhibit diverse bioactivities ranging from immunosuppressive (e.g., FR9010483) and cytotoxic agents (e.g., Madangamine A) to the well-known opioid receptor agonists (e.g., Morphine). The complex stereochemistry of these alkaloids often plays decisive roles for bioactivity and selectivity, asking for synthetic routes that address stereochemical features. In this context, ketomorphan isomers **1a-d** are promising intermediates for the synthesis of chiral, morphan-inspired compounds [2-3], considering the broad orthogonal transformation possibilities of the given functionalities.[4] However, complete stereo-control of this building block has not been fully achieved, partially due to the poor synthetic accessibility of the *syn*-diastereomers **1c-d**. Here, the synthesis, isolation and enrichment of previously uncharacterized *syn*-ketomorphans **1c-d** is described. In combination with robust optical resolution strategies, all ketomorphan isomers **1a-d** can be isolated in high enantiopurities on multi-gram scale, enabling the synthesis of chiral, morphan-derived compound libraries.

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# DISCOVERY OF NOVEL TRICYCLIC-BASED AMINES AS SELECTIVE CHOLINESTERASE INHIBITORS AND N-METHYL-D-ASPARTATE RECEPTOR ANTAGONIST

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Alzheimer's disease (AD) is a complex disorder.[1] Because of it, there are several strategies for the development of novel derivatives intended not just for its treatment but also to better the understanding of this illness with still unknown etiology. Combination of two or more therapeutic interventions into one molecule is believed to provide great benefits for AD.[2] The fluoren-9-amine analogues were designed with currently used drugs in mind.[3] Cholinesterase (ChE) inhibitors and *N*-methyl-D-aspartate (NMDA) receptor antagonist are used in symptomatic therapy of AD.[2] An initial assessment of the biological profile of novel derivatives included determination of the ChE inhibition and NMDA receptor antagonism at the GluN1/GluN2A and GluN1/GluN2B subunits, along with a cytotoxicity profile in the Chinese hamster ovary (CHO-K1) cell line.[3] All tested compounds displayed selective butyrylcholinesterase (BChE) inhibition and antagonistic activity at NMDA receptors. Enzyme kinetic study showed that prepared derivatives are competitive inhibitors of BChE and according to the docking study, the tricyclic core of new derivatives interact within the anionic site of the enzyme in a similar was as a well know ChE inhibitor, and also a template drug, tacrine. Moreover, the *in silico* prediction suggested oral availability and permeation through the blood-brain barrier for all of the novel analogues, making them interesting compounds for the treatment of AD.

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# UNEXPECTED ISOMERIZATION OF OXETANE-CARBOXYLIC ACIDS

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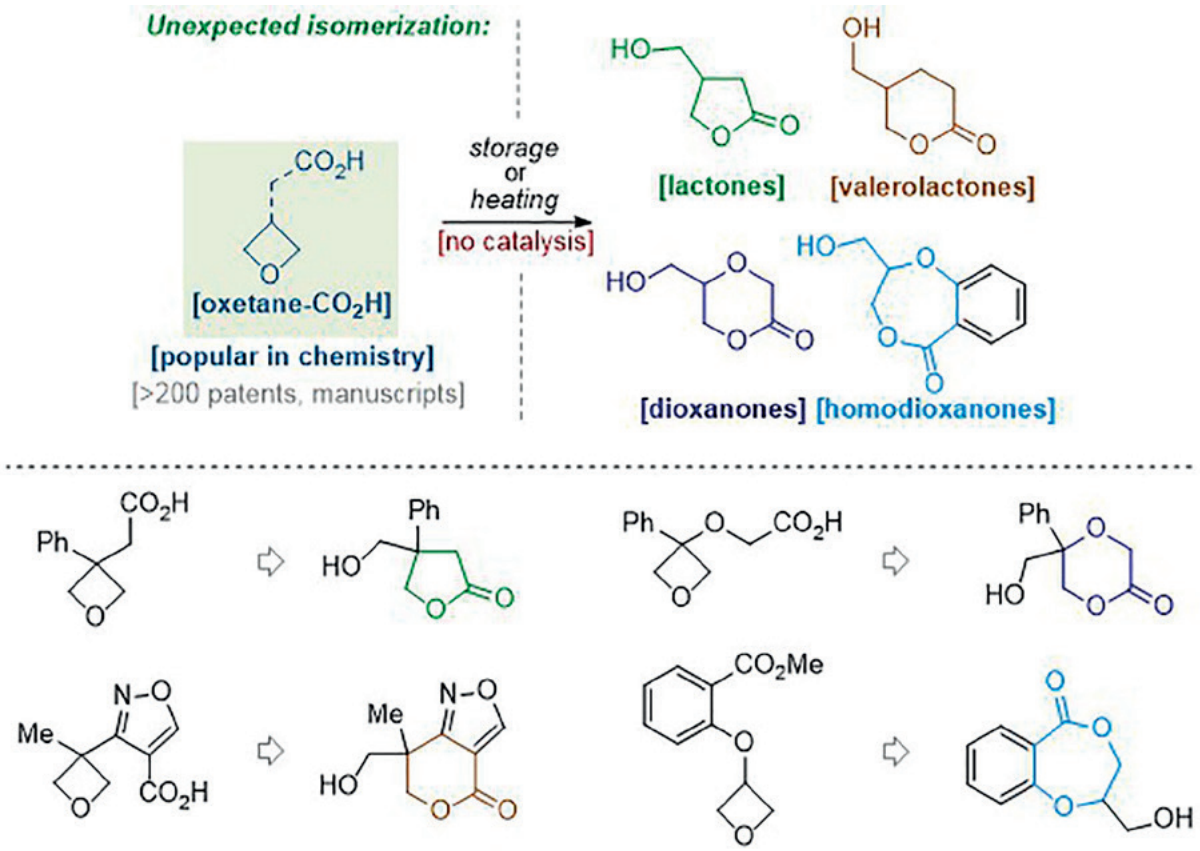
During the past decade, oxetanes have been playing an important role in chemistry as bioactive compounds and valuable starting materials in synthesis. Oxetane-carboxylic acids have been used in more than 200 patents.[1] We unexpectedly discovered that many of these molecules were unstable. Some of them isomerized into lactones under simple storage at room temperature, others - under slight heating. Chemists should keep in mind the high instability of these molecules, as this could dramatically affect the reaction yields and lead to negative results (especially in those reactions that require heating).Here, we want to disclose this previously unknown phenomenon in the literature.[2,3]

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# SYNTHESIS AND CHARACTERIZATION OF A NOVEL MASTL INHIBITOR MKI-2 TARGETING MASTL-PP2A IN BREAST CANCER CELLS

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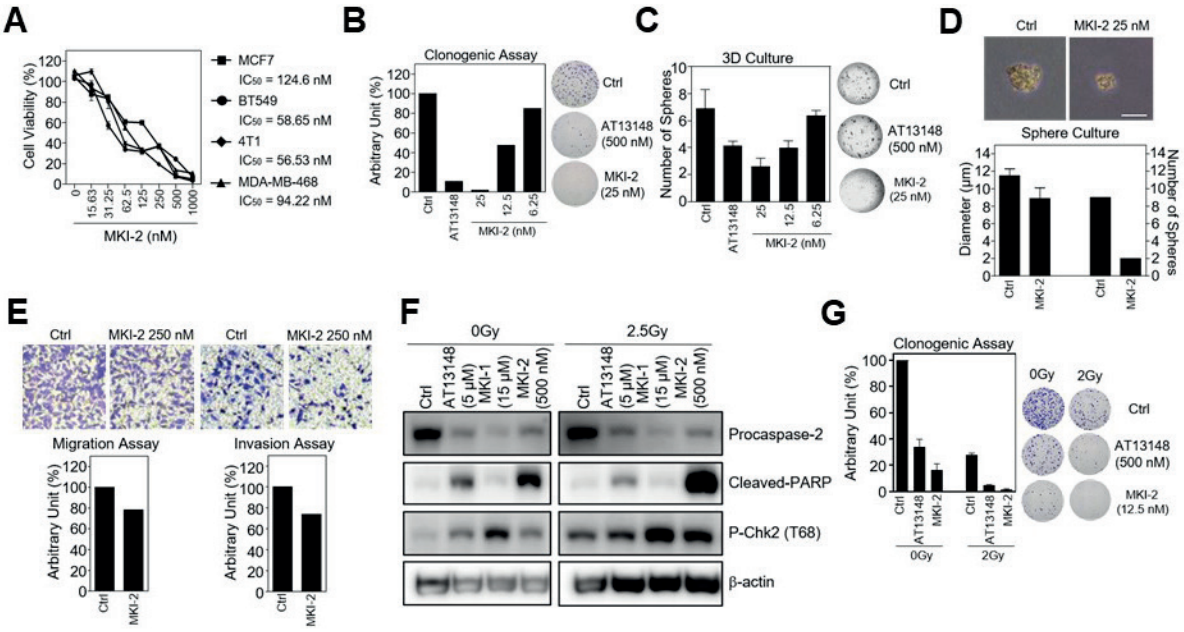
Microtubule-associated serine/threonine kinase(MASTL), also known as Greatwall kinase, is a promising target for selective anticancer therapy. Although it is an important anticancer target, no potent mastl inhibitor has been reported to date. Here we discovered a new potent and selective MASTL inhibitor, MASTL-2 kinase inhibitor (MKI-2), identified in silico as part of a drug discovery program. Our data showed that MKI-2 inhibited recombinant MASTL activity and cellular MASTL activity with $IC_{50}$ values of 37 nM and 142 nM, respectively, in breast cancer cells. Additionally, MKI-2 inhibited MASTL kinase rather than other AGC kinases, such as ROCK1, AKT1, PKAAC, and p70S6K. Moreover, MKI-2 exerted various antitumor activities by inducing mitotic catastrophe resulting from the modulation of the MASTL-PP2A axis in breast cancer cells. MKI-2 treatment showed phenocopies with MASTL-null oocytes in mouse oocytes, which was used as a model to validate MKI-2 activity. Therefore, our study provided a novel potent and selective MASTL MKI-2 inhibitor targeting the oncogenic axis MAST-PP2A in breast cancer cells.

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# IDENTIFICATION OF NOVEL, POTENT AND ORALLY AVAILABLE ALLOSTERIC SHP2 INHIBITORS FOR CANCER THERAPY

**Alessia Petrocchi, Danilo Fabbrini, Alessio Sferrazza, Ilaria Rossetti, Alina Ciammaichella, Esther Torrente, Federica Ferrigno, Monica Bisbocci, Antonella Cellucci, Martina Nibbio, Annalise Di Marco, Francesca Puca, Valentina Fodale, Jerome Amaudrut, Cristina Alli, Vincenzo Pucci, Christian Montalbetti, Alessandro Carugo, Carlo Toniatti**

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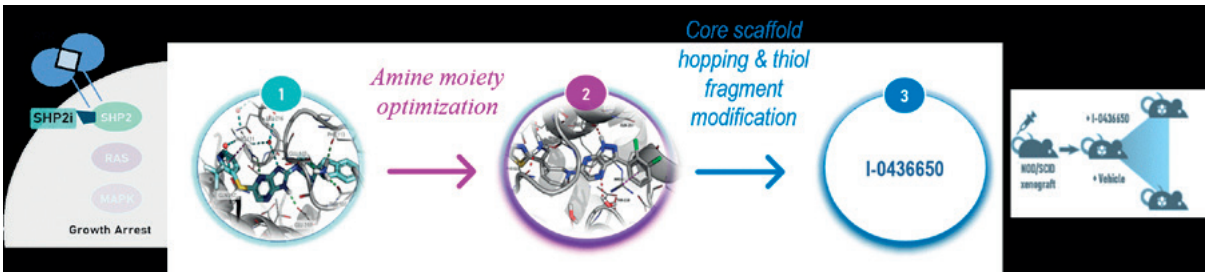
The protein tyrosine phosphatase SHP2 (Src homology-2 domain-containing protein tyrosine phosphatase-2) is a critical regulator of multiple signal transduction pathways such as RTK/RAS/ERK, and PD-1/PD-L1[1]. Therefore, inhibition of SHP2 is considered a promising therapeutic strategy to target RAS-driven cancers and to modulate immune signaling pathways within the tumor microenvironment. The first report of a small molecule capable to inhibit SHP2 activity through an allosteric mechanism triggered the development of various SHP2 inhibitors which are currently in clinical development, both in monotherapy and in combination with inhibitors of $KRAS^{G12C}$, BRAFV600E, CDK4/6, MEK, EGFR, ERK, and PD-1.[2]




We present the identification and development of multiple novel series of SHP2 inhibitors starting from imidazopyrazines[3] and azabicyclic analogs[4], culminating with the discovery of I-0436650, a potent selective and orally bioavailable SHP2i. Imidazopyrazines[3] were first confirmed to be SHP2 allosteric inhibitors by co-crystallization studies. Additional series bearing a unique primary amine were then developed. This optimization paved the way towards an orally bioavailable series to which our preclinical candidate I-0436650 belongs. I-0436650 is a potent and specific inhibitor of the SHP2 enzyme ($IC_{50}$ = 4 nM) capable of achieving dose-dependent inhibition of the RAS/MEK/ERK signaling pathway. I-0436650 exhibits significant antiproliferative activity against multiple RAS and EGFR mutant cancer cell lines and its combination with several other pharmacological agents results in synergistic tumor growth inhibition. Furthermore, I-0436650 is strongly efficacious at well tolerated doses in human xenograft models both as monotherapy and combination therapy.

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# AROMATIC ISOSTERE OF INTRAMOLECULAR H-BONDING IN SEROTONIN 2B-RECEPTOR ANTAGONISTS

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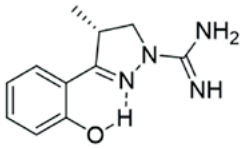
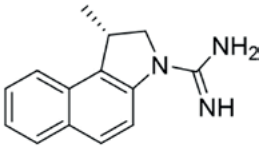
Intramolecular H-bonding is a prominent feature of the phenolic 5-$HT_{2B}$ antagonists described in WO2016/207231A1. The H-bonding can be detected by NMR and is evident from X-ray crystallography data.

