ACCORD

INTERDISCIPLINARY CONFERENCE ON DRUG SCIENCES

Warsaw
May 23-25 2024

MEDICAL UNIVERSITY OF WARSAW

ABSTRACT BOOK

MEDICAL UNIVERSITY OF WARSAW

FACULTY OF PHARMACY

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Welcome words

Dean of the Faculty of Pharmacy Medical University of Warsaw

I would like to extend a warm welcome to all Participants of the Interdisciplinary Conference on Drug Sciences, ACCORD 2024.

Following the enormous success of the first edition held in 2022, we are now approaching the second edition of the Conference. This year, the conference theme 'Pharmaceutical innovations across the borders' provides us with the opportunity to explore scientific advancements in the field of drug science and beyond traditional disciplinary boundaries.

The conference will be held at the modern facilities of the Okęcie Airport Hotel, conveniently located near the Banacha Campus of the Medical University of Warsaw and the city center. I trust that the venue's atmosphere, together with engaging lectures, fruitful discussions, and insightful panels, will significantly contribute to the advancement of the field. I wish all Participants successful meetings and an enjoyable time.

Piotr Luliński,
Ph.D., D.Sc.
Dean
Faculty of Pharmacy
Medical University of Warsaw

Chair of the Board of Discipline of Pharmaceutical Sciences of the Faculty of Pharmacy Medicinal University of Warsaw

On behalf of the Board of Pharmaceutical Sciences at Medical University of Warsaw, I warmly welcome you at the ACCORD2024 conference. It is our pleasure and pride to host again international participants: the world- recognized, international speakers as well as the remarkable audience from various countries.

Pharmaceutical sciences are truly interdisciplinary. Therefore, the motto of the conference "Pharmaceutical innovations across the borders" refers not only to its international character but also expresses our belief it is a great chance for academic and industrial scientists from various fields to get together, take up new, or expand existing collaborations. And all this in a beautiful scenario of spring-blossoming and rapidly developing city of Warsaw.

Prof. Grzegorz Nałęcz-Jawecki,
Ph.D., D.Sc.
Chair of the Board of Discipline
of Pharmaceutical Sciences
Faculty of Pharmacy
Medical University of Warsaw

Chair of the Scientific Committee ACCORD 2024

Across the borders," this is the motto of the current edition of ACCORD Conference. During the few days we want not only to discuss the latest achievements of the pharmaceutical sciences but also to build a bridge between disciplines and what more important between people.

Drug development is a team game that uses the knowledge and experience of various fields of science, from biology and physics through chemistry and medicine, as well as mathematics and computer science. Closing scientific disciplines in information bubbles slows down research and reduces its application potential. The situation is similar in the case of limited cooperation between universities and industry. Our ambition as the organizers of the Conference is to create a space of understanding between scientists and professionals from various scientific disciplines, different countries and various organizations working on new drugs and therapies.

I wish you'll enjoy the fruitful meeting.

Prof. Przemysław Dorożyński,
Ph.D., D.Sc.
Chair, Scientific Committee
ACCORD 2024
Faculty of Pharmacy
Medical University of Warsaw

Chair and Deputy-Chairs of the Organizing Committee

We are delighted to welcome you to the second edition of the Interdisciplinary Conference on Drug Sciences, ACCORD 2024. The conference theme, 'Pharmaceutical innovations across the borders', underscores the importance of interdisciplinary approaches that transcend traditional scientific boundaries and promote collaboration between academia and industry. As in the previous edition, distinguished experts from both academia and industry will deliver inspiring lectures, followed by engaging discussions on the theme 'Progress in Pharmaceutical Sciences: Reality or Illusion? Additionally, this year, we are introducing scientific workshops designed to inspire participants to explore new scientific directions.

We eagerly anticipate the conference sparking lively discussions, inspiring fresh ideas, and fostering meaningful partnerships among all attendees. On behalf of the Organizing Committee, we extend our warmest wishes for a successful and fruitful conference experience.

Joanna Giebułtowicz, Ph.D., D.Sc.
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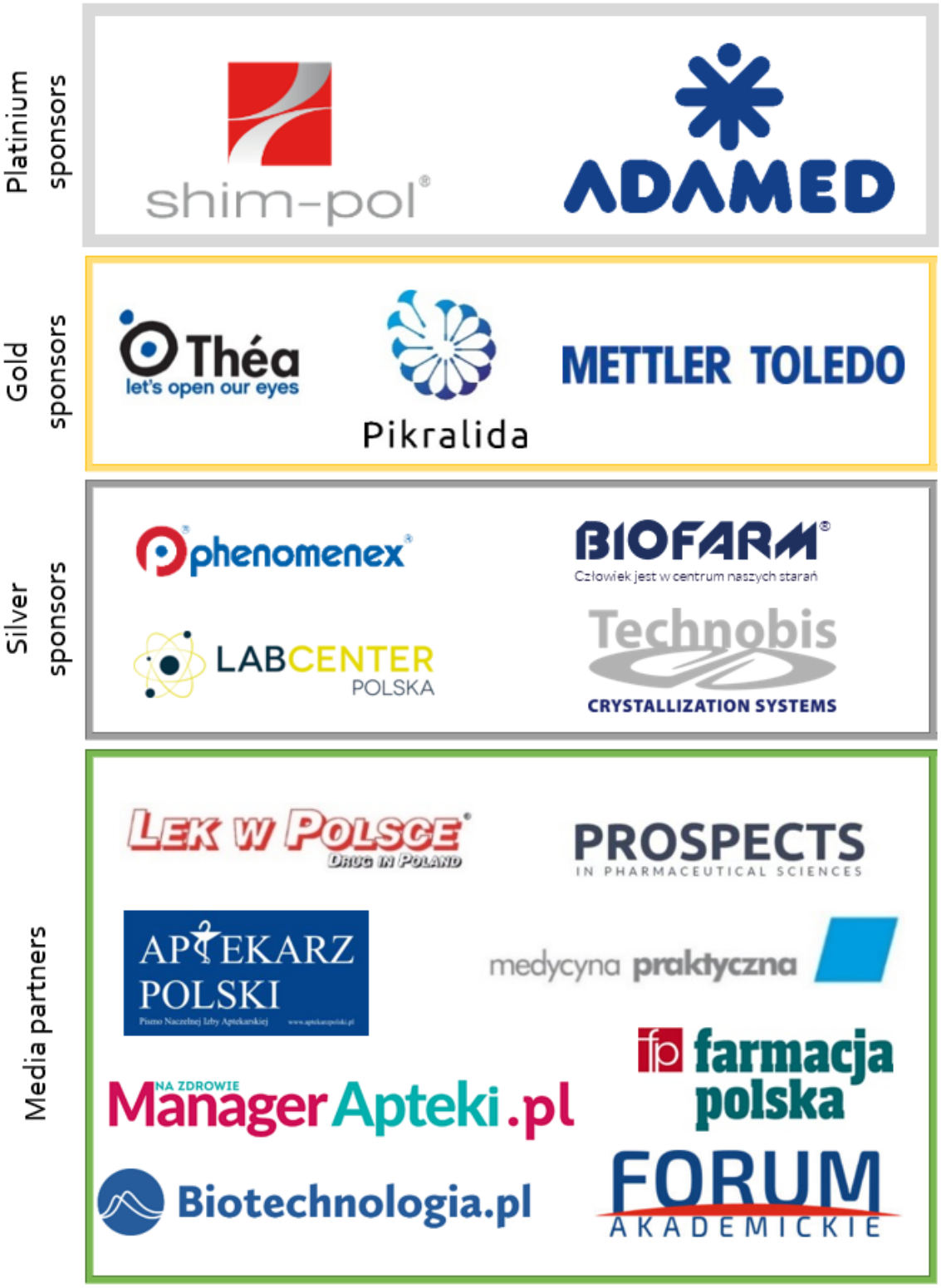

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18.	Prof. Paweł Zajdel	Jagiellonian University Medical College

Scientific Programme

IL	**Invited Lecture**
SL	**Sponsored Lecture**
SP	**Short Presentation**

Thursday, May 23rd, 2024

12:00 – 13:30		**Registration & lunch**
13:30 – 14:15		**Opening ceremony & Biniecki Awards presentation** Chairpersons: P. Dorożyński, P. Luliński, M. Dawidowski
14:15 –14:30		**Break**
Session 1		Chairpersons: M. Dawidowski, M. Wróbel
14:30 – 15:00	**IL.01**	**Alexander Domling** *Automation + Miniaturization = Acceleration*
15:00 – 15:30	**IL.02**	**Tad Holak** *Application of biomolecular NMR in drug discovery*
15:30 – 16:00		**Coffee break**
Session 2		Chairpersons: S. Michorowska, A. Kutner
16:00 – 16:30	**IL.03**	**Natacha Rochel** *Transcriptional signaling of nuclear receptors*
16:30 – 17:00	**IL.04**	**Judith Simcox** *Identifying the function and regulation of plasma lipids*
18:00 – 21:00		**Welcome Reception in Aviator**

Friday, May 24th, 2024

8:30 – 9:00		**Registration & coffee**
Session 3		Chairpersons: E. Pindelska, A. Krause
9:00 – 9:30	**IL.05**	**Fabrizia Grepioni** *Nature-inspired crystal engineering*
9:30 – 10:00	**IL.06**	**Susan Reutzel-Edens** *Inspiring medicines design through solid-state chemistry*
10:00 – 10:30	**IL.07**	**Magdalena Chrzanowska** *Towards targeting endothelial Rap1B to overcome vascular immunosuppression in cancer*
10:30 – 10:50	**SL.01**	**Magdalena Tyszkiewicz (Pikralida)** *Exploration of MMP inhibition in neurological disorders: results of phase I, single and multiple dose studies of oral PKL-021 in healthy volunteers*
10:50 – 11:30		**Coffee break**
Session 4		Chairpersons: A. Kutner, J. Giebułtowicz
11:30 – 12:00	**IL.08**	**Martin Schepelmann** *The manifold roles of the calcium-sensing receptor in non-calciotropic tissues – a new drug target*
12:00 – 12:20	**SL.02**	***Marcin Gawryś (Shim-Pol)*** *Radicalize Your Mass Spectrometer to Solve Unanswered Questions…*
12:20 – 13:00		**Lunch**
Session 5		Chairpersons: P. Dorożyński, P. Zajdel
13:00 – 14:30		***Discussion panel – Progress in Pharmaceutical Sciences: Reality or Illusion?***
Poster Session 1		Chairpersons: J. Turło, S. Polak
14:30 – 15:30	**PSP1**	*PSP.01 - PSP.16* *Poster Spotlight Presentations*
15:30 – 16:30	**PPP1**	*PPP.01 - PPP.40* *Printed Poster Presentations*
16:30 – 17:00		**Coffee break**
Session 6		Chairpersons: M. Czerwińska, J. Giebułtowicz, M. Dawidowski
17:00 – 17:30	**IL.09**	**Gerhard Wolber** *In silico drug design: Pyrod and dynophores as powerful computational microscopes to understand protein function*
17:30 – 17:45		*Biniecki Awardee Presentation*
17:45 – 18:15	**IL.10**	**Cecilia Cristea** *Electrochemical (bio)sensors – a promising tool in pharmaceutical field*
20.00 – 24.00		**Conference dinner**

Saturday, May 25th, 2024

8:30 – 9:00		**Registration & coffee**
Session 7		Chairpersons: P. Zajdel, T. Pawiński
9:00 – 9:30	IL.11	**Anna Birna Almarsdottir** *Patient participation in research on medicines use and pharmaceuticals*
9:30 – 10:00	IL.12	**Stephan Sieber** *Breaking bacterial resistance by chemical strategies*
10:00 – 10:30	IL.13	**Ewa Szczurek** *Discovering highly potent antimicrobial peptides with deep generative model HydrAMP*
10:30 – 10:45	SP.01	**A. Maj** *New active and selective phosphoinositide 3-kinase δ inhibitor the cpl302415 – transfer into the flow synthesis*
10:45 – 11:00	SP.02	**G. Klejborowska** *Recent progress in the synthesis of ferroptosis inhibitors and their biological Assessment*
11:00 – 11:30		**Coffee break**
Session 8		Chairpersons: P. Luliński, M. Sobiech
11:30 – 12:00	IL.14	**Yahya Choonara** *Designing Patient-Centric Therapeutics Using Advanced Drug Delivery: From Molecular Pharmaceutics to Novel Medicines*
12:00 – 12:20	SL.03	**M. Przybylak, M. Krzyżostaniak *(Mettler-Toledo)*** *Know more by doing less – from the synthesis laboratory to manufacturing*
12:20 – 12:35	SP.03	**R. Dell Acqua** *Platinum(ii)-peptide nucleic acid conjugates as antibacterials*
12:35 – 12:50	SP.04	**J. Plewka** *Anti-pd-l1 macrocyclic peptide pac65: comparable potency to monoclonal antibodies with potential for oral administration.*
12:50 – 13:00	SP.05	**R. Terrazas Mallea** *Automated microfluidics-assisted bioprinting of double-emulsion droplets for the Culture of spheroids*
13:00 – 13:10	SP.06	**K. Medyńska** *The impact of plant compounds with anticancer properties on liposomal doxorubicin in triple negative breast cancer oncological therapy*
13:10 – 14:00		**Lunch**
Poster session 2		Chairpersons: W. Maruszak, K.Filip
14:00 – 15:00	PSP2	*PSP.17 - PSP.31* *Poster Spotlight Presentations*
15:00 – 16:00	PPP2	*PPP.41 - PPP.80* *Printed Poster Presentations*
16:00 – 16:30		***Closing ceremony and poster awards presentation*** Chairpersons: P. Dorożyński, J. Giebułtowicz

Keynote Speakers & Panelists

Anna Birna Almarsdóttir

Professor, Department of Pharmacy, Social and Clinical Pharmacy, University of Copenhagen, Denmark

Keynote Speaker

Professor Almarsdóttir received her Ph.D. in Health Policy and Administration from the University of North Carolina at Chapel Hill, USA (UNC) in 1994. She is trained as a pharmacist in Iceland and holds an M.S. in Pharmacy Administration from UNC. She has previously held professorships at the University of Southern Denmark and the University of Iceland. She is currently a Professor of Social and Clinical Pharmacy in the Research Group for Social and Clinical Pharmacy, University of Copenhagen. The group is also designated a WHO Collaborating Center for Research and Training in the Patient Perspective on Medicines Use. Her research interests include pharmaceutical policy analysis, developing new clinical pharmacy services, and patient involvement in drug therapy decision-making. Her methods interests are mainly questionnaire construction, psychometric testing, and triangulation of qualitative and quantitative research methods. Prof. Almarsdóttir teaches social pharmacy and clinical pharmacy and supervises PhD students and Master's projects. She is especially interested in novel modes of teaching students and practitioners.