The phenolic moiety in drug compounds can improve aqueous solubility, but can also cause certain liabilities related to DMPK/ADME properties, e.g. increased oxidative metabolism and conjugation.

Based on the 3-D structures (NMR and XRD) of the phenolic 5-$HT_{2B}$ antagonists, a new isosteric class of 5-$HT_{2B}$ antagonists was designed, the tri-cyclic naphthyl derivatives (WO2020254322A1), in which the phenol-pyrazoline hydrogen bridge is replaced with aromatic carbon bonds.




phenolic
5-$HT_{2B}$ antagonists




tri-cyclic naphthyl
derivatives

The synthetic methods of the compound classes will be described along with biological data that shed light on the structural integrity of the ligand binding mode and other properties of the aromatic isostere in relation to the phenolic H-bonded structure.

Overall, data favor the phenol-pyrazoline structure, indicating some “phenol protection” conveyed by the intramolecular H-bonding.

# DEVELOPMENT OF ZIKA PROTEASE INHIBITORS BY FRAGMENT-BASED DESIGN

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Zika virus is a mosquito-borne flavivirus that has been associated with severe neurological conditions including microcephaly and Guillain-Barré syndrome. Its ability to spread rapidly was highlighted in the 2015 epidemic across predominantly South and Central America, and evidence suggests a re-emergence is increasingly likely due to urbanisation, intercontinental travel, and a warming climate.[1] Despite this threat, there are currently no approved medical interventions (therapeutic or prophylactic) against the Zika virus. The Zika NS2B-NS3 protease is a highly attractive drug target, and its structural similarity to the NS2B-NS3 protease of other flaviviruses, including that of Dengue virus, opens the door towards potential pan-flaviviral protease inhibitors. However, the NS2B-NS3 protease is a difficult-to-drug target, owing predominantly to the highly acidic nature of its shallow binding site. As such, high affinity inhibitors often show undesirable physicochemical properties, and few promising small molecule inhibitors have been reported.[2]

Fragment-based drug design (FBDD) is a robust method of developing efficient inhibitors against difficult-to-drug targets.[3] Following a fragment-based approach, we have identified and characterised a series of fragments that bind at the protease active site. SAR-by-catalogue, computational design and ligand-based design have been used in parallel to yield a series of novel monobasic small molecules that show inhibitory activity in a functional enzymatic assay.

This presentation will discuss fragment screening, hit selection and the development of novel small molecule inhibitors of Zika NS2B-NS3 protease. There will be an emphasis on the development and optimisation of biophysical binding assays suitable for weak affinity compounds early in the drug discovery campaign against Zika NS2B-NS3 protease. Ongoing work will be described including further inhibitor elaboration as well as the application of a new PAC-FragmentDEL screen to identify pan-flaviviral fragment hits that may provide promising starting points for the development of novel antivirals against both Zika and Dengue viruses.[4]

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# APPROACHES FOR DEVELOPING ANTIVIRALS AGAINST FATAL HUMAN CORONAVIRUSES

**Thanigaimalai Pillaiyar (1), Philipp Flury (1), Nadine Krüger (2), Katharina Sylvester (3), Michael Gütschow (3), Christa E. Müller (3), Stefan Laufer (1)**

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In humans, coronaviruses cause multiple respiratory and other organ infections that can range from mild to lethal. Beta-coronaviruses, such as SARS-CoV-1, Middle-East respiratory syndrome (MERS)-CoV, and, in particular, SARS-CoV-2 (nCoV-19), are extremely dangerous because they can be easily transmitted from person to person. SARS-CoV-1, which first appeared in 2003, had affected 8422 people in 32 countries, with 916 of them dying, for a fatality rate of 10-15%. MERS-CoV, which appeared in 2012, affected a total of 1401 individuals worldwide, 543 of whom died, with a mortality rate of ~39%.

The new coronavirus, known as SARS-CoV-2 (nCoV-19), is responsible for the current COVID-19 pandemic. As of January 12, 2023, the outbreak of SARS-CoV-2 has claimed more than 6.5 million lives and infected more than 670 million people around the world. Public life had come to a halt as many governments imposed social distancing strategies and travel bans to prevent the further spread of the virus. The development of antiviral drugs that are effective against SARS-CoV-2 is a major priority in the battle against COVID-19, especially with the emergence of variants that may elude vaccines [1].

Proteases play important roles in various stages of viral replication. Cathepsin L, a human lysosomal cysteine protease, assists in the proteolytic activation of the SARS-CoV-1 and -2 spike proteins and enhances viral entry, a critical stage in viral replication. The papain-like protease ($PL^{pro}$) and, in particular, the main protease ($M^{pro}$, also known as $3CL^{pro}$), are required for the conversion of precursor polyproteins into functional viral proteins [1,2]. The recent progress of our work in the identification and optimization of these protease inhibitors by enzyme inhibition and mechanism, structure–activity relationships, antiviral activity, X-ray structure determination, and the in vitro ADME will be presented. Selected compounds inhibit SARS-CoV-1 and -2, as well as MERS-CoV, implying that they could be used to treat a broader range of coronavirus infections [2,3].

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# SYSTEMATIC STUDY OF 1,2,3-TRIAZOLYL STEROLS FOR THE DEVELOPMENT OF NEW DRUGS AGAINST PARASITIC NEGLECTED TROPICAL DISEASES

**Exequiel Porta (1), Maria Sol Ballari (2), Renzo Carlucci (2), Shane Wilkinson (3), Guoyi Ma (4), Babu Tekwani (4), Edubiel Alpizar Sosa (1), David Nes (5), Guillermo Labadie (2)**

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*3) Queen Mary University of London, Mile End Road, London, E1 4NS, UK.*
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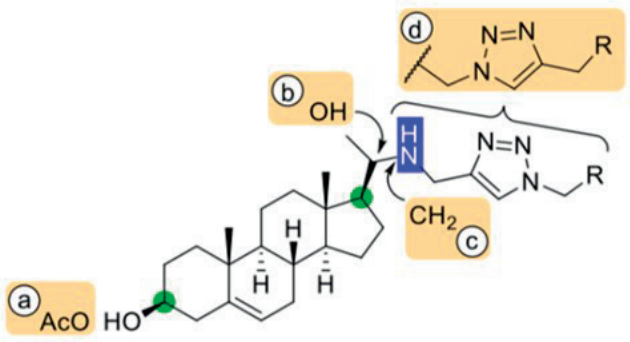
*5) Department of Chemistry & Biochemistry, Texas Tech University, Lubbock, TX 79409, USA.*

Neglected tropical diseases (NTDs) are a group of 20 conditions that primarily afflict tropical regions, where they affect over 1 billion people living in impoverished communities. This study presents the synthesis, characterization, biological activity, and molecular evaluation of a series of thirty five 1,2,3-triazolylsterols, prepared via a stereocontrolled synthesis methods inspired by azasterols with established antiparasitic activity. Ten of these synthesized compounds are chimeras/hybrids of 22,26-azasterol (AZA) and 1,2,3-triazolyl azasterols. The entire library (and their intermediates) of synthesized compounds was assayed against *Leishmania donovani*, *Leishmania mexicana*, *Trypanosoma cruzi*, and *Trypanosoma brucei*, causative agents of visceral leishmaniasis, cutaneous leishmaniasis, Chagas disease, and sleeping sickness, respectively.

Most of the compounds exhibited submicromolar/nanomolar concentrations of activity in these parasites, with high selectivity index relative to their cytotoxicity against mammalian cells (none were active in these cells at concentrations below 10 μM). The analogs demonstrating high activity and selectivity index (SI) against *L. donovani* ($IC_{50}$ 0.78 μM), *L. mexicana* ($IC_{50}$ 1.31 μM), *T. brucei* ($IC_{50}$ 0.12 μM), and *T. cruzi* ($IC_{50}$ 0.33 μM), as well as those with broad-spectrum antiparasitic activities against all the four kinetoplastid parasites, may be promising leads for further development as selective or broad-spectrum antiparasitic drugs. The activities exhibited by the hit compounds surpass the control drugs by up to an order of magnitude. Furthermore, *in silico* physicochemical properties analysis was conducted to rationalize the activities and the SAR against these parasites.

The best-performing compounds were used to elucidate the molecular target, through enzyme inhibition assays against recombinant *L. mexicana* Sterol 24-C-methyltransferase (*Lm*SMT), docking calculations, and biological activity assays (and sterol profiles by GC-Ms) against a panel of *L. mexicana* resistant to polyene antifungals amphotericin B (AmBR) and nystatin (NysR), with specific mutations in enzymes involved in the sterol pathway. Time-to-kill and dose-to-kill assays were performed to determine cell viability of wild-type, different mutants of *L. mexicana,* and SMT-overexpressed *L. mexicana*. A comprehensive description of these details, along with the results obtained, will be provided during the Symposium.

Overall, this study provides significant insights into the design and development of novel antiparasitic agents for treating parasitic neglected tropical diseases.

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## NOTES

# List of Abstracts

| | | |
|---|---|---|
| ABDEL-JALIL Raid Jamil | ASYMMETRIC SYNTHESIS AND THEORETICAL INVESTIGATION OF 4,5-DIHYDRO-1H-[1,2,4]-TRIAZOLE USING DFT CALCULATION AND X-ray CRYSTALLOGRAPHY | P002 |
| ACH Bram | DEVELOPMENT OF A NEW CARBON-FOR-NITROGEN SWAPPING PARADIGM AS A RAPID AZA-ANALOGING TOOL | P003 |
| AL-KHDHAIRAWI Amjad Ayad Qatran | DEAROMATIVE GOLD(I)-CATALYZED (3+2) CYCLOPENTANNULATION OF INDOLES | P004 |
| ALTINTOP Mehlika Dilek | A NEW SERIES OF HYDRAZONE-BASED ANTI-INFLAMMATORY AGENTS ENDOWED WITH SELECTIVE COX-1 INHIBITORY POTENCY | P005 |
| ALVAREZ Guzman | PRECLINICAL STUDIES AND DRUG COMBINATION OF LOW-COST MOLECULES FOR CHAGAS DISEASE AND LEISHMANIASIS | P006 |
| ALYAMI Bandar Ali A | DESIGN, SYNTHESIS, IN-VITRO CARBONIC ANHYDRASE-II / IX, CHOLINESTERASE AND MONOAMINE OXIDASE INHIBITION STUDIES OF C-3 PREGNENOLONE- SULFAMOYLPHENYL THIOUREA DERIVATIVES | P007 |
| AMAMEA Landry | THE TREATMENT OF VIRUS INFECTION-INDUCED INFLAMMATIONS WITH NOVEL PIEZO1-AGONISTS | P008 |
| ANSELMI Michele | DISCOVERY OF SMALL-MOLECULE INHIBITORS OF MICRORNA-21 BIOGENESIS | P009 |
| ANTONENKO Anastasiia | OaAEP1 LIGASE-ASSISTED CHEMOENZYMATIC TOTAL SYNTHESIS OF CYANOBACTERIAL METALLOTHIONEIN SmtA | P010 |
| ARBACIAUSKIENE Egle | BIOISOSTERIC REPLACEMENTS IN DEVELOPMENT OF SECOND-GENERATION 2,6-SUBSTITUTED 2H-PYRAZOLO[4,3-c]PYRIDINES | P011 |
| ARNOLD Malte | A SYSTEMATIC APPROACH TOWARDS POTENT CK1δ INHIBITORS | P012 |
| AZZOUZ Jihed | DESIGN AND SYNTHESIS OF NOVEL RNA LIGANDS AS INHIBITORS OF ONCOGENIC MICRORNAS PRODUCTION | P013 |
| BACH Anders | TARGETING PROTEIN-PROTEIN INTERACTIONS INVOLVED IN OXIDATIVE STRESS USING FRAGMENT-BASED DRUG DISCOVERY | P014 |
| BALOURDAS Dimitrios-Ilias | DARPINS AS BIOLOGICAL TOOLS TO REACTIVATE WILD-TYPE AND MUTANT p53 IN CANCER CELLS | P015 |
| BARTOLIC Marija | QUINUCLIDINIUM BASED O-ALKYL OXIMES AS POTENTIAL ANTICHOLINESTERASE AGENTS IN SYMPTOMATIC TREATMENT OF ALZHEIMER'S DISEASE | P016 |
| BASARIC Nikola | SYNTHESIS AND PHOTOCHEMISTRY OF BODIPY COMPOUNDS WITH ANTIPROLIFERATIVE ACTIVITY AND POTENTIAL THERANOSTIC APPLICATIONS | P017 |
| BAYRAK Nilufer | NEW ISOQUINOLINEQUINONE ANALOGS AS POTENTIAL ANTICANCER AGENTS | P018 |
| BEHBEHANI Haider | PALLADIUM-CATALYZED HIGH PRESSURE-ASSISTED STRATEGY FOR SYNTHESIZING DIAZA-DIBENZO[A,E]AZULENE AND DIAZA-BENZO[A]FLUORENE DERIVATIVES VIA CROSS-DEHYDROGENATIVE COUPLING AND THEIR CYTOTOXIC ACTIVITY | P019 |
| BENDALE Prachi | LIGHTING UP THE BAD BUGS: FLUORESCENT LABELLING OF THE BACTERIAL RESISTANCE FACTOR ARNT WITH COMMERCIAL FLUOROPHORES | P020 |
| BENEK Ondrej | 2-(BENZYLOXY)ARYLUREA INHIBITORS OF MPTP DO (NOT) TARGET HUMAN CYCLOPHILIN D | P021 |
| BESSET Tatiana | SYNTHESIS OF FLUORINATED MOLECULES USING MODERN SYNTHETIC TOOLS | IL04 |
| BEVERNAEGE Kevin | SITE SELECTIVE GOLD(I)-CATALYSED BENZYLIC C-H AMINATION VIA AN INTERMOLECULAR HYDRIDE TRANSFER TO TRIAZOLINEDIONES | P022 |
| BIELAWSKA Anna | THE EFFECT OF A NOVEL 4-THIAZOLIDINONE DERIVATIVE IN COMBINATION WITH ANTI-HER2 ANTIBODIES ON AUTOPHAGY IN HUMAN AGS GASTRIC CANCER CELLS | P023 |

| | | |
|---|---|---|
| BIELAWSKI Krzysztof | THE IN VITRO CYTOTOXIC AND APOPTOTIC ACTIVITY OF NEW THIOPYRANO[2,3-d]THIAZOLES | P024 |
| BLOCHOUSE Estelle | VOCs ART : VOC-BASED PROBES FOR EXPLORATION, DETECTION AND MONITORING OF PATHOLOGIES | P025 |
| BODE Jeffrey | SYNTHETIC AND MEDICINAL CHEMISTRY FOR BIOLOGICS | IL01 |
| BORGARELLI Carlotta | NOVEL FAR-RED FLUORESCENT 1,4-DIHYDROPYRIDINES FOR L-TYPE CALCIUM CHANNELS IMAGING | P026 |
| BORSARI Chiara | DEVELOPMENT OF SELECTIVE mTOR INHIBITORS FOR THE TREATMENT OF CANCER AND NEUROLOGICAL DISORDERS | OC05 |
| BOSAK Anita | N-OCTYLAMINO DERIVATIVES OF 4-AMINOQUINOLINE AS POSSIBLE MULTIFUNCTIONAL AGENTS FOR ALZHEIMER'S DISEASE TREATMENT | P027 |
| BOSSERT Michael | DIAGONALLY PROTECTED 1-METHYLCYCLOBUTANE-1,3-DIAMINES IN ABROCITINIB ANALOGUES SYNTHESIS | P119 |
| BOSSERT Michael | THERMAL [2+2] SYNTHESIS OF 3-OXOCYCLOBUTYL BORONATES VIA KETENIMINIUM SALTS | P216 |
| BOUJUT Margot | OPTIMIZATION OF SELECTIVE AND POTENT $A_{2A}R$ NEGATIVE ALLOSTERIC MODULATORS AS IMMUNOTHERAPY FOR HIGH ADENOSINE CANCERS' TREATMENT | P030 |
| BREINBAUER Rolf | REDOX-SENSITIVE PROBES FOR THE ACTIVITY-BASED PROTEIN PROFILING OF OXIDOREDUCTASES | P031 |
| BREITENBUCHER James | BIAS AND THE MEDICINAL CHEMIST'S RELATIONSHIP WITH ADDITIVITY | P032 |
| BRICELJ Alesa | HETEROBIFUNCTIONAL E3 LIGASE RECRUITERS ENABLE PAN-DEGRADATION OF INHIBITOR OF APOPTOSIS PROTEINS | P033 |
| BRKLJACA Zlatko | ACTIVITY AND ENANTIOSELECTIVITY IN BIOCATALYSIS – INFLUENCE OF ORGANIC SOLVENTS AND ACTIVE SITE MUTATIONS | P034 |
| BROCKLEHURST Cara | MICROCYCLE: A MACHINE LEARNING DRIVEN, AUTOMATED AND INTEGRATED PLATFORM FOR DRUG DISCOVERY | IL03 |
| BRUUN Tanja | REGENERATIVE CARDIAC PHARMACOLOGY – DESIGN, SYNTHESIS, AND BIOLOGICAL EVALUATION OF NOVEL SMALL MOLECULE COMPOUNDS | P035 |
| BULJAN Andela | TOTAL SYNTHESIS AND BIOLOGICAL PROFILING OF N-METHYLATED MARINOAZIRIDINE A | P036 |
| CAR Zeljka | PREPARATION OF DISUBSTITUTED FERROCENYL DESMURAMYL PEPTIDES AND THEIR MANNOSYLATED ANALOGUES - NOVEL POTENTIAL ADJUVANTS | P037 |
| CARREIRA Monica | DEVELOPMENT OF NOVEL DRUG CANDIDATES FOR SELECTIVE INHIBITION OF BCL-2 PROTEIN–PROTEIN INTERACTIONS | P038 |
| CASTIELLO Carola | DEVELOPMENT OF FIRST-IN-CLASS DUAL LSD1-PRMT5 INHIBITORS FOR THE TREATMENT OF ACUTE MYELOID LEUKEMIA | P039 |
| CAVALLARO Palmira Alessia | A WAY TO BROAD SPECTRUM ANTIVIRAL AGENTS: SMALL MOLECULES BASED ON PIPERAZINE CORE AS POTENTIAL FLAVIVIRIDAE NS3 PROTEASE INHIBITORS | P220 |
| CAVALLARO Palmira Alessia | A WAY TO BROAD SPECTRUM ANTIVIRAL AGENTS: SMALL MOLECULES BASED ON PIPERAZINE CORE AS POTENTIAL FLAVIVIRIDAE NS3 PROTEASE INHIBITORS | P137 |
| CHAI Christina | A REMARKABLE NEW MODALITY FOR THE ANTI-INFLAMMATORY PROPERTIES OF THE NATURAL PRODUCT ANDROGRAPHOLIDE VIA THE DEGRADATION OF MAPK-ACTIVATED PROTEIN KINASE 2 | P042 |
| CHESNOKOV Gleb | SYNTHETIC STUDIES TOWARDS ARTATROVIRENOLS A AND B | P043 |
| CHODOUNSKA Hana | ISOSTERIC REPLACEMENT OF CARBOXYLIC ACID MOIETY TARGETTING IMPROVED PERMEABILITY AND STABILITY | P044 |

| | | |
|---|---|---|
| DONCKELE Etienne | TAKING MOLECULAR GLUE DEGRADERS TO NEW HEIGHTS | IL24 |
| DORMAN György | 3CL PROTEASE, A PROMISING TARGET FOR ANTI-COVID-19 THERAPY. EXPRESSION, PURIFICATION, AND ASSAY DEVELOPMENT | P066 |
| DUNKEL Petra | DEVELOPMENT OF QUINOLINE PHOTOCAGES FOR BIOLOGICAL APPLICATIONS | P067 |
| DYBECK Lovisa | DEVELOPMENT OF A COUNTER ION DIRECTED STEREOSELECTIVE AZA-PRINS MULTICOMPONENT REACTION | P068 |
| ESCOLANO Carmen | ANTI-ALZHEIMER PROPERTIES OF IMIDAZOLINE I2 RECEPTOR LIGANDS | P069 |
| ESHAK Floriane | EPITOPE IDENTIFICATION USING IN-SILICO APPROACHES, A CASE STUDY: NANOBODIES BINDING TO mGlu5 RECEPTOR | OC09 |
| ESPINOSA Victoria | PHARMACOPHORIC ANALYSIS OF ANTICANCER ACTIVITY OF ISOPRENOIDS AND TERPENES ISOLATED FROM SCHINUS MOLLE L. | P070 |
| FABBRIZI Emanuele | FIRST-IN-CLASS SELECTIVE INHIBITORS OF THE LYSINE ACETYLTRANSFERASE KAT8 | P071 |
| FERJANCIC BENETIK Svit | DESIGN, SYNTHESIS AND EVALUATION OF NOVEL INDAZOLE BChE/p38$\alpha$ MAPK DUAL INHIBITORS FOR THE TREATMENT OF ALZHEIMER'S DISEASE | P072 |
| FERNANDEZ AGUADO David | A NOVEL COPPER-CATALYZED Csp3 -Csp3 CROSS-COUPLING REACTION USING READILY AVAILABLE STARTING MATERIALS | P073 |
| FERRARA Isabella | SEMI-SYNTHESIS OF THIOGLYCOSIDE DERIVATIVES OF THE NATURAL PRODUCT ANTIBIOTIC FIDAXOMICIN | P074 |
| FLEETWOOD Thomas | PRACTICALLY-ACCESSIBLE N-TRIFLUOROMETHYLATION METHODS FOR USE IN ORGANIC SYNTHESIS AND MEDICINAL CHEMISTRY | P075 |
| FLEISCHER Ivana | MAKING AND BRAKING OF C-S BONDS USING METAL CATALYSIS | IL15 |
| FRANCOTTE Pierre | DESIGN OF POTENTIATORS TARGETING THE ALLOSTERIC POCKET OF THE KAINATE SUBTYPE GLUTAMATE RECEPTORS | P076 |
| FRIAS RODRIGUEZ Maria | AUTOMATED CONTINUOUS FLOW CHEMISTRY APPLICATION IN THE PHARMACEUTICAL INDUSTRY: PHOTOCHEMICAL C-N, C-O AND C-C CROSS COUPLINGS REACTIONS | P077 |
| FUKUDA Takeshi | DS42450411: A POTENT ORALLY ACTIVE HEPCIDIN PRODUCTION INHIBIROR FOR ANEMIA OF CHRONIC DISEASE | P078 |
| GAIDAR Rita | A NEW AI-DRIVEN APPROACH TO HIT IDENTIFICATION | P079 |
| GANTER Benedikt | SYNTHESIS OF MODIFIED T-1106-5´-TRIPHOSPHATES AS POTENTIAL INHIBITOR OF THE SARS-CoV-2 RdRp | P080 |
| GARCIA MANCHENO Olga | DIRECT ACCESS TO UNNATURAL DRUG-LIKE MOLECULES BY PHOTOCATALYTIC LATE-STAGE FUNCTIONALIZATION | OC07 |
| GEE Alexander | CHEMOTYPE EXPLORATION FOR POTENTIAL TAU AGGREGATION THERAPEUTICS | P081 |
| GEORAIEVA Maya | SYNTHESIS, IN SILICO DRUG LIKENESS ESTIMATION, IN VITRO NEUROTOXICITY AND NEUROPROTECTION ON SUBCELLULAR LEVEL AND IN VITRO MAO-B INHIBITORY EFFECTS OF NEW THEOPHYLLINE-7-THIOSEMICARBAZIDES | P082 |
| GERNINGHAUS Joshua | DESIGN, SYNTHESIS AND EVALUATION OF MACROCYCLIC DIAMINOPYRIMIDINES AS DUAL INHIBITORS FOR EPH AND GAK | P083 |
| GIBAE Kim | SWITCHING AGONIST TO ANTAGONIST: STRUCTURE-BASED DESIGN AND SYNTHESIS OF NOVEL NUCLEOSIDE $A2_A$ ADENOSINE RECEPTOR ANTAGONIST AS POTENTIAL IMMUNE-ONCOLOGY AGENTS | P084 |
| GILLARD Martin | DEVELOPING Ph-ACTIVATABLE RUTHENIUM (II) FLUORESCEIN PHOTOSENSITIZERS TOWARDS THERANOSTIC APPLICATIONS | P085 |
| GODOY Fernando | SYNTHESIS AND CHARACTERIZATION OF NEW ALKYL AMINES OF RE (I). STUDY AS A POSSIBLE OPTICAL MERCURY(II) SENSOR | P086 |

| | | |
|---|---|---|
| JANSEN Charlotte Uldahl | A NEW POTENT REAGENT FOR COMBATING BACTERIAL BIOFILM INFECTIONS | P110 |
| JEANTY Matthieu | PHOTOCATALYZED MINISCI REACTION AS A KEY STEP TO ACCESS NEW N-HETEROCYCLES | P111 |
| JOHANSSON Anders | NOVEL MODES OF ENZYME INHIBITION | IL26 |
| JOHANSSON Niklas | AN ALTERNATIVE APPROACH IN ANTIMALARIAL DRUG DESIGN BY INHIBITING MEMBRANE-BOUND PYROPHOSPHATASES | P112 |
| JORG Manuela | DEVELOPMENT OF TOOLS FOR THE TARGETED DEGRADATION OF GPCRs | P113 |
| JOSA CULLERE Laia | PHOTOSWITCHING HDAC INHIBITORS FOR THE SPATIOTEMPORAL TARGETING OF CANCER CELLS WITH LIGHT | P114 |
| JUN Joonhong | DISCOVERY AND DEVELOPMENT OF JNK3 INHIBITORS AS THERAPEUTICS FOR ALZHEIMER'S DISEASE | P095 |
| JUNG Erik | NEW FIDAXOMICIN ANTIBIOTICS: COMBINING METABOLIC ENGINEERING AND SEMISYNTHESIS | P116 |
| JUNG Kwan-Young | SYNTHESIS AND CHARACTERIZATION OF A NOVEL MASTL INHIBITOR MKI-2 TARGETING MASTL-PP2A IN BREAST CANCER CELLS | P276 |
| KAMEI Yuji | STAPLED-PEPTIDE PROTACs SYNTHESIZED BY HYPERVALENT IODINE REAGENTS | P118 |
| KANG Suk Woo | ASYMMETRIC ENE-REDUCTION OF ALPHA,BETA-UNSATURATED COMPOUNDS BY F420-DEPENDENT OXIDOREDUCTASES A (FDOR-A) ENZYMES FROM MYCOBACTERIUM SMEGMATIS | P029 |
| KANG Suk Woo | ASYMMETRIC ENE-REDUCTION BY F420-DEPENDENT OXIDOREDUCTASES B (FDOR-B) ENZYMES FROM MYCOBACTERIUM SMEGMATIS | P261 |
| KESIC Jelena | SYNTHESIS AND ANTIPROLIFERATIVE ACTIVITY OF NOVEL C-5 CLEISTANOLATE ANALOGUE | P121 |
| KIM Minjae | DESIGN AND SYNTHESIS OF TRUNCATED ADENOSINE DERIVATIVES AS $A_3$ ADENOSINE RECEPTOR ANTAGONISTS | P123 |
| KIM Minji | SYNTHESIS AND BIOLOGICAL EVALUATION OF 2-PHENYLBENZIMIDAZOLE DERIVATIVES AS NOVEL ANTI-INFLAMMATORY AGENTS | P124 |
| KIM Sanghee | CONCISE ENANTIOSELECTIVE TOTAL SYNTHESIS OF SALINOSPORAMIDES | P125 |
| KIM Youyoung | DISCOVERY OF NOVEL SYNTHETIC TOLL-LIKE RECEPTOR 7 AND 8 DUAL AGONISTS AS VACCINE ADJUVANT | P126 |
| KIM Dong-Su | IDENTIFICATION OF HIT COMPOUNDS FOR DENGUE VIRUS INHIBITORS THROUGH LIBRARY SCREENING | P122 |
| KIRSCHNER Tonia | UNLOCKING NEW THERAPEUTIC OPPORTUNITIES: COVALENT INHIBITION OF KRasG13C FOR PRECISION CANCER TREATMENT | P127 |
| KOH Ming Joo | MULTICOMPONENT ALKENE CROSS-COUPLING BY BASE METAL CATALYSIS | IL20 |
| KONDRATOV Ivan | 3-$CF_3$/$C_2F_5$-SUBSTITUTED PROLINE ANALOGS: SYNTHESIS AND THE PROTEOLYTIC STABILITY OF THEIR AMIDE DERIVATIVES | P128 |
| KONECNY Jan | DISCOVERY OF N-SUBSTITUTED-5H-DIBENZO[a,d][7]ANNULEN-5-AMINES AS POTENT N-METHYL-D-ASPARTATE RECEPTOR ANTAGONISTS FOR THE TREATMENT OF NEUROLOGICAL DISORDERS | P129 |
| KONOPKA Anna | AUTOMATED SYNTHESIS OF PROTEIN DEGRADERS FOR DRUG DISCOVERY | P130 |
| KONSTANTINIDOU Evgenia | DEVELOPMENT OF SMALL MOLECULE CHEMICAL PROBES TO INVESTIGATE FABP5 FUNCTION | P131 |
| KORABECNY Jan | NOVEL ANTITUBERCULAR AGENTS BASED ON 5H-PYRROLO[3,2-d]PYRIMIDINES | P132 |
| KORKALAINEN Minna | HUMAN AGO2 INHIBITOR SCREENING ASSAY | P133 |