Title of the lecture presented at the conference:

"Patient engagement in drug research and development – concepts and issues".

Dario Braga

Professor of Chemistry, Department of Chemistry G. Ciamician the University of Bologna

Panelist

Dario Braga is author or co-author of about 500 publications and of several patents on crystal forms. Dario Braga is an expert in solid state techniques and methods.The current scientific interests of his group span from the investigation of crystal polymorphism of APIs and of organic and organometallic molecules, the screening of crystal forms (polymorphs, hydrates, salts, co-crystals etc.) and the preparation, mainly by mechanochemical methods, of ionic co-crystals with the focus on chiral resolution via metal atom coordination. In 2005, together with his group, he founded the academic spinoff company PolyCrystalLine. He is recipient of the Raffaello Nasini medal awarded by the Italian Society of Chemistry, the Federchimica prize, and the Gold Medal of the Italian Crystallographic Association. Dario Braga is currently Member of the Science Institute of the Academy of Science and President of the Institute of Advanced Studies of the University of Bologna.

Yahya Choonara -

Professor, Chair and Head of the Department of Pharmacy and Pharmacology, University of Witwatersrand, Johannesburg, South Africa (SA)

Keynote Speaker

Professor Yahya Choonara is internationally recognised as an outstanding global Pharmaceutical Scientist working at the forefront of the pharmaceutical sciences to produce life-saving medicines that have an impact on global health. As a Pharmacist and a South African National Research Foundation (NRF) Chair he leads the Wits Advanced Drug Delivery Platform (WADDP), Africa's only fully integrated research unit in its domain focused on designing Nanomedicine, Advanced Drug Delivery Systems, Biomaterials and Regenerative Medicines. He has a track record of >370 publications and 28 granted international patents in the field and has trained >110 postgraduate students and postdoctoral fellows.

Title of the lecture presented at the conference:

"Designing Patient-Centric Therapeutics Using Advanced Drug Delivery: From Molecular Pharmaceutics to Novel Medicines".

Magdalena Chrzanowska

Professor, Versiti Blood Research Institute, Medical College of Wisconsin, USA

Keynote Speaker

The Prof. Magdalena Chrzanowska received her PhD from Cell and Cardiovascular Biology, at the University of North Carolina at Chapel Hill. Her laboratory has studied the function of small G proteins in the cardiovascular system. Prof. Chrzanowska uses transgenic mouse and zebrafish models for *in vivo* studies and a variety of biochemical, molecular, and microscopy approaches to interrogate signaling by small GTPases in vascular cells *ex vivo*. Using Rap1b-knockout mice Prof. Chrzanowska has recently revealed a novel role of Rap1b *in vivo*: regulation of angiogenesis. In addition to promoting angiogenesis, her studies indicated that Rap1b is required for the maintenance of normal blood pressure as Rap1b-knockout mice are hypertensive and develop cardiac hypertrophy. Prof. Chrzanowska is currently utilizing tissue-specific knockout mice to understand which physiological processes are involved in the regulation of blood pressure and normal cardiac function regulated by Rap1.

Title of the lecture presented at the conference:

"Towards targeting endothelial Rap1B to overcome vascular immunosuppression in cancer".

Cecilia Cristea

Professor in Analytical Chemistry, Department of Analytical Chemistry, Faculty of Pharmacy, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

Keynote Speaker

Prof. Cristea is the Director of the Drugs Bioanalysis and Nanotechnologies Research Center – BioNanoMed. The Research center is using an interdisciplinary approach to identify and analyze drugs, metabolites, biomarkers, drugs of abuse, and pathogen bacteria from biological fluids, and environmental and food samples. In this regard, different analytical and physical techniques are used. After graduating from the Faculty of Chemistry and Chemical Engineering of Babes Bolyai University, she also graduates from the Faculty of Pharmacy from "Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj-Napoca. She received her Ph.D. in chemistry at the Rennes 1 University, France, and Babes Bolyai University, Cluj-Napoca, Romania. Professor Cristea is a member of the Scientific Council of the Bioelectrochemical Society (BES) and Associate Editor of the Microchemical Journal. Her research interests are in the development and implementation of advanced molecular materials for electrochemical sensing devices for biologically relevant molecules and the design of new (bio)sensors and biomimetic approaches for biomedical applications, pharmaceutical, food, and environmental analysis. The selection of aptamers for biosensing purposes but also for targeted drug delivery is a new research direction of her interest.

Title of the lecture presented at the conference:

"Electrochemical (bio)sensors - a promising tool in the pharmaceutical field".

Alexander Dömling

Professor and Chair, Drug Design Group, Professor and ERA Chair, Drug Design Group, Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry and Czech Advanced Technology and Research Institute, Palacký University in Olomouc, Olomouc, Czech Republic

Keynote Speaker

Alex Dömling studied Chemistry & Biology at the Technische Universitat Munchen (TUM). He performed his Ph.D. with Ivar Ugi and his postdoc – funded by a Feodor Lynen stipend from the Alexander von Humboldt Foundation – with double Nobel Laureate Barry Sharpless. After his habilitation at TUM, he became a professor at the University of Pittsburgh, then chair of the Department of Drug Design at the University of Groningen, and most recently ERA Chair at Palacky University. He also started several biotech companies. He is the author of over 300 publications and over 70 patent applications. His current lab works according to the mantra 'Automation + Miniaturization = Acceleration' on the ERC-funded project of engineering an autonomous drug discovery platform called AMADEUS. Alexander Dömling is a world-renowned researcher in the area of miniaturization, automation of synthetic chemistry, and multicomponent reaction chemistry. Professor Dömling applies multicomponent reaction chemistry to solve problems in drug discovery and related areas. Notably, he introduced the Acoustic Droplet Ejection technology platform to perform precise high throughput synthetic chemistry and demonstrated its applicability to multiple different chemistries and chemical biology projects.

Title of the lecture presented at the conference:

"Automation + Miniaturization = Acceleration".

Fabrizia Grepioni

Professor of Chemistry, Department of Chemistry, University of Bologne, Italy

Keynote Speaker

The scientific interests of Prof. Grepioni include crystal engineering (design, synthesis, investigation and applications of molecular crystalline materials). In particular, the research is focused on (1) Synthesis and properties of new crystal forms (polymorphs, solvates, molecular and ionic co-crystals, coordination compounds with bio-compatible metals) of organic molecules of pharmaceutical and agrochemical interest; (2) Supramolecular gels with d-block metals; (3) Luminescence of molecular solids via functionalization or co-crystallization; (4) Crystal engineering of molecular organic semiconductors; (5) Structural determination from single crystal and powder data. Techniques used by Prof. Grepioni include solution and solvent-free syntheses, X-ray diffraction, DSC, TGA, and hot-stage microscopy. Prof. Grepioni co-organized 10 successful International Workshops on Crystal Forms. Prof. Grepioni is also a co-founder of PolyCrystalLine s.p.a. company, which is specialized in solid-state problems involving polymorphs, solvates/hydrates, co-crystals, and salts, especially concerning APIs.

Title of the lecture presented at the conference:

"Nature inspired crystal engineering".

Tad Holak

Professor of Biochemistry, Faculty of Chemistry, Jagiellonian University, Cracow, Poland

Keynote Speaker

Professor Tad A. Holak is a collaborator of Nobel Prize laureate, Prof. Robert Huber and Prof. A. Dömling. Between 1988 and 2011, he was the group leader of the Biological NMR Structure Group at the Max Planck Institute for Biochemistry, Martinsried (Germany). Since 2012, he has also been the head of the Chemical Biology and Drug Discovery Group at the Jagiellonian University (Krakow, Poland). During this time, he has successfully led several projects funded by the National Science Center of Poland, the Foundation for Polish Science, and EU funds. Professor Holak's research focuses on structural studies of proteins involved in cancer and other human diseases. He and his collaborators have developed novel small-molecule inhibitors of PD-1/PD-L1 and Mdm2-p53 protein- protein interactions as potential new treatments for human cancers. Professor Holak has co-authored more than 230 scientific papers, including articles published in Nature, PNAS, JACS, and the EMBO Journal.

Title of the lecture presented at the conference:

"Monitoring the antagonist-protein and protein-protein interactions with NMR: Inhibitors of p53/Mdm2 and immune checkpoints PD-1/PD-L1".

Krzysztof Jóźwiak

Professor, Director of the National Science Centre

Panelist

Dr Krzysztof Jóźwiak is a Full Professor at the Faculty of Pharmacy, Medical University of Lublin. He conducts research in molecular pharmacology and drug chemistry of substances acting on membrane receptors with potential applications in the treatment of neurodegenerative and civilization diseases. Co-author of over 100 publications, two international patents, and over a dozen plenary papers presented at international conferences. Editor and co- author of the monograph Drug Stereochemistry: Analytical Methods and Pharmacology (2013). Manager of NSC/MSC research grants, winner of FNP Focus and FNP Team programs, participant in a consortium carrying out a project commissioned by the National Institutes of Health, USA.

He has held two postdoctoral fellowships, the first at the National Institute on Aging, NIH, Baltimore, USA (2001-2004), the second at the Biomodeling Laboratory of the International Institute of Molecular and Cellular Biology, Warsaw, Poland (2005-2007). Visiting professor at Università di Bologna, Italy (2023). Winner of team and individual scientific awards from the Minister of Health of the Republic of Poland. In 2012, he was awarded the UCB-Ehrlich Award for Excellence in Medicinal Chemistry by the European Federation of Medicinal Chemistry Societies.

From 2012 to 2020, he was a member of the Council of the National Science Center, where he served, among other things, as chairman of the Life Sciences Committee. As of October 2023, the Director of the National Science Center.

Anna Krause

PhD, Head of R&D, Member of the Board

Panelist

Anna Krause is a pharmaceutical scientist with more than 15 years of experience in the industry. She is an expert in managing research and development projects for innovative and generic drugs. Anna Krause worked at GlaxoSmithKline for several years and co-founded PozLab sp. z o.o. after the Poznań site's closure, where she served as President for eight years. For over five years, she managed the Quality and Control Department and was a Qualified Person. From September 2018 to February 2020, Anna worked as Head of Drug Development at Molecure SA, where she was responsible for the CMC area and preclinical development of drug candidates. Anna is a co-founder and board member at Pikralida sp. z o.o., and leads the R&D department.

Anna Krause has co-authored many research and development projects funded by NCBR, NCN, European Commission, and FNP organizations. She is also a lecturer in "Industrial Pharmacy" postgraduate studies conducted by the Pharmaceutical Department of the Medical University of Gdańsk.

Susan Reutzel-Edens

PhD, President SuRE Pharma Consulting, LLC

Keynote Speaker & Panelist

Dr. Susan M. Reutzel-Edens, President of SuRE Pharma Consulting, LLC, earned her Ph.D. in Chemistry at the University of Minnesota under the direction of the late Professor Margaret C. Etter. Over nearly 30 years at Eli Lilly and Company, she led a team of cross-functional scientists charged with finding commercially-viable crystalline forms for small- molecule drug products and served in a strategic scientific leadership role championing R&D innovation across the Small Molecule Design & Development organization. In 2021, she joined the Executive Leadership Team of the Cambridge Crystallographic Data Centre, where for two years she provided strategic scientific oversight of R&D innovation as Head of Science.