| | | |
|---|---|---|
| TAYLOR Garrett | DEVELOPMENT OF $P2X_7$ RECEPTOR ANTAGONISTS FOR THE TREATMENT OF NEURODEGENERATIVE DISEASES | P243 |
| TAYLOR Malcolm | REFINING THE DRUG DISCOVERY PARADIGM FOR SCHISTOSOMIASIS | P244 |
| TORRES Endika | DEVELOPMENT OF AN ITERATIVE PIPELINE FOR THE DISCOVERY OF SELECTIVE MULTITARGET TYROSINE KINASE INHIBITORS | P245 |
| TRACEY Luu | DEVELOPMENT OF DUAL-FUNCTIONALIZED FLUOROPHORES TO MONITOR ANTIBODY DELIVERY SYSTEMS | P246 |
| TRAN Eric | EXPLORATION OF PHENOXAZINE DERIVATIVES FOR THE TREATMENT OF TUBERCULOSIS | P247 |
| TURVEY samuel | IDENTIFYING SMALL MOLECULE MODULATORS OF IR-IGF1R HYBRID FORMATION | P248 |
| TUYUN Amac Fatih | SULFONAMIDE-BASED AZANAPHTHOQUINONE HYBRID MOLECULES: PROMISING ANTIMICROBIAL AGENTS | P057 |
| TUYUN Amac Fatih | IDENTIFICATION OF PLASTOQUINONE ANALOGS TO BE EFFECTIVE IN BREAST CANCER TREATMENT | P250 |
| VAKALOPOULOS Alexandros | DISCOVERY OF IMIDAZO[1,2-A]PYRIDINE CARBOXAMIDE BAY 1165747, A LONG-ACTING SOLUBLE GUANYLATE CYCLASE STIMULATOR FOR RESISTANT HYPERTENSION | OC06 |
| VALAKA Anna | DEVELOPMENT OF A METHODOLOGY FOR TRACELESS AFFINITY LABELLING OF BRUTON'S TYROSINE KINASE IN LIVE CELLS | P251 |
| VALENTINI Alice | DISCOVERY OF POTENT TETRAZOLE FREE FATTY ACID RECEPTOR 2 ANTAGONISTS | P252 |
| VASSILIOU Stamatia | SYNTHESIS OF UNSYMMETRICAL SQUARAMIDES AS POTENTIAL BIOLOGICAL TARGETS AND DETAILED CONFORMATIONAL ANALYSIS | P253 |
| VAUZEILLES Boris | SYNTHESIS OF MOLECULAR PROBES FOR THE DETECTION OF PATHOGENS AND REACTIVE OXYGEN SPECIES | P254 |
| VAZQUEZ Santiago | IN VIVO EVALUATION OF SOLUBLE EPOXIDE HYDROLASE INHIBITORS IN MURINE MODELS OF ALLODYNIA, CHEMOTHERAPY-INDUCED NEUROPATHIC PAIN AND VISCERAL PAIN | P255 |
| VENTURA Maria Rita | SYNTHESIS OF (OLIGO)ARABINOSIDES TO STUDY NEW DRUG TARGETS FOR TUBERCULOSIS TREATMENT | P256 |
| VODOLAZHENKO Mariia | STEROIDS WITH UNNATURAL CONFIGURATION | P257 |
| VOETS Lauren | DESIGN, DEVELOPMENT AND EVALUATION OF NOVEL ANTICANCER DRUGS TARGETING PROTEIN KINASE D2 (PKD2) | P258 |
| VOLOCHNYUK Dmytro | ELECTROPHILIC [4+1]-CYCLIZATION OF PICOLINES – EFFICIENT AVENUE TOWARDS 6-AZAINDOLE AND 6-AZABENZOFURANES | P142 |
| VOLOCHNYUK Dmytro | EFFICIENT ONE-STEP APPROACH TO ANNULATED TETRAHYDROFURANS: SUPPORTING THE SEARCH FOR NEW POTENT CCR2 RECEPTOR ANTAGONISTS | P260 |
| VOLOCHNYUK Dmytro | COCKTAILS FOR 19F NMR FRAGMENT SCREENING: FROM THEORY TO APPLICATIONS USING ENAMINE'S STOCK CHEMICAL SPACE | OC08 |
| VULPETTI Anna | NOVEL, REVERSIBLE DCAF1 LIGANDS EXPAND THE TPD-TOOLBOX | IL21 |
| WADA Hiroki | THE SYGNATURE CHARMeD PROTAC PLATFORM | P120 |
| WADA Hiroki | NEXT STOP GLUE CITY - NOVEL MOLECULAR GLUE PLATFORM | P262 |
| WILDING Birgit | DISCOVERY OF THE CLINICAL CANDIDATE BI 1810631 (ZONGERTINIB), A SELECTIVE HER2 INHIBITOR FOR THE TREATMENT OF HER2 EXON 20 INSERTION DRIVEN TUMORS | OC10 |



# List of Authors

| | | |
|---|---|---|
| BAUER | M. | IL26 |
| BAUM | A. | OC10 |
| BAY | Y. | P076 |
| BAYRAK | N. | P057, P250, P018 |
| BEAUMONT | K. | P172 |
| BECK-SICKINGER | A. | P051 |
| BEDIA-DIAZ | G. | P054 |
| BEERBAUM | M. | P127 |
| BEHBEHANI | H. | P019 |
| BEKIĆ | S. | P109 |
| BELLINA | F. | P009 |
| BELSITO | E. L. | P220, P137 |
| BENDALE | P. | P020 |
| BENEK | O. | P021 |
| BENKOVA | M. | P274 |
| BENZ | J. | IL05 |
| BERECZKI | I. | P066 |
| BERGER | B.-T. | P203 |
| BERGER | L. M. | P083 |
| BERGSTROM | F. | IL26 |
| BERNDT | S. | OC03 |
| BERTOLINI | S. | P106 |
| BERTORELLI | R. | P222 |
| BERTOZZI | F. | P009 |
| BESSET | T. | IL04 |
| BEST | S. | P173 |
| BEUS | M. | P101 |
| BEVERNAEGE | K. | P022 |
| BIALY | L. | IL11 |
| BIELAWSKA | A. | P024, P023, P152 |
| BIELAWSKI | K. | P024, P023, P152 |
| BIGNAMI | F. | P106 |
| BILLEN | M. | P258 |
| BIRNER-GRÜNBERGER | R. | P031 |
| BISBOCCI | M. | P277, P225 |
| BLAISING | J. | P173 |
| BLAKSKJÆR | P. | P195 |
| BLOCHOUSE | E. | P025 |
| BODE | J. W. | P048, IL01, P130 |
| BOHEME | T. | IL11 |
| BOIJE AF GENNÄS | G. | P112 |
| BOJANIĆ | K. | P036, OC02 |
| BON | C. | IL03 |
| BONALDI | T. | P039 |
| BONDARENKO | S. S. | P260 |
| BORBÁS | A. | P066 |
| BORDI | S. | P048, P130 |
| BORGARELLI | C. | P026 |
| BORGES | F. | P167 |
| BORGOGNO | M. | P169 |
| BORJESSON | U. | IL26 |
| BORRELL | J. I. | P245 |
| BORSARI | C. | OC05 |
| BORTOLAMI | M. | P201 |
| BORYSOV | O. V. | P105 |
| BOSAK | A. | P016, P168, P027, P185 |
| BÖSE | D. | OC10 |
| BOŠKOVIĆ | J. | P063 |
| BOSSERT | M. | P216, P119 |
| BOTTELBERGS | A. | IL12 |
| BOUCHE | L. | P173 |
| BOUJUT | M. | P030 |
| BRANCALE | A. | P244 |
| BRANDHOFER | T. | OC07 |
| BRANDT | P. | IL26 |
| BRAZZOLOTTO | X. | P136, P191 |
| BREA | J. | P160, P255, P069 |
| BREINBAUER | R. | P031 |
| BREITENBUCHER | G. | P032 |
| BRELA | M. | P237 |
| BRENNECKE | B. | IL05 |
| BRICELJ | A. | P033 |
| BRIEM | H. | IL14 |
| BRKLJAČA | Z. | P034 |
| BROCKLEHURST | C. | IL03 |
| BROSSE | N. | P148 |
| BROUARD | I. | P115 |
| BROZOVIĆ | A. | P270 |
| BRUUN | T. | P035 |
| BUCKI | A. | P087 |
| BUCKLEY | G. | P204 |
| BULJAN | A. | P036 |
| BULTYNCK | P. G. | P026 |
| BURRIS | T. P. | P054 |
| BUŠLJETA | M. | P194 |
| CAI | W. | P155 |
| CALDWELL | N. | P244 |
| CALLEBAUT | B. | P055 |
| CALVÓ-TUSELL | C. | P255 |
| CAMPONESCHI | A. | P251 |
| CANARD | B. | P080 |
| CANCEDDA | L. | P169 |
| CANCHO-GRANDE | Y. | OC03 |
| CANNONI | E. | P025 |
| CANO | C. | P093 |
| CANTÜRK | Z. | P188 |
| CAPELLI | A. M. | P106 |
| CAPUANO | B. | P230, P279, P186 |
| CAR | I. | OC02 |
| CAR | Ů | P037, P175, P194 |

| | | |
|---|---|---|
| CARDOSO | J. | P231 |
| CARLUCCI | R. | P281 |
| CARMALI | S. | P020 |
| CARNEIRO | A. | P167 |
| CAROLI | J. | P039 |
| CARREIRA | E. M. | IL05 |
| CARREIRA | M. | P038 |
| CARTER | G. P. | P247 |
| CARUGO | A. | P277 |
| CARZANIGA | L. | P106 |
| CASTIELLO | C. | P039, P207 |
| CASTRO RUSSO | R. | P166 |
| CAVALLARO | P. A. | P271, P220, P137 |
| CAVALLO | L. | P022 |
| CELIA | F.-R. | P213 |
| ĆELIĆ | A. | P109 |
| CELLUCCI | A. | P277, P225 |
| ČERMÁKOVÁ | K. | P053 |
| CHACHRA | S. | P093 |
| CHAE | C. H. | P276 |
| CHAI | C. L. | P042, P047 |
| CHALYK | B. | P275 |
| CHANDRASHEKARAN | I. | P279, P131 |
| CHANTEGREIL | F. | P191 |
| CHÂTRE | R. | P025 |
| CHAVARRIA | D. | P167 |
| CHEFF | D. | P266 |
| CHEŁMINIAK-DUDKIEWICZ | D. | P229 |
| CHEN | Y. | P272 |
| CHEN | Z. | P204 |
| CHENG | Q. | P266 |
| CHERNYKH | A. V. | P119, P128 |
| CHESNOKOV | G. | P043, P074, P179 |
| CHETTA | P. | OC10 |
| CHEUNG | C.-Y. | P247 |
| CHIARAMONTE | N. | P223 |
| CHODOUNSKA | H. | P044 |
| CHOI | H. | P045 |
| CHOI | H. M. | P124, P091, P046 |
| CHOI | M. | P122 |
| CHOO | M.Z.Y. | P047 |
| CHOUDHARY | J. | P157 |
| CHOUNG | O.-H. | P048 |
| CHRIST | C. | IL14 |
| CHRISTENSEN | A. B. | P195 |
| CHRISTOPOULOU | K. | P215 |
| CHRIVIA | J. | P054 |
| CHUNG | K. L. | P224 |
| CHVOJKOVA | M. | P239 |
| CIAMMAICHELLA | A. | P277, P225 |
| ČIČAK | M. | P001 |
| CLAES | S. | P052 |
| COBOS | E. J. | P255 |
| COCK | T.-A. | P172 |
| CODONY | S. | P255 |
| COHEN | M. | P038 |
| COIN | G. | P048, P130 |
| COLLASON | B. | P192 |
| COLLIN | L. | IL05 |
| COLLINS | B. | P020 |
| COLOTTI | G. | P207 |
| COLSON | T. | P076 |
| CONCEICAO | C. A. | P256 |
| CONSORTIUM | A. | P149 |
| COOK | G. | P247 |
| COOTE | S. | IL25 |
| CORDEIRO-DA-SILVA | A. | P104, P212 |
| CORONA | A. | P102 |
| CORPAS | R. | P255 |
| COSTA | P. | P231 |
| COSTA | V. | P189 |
| COSTAFROLAZ | J. | P116 |
| COSTI | M. | P104, P212 |
| COSTI | R. | P205, P102, P201, P158 |
| ČOŽ-RAKOVAC | R. | OC02 |
| CRAMER | A. | P166 |
| CRNČEVIĆ | D. | P218, P233, P049 |
| CRNOLATAC | I. | P034 |
| CRONIN | L. | IL23 |
| CRUCES | J. | P038 |
| CRUCITTI | D. | P167 |
| CSEKEI | M. | P139 |
| ČUDINA | O. | P063 |
| CURTI | C. | P090, P163 |
| CURTIS | G. | P279, P131 |
| CZARNOMYSY | R. | P024 |
| CZOPEK | A. | P089 |
| DAENEN | M. | P050 |
| DAILLER | D. | P116 |
| DAIPULE | K. | P241 |
| DANIELSSON | J. | IL26 |
| DANON | J. J. | P243 |
| DANTE | R. | P071 |
| DARNHOFER | B. | P031 |
| DARVENIZA | L. | P051 |
| DAS DORES PEREIRA | R. | P166 |
| DATSENKO | O. P. | P216 |
| DAVIDS | T. | P054 |
| DAVIS | H. | IL03 |
| DE BORGGRAEVE | W. M. | P258, P026 |

| | | |
|---|---|---|
| DE FUSCO | C. | IL26 |
| DE GEEST | B. G. | P249 |
| DE HARO GARCIA | T. | PR02 |
| DE JONGHE | S. | P052 |
| DE MEI | C. | P222 |
| DE MELO OLIVEIRA | N. F. | P166 |
| DE OLIVEIRA SANTOS | L. L. | P166 |
| DE SANTIS | R. | P102 |
| DE SANTO | M. | P271, P220, P137 |
| DE VERA | I. M. S. | P054 |
| DE VIVO | M. | P169 |
| DEBNATH | A. | P201 |
| DECOTTIGNIES | A. | P050, P085 |
| DEFRANCQ | E. | P050 |
| DEGROOTE | F. | P055 |
| DEHAEN | W. | P052 |
| DEJEU | J. | P050 |
| DEMEYERE | E. | P056 |
| DENIJS | E. | P249 |
| DENOO | B. | P055 |
| DERNOVŠEK | J. | P059 |
| DESCAMPS | C. | P148 |
| DEUTSCHER | R. C. | P060 |
| DHUMALE | P. | P195 |
| DI ANTONIO | M. | P061 |
| DI BELLO | E. | P039, P207 |
| DI GIORGIO | A. | P013 |
| DI MARCO | A. | P277 |
| DI MATTEO | P. | P201 |
| DI SANTO | R. | P205, P102, P201, P158 |
| DIAZ | J. F. | P190 |
| DIBCHAK | D. | P275 |
| DITTMANN | S. | P074, P116 |
| DIXON | D. J. | IL06 |
| DJAKOVIĆ | S. | P062 |
| DNYANDEV | J. B. | P084 |
| DO HUU | N. | P148 |
| DOAK | B. | P279, P131, P186 |
| DOBRIČIĆ | V. | P063 |
| DOHERTY | C. | P064 |
| DOKLI | I. | P065, P034 |
| DOLEZAL | M. | P144 |
| DOLŠAK | A. | P232 |
| DONCKELE | E. | IL24 |
| DONOVAN | K. A. | P033 |
| DORMÁN | G. | P066 |
| DORST | A. | P116 |
| DÖTSCH | V. | P015 |
| DREANO | L. | P112 |
| DRIZARD | N. | P148 |
| DRUZHENKO | T. V. | P119 |
| DUBIEL | M. | P268 |
| DUCA | M. | P013, P009 |
| DUDENKO | D. V. | P028 |
| DUNKEL | P. | P067 |
| DYBECK | L. | P068 |
| DYMOCK | B. | P172 |
| EBERL | L. | P208, P179 |
| EDELMANN | M. R. | IL05 |
| EDITA | P. | P234 |
| EID | R. | P025 |
| EK | M. | IL26 |
| ELENA | H. | P234 |
| ELIAS | B. | P050, P085 |
| ELMORE | C. S. | P182 |
| EMANUELE | F. | P071 |
| ENTRENA | J. M. | P255 |
| ERTL | P. | IL03 |
| ERTORUN | Ļ. | P005 |
| ESCAMILLA-AYALA | D. A. | P026 |
| ESCOLANO | C. | P069 |
| ESHAK | F. | OC09 |
| ESPANO | E. | P122 |
| ESPINOSA | V. | P070 |
| ESPOSITO | F. | P102 |
| ESPOSITO | G. | P201 |
| ESTÉVEZ | F. | P115 |
| ESTRADA-TEJEDOR | R. | P245 |
| ETTMAYER | P. | OC10 |
| FABBRINI | D. | P277, P225 |
| FAGGIONI | G. | P102 |
| FARROW | R. | P172 |
| FASSARI | B. | P207 |
| FAVA | M. | P271 |
| FEHÉR | î | P269 |
| FEIXAS | F. | P255 |
| FELBER | J. G. | P266 |
| FERJANČIČ BENETIK | S. | P072 |
| FERNANDES | C. | P167 |
| FERNANDEZ AGUADO | D. | P073 |
| FERNÁNDEZ-RUIZ | J. | P178 |
| FERRARA | I. | P074 |
| FERREIRA | M. | P189 |
| FERRIGNO | F. | P277, P225 |
| FESSARD | T. | P219 |
| FEZZARDI | P. | P225 |
| FIERZ | B. | P155, P118 |
| FIGUEROLA-ASENCIO | L. | P178 |

| | | |
|---|---|---|
| FILATOV | Y. | OC08 |
| FILIMONOVA | K. | P275 |
| FINDRIK BLAŽEVIĆ | Z. | P034 |
| FINGER | V. | P274, P132 |
| FINGERLE | J. | IL05 |
| FIONI | A. | P106 |
| FIORAVANTI | R. | P207 |
| FIORENTINO | F. | P039, P071 |
| FISCHER | L. | P139 |
| FISHWICK | C. | P248 |
| FLAUAUS | C. | P268 |
| FLEETWOOD | T. | P075 |
| FLEISCHER | I. | IL15 |
| FLORES | E. | P183, P086 |
| FLURY | P. | P280 |
| FODALE | V. | P277 |
| FOLLMANN | M. | OC06 |
| FONTINHA | D. | P206 |
| FORD | B. | P093 |
| FORDE-THOMAS | J. | P244 |
| FRAME | L. | P244 |
| FRANCESCONI | V. | P104, P212 |
| FRANCOTTE | P. | P076 |
| FRED WONG | W. | P047 |
| FREDLUND | L. | IL26 |
| FREIRE BENTO | A. | P106 |
| FRIAS | M. | P077 |
| FUCHS | J.E. | OC10 |
| FUKUDA | T. | P078 |
| GADEMANN | K. | P074, P208, P235, P179, P116, P043 |
| GAHLAWAT | S. | P182 |
| GAIDAI | A. | P211 |
| GAIDAR | R. S. | P079 |
| GAIO | P. | P166 |
| GALAVSKYY | S. O. | P119 |
| GAN | P. X. L. | P042 |
| GANTER | B. | P080 |
| GAO | Y. | P272 |
| GARBO | S. | P207 |
| GARCÍA | C. | P115 |
| GARCÍA MANCHEÑO | P. D. O. | OC07 |
| GARCIA-HUIDOBRO | J. P. | P165 |
| GARRETT | T. R. | P243 |
| GASTON-MATHÉ | Y. | P148 |
| GAWLIŃSKA | M. | P191 |
| GAZZI | T. | IL05 |
| GEE | A. | P081 |
| GEE | Y. S. | P247 |
| GEFFROY | C. | P025 |
| GEIGER | T. M. | P060, P267 |
| GEORGAKI | E.-G. | P156 |
| GEORGIADIS | D. | P156 |
| GEORGIEVA | M. | P082 |
| GEORGIOU | E. A. | P215 |
| GERNINGHAUS | J. | P083 |
| GERSTBERGER | T. | OC10 |
| GERTSCH | J. | IL05 |
| GHEYOUCHE | E. | P148 |
| GHIDINI | E. | P106 |
| GIAMMARINO | F. | P220, P137 |
| GIANNOPOULOU | A. F. | P215 |
| GIBAE | K. | P084 |
| GILBERT | I. | P244 |
| GILLARD | M. | P085 |
| GILLES | P. | P258 |
| GINSBURG-MORAFF | C. | IL03 |
| GIOÉ-GALLO | C. | P160 |
| GIROTTO | S. | P009 |
| GIROUD | M. | IL05 |
| GIVSKOV | M. | P110 |
| GLAVAŠ-OBROVAC | L. | P259 |
| GŁUCH-LUTWIN | M. | P087 |
| GOBBI | L. | IL05 |
| GOBEC | S. | P052, P136, P232, P072 |
| GODOY | F. | P183, P086 |
| GODYŃ | J. | P191, P087 |
| GOLDMAN | A. | P112 |
| GOMEZ | L. | P032 |
| GÓMEZ | A. | P086 |
| GÓMEZ-CAÑAS | M. | P178 |
| GONZÁLEZ-CANO | R. | P255 |
| GÓRAL | I. | P087 |
| GORECKI | L. | P140 |
| GORNOWICZ | A. | P023 |
| GOSLING | D. | IL03 |
| GOSS | R. | IL13 |
| GOUILLER | A. | P030 |
| GOUPIL | A. | OC09 |
| GRANTZ ŠAŠKOVÁ | K. | P240 |
| GRAUWEN | K. | IL12 |
| GRAZ | M. | P110 |
| GRBČIĆ | P. | OC02 |
| GRETHER | U. | IL05 |
| GREVEN | S. | OC03 |
| GRIER | K. | P088 |
| GRIESSER | T. | P116 |
| GRIFFETT | K. | P054 |
| GRIMALDI | B. | P222 |
| GRINAN-FERRE | C. | P069, P255 |
| GRØTLI | M. | P251 |

| | | |
|---|---|---|
| GRYGORENKO | O. | P138, P128 |
| GRYNYOVA | A. | P275 |
| GRYZŁO | B. | P089 |
| GUAZZETTI | D. | P090, P163 |
| GUBA | W. | IL05 |
| GUBERMAN | M. | IL05 |
| GUCCIONE | E. | P039 |
| GUCWA | N. | P191 |
| GUERRERO | C. | P038 |
| GUESRREGEN | S. | IL11 |
| GUIBERT | M. | P030 |
| GUNZBURG | M. | P279, P186 |
| GUTIERREZ DE TERAN | H. | P242 |
| GÜTSCHOW | M. | P033, P280 |
| GWON | M. J. | P124, P091, P046 |
| GYUDONG | K. | P084 |
| HAH | J.-M. | P095 |
| HAHN | M. | IL14 |
| HAIDER | A. | IL05 |
| HAJDÚ | I. | P066 |
| HALECKOVA | A. | P021 |
| HALES | L. | P092 |
| HALL | M. D. | P266 |
| HALLAND | N. | IL11 |
| HALLATT | A. | P093 |
| HALLS | M. L. | P113, P131 |
| HAMDY | A. | OC03 |
| HAMED | H. | P030 |
| HAMED | M. M. | P241 |
| HAMMOCK | B. D. | P255 |
| HAN | S. B. | P122, P126 |
| HANKE | T. | P203, P083 |
| HANOPOLSKYI | A. I. | P260 |
| HANQUET | G. | P177 |
| HANSEN | N. J. V. | P195 |
| HARTUNG | I. | OC06 |
| HARVEY | A. | P172 |
| HASHIMOTO | M. | P078 |
| HASSANNIA | B. | P145, P223 |
| HAUPT | V. J. | P079 |
| HAUSCH | F. | P267, P060 |
| HE | Y. | IL05 |
| HEER | D. | IL05 |
| HEERING | J. | P268 |
| HEIDARI | Z. | P054 |
| HELD | J. | P058, P206 |
| HEMMINGSEN | A. V. | P094 |
| HENTSCH | A. | IL05 |
| HERASYMCHUK | M. | P138 |
| HÉRITIER | M. | P030 |
| HERMANN | G. | IL14 |
| HERNÁNDEZ-GARCÉS | J. | P115 |
| HERNANDEZ-OLMOS | V. | P268 |
| HERRMANN | J. | P241 |
| HERSCHEL | S. | P273 |
| HERTER | L. | P219 |
| HEUBEL | C. | P214 |
| HEYMANN | T. | P060 |
| HILBERT | M. | IL05 |
| HIRSCH | A. K. H. | P241 |
| HOCEK | M. | P117 |
| HOCHSTRASSER | R. | IL05 |
| HOFFMANN | B. | P148 |
| HOFFMANN | K. | P244 |
| HÖFNER | G. | P191 |
| HOLMES | E. | P244 |
| HOLOTA | S. | P152 |
| HOLOTA | Y. | P275, P174 |
| HOLOVACH | S. | P138 |
| HOLZE | J. | P232 |
| HONER | M. | IL05 |
| HOPKINS | A. | P020 |
| HOPMANN | K. H. | P182 |
| HORAK | M. | P129, P239, P274 |
| HORNSPERGER | B. | IL05 |
| HOUSSEMAN | C. | P148 |
| HRABINOVA | M. | P239, P274 |
| HRENAR | T. | P016 |
| HRYHORIEV | N. | P211 |
| HU | Y. | P272 |
| HUBER | S. | IL05 |
| HUC | I. | P139 |
| HUCHTING | J. | P080 |
| HUGHES | T. S. | P054 |
| HULLAERT | J. | P055 |
| HULTQVIST | L. D. | P110 |
| HUNT | P. A. | P170 |
| HUNTER | M. | P116 |
| HURST | D. | P178 |
| HUSBAND | J. | P002 |
| HUTTUNEN | K. | P008 |
| HWANG | H.-H. | P091, P046 |
| IACCARINO | C. | P225 |
| IALONGO | D. | P205, P102, P201, P158 |
| IBRAHIM | H. | P019 |
| IEREMIAS | L. | P162 |
| IFTIKHAR | M. | P103 |
| IGLESIAS-GUERRA | F. | P220, P137 |
| ILAŠ | J. | P059 |