Susan has contributed to the development of more than 150 compounds, is a named inventor on 12 US patents, and has published over 60 papers and book chapters on key aspects of solid form development. Her research interests span diverse topics in organic solid-state chemistry and pharmaceutical materials science, including polymorphism, materials design and engineering, crystallization, solid form screening, physical characterization, structure-property relationships, crystal structure prediction and digital design. She was elected Fellow of the Royal Society of Chemistry in 2018, and currently serves on the CrystEngComm Editorial Board, the Editorial Advisory Boards of Journal of Pharmaceutical Sciences, Molecular Pharmaceutics, and Pharmaceutical Research, and as a topic editor for Crystal Growth and Design. She also served on the inaugural Scientific Advisory Board of the Cambridge Crystallographic Data Centre and is an adjunct professor in the Industrial and Molecular Pharmaceutics Department at Purdue University.

Title of the lecture presented at the conference:

"Inspiring medicines design through solid-state chemistry".

Natacha Rochel

Professor, Institute of Genomics and Cellular Biology, Department of Integrated Structural Biology, the University of Strasburg, France

Keynote Speaker

Professor Rochel is the Leader of a research subgroup investigating the structure-function relationship of nuclear receptors and their coregulators, involved in the chemical biophysics of transcriptional signaling. Prof. Rochel's group is involved in the chemical biophysics of transcriptional signaling. The current research of Prof. Rochel-Guiberteau is related to the structural analysis of novel des- C- ring and aromatic- D -ring analogues of 1a,25- dihydroxyvitamin D3, development of novel gemini-cholesterol analogs for retinoid-related orphan receptors. Prof. Rochel has recently published a fundamental review on vitamin D and its receptor (VDR) from a structural perspective as well as on VDR function and evolution based on the lamprey ligand binding domain.

Title of the lecture presented at the conference:

"Structural biology of the vitamin D nuclear receptor: from molecular mechanisms to therapeutics".

Martin Schepelmann

Group leader of the research group "Interdisciplinary Pathophysiology" at the Institute of Pathophysiology and Allergy Research, Medical University of Vienna, Austria

Keynote Speaker

Priv.-Doz. Mag. pharm. Dr. Martin Schepelmann is a group leader at the Institute for Pathophysiology and Allergy Research, Center for Pathophysiology, Infectiology, and Immunology, at the Medical University of Vienna, Austria. After studying Pharmacy at the University of Vienna, Dr. Schepelmann moved to the UK in 2011 to pursue a PhD in Physiology as Marie Skłodowska-Curie Fellow at Cardiff University (UK), in the group of Prof. Daniela Riccardi. After a 3- year postdoc in drug discovery in the group of Prof. Andrea Brancale, Dr. Schepelmann returned to Austria in 2017 and took up a position as senior postdoc at the Medical University of Vienna in the research group of Enikö Kallay. In June 2023, he successfully completed his Habilitation in Pathophysiology and started his own research group. His research interests include the role of the Calcium-Sensing Receptor in health and disease, the effects of aging and dietary measures on colitis and colorectal cancer, and the role of nucleic acid receptors in cancer. Due to his training as both a pharmacist and physiologist, as well as his work in drug discovery, Dr. Schepelmann is especially interested in "building bridges" bridges between these disciplines. Dr. Schepelmann was awarded many international and national prizes for his work, e.g. the American Society for Bone and Mineral Research Young Investigator Award, the Science Prize of the Austrian Society for Endocrinology and Metabolism, and "Researcher of the Month" of the Medical University of Vienna.

Dr. Schepelmann is the coordinator of the "eRaDicate" Doctoral Network, funded with more than € 2.5 Mio to train 11 PhD students in anti-cancer research and drug discovery all over Europe.

Title of the lecture presented at the conference:

"The manifold roles of the Calcium-Sensing Receptor in non-calciotropic tissues – a new drug target?".

Stephan A. Sieber

Professor and Chair of Organic Chemistry II, School of Natural Sciences, Technical University of Munich, Germany

Keynote Speaker

Professor Sieber received his PhD with distinction from Philipps-University in Marburg under the guidance of Prof. Mohamed A. Marahiel, on "Nonribosomal Peptide Synthetases: Quaternary Structure and Chemoenzymatic Synthesis of Macrocyclic Peptides". His research for doctoral thesis was partly completed in the laboratory of Prof. Christopher T. Walsh at Harvard Medical School in Boston, USA. He did his postdoctoral research with Prof. Benjamin F. Cravatt at the Scripps Research Institute in La Jolla, USA, on "A Chemical-Proteomic Strategy for Targeting Cancer-Associated Enzymes". The research goal of Prof. Sieber's group is to identify unprecedented antibacterial targets beyond the scope of current antibiotics and to exploit these for chemical manipulation. To this goal, the group is applying a multi-disciplinary strategy comprising synthetic chemistry, functional proteomics, microbiology, and protein biochemistry techniques as outlined in the following sections. The research focuses on three main topics: novel antibacterial targets, natural product mode of action, and chemical proteome mining.

Title of the lecture presented at the conference:

"Breaking bacterial resistance by chemical strategies"

Judith Simcox

Assistant Professor, HF DeLuca Biochemistry Laboratory, Department of Biochemistry University of Wisconsin-Madison, USA

Keynote Speaker

Dr. Simcox received her PhD from the University of Utah. As a postdoctoral fellow, Dr. Simcox discovered that acylcarnitines are necessary for maintaining body temperature during cold exposure. The Simcox laboratory is focused on two unanswered questions: how are liver-produced lipids taken up and metabolized in brown adipocytes and how is hepatic lipid processing regulated in cold exposure? Heavy isotope and fluorescently labeled lipids are used to identify lipid importers, assess metabolic pathways of uptake, and characterize the functional importance of various lipid species in isolated brown adipocytes. In untargeted lipidomic analysis, Dr. Simcox identified several hundred hepatic lipids that are altered in cold exposure and correlate with changes in circulating lipids. The role of these lipids will be functionally characterized in cold exposure and the transcriptional programs that regulate their production and clearance identified.

Title of the lecture presented at the conference:

"Identifying the function and regulation of plasma lipids".

Marcin Sobczak

Department of Pharmaceutical Chemistry and Biomaterials Faculty of Pharmacy, Medical University of Warsaw

Panelist

Professor Marcin Sobczak is a distinguished scientist and manager with over 25 years of experience in research and teaching institutions, as well as the commercial sector. His research expertise spans medical and health sciences, exact and natural sciences, and engineering and technical sciences. He specializes in biomaterials engineering, nanomedicine, pharmaceutical chemistry, polymer physicochemistry, medicinal chemistry, and drug delivery technologies. Co-authoring around 120 articles in renowned scientific journals and several patents, Professor Sobczak has also led and executed numerous externally funded projects, receiving multiple scientific awards for his contributions.

He has served as Vice Dean for Science and Chair of the Pharmaceutical Sciences Discipline Council at the Warsaw Medical University, Director of the Military Institute of Hygiene and Epidemiology, and held other leadership positions in the field of science. Additionally, he has been a member of scientific councils of several research institutes and science and technology transfer centers. Professor Sobczak's expertise extends to serving as an expert and reviewer for organizations such as NCN, NCBR, MEiN, and the Science Evaluation Committee. He also brings valuable private sector experience, having held positions as R&D manager and project manager, as well as running his own business in the field of research and development in natural and technical sciences.

Ewa Szczurek

Associate Professor, Institute of Informatics, University of Warsaw, Warsaw, Poland

Keynote Speaker & Panelist

Prof. Ewa Szczurek is the co-director of the Institute of AI for Health at Helmholtz Munich, Germany (from Feb 2024), and leads joint labs at Helmholtz Munich and at the Faculty of Mathematics, Informatics and Mechanics of the University of Warsaw, Poland. She was a visiting associate professor at Northwestern University in the United States (2023) and a visiting fellow at the Center for Interdisciplinary Research, Bielefeld, Germany (2016). She holds Master degrees in computer science from the University of Warsaw, Poland and Uppsala University, Sweden. She obtained her doctoral degree from the Max Planck Institute for Molecular Genetics in Berlin (2011), followed by a postdoctoral fellowship in Switzerland at ETH Zurich. Prof. Szczurek was a recipient of the distinction, scientific and didactic awards from the Rector of the University of Warsaw, as well as ETH Zurich and IMPRS fellowships for her postdoctoral and doctoral research. She acts as a program committee member for the ISMB and RECOMB-CCB conferences, as well as associate editor for Genome Biology. Her research focuses on artificial intelligence, in particular probabilistic graphical models and deep generative models, and their applications in computational medicine. Her specific applications include oncology, pulmonology and the AI-driven design of antimicrobial peptides, a work for which she was recently awarded the ERC Consolidator grant.

Title of the lecture presented at the conference:

"Generative AI for antimicrobial peptide discovery"

Gerhard Wolber

Professor for Pharmaceutical Chemistry and head of the molecular drug design group at the Institute of Pharmacy at the Freie Universität Berlin

Keynote Speaker

After his studies of pharmacy at the University of Innsbruck and Computer Science at the Technical University of Vienna, he received his PhD in pharmaceutical chemistry at the University of Innsbruck. In 2003 he co-founded the molecular modeling software company Inte:Ligand acting as head of research and development. In 2008 he changed back to academia as assistant professor at the University of Innsbruck before changing to the Freie Universität Berlin in 2010. His lab consistently implements the "design-make-test" cycle in drug design by combining computational drug discovery with synthesis and biological evaluation.

Current research interests:

- Development and application of new computer-aided methods in drug design
- Tailored ligands with functional selectivity for G protein-coupled receptors
- Toll-like receptors: modulation of the innate immune response for inflammation regulation and cancer therapy
- Cytochrome P450 enzymes: Metabolism & Applications in Cancer Therapy
- Viral protease inhibitors by fragment-based de novo design

Title of the lecture presented at the conference:

"*In silico* drug design: Pyrod and dynophores as powerful computational microscopes to understand protein function".

Abstracts of invited lectures

Abstract No. IL.01

Automation + Miniaturization = Acceleration

Alexander Domling[1]

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Objectives: Drug discovery and development is a slow and highly expensive process with a very high attrition rate. A recent analysis of productivity in pharmaceutical industry revealed that 'reducing late- stage (Phase II and III) attrition rates and cycle times during drug development are among the key requirements for improving R&D productivity' (Paul et la. Nature Reviews Drug Discovery 9, 203–214 (2010).

Methods: In 2019 we introduced acoustic droplet ejection technology (ADE) as a super-fast way to perform organic synthesis on a nano scale in a highly automated fashion. [1] Meanwhile we and others could establish ADE as a general way to perform organic synthesis, applicable to a broad range of heterocyclic scaffolds, covalent inhibitors, or cyclic peptides. [2,3] We could show that despite minute amounts of compounds formed, the amount is still sufficient to perform multiple assays ranging from biophysical to phenotypical assays. [4,5] Specifically, we applied ADE nano chemistry together with high throughput protein crystallography, intact mass spectrometry, SPR, or thermoshift assays. [6,7] Recently, we also showed the compatibility of miniaturized automated chemistry with a direct-to-biology (D2B) phenotypical approach for the ultra-fast discovery of potent cellular active molecular glues. [8]

Results: ADE-supported drug discovery represents a paradigm shift from the traditional practices of parallel medicinal chemistry conducted on a millimole (mmol) or micromole (µmol) scale in the industry. ADE chemistry offers notable advantages, including high sustainability, unprecedented speed, and compatibility and seamless integration with various screening methods. The synthesis and HTS process is largely automated, contributing to safety and data reproducibility. The synthesis and high-throughput screening (HTS) capabilities allow for unprecedented speed and breadth in data collection, fostering big data-driven drug discovery. When combined with machine learning approaches, ADE-enabled high- throughput chemistry and HTS will expedite the design-make-test-analyze cycle (DMTA), towards the dream of the fully automated closed cycle automated early drug discovery.

Literature:

[1] ACS Cent. Sci. 2019, 5, 3, 451. [2] Angew Chem Int Ed Engl. 2020, 59, 12423. [3] Green Chem., 2019, 21, 225. [4] RSC Med Chem. 2021, 12, 809. [5] Green Chem., 2023, 25, 1380. [6] Angew.Chem. Int. Ed. 2021, 60, 18231. [7] Science Advances 2019, 5, DOI: 10.1126/sciadv.aaw4607. [8] Nat Commun 2023, 14, 8437.

Abstract No. IL.02

Monitoring the antagonist-protein and protein-protein interactions with NMR: Inhibitors of p53/Mdm2 and immune checkpoints PD-1/PD-L1

Tad Holak[1], Bogdan Musielak[1], Radoslaw Kitel[1]

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Objectives: Protein-protein interactions (PPIs) play a central role in virtually every cellular process. These interactions are often weak and transient. NMR spectroscopy has the unique ability to provide information about these types of interactions, making NMR ideal for fragment-based screening, where the binary binding between low-affinity fragments and target proteins is studied. This type of NMR "binary screening" does not provide information about whether a compound can inhibit or dissociate protein-protein interactions.

We have recently described an NMR-based assay for studying the effect of antagonists on protein- protein interactions. The method, called AIDA-NMR (for Antagonist Induced Dissociation Assay- NMR), belongs to the target protein-detected NMR screening methods and provides unambiguous information on whether an antagonist of a protein-protein interaction is strong enough to dissociate the complex (Bista, M. et al., 2009. Robust NMR screening for lead compounds using tryptophan-containing proteins. J. Amer. Chem. Soc. 131, 7500-7501).