| | | |
|---|---|---|
| ILIOPOULOS | D. | P041 |
| ILLUM GOLBÆKDAL | P. | P182 |
| ILYICHOVA | O. | P279, P131 |
| IMINOV | R. | P211, P105 |
| IOTTI | N. | P106 |
| ISERN | J. | P157 |
| ISHIYAMA | T. | P078 |
| ISMALAJ | D. E. | P026 |
| ISSOGLIO | F. | P256 |
| IVASECHKO | I. | P024 |
| IVONIN | S. P. | P142 |
| IZUMI | R. | OC03 |
| JACKSON | C. J. | P029, P261 |
| JAESCHKE | G. | P173 |
| JAGEROVIC | N. | P178 |
| JAMES | C. | P108 |
| JAN | M. S. | P007 |
| JANDOUREK | O. | P144 |
| JANEBA | Z. | P001 |
| JANKOVIĆ | J | P109 |
| JANNUZZI | A. T. | P250 |
| JANSEN | C. U. | P110 |
| JANSEN | F. | IL26 |
| JANSEN | J. | P033 |
| JANSSON | P. J. | P140 |
| JARHAD | D.B. | P123, P238 |
| JAYAPRAKASH | V. | P250 |
| JEANTY | M. | P111 |
| JELIĆ | D. | P265, P264 |
| JEONG | L. S. | P045, P123, P238 |
| JERABKOVA | M. | P191 |
| JEYASEKHARAN | A. | P039 |
| JOERGER | A. C. | P015 |
| JOHANNES | S. | OC03 |
| JOHANSSON | A. | IL26 |
| JOHANSSON | N. G. | P112 |
| JOHANSSON | P. | IL26 |
| JOHNSON | T. | P172 |
| JOHNSTON | H. | P173 |
| JOIN-LAMBERT | P. | P148 |
| JOŃCZYK | J. | P191 |
| JORA | B. | P255 |
| JÖRG | M. | P113, P246, P247 |
| JOSA-CULLERÉ | L. | P114 |
| JOSÉ ANTONIO | G.-V. | P227 |
| JOSEPHS | T. | P230 |
| JOVIĆ | O. | OC02 |
| JOYCE | J. | P107 |
| JOZANOVIĆ | M. | P164 |
| JUEL ENEMÆRKE | V. | P182 |
| JUN | D. | P274 |
| JUN | J. | P095 |
| JUNG | E. | P074, P116 |
| JUNG | K.-Y. | P276 |
| JUNG | Y.-S. | P181 |
| JUSOT | M. | P148 |
| KADLECOVÁ | A. | P240 |
| KAISER | F. | P079 |
| KALAMPALIKI | A. D. | P215 |
| KALAWONG | R. | P179 |
| KALESH | K. | P157 |
| KAMEI | Y. | P118 |
| KAMIŃSKI | K. | P237 |
| KANABAR-RAIVADERA | V. | P108 |
| KANG | H. | P143 |
| KANG | H. | P046 |
| KANG | J. | P122 |
| KANG | M. | P276 |
| KANG | S. W. | P029, P261 |
| KANY | A. M. | P241 |
| KAPELIUKHA | A. | P260 |
| KASSIOU | M. | P081, P243, P107, P064 |
| KASTRUP | J. | P076 |
| KATAGIRI | T. | P078 |
| KATHRIN | B. | P234 |
| KAUR | A. | P247 |
| KEEN | A. C. | P113 |
| KELLER | C. | IL05 |
| KERR | W. | P075 |
| KESIĆ | J. | P121 |
| KHATTAB | A. | P112 |
| KICIN | B. | IL05 |
| KIHLBERG | J. | P210 |
| KIM | C. | P276 |
| KIM | C. | P091 |
| KIM | D. | P238 |
| KIM | D.-S. | P122 |
| KIM | J.-K. | P122 |
| KIM | J.-S. | P276 |
| KIM | M. | P123 |
| KIM | M. J. | P124, P091, P046 |
| KIM | Sa. | P125 |
| KIM | Se. | P224 |
| KIM | Y. | P126 |
| KIM | Y.-H. | P276 |
| KIRIAZIS | A. | P112 |
| KIRSCHNER | T. | P127 |
| KLEJBOROWSKA | G. | P145, P223 |
| KLEPETÁŘOVÁ | B. | P257 |
| KLEY | J. T. | P151 |

| | | |
|---|---|---|
| KLINGL | D. Y. E. | P026 |
| KNAPP | S. | P268, P203, P083, P215, P015 |
| KNEZ | D. | P136, P072 |
| KNEŽEVIĆ | A. | P065 |
| KNIGHT | J. C. | P246 |
| KO | S.-G. | P091, P046 |
| KOBRLOVA | T. | P274 |
| KOELLING | F. | IL14 |
| KOH | M. J. | IL20 |
| KOJIĆ | V. | P121 |
| KOŁACZKOWSKI | M. | P089 |
| KOLCHEVA | M. | P274 |
| KOLMAN | V. | P001 |
| KOLYMPADI MARKOVIC | M. | P164 |
| KONDEVA-BURDINA | M. | P082 |
| KONDRATOV | I. | P128 |
| KONECNA | K. | P144 |
| KONECNY | J. | P129, P274 |
| KONOPKA | A. | P130 |
| KONSTANTINIDOU | E. | P131 |
| KONTRA | B. | P067 |
| KONVALINKA | J. | P053 |
| KORABECNY | J. | P191, P129, P239, P274, P132 |
| KORDUBAYLO | M. V. | P105 |
| KORKALAINEN | M. | P133 |
| KORN | C. | IL05 |
| KORPOS PINTYE-GYURIB | E. | P269 |
| KORTUS | S. | P274 |
| KOS | I. | P134, P135 |
| KOŠAK | U. | P136, P072 |
| KOSAR | M. | IL05 |
| KOSTAKIS | I. K. | P041, P215 |
| KOTSCHY | A. | P139 |
| KOUTSOPETRAS | I. | P219 |
| KOVAČEVIĆ | I. | P121 |
| KOVAČEVIĆ | M. | P037, P194 |
| KOVAR | O. | P239, P140 |
| KOZÁK | J. | P001 |
| KRALJ | J. | P270 |
| KRALJ | M. | P017 |
| KRALJEVIĆ PAVELIĆ | S. | OC02 |
| KRALJIĆ | K. | P264 |
| KRÄMER | A. | P203, P083 |
| KRÄMER | S. D. | IL05 |
| KRAMER ROCHA | L. | P166 |
| KRAMMER | L. | P031 |
| KRAUT | N. | OC10 |
| KRAVETS | I. O. | P028 |
| KREMEYER | J. | P202 |
| KRETZ | J. | IL05 |
| KRĘŻEL | A. | P010 |
| KRISTENSEN | A. | P076 |
| KRISTENSEN | O. | P195 |
| KRIUKOVA | A. | P148 |
| KROLL | C. | IL05 |
| KRÖNKE | J. | P033 |
| KRSTULOVIĆ | L. | P058, P259 |
| KRÜGER | N. | P280 |
| KRYŠTOF | V. | P011, P176 |
| KU | J.-M. | P143 |
| KUBIČEK | A. | P265 |
| KUBYSHKIN | V. | P216 |
| KUČAS | F. | P194 |
| KUCERA | T. | P239, P274 |
| KUCEROVA | M. | P144 |
| KUCZAK | M. | P161 |
| KUDLÁČEK | K. | P257 |
| KUDOVA | E. | P228, P044, P257 |
| KUHLMANN | J. | OC07 |
| KUHN | B. | IL05 |
| KUMAR SINGH | A. | P010 |
| KUMARASIRI | M. | P172 |
| KURZ | M. | P214 |
| KŮTOVÁ | Z. | P240 |
| KVARATSKHELIA | M. | P158 |
| KVIDO | S. | P234 |
| KWON | Y.-J. | P276 |
| LA ROVERE | D. R. M. | P026 |
| LABADIE | G. | P281 |
| LABOUILLE | S. | P148 |
| LACLEF | S. | OC02 |
| LAHAV | N. | P149 |
| LAHTELA-KAKKONEN | M. | P008 |
| LAI | S. K. | P093 |
| LAK SHIN | J. | P084 |
| LAMBONA | C. | P207 |
| LANDEK | G. | P265 |
| LANTHIER | C. | P145, P223 |
| LAPIĆ | J. | P062 |
| LAPOINTE | G. | IL03, P146 |
| LARSEN | L. K. | P195 |
| LAUČAN | M. | P016 |
| LAUFER | S. | P147, P280 |
| LAUGEOIS | M. | P148 |
| LAVIE | M. | P149 |
| LEE | Ja. | P124 |
| LEE | Je. | P150, P224 |
| LEE | J. K. | P046 |
| LEE | J. Y. | P124, P091, P046 |

| | | |
|---|---|---|
| LEE | K.-T. | P124 |
| LEE | S. | P143 |
| LEEM | J. | P276 |
| LEES | K. | P244 |
| LEGGIO | A. | P271, P220, P137 |
| LEINO | T. O. | P151 |
| LERCHEN | H.-G. | OC03 |
| LERNER | C. | P173 |
| LEROUX | F. | P177 |
| LEROY | C. | P076 |
| LESAC | A. | P065 |
| LESYK | R. | P024, P023, P152 |
| LEVANDOVSKIY | I. | P211 |
| LEVENTIĆ | M. | P259 |
| LEVRIER | C. | P172 |
| LIAO | W. | P042 |
| LILLETHORUP | I. A. | P153 |
| LIM | E. T. | P047 |
| LIN | T.-C. | P263 |
| LINDSAY | D. | P073 |
| LISTA | F. | P102 |
| LISTER | A. | P154 |
| LIU | X. | P155 |
| LLAMAS-RIZO | S. | P156 |
| LLEBARIA | A. | P114 |
| LOBELL | M. | IL14 |
| LONDON | N. | P149 |
| LONGOBUCCO | C. | P271 |
| LŐRINCZ | Z. | P066 |
| LOZA | M. I. | P069, P160, P255 |
| LOZYNSKYI | A. | P152 |
| LU | R. | P268 |
| LUCENA-AGELL | D. | P190 |
| LUKOWSKI | R. | P268 |
| LUO | D. | P279 |
| LUPTON | D. W. | P029, P261 |
| LUU | T. | P246 |
| MA | G. | P281 |
| MACCARRONE | M. | IL05 |
| MACH | L. | IL05 |
| MACHARA | A. | P240 |
| MADIA | V. N. | P205, P102, P201, P158 |
| MADL | T. | P039 |
| MADSEN | D. | P195 |
| MAES | D. | P159 |
| MAHLERT | C. | OC03 |
| MAHNASHI | M. H. | P007 |
| MAI | A. | P039, P207 |
| MAIER | M. S. | P266 |
| MAJDIČ | G. | P136 |
| MAJELLARO | M. | P160 |
| MAJERIĆ ELENKOV | M. | P034 |
| MAJOR | A. | P116 |
| MAKHANKOVA | V. G. | P119 |
| MAKSUMIC | L. | P263 |
| MAKSYMENKO | O. | P211 |
| MALARZ | K. | P161 |
| MALAWSKA | B. | P191 |
| MALINAK | D. | P181 |
| MALIZIA | A. | P169 |
| MANANDHAR | A. | P162, P252 |
| MANDRUP BERTOZZI | S. | P222 |
| MAO | T. | P267 |
| MARAKOVIĆ | N. | P168, P027 |
| MARCANTONIO | E. | P090, P163 |
| MARIE | A. | P111 |
| MARINOVIĆ | M. | P101 |
| MARIUS | L. | P234 |
| MARKOVIĆ | D. | OC02, P164 |
| MARTIN | L. | P108 |
| MARTIN | R. E. | IL05 |
| MARTINA | M. | P071 |
| MARTÍNEZ | A. L. | P255 |
| MARTINS TEIXEIRA | M. | P166 |
| MARX | L. | OC03 |
| MASCAYANO | C. | P183, P180, P165 |
| MASON | J. | P075 |
| MATARACI-KARA | E. | P057 |
| MATHEW | A. | P208 |
| MATICO | R. | IL12 |
| MATIJEVIĆ | G. | OC02 |
| MATOS | M. J. | P166, P167 |
| MATOŠEVIĆ | A. | P168, P027, P185 |
| MATTENBERGER | Y. B. | P116 |
| MATTEVI | A. | P039 |
| MATTIO | L. M. | P169 |
| MATULIS | D. | P059 |
| MATULJA | D. | OC02, P164 |
| MATZIOL | T. | P232 |
| MAULIDE | N. | IL10 |
| MAZZUCATO | R. | P106 |
| MC DOWELL | K. | P131 |
| MCCONNELL | D. B. | IL16, OC10 |
| MCGOURAN | J. | P171 |
| MCKENNA | S. | P171 |
| MCLACHLAN | M. | P172 |
| MCMILLAN | A. E. | P048 |
| MEDEN | A. | P136 |
| MEIER | C. (1) | IL05 |
| MEIER | C. (2) | P080, P209 |
| MELLADO | M. | P070 |

| | | |
|---|---|---|
| MELNYKOV | K. | OC08, P138, P217 |
| MÉNDEZ PÉREZ | M. | IL11 |
| MENDONCA MATOS DAY | P. | P244 |
| MERCEDES | R.-H. | P213 |
| MERI | S. | P112 |
| MERTLÍKOVÁ KAISEROVÁ | H. | P001 |
| MESCH | S. | P173 |
| MESSORE | A. | P205, P102, P201, P158 |
| METZNER | K. | P268 |
| MEULI | M. | P116 |
| MEYNERS | C. | P267, P060 |
| MEZEIOVA | E. | P274 |
| MIAH | A. | OC04 |
| MIHELEC | D. | P175 |
| MILČIĆ | N. | P034 |
| MILEO | V. | P106 |
| MILIŠIŪNAITĖ | V. | P011, P176 |
| MILLER | R. | IL12 |
| MILOVANOVIĆ | V. | P185 |
| MIN | K. | P095 |
| MISIACHNA | A. | P129, P239, P274 |
| MITKOV | J. | P082 |
| MOGET | N. | P177 |
| MOGK | G. | IL14 |
| MOHANTY | B. | P279 |
| MÖHLE | K. | P179 |
| MOLYNEAUX | C.-A. | P279 |
| MÓNICA | D.-G. | P227 |
| MONJARAS-FERIA | J. | P020 |
| MONTALBETTI | C. | P277, P225 |
| MONTEIRO | L. | P189 |
| MONTEITH | G. | P172 |
| MONTGOMERY | A. P. | P243 |
| MOON | C. S. | P124 |
| MOON | J. | P095 |
| MORALES | P. | P178 |
| MORALES-CHESTA | P. | P165, P183, P180 |
| MORALES-GARCIA | J. A. | P069 |
| MORDYL | B. | P087 |
| MORELLI | C. | P271 |
| MORINA | D. | P179 |
| MORISSEAU | C. | P255 |
| MOROZ | Y. S. | P028, P079, OC08 |
| MOROZA | Y. S. | P174 |
| MOROZOVOVÁ | M. | P257 |
| MOSNA | K. | P010 |
| MOSTINSKI | Y. | IL05 |
| MPAKALI | A. | P156 |
| MRACSKO | E. Z. | P173 |
| MROZEK-WILCZKIEWICZ | A. | P161 |
| MRUGALLA | F. | IL14 |
| MU | L. | IL05 |
| MUCKOVA | L. | P239 |
| MUCSI | Z. | P067 |
| MUENCH | S. | P248 |
| MUES | H. | IL03 |
| MÜHLFENZL | K. S. | P182 |
| MULARSKI | J. | P161 |
| MÜLLER | C. E. | P280 |
| MÜLLER | M. P. | P127 |
| MÜLLER | R. | P116, P241 |
| MÜLLER | S. | P268 |
| MÜLLER HERDE | A. | IL05 |
| MUNCK | S. | P026 |
| MÜNICK | P. | P015 |
| MUÑOZ-OSSES | M. | P165, P183, P180 |
| MURAMATSU | S. | P078 |
| MURATORE | M. | IL12 |
| MURGAI | A. | P033 |
| MURGO | A. | P106 |
| MURRAY | J. | P279 |
| MUSILEK | K. | P181, P021 |
| MUSIOL | R. | P161 |
| MUSTAČ | L. | P264 |
| MYKHAILIUK | P. K. | P275, P216 |
| MZIK | M. | P191 |
| NACHON | F. | P136 |
| NAHRA | F. | P022 |
| NAIK | S. D. | P123 |
| NAMASYYAYAM | V. | P140 |
| NANDITA | E. | P221, P040 |
| NASCIMENTO BARBOSA | C. L. | P166 |
| NAVARRETE-TUDELA | E. | P165, P183, P180 |
| NAVARRO-SALVO | N. | P180 |
| NAZARÉ | M. | IL05 |
| NEDELJKOVIĆ | N. | P063 |
| NEEDLE | A. A. | P144 |
| NES | D. | P281 |
| NEUMÜLLER | R.A. | OC10 |
| NG | Y. L. D. | P033 |
| NGUYEN | H. | P279 |
| NGUYEN | J. | P201 |
| NGUYEN | Q. T. N. | P042 |
| NIBBIO | M. | P277, P225 |
| NICHOLS | P. L. | P048, P130 |
| NICQUE | M. | P184 |
| NIGGENABER | J. | P127, P263 |
| NIKOLIC | K. | P069 |

| | | |
|---|---|---|
| NIKOLIĆ | M. | P063 |
| NIKŠIĆ-FRANJIĆ | I. | P192 |
| NILSSON | M. | P110 |
| NITSCHE | C. | OC11, P279 |
| NOCE | B. | P207 |
| NOEL | T. | IL02 |
| NOLAN | S. | P022 |
| NONATO RABELO | R. A. | P166 |
| NORSIKIAN | S. | IL17 |
| NOVAKOVIĆ | I. | P185 |
| NOWAKOWSKA | M. | P237 |
| NYSTRÖM | H. | P251 |
| OBREZA | A. | P072 |
| ODDI | S. | IL05 |
| ODELL | L. | P068 |
| ODŽAK | R. | P218, P233, P049 |
| OEHLRICH | D. | IL12 |
| OEREN | M. | P170 |
| OH | J. S. | P276 |
| O'HARA | F. | IL05 |
| OKSIUTA | O. | OC08 |
| OLENDER | R. | P038 |
| OLIVA | M. A. | P190 |
| OMRAN | A. | IL05 |
| OPALINSKI | I. | P025 |
| OPSENICA | D. M. | P027, P185 |
| ORMSBY | T. | P053 |
| OSMAN | J. | P186 |
| OSTAPCHUK | E. N. | P260 |
| OSTROWSKA | O. | P089 |
| OSUNA | S. | P255 |
| OTTONELLO | G. | P222 |
| OZAWA | S.-I. | P187 |
| ÖZDEMIR | A. | P188, P005 |
| OŽEGOVIĆ | A. | P065 |
| PACHER | P. | IL05 |
| PACHOLCZYK | M. | P161 |
| PADALINO | G. | P244 |
| PAEHLER | A. | P173 |
| PAGE | C.P. | P108 |
| PAHNKE | J. | P140 |
| PAIVA-MARTINS | F. | P189 |
| PALAO | E. | P077 |
| PALENCA | I. | P201 |
| PALLAS | M. | P069, P255 |
| PALMIERI | M. | IL03 |
| PALOMBA | M. | P222 |
| PALOMO | V. | P190 |
| PANEK | D. | P191, P087 |
| PANOSETTI | M. | P009 |
| PANOSSIAN | A. | P177 |
| PAPAKOTSI | P. | P041 |
| PAPOT | S. | P025 |
| PAPP | F. | P269 |
| PARIS-OGAYAR | R. | P190 |
| PARK | H. | P095 |
| PARK | S. | P125 |
| PARK | W. | P122 |
| PARNES | T. | P038 |
| PAŠALIĆ | L. | P193 |
| PASHENKO | A. E. | P028, P211, P217, P134, P135 |
| PASIEKA | A. | P191 |
| PASQUA | L. | P271 |
| PATACCHINI | E. | P205, P102, P201, P158 |
| PATEL | J. | P226 |
| PATEROVA | P. | P144 |
| PATERSON | B. | P051 |
| PAUL | N. | P213 |
| PAVADAI | E. | P204 |
| PAVEL | M. | P234 |
| PAVIĆ | K. | P101, P206 |
| PAVLOVIC | B. | P214 |
| PAVLOVIĆ SAFTIĆ | D. | P192 |
| PAZ | J. | P038 |
| PEARL | E. | P048, P130 |
| PEIFER | C. | P012 |
| PEJCHAL | J. | P191 |
| PEJOSKI | D. | P030 |
| PEM | B. | P193 |
| PÉREZ | B. | P255 |
| PÉREZ | C. | P255 |
| PERKOVIĆ | I. | P101, P206 |
| PERRON | Q. | P148 |
| PESSANHA DE CARVALHO | L. | P058, P206 |
| PETER | K. | P033 |
| PETERSEN | L. K. | P195 |
| PETERSEN | R. G. | P195 |
| PETRI | E. | P109 |
| PETROCCHI | A. | P277, P225 |
| PETROVIĆ PEROKOVIĆ | V. | P037, P175, P194 |
| PETRUCCI | R. | P201 |
| PETTERSSON | L. | P278 |
| PFAFF | P. | IL05 |
| PHAM | L. B. H. | P150, P224 |
| PHAM | M. | P054 |
| PHELPS | J. | P279 |
| PHILIPP | L. | P234 |
| PHILIPPE | J. | P111 |
| PIALIC | L. | P173 |

| | | |
|---|---|---|
| PIANTANIDA | I. | P192 |
| PICKERING | D. | P076 |
| PIETRAK | B. | IL12 |
| PIGA | M. | P269 |
| PIGNATA | L. | P039 |
| PILLAIYAR | T. | P280 |
| PINTO | E. | P231 |
| PIROTTE | B. | P076 |
| PLÜCKTHUN | A. | P015 |
| PLUM | G. | P148 |
| POATER | A. | P022 |
| POCZKA | L. | P266 |
| PODESCHWA | M. | IL11 |
| PODUST | L. | P201 |
| POHLKI | F. | IL07 |
| POINOT | P. | P025 |
| POJE | G. | P101, P206 |
| POLISHCHUK | O. | P216 |
| PONGRAC | P. | P264 |
| PONTING | D. J. | P170 |
| POPŁAWSKA | B. | P023 |
| POPOVIC | M. | P017 |
| POPSAVIN | M. | P121 |
| POPSAVIN | V. | P121 |
| PORTA | E. | P281, P157 |
| PORTER | C. | P131 |
| PORTO | S. | P166 |
| POSADA | L. | P202 |
| POSTMUS | A. | IL05 |
| POULSEN | T. | IL19 |
| POUWER | R. | P172 |
| PRCHAL | L. | P239, P274 |
| PRIMOŽIČ | I. | P016, P233 |
| PROJ | M. | P052, P072 |
| PROSCHAK | E. | P268 |
| PRUDENCIO | M. | P206 |
| PRYSIAZHNIUK | K. | P216 |
| PUCA | F. | P277 |
| PUCCI | V. | P277, P225 |
| PUIG DE LA BELLACASA | R. | P245 |
| PULKRABKOVA | L. | P274 |
| PUNČEC | M. | P236 |
| QUAIA | R. | P009 |
| QVORTRUP | K. | P110, P153, P094, P088 |
| RACINE | S. | IL03 |
| RACKELMANN | N. | IL11 |
| RADIĆ STOJKOVIĆ | M. | P270 |
| RADOJČIĆ REDOVNIKOVIĆ | I. | P062 |
| RADOŠEVIĆ | K. | P062 |
| RADOVANOVIĆ | V. | P265 |
| RAEVSKII | O. V. | P028 |
| RAHMANI | R. | P172 |
| RAIG | N. D. | P203 |
| RAJESWARAN | W. | P204 |
| RAJIĆ | Z. | P101, P058, P206 |
| RAMIĆ | A. | P016 |
| RAMOS | R. | P006 |
| RASHEED | S. | P241 |
| RASHID | U. | P007 |
| RAUCCI | A. | P039, P207 |
| RAUH | D. | P205, P127, P263 |
| RAYL | M. | P054 |
| READ | K. | P244 |
| REBELO | R. | P189 |
| REBSTOCK | A.-S. | OC03 |
| REDLICH | G. | OC06 |
| REGGIO | P. | P178 |
| REINAUD | O. | P192 |
| RELITTI | N. | P225 |
| REN | J. | P208 |
| REPITY | M. L. | P060 |
| RESENDE | D. | P231 |
| REXEN ULVEN | E. | P162, P252 |
| ŘEZNÍČKOVÁ | E. | P011, P176 |
| RIBIĆ | R. | P037, P175, P194 |
| RICHTER | H. | IL05 |
| RIJONO | D. | P209 |
| RINKEN | A. | P160 |
| RITTER | M. | IL05 |
| RIU | F. | P210 |
| RIZZI | A. | P106 |
| RIZZO | M. | P104, P212 |
| ROÀ | E. | P219 |
| ROBINSON | R. | IL03 |
| RODIĆ | M. V. | P121 |
| RODRÍGUEZ | R. | P038 |
| RODRÍGUEZ-FRANCO | M. I. | P255 |
| ROGERS-EVANS | M. | IL05 |
| ROH | J. | P132 |
| ROH | K. | P143 |
| ROJE | M. | P036 |
| ROMBACH | D. | IL05 |
| ROOD | C. | P054 |
| ROSENFELD | R. | P038 |
| ROSENGREN | B. | IL26 |
| ROSENKILDE | M. | P153, P094 |
| ROSSETTI | I. | P277, P225 |
| ROSZCZENKO | P. | P024 |
| ROTILI | D. | P207 |

| | | |
|---|---|---|
| ROUMELIOTIS | T. | P157 |
| RUCK | R. | IL08 |
| RUDENKO | T. V. | P275 |
| RUF | S. | P214 |
| RUGGERI | M. | P169 |
| RUPPITSCH | L. A. | P210 |
| RURKA | P. | P161 |
| RUSANOV | E. B. | P142 |
| RUSSO | D. | P009 |
| RUTH | P. | P268 |
| RYABUKHIN | S. V. | P028, P142, P211, P105, P217, P138, OC08, P134, P135, P174, P119, P260 |
| RYCKAERT | B. | P055 |
| SAAVEDRA | E. | P115 |
| SABLJIĆ | A. | P218, P233, P049 |
| ŠÁCHA | P. | P053 |
| ŠAČKUS | A. | P011, P176 |
| SADIQ | A. | P007 |
| SAITER | J. | P177 |
| SAKAČ | N. | P164 |
| ŠAKIĆ | D. | P164 |
| SAŁAT | K. | P136, P191 |
| SALOMÉ | C. | P219 |
| SÁNCHEZ | F. | P221, P040 |
| SANDER | P. | P116 |
| SANDNER | P. | OC06 |
| SANFELIU | C. | P255 |
| SANG KOOK | L. | P084 |
| SAN-MARTIN-GALINDO | P. | P226 |
| SANTORO | V. | OC10 |
| SANTOS | P. G. | P189 |
| SANTOS-CABALLERO | M. | P255 |
| SANZ GARCIA | J. | P148 |
| SARIKAKI | G. | P041 |
| SARLAH | D. | IL09 |
| SAROTT | R. | IL05 |
| SASAKI | R. | P187 |
| SAVAGE | P. | P107 |
| SAVARDI | A. | P169 |
| SAVIĆ | M. | P109 |
| SAVIJOKI | K. | P226 |
| SCANLON | M. | P230, P279, P131, P186 |
| SCAPOZZA | A. | P030 |
| SCAPOZZA | L. | P030 |
| SCARPA | S. | P205 |
| SCARPELLI | R. | P222 |
| SCARPELLINI | C. | P145, P223 |
| SCHADE | D. | P273 |
| SCHAEFER | J. | P015 |
| SCHÄFER | B. | P015 |
| SCHÄFER | S. C. | P060 |
| SCHÄFLE | D. | P116 |
| SCHARN | D. | OC10 |
| SCHELLENBERG | M. | P235 |
| SCHIBLI | R. | IL05 |
| SCHIMECZEK | M. | IL14 |
| SCHMIDTKO | A. | P268 |
| SCHOLS | D. | P052 |
| SCHOLZEN | K. C. | P266 |
| SCHROEDER | M. | IL21 |
| SCHULER | S. M. M. | P241 |
| SCHULTZ-KNUDSEN | K. | P162, P252 |
| SCHULZ | T. | P263 |
| SCIPIONE | L. | P205, P102, P201, P158 |
| SCOTT | C. | P029, P261 |
| SEDIĆ | M. | OC02 |
| SEDLÁČEK | J. | P240 |
| SEGALL | M. D. | P170 |
| ŠEGAN | S. | P027 |
| SEGLER | M. | IL18 |
| SEGUELLA | L. | P201 |
| SELEN | E. | P221 |
| SELISKO | B. | P080 |
| SEONG | S. | P122 |
| SERNA | E. | P006 |
| SESTAK | V. | P191 |
| SFERRAZZA | A. | P277, P225 |
| SHANKAR KASHYAP | R. | P258 |
| SHANNON | J. | P173 |
| SHARMA | S. | IL12 |
| SHARMA | V. | P201 |
| SHEMY | A. | P258 |
| SHEN | M. | P266 |
| SHENVI | R. | IL27 |
| SHILKAR | D. | P250 |
| SHULHA | S. | P216 |
| SIEBER | S. | P179 |
| SIEGER | P. | P151 |
| SIEVERS | S. | P074, P116 |
| SILVA OLIVEIRA | F. M. | P166 |
| SILVIA | P.-S. | P213 |
| ŠIMANOVIĆ | A. | P065 |
| SIMÃO MACHADO | F. | P166 |
| ŠIMKOVÁ | A. | P053 |
| SIMMONS | K. | P248 |
| SINGH | P. K. | P226 |
| SJÖ | P. | P210 |
| SKALTSOUNIS | A-L. | P041 |