Methods: The application of NMR to the study of PPIs is illustrated by our studies of the interaction between small molecules and Mdm2 and small molecules and PD-L1 (Musielak et al., 2020. Competition NMR for Detection of Hit/Lead Inhibitors of Protein-Protein Interactions. Molecules 25, 3017).

Results: We have developed a competition NMR experiment, AIDA-NMR, that directly shows whether an antagonist releases proteins from their PPI interaction.

In almost all human cancers, the effectiveness of the p53 pathway is compromised, in part by overexpression of Mdm2. Restoring the impaired function of the single gene, p53, offers new avenues for cancer therapy.

Blockade of the PD-1/PD-L1 immune checkpoint pathway has led to unprecedented results in cancer treatment in recent years. We will describe the NMR experiments used to study the interaction of small molecule compounds with PD-L1. These compounds can effectively block the PD-1/PD-L1 interaction.

Conclusions: We believe that AIDA-NMR is a valuable complement to the well-known binary ligand-protein SAR-by-NMR assays, but also to the saturation transfer difference (STD) NMR experiment. Compared to STD, AIDA-NMR avoids the weaknesses of this experiment because AIDA-NMR provides control for compound aggregation and protein instability, two situations that lead to false positives.

Abstract No. IL.03

Structural biology of the vitamin D nuclear receptor: from molecular mechanisms to therapeutics

Natacha Rochel[1]

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Objectives: The biologically active form of vitamin D (1,25D3) plays a key role in calcium homeostasis, as well as in cell proliferation and differentiation. These activities are mediated by its nuclear receptor VDR, a ligand-regulated transcription factor, and loss-of-function variants impairing 1,25D3 binding induce Hereditary Vitamin D-Resistant Rickets. VDR natural ligands and analogues have been extensively investigated as anticancer agents. .

Methods: Using structural methods in combination with biophysical and functional assays, we characterized the mechanisms of action of VDR ligands.

Results: Based on the exploitation of the structural knowledge about VDR-ligand interactions, we have developed novel safer and disease-tailored selective analogs. In addition, VDR antagonists and inhibitors have potential applications for the treatment of hypercalcemia or hypercalciuria associated with high vitamin D levels, characteristic of several rare and refractory disorders. In the recent years, we came interested in the design and characterization of VDR antagonist ligands. Structure-function studies revealed that the studied antagonists act via different mechanisms.

Abstract No. IL.05

Nature inspired crystal engineering

Fabrizia Grepioni[1]

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Objectives: In this talk results will presented obtained by applying crystal engineering to develop nature- inspired organic and organic/inorganic materials, with the intent of tackling challenges in the health and environmental fields. A number of examples will be shown to demonstrate that co-crystallization of organic/inorganic systems with organic compound can be successfully used to attain preparation of novel antibacterial agents to tackle antimicrobial resistance. The fundamental idea is that a crystal engineering approach based on the choice of intermolecular interactions between organic and organic/inorganic compounds allows to obtain materials with collective properties that are different, and often superior to those of the separate components. It is also demonstrated that the success of this strategy depends crucially on cross-disciplinary synergistic exchange with expert scientists in the areas of bioinorganics and microbiology application-oriented developments of these novel materials.

Abstract No. IL.06

Inspiring medicines design through solid-state chemistry

Susan Reutzel-Edens[1]

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Objectives: Drug product design generally begins with identifying, oftentimes through crystallization screening, solid forms in which to isolate and store the drug substance. When performed under different conditions, crystallization can yield different forms (polymorphs, solvates) with different particle sizes and shapes, thus providing an opportunity to engineer drug crystals to desired specifications. To meet the design requirements for a drug product, a solid form must be selected from potentially numerous options discovered through crystallization screening. However, the path to even one commercially- viable form can, in some cases, be lengthy and difficult.

Methods: Experimental solid form screening, crystal structure prediction, free energy calculations

Results: Under immense pressure to shorten development timelines and reduce costs, the pharmaceutical industry is keen on right-sizing the time and effort spent on finding suitable solid forms.

Conclusions: In this presentation, the use of crystal structure prediction, a computational workflow to calculate crystal structures starting from a molecular diagram, to enhance the effectiveness of experimental solid form screens and achieve molecular-level understanding of structures and their properties is explored. Recent advances in predicting crystal form stability under real-world conditions, which have redefined the state-of-the-art in crystal modeling, are also discussed.

Abstract No. IL.07

Towards targeting endothelial Rap1B to overcome vascular immunosuppression in cancer

Magdalena Chrzanowska[1], Guru Prasad Sharma[1], Ramoji Kosuru[1], Behshid Ghadrdoost Nakhchi[1]

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Objectives: The tumor microenvironment (TME), a complex interplay between cancer cells and host tissue, determines tumor growth and metastasis. Elevated VEGF levels in the TME promote angiogenesis and contribute to EC anergy, suppressing leukocyte recruitment and hindering the infiltration of cytotoxic CD8+ T cells, a key component of adoptive T-cell therapies. Understanding how elevated VEGF modulates this response is crucial for improving cancer treatment outcomes. We investigated the role of small GTPase Rap1B in tumor vasculature and tumor growth, and in mechanisms through which VEGF modulates tumor EC responses.

Methods: B16F10 melanoma tumors were grown in EC-specific Rap1B-knockout mice and controls. TME composition, EC and T-cell activation were assessed via flow cytometry. CD8+ T-cell depletion's effect on tumor growth was tested using anti-CD8 or control monoclonal antibodies. TNF-α's impact on gene expression in siRap1B and siControl ECs was analyzed through bulk RNA sequencing, principle component analysis, differential gene expression, and gene set enrichment analysis using Reactome database. TNF-α-induced NFκB activity was measured by luciferase assay, and the adhesion of Jurkat T cells to TNF-α-treated ECs was determined through fluorescence. The effect of TNF-α, with or without VEGF-A, on cell adhesion molecule expression was evaluated by Western blot.

Results: EC Rap1B deletion restricts tumor growth and tumor angiogenesis, enhances leukocyte recruitment, and leads to increased $CD8^+$ T cell activity. Notably, depletion of $CD8^+$ T cells restored tumor growth in Rap1B-deficient mice, highlighting the critical role of $CD8^+$ T cells in this process. Mechanistically, Rap1B deficiency leads to upregulated TNF-α signaling, increased NF-κB transcription and proinflammatory chemokine and cell adhesion molecule expression. Furthermore, EC Rap1B deletion prevents VEGF-induced immunosuppressive downregulation of cell adhesion molecules, demonstrating that Rap1B is essential for VEGF-A-suppressive signaling.

Conclusions: We identify EC Rap1B as a novel vascular target for cancer immunotherapy, with potential for combination with immune checkpoint inhibitors. The parallels between Rap1 and closely related oncogene Ras signaling mechanisms suggest that targeted therapies could be adapted to disrupt abnormal vasculature and improve immunotherapy outcomes. Our results provide new insights into the molecular mechanisms of vascular immunosuppression and highlights the potential of targeting EC Rap1B to enhance anti-tumor immune responses and improve cancer treatment outcomes.

Abstract No. IL.08

The manifold roles of the Calcium-Sensing Receptor in non-calciotropic tissues – a new drug target?

Martin Schepelmann[1]

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Objectives: The Calcium-Sensing Receptor (CaSR) is known for being chiefly expressed in calciotropic tissues such as the parathyroids and the kidneys and has been a known drug target for secondary hyperparathyroidism. Positive allosteric CaSR modulators (PAMs) such as cinacalcet (Mimpara/Senispar) have been on the market and in clinical use for this indication now for 20 years. These drugs act by increasing the affinity of the CaSR for its natural ligands (in this context: Ca^{2+}) in the parathyroid glands, thus reducing the secretion of parathyroid hormone. However, the CaSR is also expressed in many other tissues, such as the vasculature, the lung, or the colon. In these tissues, the role of the CaSR has long been unclear. However, more and more evidence is emerging for crucial physiological and pathophysiological roles of this receptor in these non-calciotropic tissues that could qualify the CaSR as a novel drug target.

Methods: We have investigated the roles of the CaSR in the vasculature, the lung, and the colon using various techniques such as transgenic mouse models, pharmacological intervention studies, molecular biology etc.

Results: We demonstrate novel roles for the CaSR in health and disease, e.g. in vascular contractility regulation, direct vascular-mediated effects on mineral ion homeostasis, airway inflammation, and colitis. A specific interest lies here on the repurposing of the calcilytics, negative allosteric CaSR modulators (NAMs), after their failed development as anti-osteoporosis drugs.

Conclusions: Recent studies suggest that the CaSR might be a novel target for diseases such as inflammatory bowel disease and asthma. Results on the physiological roles for the CaSR in blood vessels could contribute to explaining certain disease effects and drug side effects in chronic kidney disease.

Acknowledgements: Funded by the European Union (264663, 675228, 101119427), the Austrian Science Fund (P 29948-B28) and the Herzfelder Family Fund (P 32840-B).

Abstract No. IL.09

In silico drug design: Pyrod and dynophores as powerful computational microscopes to understand protein function

Gerhard Wolber[1]

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Objectives: 3D pharmacophore models are three-dimensional ensembles of chemically defined interactions of a ligand in its bioactive conformation. They provide an elegant way to decipher chemically encoded ligand information and have become a valuable tool in drug design. Static 3D pharmacophores - although valuable tools for fast and efficient virtual screening *in silico* - represent only a static view of protein-ligand interactions. Therefore, we aim to combine 3D pharmacophore models with molecular dynamics simulations to explore physiologically relevant information in dynamic interaction patterns.

Methods: *Pyrod* and *Dynophores* were used in this study. *Pyrod* is a software used for developing dynamic interaction fields, while *Dynophores* is a tool used for dynamic pharmacophore modeling. We obtained crystal structures and homology models from publicly available databases and developed 3D pharmacophore models to identify key ligand-receptor interaction patterns. Molecular dynamics simulations were conducted to investigate the dynamic behavior of receptor-ligand complexes, providing insights into conformational changes and dynamic interaction networks.

Results: We showcase the use of 3D pharmacophore modeling combined with molecular dynamics simulations to reveal the dynamic interaction patterns of ligands. The approach integrates insights into dynamic ligand binding mechanisms, which are critical for understanding receptor activation and modulation. The study demonstrates how novel bioactive compounds with new scaffolds were identified using this workflow, which were subsequently experimentally validated. *Pyrod* and dynophores have been shown to be effective computational tools for elucidating protein function and guiding structure-based drug design. This has been demonstrated particularly in the context of G protein-coupled receptors (GPCRs), Toll-like receptors, and inhibitors of ribosomal assembly.

Conclusions: In this study, we utilized 3D pharmacophore models and molecular dynamics simulations to investigate dynamic interaction patterns of protein-ligand interactions for the purpose of guiding the development of therapeutics through *in silico* drug design. Our approach unveiled physiologically relevant information on ligand binding mechanisms, which we then used for high-throughput virtual screening resulting in the discovery of novel, bioactive molecules. The use of *Pyrod* and *Dynophores* proves to be a powerful computational strategy for understanding protein function and facilitating rational drug design efforts.

Abstract No. IL.10

Electrochemical (bio)sensors - a promising tool in the pharmaceutical field

Cecilia Cristea[1]

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Objectives: Making use of one of the cleanest and simplest reagents, electron, electrochemistry enables the monitoring of electrochemically active targets or probes without any or little prior separation. Beside this, the latest discoveries in the field of nanomaterials have been introducing a wide range of electrode materials which could be further modified with bioelements or biomimetic receptors, assuring highly sensitive and selective detection of a wide range of analytes going from small molecules like antibiotics or analgesics to larger molecules like proteins.

Methods: The surface of electrodes modified with nanostructured or composite materials can increase the analytical performance of (bio)sensors and opens the way for sensors miniaturization and portability contributing also to the stability and improvement in the immobilization of bioreceptors and in the end, enhancing the overall analytical performance of the sensors in terms of sensitivity and electro- conductivity.

Results: Another direction developed in recent years showing a tremendous interest in the biomedical field is the development of wearable sensors for on-body and on-site detection of a large range of targets. While early wearable sensors have focused on healthcare and fitness, in the last years, there have been developed a lot of similar approaches for security, environmental and forensic applications. Still, many challenges must be faced for the integration of wearable systems and sensors into point-of-use or portable devices. Efficient integration methods assisted by the development of stretchable components could guarantee technological advances in the fabrication of wearable sensors. Miniaturization of potentiostats and minimal samples preparation are other requirements that must be fulfilled. Several immuno- and bioassays are commercially available today to a wide audience. By simply changing the bioelement type, other biosensors have been developed, for instance, those based on affinity or on immunological recognition.

Conclusions: This talk will underline the importance of electrochemical (bio)sensing for pharmaceutical applications under the frame of nowadays requirements, highlighted by recent examples of nanomaterials-based sensing tools applied for the quantification of different targets/drugs from biomedical or environmental samples.

Abstract No. IL.11

Patient engagement in drug research and development – concepts and issues

Anna Birna Almarsdóttir[1]

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Objectives: Until recently, researchers within pharmaceutical sciences saw patients mostly as clinical trial participants with their contribution limited to data provision. It is now becoming evident that patients' contributions based on knowledge of living a life with their disease and treatment is of great importance to drug developers and clinical researchers. The objective is to shed light on the landscape of patient engagement in drug research and development.

Methods: The concepts of patient perspective, patient insights, patient involvement, patient engagement are important in understanding the landscape of patient engagement as patients can both be seen as data providers and collaborators. An overview will be provided of what is known about the patient perspective on medicines use and how patients or citizens can be included in drug development. The overview is based on the main sources that have provided input or guidance to patient engagement, and examples of existing applications and guidance.

Results: Researchers have found important to make a clear delineation between research *on* and *with* patients. Research *on* patients has them as clinical trial participants or providing data on themselves (research subjects). Conversely, the area of patient engagement refers to the research or development carried out with patients where they have roles as advisor, reviewer, co-researcher, or driving force. The main issues that researchers need to deal with are firstly, who can represent patients and in which capacity. Patient can be experts, advisors, and patients talking for themselves. The first two categories are useful as representatives of a larger body of patients and requires training, whereas the last one are persons who do not take on themselves to represent others. A second issue is the meaning of patient engagement in the research process. From the patient perspective, it means involvement in decisions at various stages of the process, not forgetting their potential contribution early on (i.e. in the design of study). Patient representatives object to just „ticking the box" regarding regulatory requirements, and do no view their role as solely to ensure patient recruitment into clinical trials.

Conclusions: Patient engagement is about partnerships between researchers and patients along the drug development process. The same standards that apply to research *on* patients do not apply here. To be purposeful, patient engagement must be based on established standards and qualified methods to obtain meaningful input from patients into the drug development process.

Abstract No. IL.12

Breaking bacterial resistance by chemical strategies

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Multiresistant bacterial pathogens such as Methicillin-resistant *Staphylococcus aureus* (MRSA) are responsible for a variety of severe infections that pose a significant threat to global health. To approach this challenge new chemical entities with an unprecedented mode of action are desperately needed. This presentation will cover our latest efforts to identify new anti-bacterial targets and corresponding chemical inhibitors. A proteome mining approach will be presented to identify cofactor-dependent enzymes as novel antibiotic targets. Small molecule cofactor mimics infiltrate the bacterial metabolic machinery leading to their incorporation in cofactor-dependent enzymes. Their analysis via mass-spectrometry revealed the function of uncharacterized proteins in important bacterial pathways as well as the identification of novel antibiotic hits along with their mode of action.

In a separate approach we identified new synthetic or natural product derived compound classes that effectively kill pathogenic bacteria. Chemical synthesis of improved derivatives led to the identification of active molecules with nanomolar potency and suitable metabolic stability. The mode of action was investigated by diverse methodologies including affinity based protein profiling (AfBPP). For example, one compound stimulates a signal peptidase correlating with enhanced secretion of extracellular proteins. These included essential cell-wall remodeling enzymes whose dysregulation likely explains the associated antibiotic effects.

Abstract No. IL.13

Generative AI for antimicrobial peptide discovery

Ewa Szczurek[1]

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Objectives: Antimicrobial peptides emerge as compounds that can alleviate the global health hazard of antimicrobial resistance, prompting a need for novel computational approaches to peptide generation.

Methods: Recently, we proposed HydrAMP, a conditional variational autoencoder that learns lower-dimensional, continuous representation of peptides and captures their antimicrobial properties. The model disentangles the learnt representation of a peptide from its antimicrobial conditions and leverages parameter-controlled creativity. HydrAMP is the first model that is directly optimized for diverse tasks, including unconstrained and analogue generation and outperforms other approaches in these tasks. An additional preselection procedure based on ranking of generated peptides and molecular dynamics simulations increases experimental validation rate.

Results: HydrAMP outperforms competitor approaches for AMP generation. Wet-lab experiments on five bacterial strains confirm high activity of nine peptides generated as analogues of clinically relevant prototypes, as well as six analogues of an inactive peptide.

Conclusions: HydrAMP enables generation of diverse and potent peptides, making a step towards resolving the antimicrobial resistance crisis.

Abstract No. IL.14

The Design of 21st Century Therapeutics: Advancing Drug Delivery, Nanomedicine, and Regenerative Medicine

Yahya E. Choonara[1]

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Advances in medicines/therapeutics in the 21st century are driven largely by innovations in drug delivery and nanomedicine. In merging these two areas, regenerative medicine has also grown and given rise to a new era of patient-centric therapeutics aimed at delivering targeted treatment towards the pathology of disease or for patient convenience. Treating certain diseases remains a significant burden, particularly for low-middle-income countries (LMICs) where the burden of disease also has socio-economic repercussions in addition to poor healthcare outcomes. Research into advanced drug delivery, nanomedicine, and tissue engineering can resolve these challenges and change clinical practice or the fate of chronic diseases. A concise incursion into innovative prototypes developed to optimally treat diseases will be outlined and how the molecular features of illnesses can be exploited to design 21st- century medicines/therapeutics. This includes cancer nanomedicine, ocular therapeutics and tissue engineering applications with a focus on 3D-biomimetic platforms in neurotrauma. Conventional treatments are limited by restrictive bio-barriers and do not provide adequate architecture essential for recovery. Several advances also reveal that engineered biomaterials can interact with organ/tissue systems at a molecular level to revolutionize therapeutics by stimulating, responding to, and interacting with target sites to induce physiological responses while minimizing side effects.

Abstracts of Printed Poster Presentations

Abstract No. PPP.01

The influence of H/D exchange on the thermal and structural properties as well as molecular dynamics (at ambient and high pressure) of the selected active pharmaceutical ingredient

Paulina Jesionek[1], Barbara Hachuła[2], Dawid Heczko[3], Karolina Jurkiewicz[4], Magdalena Tarnacka[4], Marcin Zubko[5], Marian Paluch[4], Kamil Kamiński[4], Ewa Kamińska[1]

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Objectives: We aimed to thoroughly characterize the thermal properties, internal (atomic) structure, and relaxation dynamics of neat melatonin (MLT) and its partially deuterated derivative (MLT-d_2). An important aspect was the dielectric measurements carried out on both substances (prepared in the amorphous form) at high-pressure conditions - reflecting those accompanying the tableting process.

Methods: MLT and MLT-d_2 were analyzed by using Fourier Transform Infrared (FTIR), Differential Scanning Calorimetry (DSC), X-ray Diffraction (XRD), and Broadband Dielectric Spectroscopy (BDS).

Results: The performed experiments indicated i.e., *i*) the presence of an additional thermal event in the thermogram of MLT-d_2; *ii*) a temperature-dependent variations in the structure of both examined substances; *iii)* an excellent physical and chemical stability of MLT and MLT-d_2 at high pressure; *iii)* a change in the shape i.e., the broadening of the α-relaxation peak with compression and also *iv)* a discrepancy in the pressure evolution of T_g, as well as the temperature dependence of the activation volume, DV_a, for both compounds, which are most likely due to different effects of compression/densification on the hydrogen and deuterium bond dynamics in MLT and MLT-d_2, respectively.

Conclusions: Our research revealed that such a slight modification of the compound as a hydrogen/deuterium (H/D) substitution can affect the thermal and structural properties as well as high- pressure relaxation dynamics of the investigated compounds. It should also be mentioned that the results presented in this paper are a preliminary stage for further fundamental research, including solubility studies on neat and deuterated active substances in various pH- and biorelevant media.

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Keywords: melatonin, H/D substitution, high-pressure studies

Abstract No. PPP.02

Synthesis of new dimers of dipyridothiazines with anticancer action

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Objectives: Phenothiazine derivatives are a group of heterocyclic compounds with a wide spectrum of biological properties, including anticancer, antibacterial, antiviral and immunomodulatory properties [1]. Research conducted for many years has shown that modified phenothiazines with pyridine rings in their structure have promising antiproliferative and anticancer effects related to the activation of the mitochondrial apoptosis pathway [2-5]. Taking into account the above literature reports, the goals of our project were formulated, in which it was decided to obtain a group of dipyridothiazine dimers with anticancer activity by chemical transformation.

Methods: Conditions for efficient reactions for the synthesis of new derivatives using selected dipyridothiazines and linkers in the presence of a strong base and DMF were developed. The structures of the new derivatives were clearly confirmed by NMR spectroscopic techniques and HR MS mass spectrometry. Next, the obtained dimers were tested for anticancer activity against breast (MCF7) and colon (SW480) cancer lines, using normal muscle (L6) cell lines and a reference drug (doxorubicin).

Results: Conditions for the synthesis of dipyridothiazine derivatives were developed, obtaining sixteen new derivatives with the structure of dipyridothiazine dimers. The structure of the new molecules was confirmed using NMR and 2D NMR spectroscopy and their purity using HR MS spectrometry. The obtained compounds were subjected to cytotoxicity tests (using the MTT tests) against cancer cell lines: MCF7, SW480 and normal cells L6, obtaining promising results.

Conclusions: The biological results indicate the promising anticancer potential of selected derivatives and selectivity also. The activity is depended on the location of nitrogen atoms in the dipyridothiazine system and the type of linker connecting the dipyridothiazine units. The research was carried out with the support of Metropolitan Science Support Fund program, Grants 2023, 2024.

Further studies of the mechanism of anticancer activity have been planned.

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Abstract No. PPP.03

A systemic insight into liposomal nanoparticle formulations of annamycin

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Objectives: Anthracycline inhibitors of topoisomerase II have strong cytotoxic effects and are used in the treatment of many cancers. Annamycin, *2'-iodo-3'-hydroxy-4'-epi-4-demethoxy-doxorubicin*, is a highly lipophilic non-cardiotoxic anthracycline, resistant to MDR efflux. Its liposome formulation further decreases the cardiotoxicity, systemic toxicity, whereas increases plasma stability, bioavailability, absorption by cancer cells. Liposomes used in the current drug formulation are heterogenous multilayer mm-sized lipid vesicles, absorbed quickly by RES. We developed long-circulating pegylated unilamellar small nm-sized nanoliposomes as annamycin carriers, significantly improving their properties.

Methods: Liposomes were prepared by extrusion method combined with thin lipid film formation and annamycin was encapsulated in the lipid bilayer or actively in the particle core through ion gradient. The studied lipids were: SPC, DMPC, DMPG, DSPG, and others. Screening of the proposed formulations against stability (aggregation, precipitation), drug crystallization/aggregation, and encapsulation efficiency such parameters as lipid composition and drug : lipid weight ratio were optimized in the process. Hydrodynamic size distribution, dispersity, and colloidal stability were dissected by dynamic light scattering spectroscopy.

Results: We identified highly stable liposomal nanoparticles with diameters below 150 nm with very high efficiency of annamycin encapsulation above 95% and drug : lipid weight ratios as high as 1:20. The novel optimized formulations may be tested in preclinical and clinical programs.

Conclusions: In perspective, our long-circulating nanoparticle formulations with enhanced permeability and retention effect (EPR) may be used for development of combinatorial therapies to induce potential drug synergism on the one hand and to hamper MDR effects on the another.

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Abstract No. PPP.04

Anticancer effects of IL-13RA2-targeting cytotoxins - *in vitro* studies

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Objectives: Interleukin 13 receptor alpha 2 (IL-13RA2) is a membrane-bound protein expressed in over 75% of gliomas and absent in normal brain tissues. Also, other types of tumors overexpressed IL-13RA2, including melanomas, ovary and pancreatic cancer, making it an attractive target for cytotoxic therapeutics. We examined the anticancer potential of two immunotoxins targeting IL-13RA2: WPD 101a.1 and WPD 101a.2, both consisting of optimized IL-13 ligand fused to modified derivative of bacterial toxin, in glioblastoma, breast cancer and prostate cancer *in vitro* model.

Methods: Cancer cell lines representing glioblastoma (U-251, LN-229 and SNB-19), breast cancer (MDA- MB-231, T47D) and prostate cancer (PC-3) were used. IL-13RA2 expression was determined by immunocytochemical assay. Cell viability (MTS), protein synthesis (SRB), cell proliferation (BrdU), apoptosis induction (HOECHST 33342 staining) and colony formation (Clonogenic assay) were investigated after immunotoxins treatment.

Results: In cells with high and moderate expression of the IL-13RA2 both immunotoxins decreased significantly in a dose- and time-dependent manner cell viability and the clonogenic potential *via* apoptosis induction. Anticancer activity of WPD 101a.1 and WPD 101a.2 was significantly lower in the case of cells with low IL-13RA2 expression level.

Conclusions: Our studies indicate, that both immunotoxins showed anti-tumor activity against various *in vitro* cancer models and their effectiveness depends on the number of receptors on the cell surface. Both WPD 101a.1 and *WPD 101a.2* might be considered as candidates for effective targeted therapy.

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Abstract No. PPP.05

Berubicin – effective drug candidate against primary and metastatic brain tumors

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Objectives: Brain metastasis (BM) are the most recurrent complication of systemic cancer and about 10 times more common than primary brain cancer. It is estimated that 40 % of patients with systemic neoplastic disease will develop BM. Unfortunately, secondary brain tumor is associated with extremely poor prognosis. Average survival rate for patients with BM is only 8 months. Standard treatment involves surgery resection and radiotherapy but effective and universal chemotherapy for BM is not existing. Berubicin (BER) is a novel chemotherapeutic agent that has potential to be an answer for the urgent need of a potent drug for BM. BER is a 4'-o-benzyllated doxorubicin (DOXO) derivative. BER is not only 2-3 times more potent than DOXO, but has an unique ability to cross the blood brain barrier. In the clinical trials BER, showed high effectiveness for the treatment of primary brain tumors in adults. We investigated the potential of BER as a chemotherapeutic in the BM treatment.