| | | |
|---|---|---|
| SKRYDSTRUP | T. | P182 |
| SLADIĆ | D. | P185 |
| SLAVIK | R. | IL05 |
| SLAVIKOVA | B. | P228, P044 |
| SLECHTA | P. | P144 |
| SLØK | F. A. | P195 |
| SŁUGOCKA | E. | P087 |
| SMALIY | R. | P134, P135 |
| SMOLARKIEWICZ-WYCZACHOWSKI | A. | P229 |
| ŠMUC | T. | OC02 |
| ŠMUC | T. | P236 |
| SMYRNOV | O. K. | P217 |
| ŚNIECIKOWSKA | J. | P089 |
| SOETHOUDT | M. | IL05 |
| SOLCA | F. | OC10 |
| SOLDERMANN | N. | IL22 |
| SOMMER | A. | OC03 |
| SONG | H. | P204 |
| SONG | Y. | P230 |
| SOREGI | P. | P139 |
| SOSIČ | I. | P033 |
| SOUČEK | R. | P257 |
| SOUKUP | O. | P191, P129, P239, P140, P274 |
| SOUSA | E. | P231 |
| SOVA | M. | P232 |
| ŠPRUNG | M. | P218, P233, P049 |
| STANCHO | S. | P234 |
| STARK | H. | P268 |
| STEEGBORN | C. | P207 |
| STEEL | P. | P157 |
| STEFAN | K. | P140 |
| STEFAN | S. M. | P140 |
| STEINEBACH | C. | P033 |
| STEINER | T. | P235 |
| STEINHILBER | D. | P268 |
| STELTE-LUDWIG | B. | OC03 |
| STEPANIĆ | V. | P236, P036, P034, OC02 |
| STEVEN | F. | IL05 |
| STINGLHAMER | M. | OC07 |
| STOIKA | R. | P024 |
| STOJAN | J. | P136 |
| STOLAREK | M. | P237 |
| STRAŠEK BENEDIK | N. | P232 |
| STRATIKOS | E. | P156 |
| STRAVOPODIS | D. J, | P215 |
| STREET-JEAKINGS | R. | P244 |
| STRMEŇ | T. | P257 |
| STROMSTEDT | M. | IL26 |
| STRUBEL | A. | P015 |
| STUCHBURY | G. | P172 |
| SUAREZ | J. | IL12 |
| SUBOVÁ | D. | P240 |
| SÜESS | C. | P030 |
| SUGIARTO | W. O. | P060 |
| SUH | J. H. | P122 |
| SUMMA | M. | P222 |
| SUMMERER | D. | P263 |
| SUNG | K. | P238 |
| ŠUŠAK | P. | P016 |
| SUTTON | M. | P020 |
| ŠVACO | P. | P034 |
| ŠVAJGER | U. | P232 |
| SVOBODOVA | B. | P239, P140 |
| SVOBODOVÁ | L. | P240 |
| SYKES | D. | IL05 |
| SYLVESTER | K. | P280 |
| SZCZUBIAŁKA | K. | P237 |
| SZÉLES | M. | P241 |
| SZEWCZUK | O. | P024 |
| 'T HART | P. | P202 |
| TADDIO | M. F. | IL05 |
| TAFANI | M. | P207 |
| TAGLIAZUCCHI | L. | P104, P212 |
| TAJMOUATI | H. | P148 |
| TALAGAYEV | V. | P232 |
| TALMAN | V. | P035 |
| TAN | K. | IL03 |
| TANAKA | Na. | P078 |
| TANAKA | No. | P187 |
| TANDARIC | T. | P242 |
| TANG | L. | P034 |
| TARANTA | A. | P195 |
| TARDY | S. | P030 |
| TARKHANOVA | O. O. | P079 |
| TAYLOR | M. | P244 |
| TEKWANI | B. | P281 |
| THAM | M. | P086 |
| THAO | C. | P148 |
| THEODOROPOULOU | N. | P253 |
| THOM | S. | P173 |
| THOMAS | D. | OC06 |
| THOMAS | L. | P234 |
| THOMAS | P. | P111 |
| THOMPSON | P. | P092 |
| THORN-SESHOLD | J. | P266 |
| THORN-SESHOLD | O. | P266 |
| THORUM NEUMANN | K. | P182 |
| TIAN | L. | P272 |
| TJADEN | A. | P268 |
| TOLKER-NIELSEN | T. | P110 |

| | | |
|---|---|---|
| TOMAŠIČ | T. | P059, P269 |
| TONELLI | M. | P104, P212 |
| TONIATTI | C. | P277, P225 |
| TORRENTE | E. | P277, P225 |
| TORRES-URTIZBEREA | E. | P245 |
| TRAMONTANO | E. | P102 |
| TRAN | E. | P247 |
| TRAN | Q. T.N. | P047 |
| TRESADERN | G. | IL12 |
| TREU | M. | OC10 |
| TRIPATHI | S. K. | P045 |
| TRIPODI | M. | P207 |
| TROIAN-GAUTIER | L. | P085 |
| TRONTELJ | J. | P136 |
| TUCK | K. | P051 |
| TUDINO | V. | P102, P201 |
| TUMIR | L.-M. | P270 |
| TURCHENIUK | V. | P260 |
| TURCU | A. L. | P255 |
| TURELLI | L. | P219 |
| TURKU | A. | P112 |
| TURVEY | S. | P248 |
| TUYUN | A. F. | P057, P250, P018 |
| TYAGI | M. | P210 |
| TYLER | J. | P054 |
| TZOURAS | N. | P022 |
| UEDA | K. | P078 |
| ULLMER | C. | IL05 |
| ULMER | W. | IL03 |
| ULVEN | T. | P162, P252 |
| URBANČIČ | D. | P059 |
| UZELAC | L. | P017 |
| VACCARO | F. | P106 |
| VAKALOPOULOS | A. | OC06 |
| VALAKA | A. | P251 |
| VALENTE | S. | P039, P207 |
| VALENTINI | A. | P252 |
| VALES | K. | P239 |
| VALKO | M. | P274 |
| VALKOVA | I. | P082 |
| VAN DEN BOSCH | P. L. | P026 |
| VAN DER STELT | M. | IL05 |
| VAN DER VEKEN | P. | P145, P223 |
| VAN HOOF | M. | P052 |
| VAN KASTEREN | S. | P171 |
| VAN LINT | J. | P258 |
| VAN LOY | T. | P052 |
| VAN OPENDENBOSCH | N. | IL12 |
| VAN SCHOUBROEK | B. | IL12 |
| VANDEN BERGHE | T. | P145, P223 |
| VARGA | Z. (1) | IL05 |
| VARGA | Z. (2)+C1493 | P269 |
| VASKOVA | M. | P021 |
| VASSILIOU | S. | P253 |
| VAUZEILLES | B. | P254 |
| VÁZQUEZ | S. | P255 |
| VECCHIO | D. | P222 |
| VEGA-HOLM | M. | P220, P137 |
| VEGA-PÉREZ | J. M. | P220, P137 |
| VENTURA | M. | P204 |
| VENTURA | M. R. | P256 |
| VEPRINTSEV | D. B. | IL05 |
| VERONESI | M. | P009 |
| VERÓNICA | A. | P213 |
| VERSINI | A. | P235 |
| VICENTI | I. | P220, P137 |
| VIDILASERIS | K. | P112 |
| VIOLLIER | P. | P116 |
| VIŠNJEVAC | A. | P192 |
| VITALI FORCONESI | G. | P169 |
| VODOLAZHENKO | M. | P257 |
| VOET | A. | P258 |
| VOETS | L. | P258 |
| VOLOCHNYUK | D. M. | P028, P174, P119, P211, P217, P260, P138, OC08, P134, P135, P142, P105 |
| VOLOSHCHUK | V. V. | P142 |
| VOLOVENKO | O. | P211 |
| VOSS | S. | P279 |
| VRČEK | V. | P164 |
| VUJIĆ | Z. | P063 |
| VULPETTI | A. | IL21 |
| WADA | H. | P120, P262 |
| WADE | R. | P104, P212 |
| WAGNER | G.K. | P020, P108 |
| WALKER | J. | P054 |
| WALLACE | O. | IL24 |
| WALLÉN | E. A. A. | P151 |
| WALZ | M. | P267 |
| WANG | H. | IL05 |
| WANNER | B. M. | P048, P130 |
| WANNER | K. | P191 |
| WANNINAYAKE | U. S. | P054 |
| WARMUTH | J. D. | P127 |
| WASER | J. | P118, P155 |
| WATANABE | K. | P078 |
| WEI | F. | P272 |
| WEINDL | G. | P232 |
| WEISNER | J. | P263 |
| WENGLÉN | C. | P278 |

| | | |
|---|---|---|
| WERRY | E. L. | P243 |
| WEST | N. | P247 |
| WESTPHAL | M. | IL05 |
| WESTWELL | A. | P244 |
| WICHUR | T. | P087 |
| WIĘCKOWSKA | A. | P191, P087 |
| WIĘCKOWSKI | K. | P089 |
| WIEDMANN | M. | OC03 |
| WIESE | J. N. | P127 |
| WIJESEKERA | H. | P172 |
| WILDING | B. | OC10 |
| WILKINSON | S. | P281 |
| WILL | M. | IL11 |
| WILLEMS | S. | P263 |
| WILLIAMS | M. | P279 |
| WINNE | J. | P003, P022, P055, P056, P103, P159, P184, P004, P249 |
| WINTER | G. | PR01 |
| WITT | L. | P012 |
| WITTINE | K. | P264 |
| WITTROCK | S. | OC03 |
| WITTWER | M. | IL05 |
| WOELFLINGSEDER | L. | OC10 |
| WOLBER | G. | P232 |
| WONG | W. S. F. | P042 |
| WOONGSUB | B. | P084 |
| WU | W. W. X. | P048 |
| WUNDER | F. | OC06 |
| WYLER | R. | IL03 |
| XHAARD | H. | P112 |
| XIAO | J. | P272 |
| XIYAN | H. | P084 |
| XU | Yua. | P272 |
| XU | Yuz. | P204 |
| YADA | M. | P187 |
| YALUFF | G. | P006 |
| YANG | H. | P279, P186 |
| YEH | M. | P172 |
| YILDIRIM | H. | P057, P250 |
| YILDIZ | M. | P057, P250 |
| YIN | Y. | IL12 |
| YLI-KAUHALUOMA | J. | P151, P035, P112, P226 |
| YOON | Y. N. | P276 |
| YU | X. | IL12 |
| YUAN | T. | P004 |
| YUDIBETH | S.-L. | P227 |
| ZADRAVEC | R. | P265 |
| ZAHN | S. | OC10 |
| ŽAKELJ | S. | P136 |
| ZAKOŠEK PIPAN | M. | P136 |
| ZANARDI | F. | P090, P163 |
| ZANDONA | A. | P168, P027 |
| ZDAROVA KARASOVA | J. | P191 |
| ZEISEL | L. | P266 |
| ZEMANOVA | L. | P021 |
| ZENG | Y. | P272 |
| ZERVA | A. | P203 |
| ZHANG | J. | P204 |
| ZHAO | P. | P178 |
| ZHENG | M. | P267 |
| ZHU | W. F. | P268 |
| ZHUBI | R. | P083 |
| ZIDAR | N. | P059, P269 |
| ZIEGLER-BOROWSKA | M. | P229 |
| ŽIHER | D. | P264 |
| ZIMČÍK | P. | P229 |
| ZIOLA | P. | P161 |
| ZLATIC | K. | P017 |
| ZLATKOV | A. | P082 |
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| ZOGRAFOU-BARREDO | N. A. | P093 |
| ZONJIĆ | I. | P270 |
| ZORE | M. | P226 |
| ZSIGMOND | î | P066 |
| ZUBRIENE | A. | P059 |
| ŽUKAUSKAITĖ | A. | P011, P176 |
| ŽUNEC | S. | P027 |
| ZWERGEL | C. | P207 |
| ZWILLINGER | M. | P139 |

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is accurate at the time of printing.