Methods: Cell lines representing brain metastatic site of various cancer types were used: DU145 (prostate cancer), COLO792 (melanoma), MA-MEL-45A (melanoma), COLO668 (lung cancer), MDA- MB- 361 (breast cancer). Cell viability (MTS), cell proliferation (BrdU) and apoptosis were assessed after BER treatment.

Results: According to MTS and BrdU assays, BER reduced cell viability and proliferation in a dose-and time-dependent manner. For each cell line IC_{50} value was calculated and estimated: 14.14 nM (for DU- 145), 84.12 nM (for COLO792), 47.89 nM (MA-MEL-45A), and 26.02 nM (COLO668). IC_{50} values were significantly lower than that for DOXO treatment. Cancer cells were eliminated via apoptosis induction.

Conclusions: BER inhibits viability and proliferation of brain metastatic cells with high potency, regardless of the primary tumor origin. Our results indicate that BER could be consider as a drug- candidate for secondary brain tumors therapy.

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Abstract No. PPP.06

Application of capillary electrophoresis (CE) as an innovative method for determining Bimatoprost, Latanoprost and Travoprost in pharmaceutical preparations.

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Objectives: The term glaucoma refers to a group of optic neuropathies. These include progressive degradation of retinal ganglion cells and dissection of the optic nerve head. The above changes are associated with defects in the peripheral visual field, which may ultimately lead to blindness. It is believed that the increased intraocular pressure (IOP) occurring in this disease is one of the main factors in the development of neuropathy. It causes ischemia of the optic disc, leading to damage to the retinal nerve fibers. This, in turn, manifests itself in changes in the field of vision. The most commonly used drugs that provide the best results in lowering intraocular pressure are Bimatoprost, Latanoprost and Travoprost. Therefore, glaucoma treatment is mainly based on the use of drugs that lower intraocular pressure. The most effective and most frequently used drugs that provide the best results in lowering intraocular pressure in the treatment of glaucoma are Bimatoprost, Latanoprost and Trawoprost. From a chemical point of view, these drugs are analogues of prostamide F2a (the main active substance of Bimatoprost) and analogues of prostaglandins PGF2α (the main ingredients of Latanoprost and Travoprost).

Methods: The investigated material included solutions of ophthalmic drugs containing Bimatoprost, Latanoprost and Trawoprost as active substances. The capillary electrophoresis method from Beckman Coulter equipped with a UV-vis detector was used for the tests. The separation parameters were achieved using a capillary with a length of 30 cm and an internal diameter of 75 µm, a 100 mM phosphate running buffer (pH 2.0), capillary temperature 20°C, voltage -15 kV.

Results: The developed method has been optimized and validated. Good results were obtained for different aspects including stability of the solutions, linearity, and precision. Detection and quantification limits for Bimatoprost, Latanoprost i Trawoprost were reached at the level of 3.9 µg/mL, 4.2 µg/mL, 4.6 µg/mL and 11.6 µg/mL, 11.9 µg/mL, 12.4 µg/mL respectively. Preliminary investigations of the concentrations of Bimatoprost and other substances in liquid drug forms (eye drops) showed close compliance with the contents of these substances declared by the manufacturers.

Conclusions: This method has been successfully used to the measuring concentrations of Bimatoprost, Latanoprost i Trawoprost in order to determine the content of these compounds in in medicinal preparations. The sample preparation procedure is very simple and not time consuming. The speed of obtaining the result of the analysis was only 11 minutes.

Abstract No. PPP.07

Synthesis and characterization of cocrystals and salts of antifolate pyrimethamine with vanillic acid

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Objectives: Pyrimethamine (PYR), antifolate used as an antiparasitic agent, is known to form salts with monohydroxybenzoic acids. [1] To explore the possibility of application of substituted hydroxybenzoic acids for the formation of stable solid assemblies with pyrimethamine, we designed and prepared the associates of PYR with vanillic acid (VANA, 4-hydroxy-3-methoxybenzoic acid).

Methods: All the pyrimethamine/vanillic acid associates were obtained by solution crystallization (slow evaporation) from various solvents. Wide range of crystallization conditions, including type of the solvent, temperature, and stoichiometry of used reagents was tested. Obtained monocrystals were studied and thoroughly characterized by SC-XRD, and thermal analyses (TGA and DSC).

Results: Four new associates of pyrimethamine with vanillic acid were obtained, including, depending on the crystallization conditions, solvated and non-solvated cocrystals and salts. The formation of the specific associates was proved by SC-XRD. Thorough analysis of obtained crystal structures enabled identification of weak interactions stabilizing the formation of the PYR/VANA adducts. TG and DSC studies proved changes in thermal stabilities of obtained associates in comparison to the used reagents.

Conclusions: The results confirm great potential of pyrimethamine to for solid state assemblies with substituted hydroxybenzoic acids. Moreover, the study revealed how interplay of different crystallization conditions induces formation of different PYR/VANA products.

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Keywords: pyrimethamine, hydroxybenzoic acids, crystal engineering

Abstract No. PPP.08

Magnetic nanoparticles coated with hydrazide starch, as a potential pH-sensitive carrier - synthesis and characterisation

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Objectives: Design, synthesize and characterize a pH-sensitive biopolymer-based carrier for anticancer drugs.

Methods: The polymer shell synthesis method was previously optimized to obtain magnetic nanoparticles coated with hydrazidomethyl starch that can bind doxorubicin. The synthesis of hydrazidomethyl starch was carried out in three stages. Starting with the synthesis of the sodium salt of carboxymethyl starch (CMS-Na), followed by its hydrolysis to carboxymethyl starch (CMS). In the final step, the carboxymethyl starch was reacted with hydrazine. Next, the magnetic nanoparticles obtained by alkalinizing a solution containing iron (II) and (III) ions were covered with starch. The starch layer covering the magnetic core of the nanoparticles was then modified into hydrazidomethyl starch using the same method as for the pure polymer. Then, the modified starch was used to attach doxorubicin (DOX) with the formation of a hydrazone bond. The percentage of loaded and released drug was examined by spectrofluorimetry.

Results: The resulting magnetic nanoparticles coated with hydrazidomethyl starch contains hydrazide groups in its structure that can form a pH-sensitive hydrazone bond with doxorubicin. Each synthesis stage was characterized using techniques such as FTIR-ATR spectroscopy, thermal analysis, XRD, and SEM imaging. The drug loading content (DLC) was average 19,3%. Subsequently, the prepared carrier- drug system was tested in solutions mimicking different pH environments, including those resembling cancer cells, healthy cells, and their subcellular components, to assess drug release from the carrier. The findings revealed that drug release from the carrier was over four times higher in a slightly acidic environment compared to a neutral one.

Conclusions: The obtained materials were able to attach an anticancer drug using a pH-sensitive hydrazone bond. The results underscores the potential of the developed carrier system for targeted drug delivery in cancer therapy.

Abstract No. PPP.09

Co-crystals of genistein with coformers containing N-heterocyclic aromatic unit. Crystal structures, solubility studies, spectral and thermal characterization.

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Objectives: Genistein is one of the natural isoflavonoids, low molecular weight polyphenolic compounds, which are known to exhibit various biological activities. Due to its health-promoting effects such as antioxidation, anti-inflammatory, antitumor and antibacterial properties genistein provides a huge potential for applications in various branches of medicine. It is considered a promising agent in treatment of cancer, diabetes, obesity and genetic diseases. Similarly to most flavonoids, genistein is classified as Class II agent in the Biopharmaceutical Classification System (BCS). Its low water solubility leads to poor bioavailability, which limits its therapeutic effectiveness. One of the method of increasing the solubility and bioavailability, which are key factors determining drug effectiveness, is synthesis of co-crystals consisting of an active substance and a suitable coformer. The aim of conducted research was to obtain novel crystal forms of genistein which exhibit enhanced solubility.

Methods: Herein, we present eight newly synthesized co-crystals of genistein with coformers containing *N*-heterocyclic aromatic unit: 4-methylpyridine; 3,4-dimethylpyridine; 3,5dimethylpyridine; 2,3dimethylpyridine; 2,4-dimethylpyridine; 2,5-dimethylpyridine; 2,6dimethylpyridine and 4dimethylaminopyridine. New multicomponent crystalline solids was identified by solvent-drop grinding approach and isolated afterwards by slow evaporation methods and characterized by singlecrystal X-ray diffraction, powder X-ray diffraction, thermal analysis, infrared spectroscopy, solubility and permeability studies.

Results: Obtained co-crystals revealed the presence of an O-H·Narom heterosynthons between O7 and O4′ hydroxyl moieties of genistein and the pyridyl ring of a conformer. Hirshfeld surface analysis and the associated two-dimensional (2D) fingerprint plots revealed that genistein has significant hydrogen bond donor capability when cocrystallized. Investigated cocrystals exhibited higher solubility than the pure genistein. An increasing of the rate of permeability of genistein in cocrystal forms compared to pure genistein have been observed on the basis of biological permeability studies.

Conclusions: The improved permeation rate and solubility of studied co-crystals of genistein, allows us to preliminarily assume that the bioavailability of the substance present in cocrystal will be improved over its uncomplexed form.

Abstract No. PPP.10

Metabolic Profiling Indicates Predictive Role of Carnitine and Acylcarnitines in Rivaroxaban Efficacy for Thrombus Dissolution in Atrial Fibrillation

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Objectives: Traditional anticoagulants, vital for managing thromboembolic complications in atrial fibrillation (AF), are increasingly being replaced by novel oral anticoagulants like rivaroxaban, improving lifespan and quality of life. Despite the approval of once-daily rivaroxaban 20 mg for reducing risk of stroke and systematic embolism in nonvalvular AF, treatment is ineffective in some patients, who develop thrombus in left atrial appendage (LAA). RIVA-TWICE study demonstrated thrombus resolution in the LAA with a modified dosage regimen of rivaroxaban 15 mg administered twice daily. However, over 50% of patients remained unresponsive to this modified treatment despite the drug's plasma levels being within the therapeutic range. In our previous study, metabolic profiles of responders and non- responders were compared using untargeted metabolomics, which revealed differential abundance of metabolites in pathways such as alpha-linoleic acid, fatty acid metabolism (acylcarnitines, ACs), carnitine synthesis, and protein degradation. These findings suggested that ACs, carnitine, and its precursors may influence rivaroxaban treatment efficacy, justifying the selection of these metabolites for quantitative analysis.

Methods: The study included 15 AF patients who developed thrombus in LAA despite standard dose of rivaroxaban (20 mg once-daily), and who were subsequently switched to 2x15 mg. Targeted quantitative LC-MS analysis of selected metabolites was conducted on plasma samples collected prior to the modification of the dosage regimen.

Results: After the dosage adjustment, complete thrombus resolution in the LAA was observed in 7 (46.7%) patients, who displayed elevated levels of various ACs, such as C3, C18, iC5, iC5-OH, and C6- DC ACs, as well as carnitine and its precursors: methionine, trimethyllysine, butyrobetaine, and lysine.

Conclusions: The patients' phenotype, including carnitine, its precursors, and ACs concentrations, may predict rivaroxaban efficacy in dissolving thrombus. While these compounds may not directly impact thrombus resolution, their elevated levels can be regarded as indicative features of the patient's phenotype.

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Abstract No. PPP.11

Optimizing Saliva Metabolomics for Personalized Medicine A Path to Noninvasive Biomarker Discovery, Precision Diagnosis and Therapy Monitoring

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Objectives: The growing demand for personalized medicine has led to increased interest in non- invasively collected biofluids. Saliva has emerged as a promising alternative to blood, owing to its rich biological content, which includes numerous biomarkers found in blood, and its ability to reflect the pharmacologically active fraction of circulating drugs. Importantly, saliva collection is easy, painless, and cost-effective, making it suitable for identifying diagnostic biomarkers, prognostic indicators, and monitoring therapies for both oral and systematic diseases. However, until relatively recently, the metabolite composition of saliva has received limited attention, with the study of salivary metabolites remaining largely unexplored. One of the key areas requiring standardization is the choice of extraction solvent. Therefore, the aim of our study was to investigate the effect of this factor on saliva metabolome coverage and data quality, with the ultimate objective of identifying the optimal procedure. Additionally, different types of chromatographic columns were tested

Methods: Metabolomic analysis of saliva samples collected from six healthy individuals and extracted using four different extraction solvents (acetonitrile (ACN), acetonitrile:methanol (ACN : MeOH) (1:1, *v/v*), isopropanol (IPA) and methyl tert-butyl ether (MTBE) was performed using LC-MS/MS with four different chromatographic columns (SeQuant ZIC HILIC, Accucore Amide HILIC, Kinetex PFP, Kinetex C18).

Results: No significant effects of the extraction solvent and chromatographic column were observed for polar metabolites. Based on the number of annotated metabolites, extraction with ACN : MeOH and the use of a SeQuant ZIC HILIC column are recommended. For non-polar metabolites, the most efficient extractions were extractions with ACN and ACN : MeOH, and the C18 column yielded the greatest number of annotated metabolites.

Conclusions: Based on the collected data, monophasic extraction with ACN : MeOH and LC-MS/MS analyses using both SeQuant ZIC HILIC (for polar metabolites) and C18 (for non-polar metabolites) columns are recommended as a convenient and effective approach for saliva metabolomics analysis, ensuring satisfactory data quality.

Abstract No. PPP.12

Quality Assessment of Dietary Supplements Using Liquid Chromatography Coupled with Mass Spectrometry

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Objectives: Dietary supplements are food which are widely consumed in both the European Union (EU) and the United States of America (USA). However, due to the absence of mandatory quality testing, there is a notable dearth of data regarding the quality of these supplements. Specifically, data for Eastern Europe are scant. In response to this critical need, we conducted a comprehensive analysis of legal and illegal dietary supplements containing various substances.

Methods: Our study encompassed twenty-one legally registered supplements obtained from diverse European countries available in the Polish market. Additionally, we examined nine illegal supplements seized from a clandestine facility by the Polish Police, originating from various global locations. Employing high-performance liquid chromatography coupled with mass spectrometry as one of the most reliable analytical method, we conducted a meticulous screening for contaminants.

Results: The results of our analysis revealed the presence of 32 contaminants in the 30 dietary supplements under scrutiny. Among these, 25 contaminants were identified in 13 legal products, while 7 contaminants were found in 5 illegal preparations. Our untargeted analysis uncovered a concerning aspect— the intentional adulteration of supplements, whether legal or illegal, with pharmacologically active substances. The presence of other substances etched in small doses, indicates a low level of chemical purity of the place where they were produced.

Conclusions: Our research confirms that most of the analyzed dietary supplements are of low quality due to deliberate adulteration or produced in conditions of inadequate chemical purity. The presence in dietary supplements of substances not registered for human use or not approved for marketing in the detected combinations constitutes a serious threat to health and life. Prioritizing the prevention of intentional adulteration and ensuring consumer safety should be central to ongoing regulatory discussions on dietary supplements.

Abstract No. PPP.13

The evaluation of the quality of dietary supplements using dissolution test

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Objectives: Dietary supplements are widely used by consumers, and the number of available supplements on the Polish market is rapidly increasing. Polish law does not regulate the quality parameters of dietary supplements introduced into the market. Manufacturers are not required to present quality tests, stability tests, or tests regarding the interactions of dietary. This results in low quality of available dietary supplements. Due to the similarity of dietary supplements to medicinal products, it seems reasonable to investigate the release of substances from the dietary supplement forms. This research goal was to assess the suitability of conventional pharmacopoeial methodologies (specifically USP I or USP II) and the PhysioCell apparatus (biorelevant gastric conditions).

Methods: Our research focused on assessing the release of substances in dietary supplements containing carnitine, proline, tryptophan, tyrosine and vitamin C sourced from the EU, Switzerland, and the USA. Pharmacopoeial methods were initially employed, and samples exhibiting releases below 25% were reanalyzed using PhysioCell. Considering the various forms in which they were presented, a total of 16 capsule-form supplements and 3 tablet-form supplements were evaluated.

Results: The application of gastric emptying conditions for dietary supplements in capsules resulted in increased release of substances compared to the pharmacopoeial dissolution tests. In the case of 8 out of 16 tested supplements in capsule-form, despite the conditions used, the release was below 80%. In the case of tablet-form supplements, releases were low in both pharmacopoeial dissolution tests and those gastric emptying conditions.

Conclusions: Pharmacopoeial methods represent a straightforward and cost-effective approach to evaluating the quality of dietary supplements. While supplements are identified as low quality through pharmacopoeial assessment, PhysioCell allows to validate such conclusions. Conversely, if PhysioCell yields higher test results, it signifies that the release of substances from these dietary supplements is highly dependent on the conditions of the gastrointestinal tract.

Abstract No. PPP.14

The role of heteroaryl substituents in tuning receptor selectivity and metabolic stability: the case of 5-HT7 receptor inverse agonists with antidepressant activity

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Objectives: The type 7 serotonin receptor (5-HT7R) couples to Gs subtypes activating adenylyl cyclase to produce cAMP. Preclinical and clinical data support the hypothesis that 5-HT_7R inverse agonists may represent a valid alternative strategy for the treatment of affective disorders. Moreover, 5-HT_7R induced depressive-like behavior in mice by activation of matrix metalloproteinase 9 (MMP-9) in a Gs-dependent manner. Herein, we designed, synthesized and pharmacologically evaluated, using *in vitro* and *in vivo* methods, a series of close analogs of PZ-1129, a potent 5-HT_7R inverse agonist from a series of arylsulfonamide derivatives of (aryloxy)ethyl alicyclic amine, to improve selectivity while enhancing metabolic stability.

Methods: A series of 18 compounds was designed using computer-aided methods and synthesized according to a multistep procedures, involving mechanochemical approach. The affinity of compounds for 5-HT_7R was determined by radioligand binding experiments. The effect on 5-HT_7R constitutive activity at Gs signaling was evaluated using the ability of tested compounds to inhibit cAMP production induced by agonist 5-HT. The metabolic stability of selected compounds was tested in the rat liver microsome (RLM) assay. The impact on MMP-9 activity was assessed using *ex vivo* methods. Antidepressant properties of PZ-1657 were tested in forced swim test (FST) in male Swiss Albino mice.

Results: The study identified compound PZ-1657 as potent 5-HT7R inverse agonist as measured by decrease in cAMP accumulation, and also displaying high selectivity over 5-HT1AR. It was characterized by good *in vitro* metabolic stability. It exerted antidepressant-like properties in mice. Notably, PZ-1657 reduced the 5-HT_7R-mediated MMP-9 activity in mice hippocampus exerting potency similar to reference SB-269970.

Conclusions: The high potency and selectivity along with favorable ADMET properties justify further development of PZ-1657 as a pharmacological tool to deeply investigate the role of 5-HT_7R in affective disorders.

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Abstract No. PPP.15

Using Multivariate Orthogonal Analysis to Improve the Effectiveness of Preparing Cyclosporine A Modified Daily Disposable Contact Lenses Composed of Hilafilcon B

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Objectives: The study aimed to improve the process of loading Cyclosporine A (CyA) onto commercial contact lenses. Multivariate orthogonal analysis (OAD) was used for the investigation. Throughout the study, the kinetics of both the loading and release of CyA was managed.

Methods: The drug modification procedure was implemented on silicone hydrogel lenses (SHL, Soflens Daily Disposable, Hilafilcon B, Bausch&Lomb®).The SHLs were modified with vitamin E and Cyclosporine A. The proper SHL modification procedure was preceded by the 24-hour application of vitamin E. The lenses were dried and subsequently soaked in CyA solution, applying variable loading parameters (Table 1), according to the scheme of the OAD procedure designed.

In the final step, CyA drug-modified contact lenses were placed in a buffer solution (pH = 7.9 ± 0.3) that mimicked the environment of human tears, called artificial lacrimal fluid (ALF). The aim of this stage was to quantitatively monitor the CyA release process into the tear fluid. The procedure was carried out in triplicate for each set of parameters.

Table 1. The parameters of the orthogonal array procedure.

Number	Temperature	CyA [µg/ml]	Loading time [h]	VE [µg/ml]
1	5	5	24	0
2	5	10	48	25
3	5	15	72	50
4	5	20	144	100
5	10	5	48	100
6	10	10	24	50
7	10	15	144	25
8	10	20	72	0
9	15	5	72	25
10	15	10	144	0
11	15	15	24	100
12	15	20	48	50
13	20	5	144	50
14	20	10	72	100
15	20	15	48	0
16	20	20	24	25

Results: The purpose of the conducted research was to determine the optimal parameters of the loading process, allowing the creation of a drug delivery system based on SHL characterized by the longest and most balanced drug release scheme.

The poster will present crucial parameters determined by the OAD analysis, indicating the significance of the factors examined on the efficiency of the drug modification procedure.

Conclusions: On the basis of the data obtained, it was concluded that among the investigated factors, the key is the immersion time of the lens in the CyA loading solution, followed by the concentration of this solution. Another less significant factor is the temperature of the loading solution.

Abstract No. PPP.16

Magnetic nanoparticles with a chitosan-hemoglobin coating as potential photosensitive PDT drug carriers

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Objectives: The presented research focuses on synthesizing magnetic nanoparticles (MNPs) coated with modified chitosan and hemoglobin as a potential Photodynamic Therapy (PDT) drug carrier.

Methods: Three types of magnetic nanoparticles were synthesized, and their core was covered with modified chitosan, differing in the number of long-distance amino groups to which bovine serum hemoglobin was covalently bound. Then, used drug chlorin e6 was bonded to the material covalently and by physical adsorption. The efficiency of loading the drug into the obtained nanoparticles was determined (% DLE). The chemical structure of all materials was confirmed using ATR-FTIR spectroscopy, and the surface morphology was characterized using SEM and STEM (High-Resolution Electron Microscopy). The thermal stability was also checked by thermogravimetric analysis and the size of the obtained materials was examined using the Dynamic Light Scattering (DLS) method. The MNP's zeta potential was tested to determine whether the materials can interact with cell membranes. Finally, the ability of the synthesized nanoparticles to produce species of singlet oxygen was also determined.

Results: The results show the dependence between the number of chitosan-derived amino groups on MNPs and the amount of bound hemoglobin. This also translates into the amount of bound drug - the material with the largest amount of hemoglobin gives the highest %DLE value. The material with the highest drug content has a positive zeta potential, which confirms that it can interact with cell membranes. The most important information in the context of using the material in PDT is the fact that it is capable of generating singlet oxygen.

Conclusions: The tested properties of the obtained material confirm that it can be an effective carrier of the drug chlorin e6 in PDT. This confirms the ability of the drug to bind to the material and, above all, the fact of generating singlet oxygen

Abstract No. PPP.17

Magnetic nanoparticles coated with chitosan enriched in free dihydroxyboryl groups for fast binding of alpha-1-acid glycoprotein

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Objectives: The presented research focuses on the synthesis of magnetic nanoparticles (MNPs) coated with modified chitosan enriched free dihydroxyboryl groups for fast binding of glycoproteins.

Methods: The first stage of the project included the chemical modification of chitosan and then the introducing of dihydroxyboryl groups derived from boronic acids capable of quickly binding sugars to its structure. The obtained new polymer material was characterized in terms of structure and surface morphology (ATR-FTIR, SEM, TEM, XRD analyses). Using thermogravimetric analysis and differential scanning calorimetry, the thermal stability of the material was determined. The hydrophilic/hydrophobic nature of the modified chitosan was also examined by measuring the contact angle The next stage of the research included the synthesis of magnetite nanoparticles covered obtained chitosan materials with free dihydroxyboryl groups. The magnetic material was characterized and then used to bind alpha-1-acid glycoprotein.

Results: Chitosan-coated magnetic nanoparticles with free dihydroxyboryl groups show the ability to bind alpha-1-acid glycoprotein. The amount of bound glycoprotein is depended by the type of coating the nanoparticle and the pH of the environment.

Conclusions: The obtained materials, due to their ability to bind glycoproteins, could be used in diagnostic tests for glycoprotein uptake. Additionally, they could be used in many other applications, such as: capture of ligands or creation of diagnostic tests. Moreover, the fact that viruses have glycoproteins in their structure, these materials may find potential use as materials for the deactivation of pathogens by selective and rapid binding of glycoproteins.

Abstract No. PPP.18

A simple LC-MS/MS method for determination of calcineurin inhibitors in Mitra™ and Capitainer™ microsampling systems – a pilot comparative study

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Objectives: The calcineurin inhibitors - tacrolimus (TAC) and cyclosporin A (CSA) are the cornerstones of current immunosuppressive therapy. Due to the narrow therapeutic index and high inter- and intraindividual variability, therapeutic drug monitoring is necessary for appropriate dose adjustment. Routinely, the whole blood is the suitable matrix for the mentioned drug determination. LC-MS/MS (liquid chromatography-tandem mass spectrometry) or immunochemical assays (IAs) are used for that process. New microsampling techniques, based on volumetric-absorptive technology with a fixed amount of capillary blood collection, are attractive for home-based self-sampling processes, especially in the pediatric population. The study aimed to simultaneously develop a TAC and CSA determination method in two new microsampling devices - Mitra™ (Neoteryx, Trajan, USA) and Capitainer™ (Capitainer AB, Sweden).

Methods: In the study, the devices for 10µL capillary blood collection have been used. The extraction process was optimized using different solvents and various drying times. The assay was based on a previously validated method for TAC determination in Mitra™ samples using Nexera X2-Shimadzu 8050 LC-MS/MS system. Analytical methods were validated according to EMA (European Medicines Agency) guidelines in the following calibration ranges: 0.5 - 60 ng/ml for TAC and 1 - 500 ng/ml for CSA. During validation, accuracy, precision, carry-over effect, matrix effect, and short- and long-term stability were examined.

Results: The sample preparation process was optimized: the 2-hour time of sampler drying after blood collection, the extraction solvents (water for Mitra™, and methanol: water mixture for Capitainer™ device), and supporting of extraction using sonification. The extract was purified using a precipitation mixture following SALLE (salt-assisted liquid-liquid extraction) methodology (this step reduced the matrix effect in the case of Capitainer™ samples). The validation criteria were fulfilled - mean precision, accuracy, absolute recovery, carry-over, and percentage short- and long-term were within the acceptance range. No hematocrit effect was observed.

Conclusions: The bioanalytical method for the simultaneous determination of TAC and CSA has been successfully validated. After evaluation, it can be concluded that the Mitra™ device has proved to be the better choice for clinical application due to more favourable validation parameters and an extension process than Capitainer™ device. In the next step, the devices will be evaluated according to clinical utility in the routine TDM process.

Abstract No. PPP.19

HPLC-DAD *versus* LC-MS/MS methods for (val)ganciclovir therapeutic drug monitoring in clinical practice – comparative study

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Objectives: Ganciclovir is an antiviral medication primarily used to treat infections caused by cytomegalovirus (CMV), particularly in immunocompromised individuals, such as transplant recipients and patients with HIV/AIDS. Cytomegalovirus (CMV) is one of the most common viral infections in neonatal population. The mechanism of action is based on inhibiting viral replication. Currently, GCV is available as intravenous (IV) injection and oral capsules (as prodrug- valganciclovir, VGCV). Therapy with GCV is associated with significant side effects, including bone marrow suppression, which can lead to decreased production of red and white blood cells and platelets. Therefore, Therapeutic Drug Monitoring (TDM) is required, especially in neonates and children. Additionally, this approach caused to conduct the pharmacotherapy based on PK/PD (pharmacokinetic-pharmacodynamic) modeling, taking into account the viral load level.

Methods: The serum/plasma GCV levels were determinated simultaneously using HPLC-DAD (high performance liquid chromatography with diode array detection) and LC-MS/MS (liquid chromatography-tandem mass spectrometry) analytical techniques. The both methods were validated previously, according to EMA (European Medicines Agency) guidelines. The acyclovir has been used as internal standard, as alternative to deuterated GCV (especially in LC-MS/MS) due to better compensation of matrix effects, and higher stability as well. For statistical analysis using several tools - Passing Bablok regression, Bland-Altman bias, correlation and normality test- 120 paired samples were used. The samples were obtained from patients under GCV (intravenous) or VGCV (orally) treatment in The Children's Memorial Health Institute in Warsaw. The study was positively approved by local Bioethics Comittee.

Results: The correlation between paired results ranged from 0.977 (0.956-0.993). The Passing-Bablok regression equation was: HPLC=0.879(MS)+0.076. The slope was not in the acceptance range, but zero was in the intercept calculated range, therefore criteria were fulfilled for this parameter. The mean percentage bias calculated using the Bland-Altman plot between paired results was -12.08% (HPLC versus MS).

Conclusions: After cross- and clinical validation, it might be concluded that methods (HPLC-DAD versus LC-MS/MS) are not equivalent in clinical practice. Due to high selectivity, the LC-MS/MS method should be considered the reference method for (V)GCV determination, especially in low drug levels.

Abstract No. PPP.20

Loading of enzymatic cargo into extracellular vesicles derived from lung cancer cells

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Objectives: Extracellular vesicles (EVs) are nanosized membrane-bound structures secreted by different cell types that have gained scientific interest because they offer advantages in the field of novel diagnostics and targeted delivery systems. The aim of the study was to develop a method for loading of glucose oxidase (GOX), a flavin adenine dinucleotide (FAD)-dependent enzyme, into EVs derived from lung cancer cells (A549).

Methods: The GOX loading into A549 cells was carried out using five different techniques: (i) 18-hour incubation at room temperature, (ii) saponin permeabilization, (iii) triple freeze-thaw cycling at -80°C and room temperature, (iv) 30 cycles of 1-second pulse/1-second pause, on ice sonication, and (v) 250V, one 10ms pulse electroporation. The as-obtained GOX-loaded EVs were then separated by ultracentrifugation (100,000 x g) for 90 min at 4°C. To verify the enzymatic cargo, the uploaded and pristine EVs were analyzed for protein (BCA) and FAD levels using HPLC with spectrofluorimetric detection. Nanoparticles tracking analysis (NTA) was applied for size measurements in the uploaded EV samples. The cytotoxic effects of the as-developed constructs were studied on A549 cells based on Alamar Blue assays.

Results: EVs ranging from 100 to 150 nm were obtained after loading in all tested methods. This was also accompanied with increased FAD levels due to uploaded GOX into the EVs. The highest amount of protein was determined in the EV samples subjected to electroporation which was further used to produce the samples for cytotoxicity studies. The massive cytotoxic effects were noted for the GOX- loaded EVs on A549 cells.

Conclusions: We have developed a suitable method for encapsulating the enzymatic cargo (GOX) into the EVs derived from lung cancer cells. The results show the electroporation as a most profitable method for GOX loading into the EVs. The EVs uploaded with GOX produced serious cytotoxic effects on lung cancer cells.

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Keywords: : exosomes, delivery system, glucose oxidase

Abstract No. PPP.21

Large volume production of extracellular vesicles in a hollow fiber bioreactor

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Objectives: The hollow fiber bioreactor (HFB) is a sophisticated three-dimensional (3D) cell culture system utilizing small, semi-permeable capillary membranes known as hollow fibers. These fibers possess a molecular weight cut-off range, facilitating the exchange of nutrients and the removal of toxins. Within specially designed chambers, HFB allows for the cultivation of large cell populations, where oxygen, nutrients, and cell culture medium are delivered via a pulsatile perfusion pump mechanism. Despite its compact size, the HFB system maintains a high cell density, facilitating the large-scale production of extracellular vesicles (EVs). In this context, the goal of the study was to setup the HFB technology for large volume production of EVs derived from lung cancer cells (A549).

Methods: The A549 cells were expanded in conventional culture flasks using DMEM with 10% FBS and were subsequently used to seed on a medium-sized, hollow-fiber culture cartridge with a 20 kDa molecular weight cut-off (Fibercell Systems; Frederick, MD). The cells were adapted to bioreactor culture conditions and finally cultured in DMEM with 10% Fibercell Systems CDM-HD. Media from both conventional culture flasks and the hollow-fiber culture cartridge were collected for EV isolation according to the protocol. Subsequently, the presence of EVs in the harvested medium was confirmed using nanoparticles tracking analysis and transmission electron microscopy.

Results: Maintaining A549 cells in the 3D culture on HFB resulted in highly concentrated production of EVs showing a typical size and morphometry addressing to exosomes. No other cellular components were observed in the as-produced EV batches.

Conclusions: HFB serves as an advanced platform for three-dimensional cell culture, facilitating the production of large-scale EVs in a compact space. Our study demonstrates that maintaining lung cancer cell lines in the HFB system results in the increased production of EVs, which hold significant potential in further bioengineering studies.

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Abstract No. PPP.22

The Analysis of Phytochemical Composition of Lady's Mantle Herb Extracts and Their Bioavailability in Cosmetic Formulations.

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Objectives: Lady's mantle (*Alchemilla vulgaris*) is a plant used in traditional medicine. The European Pharmacopoeia 10 contains monographs on *Alchemillae herba* (the flowering, aerial parts of *A. vulgaris* Lady's mantle is rich in flavonoids, tannins and phenolic acids. It has antioxidant, anti-inflammatory, antimicrobial and anti-photoaging properties. Externally, it was used to rinse the mouth and throat in case of inflammation, as well as in the treatment of skin rashes, lesions and ulcers. Our research aimed to compare the quality of Lady's Mantle herbs available on the Polish market by analyzing the composition of their alcohol, glycol, and water extracts. Additionally, the antioxidant properties of these extracts were assessed. The next stage of our study involved investigating the release of active substances from hydrogel formulations containing extracts obtained from an herbal product with the highest phytochemical content.

Methods: Five herbal products available in Polish drugstores were selected for the study: one sold as a medicinal product, and four as dietary supplements. For each product, 50% ethanolic, 50% propylene glycolic, and water extracts were obtained. The content of the main components was analyzed using HPLC-DAD. Additionally, the total contents of polyphenols and flavonoids were determined. Antioxidant properties were assessed using the FRAP and DPPH radical scavenging methods. A release study of active ingredients from hydrogel-based cosmetic formulations was performed using a phosphoric buffer at pH 5.6.

Results: Among the herbal products tested, the raw material sold as a medical product turned out to be the best. It contained the most active ingredients, including phenolic acids, e.g. ellagic acid. Extracts from this raw material also showed the strongest antioxidant properties. Among the extracts, ethanol extracts contained the most phytochemicals (50%), followed by glycol and water extracts, although the differences were not significant. Active ingredients were released from hydrogels containing these extracts to a similar extent.

Conclusions: Lady's mantle extracts are rich in active ingredients with strong antioxidant properties. Release studies have shown that these extracts can be used in cosmetics and dermocosmetics.

Abstract No. PPP.23

Biodistribution of magnesium-doped iron oxide nanoparticles in lung cancerous and non-cancerous cells

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Objectives: Biocompatible iron oxide nanoparticles (NPs) are promising, non-toxic agents used in medicine. The as-synthesized mPEG-silane-coated iron(III) oxide nanoparticles doped with magnesium are found to be stable in cellular culture medium and highly monodispersive. The aim of the study was to assess their biodistribution in lung cancerous and non-cancerous cells in *in vitro* studies as it is one of the main important determinants that drives the efficacy and toxicity of nanomaterials.

Methods: The biodistribution of NPs was evaluated using Single-Cell Inductively Coupled Plasma Mass Spectrometry (SC-ICP-MS) and Transmission Electron Microscopy (TEM) method. SC-ICP-MS was employed to determine the elemental content, including iron and magnesium, in human adenocarcinomic alveolar basal epithelial (A549) and human normal bronchial epithelial (BEAS-2B) cells treated with NPs at concentrations of 0.1 and 100 $\mu g \cdot mL^{-1}$ for 24 hours. The uptake of NPs by A549 cells was assessed at various time points (15 minutes, 1, 3, 6, and 24 hours) after incubation with 250 $\mu g \cdot mL^{-1}$ of as-synthesized NPs using TEM. Additionally, the uptake was evaluated after exposure to an alternating magnetic field (AMF).

Results: The studies revealed that lung cancer cells exhibit more efficient uptake of NPs compared to normal lung cells. Moreover, higher concentrations of NPs led to increased uptake by A549 cells. A significant accumulation of NPs was observed both intracellularly and extracellularly in lung cancer cells after exposure to AMF.

Conclusions: The as-synthesized magnesium-doped iron oxide NPs demonstrate uptake by both lung cancerous and non-cancerous cells, which is additionally dose and AMF-dependent, indicating their potential for further testing in medical applications.

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Abstract No. PPP.24

The potential role of aqueous extract from fruits of *Chaenomeles japonica* and its post-digestive fractions in human neutrophils

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Objectives: *Chaenomeles japonica* (Thunb.) Lindl. ex Spach (CJ), commonly cultivated in Poland, have been also used in traditional Chinese Medicine. The fruits of CJ are rich in polyphenol compounds, especially procyanidins (Du et al., 2013; Siegien et al., 2021). The study aimed to assess the influence of phytochemicals present in CJ fruit extract on various inflammation-related factors in human neutrophils (PMN).

Methods: Firstly, we performed gastrointestinal digestion (GI) *in vitro* of aqueous CJ fruit extract, to assess potential bioavailable metabolites. We compared the activity of crude fruit extract and obtained GI fractions in PMN. We evaluated the neutrophil extracellular traps (NETs) and neutrophil alkaline phosphatase (NAP) secretion using fluorescence intensity assay. In addition, the release of proinflammatory cytokines/chemokines (tumor necrosis factor (TNF-α), interleukin(IL)-8, IL-1β) was examined.

Results: The crude CJ fruit extract as well as all obtained GI fractions decreased NET formation compared to stimulated control. At the same time, there are apparent differences in the NET as well as NAP secretion between the used GI fractions of CJ. The release of TNF-α in PMN was 62.1% for 100 µg/mL of CJ fruit extract, compared to samples treated with lipopolysaccharide. An even stronger inhibitory effect, on the same factor, was observed for two GI fractions – intestine and colon – respectively 52.1% and 52.2%, at the same concentration of 100 µg/mL.

Conclusions: In conclusion, both aqueous extract from the fruit of *C. japonica* and post-digestive fractions present inhibitory activity of cytokine and NET secretion. The gastrointestinal digestion of the extract likely affects the composition and bioactivity of the fractions, as there are noticeable differences among the effects of the GI fractions.

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Abstract No. PPP.25

Novel sulfoximine scaffolds derived from strecker reaction

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Objectives: The sulfoximine moiety has a great potential as a functional group in drug discovery. It can be viewed as isosteric e.g. to sulfone, sulfonamide or carboxamide [1], which may be insoluble due to the strong intermolecular interactions in the solid state. The sulfoximine group can provide improved solubility. Moreover, it offers unique H-bond donor/acceptor capabilities and has been shown to mimic the substrate-enzyme transition states, thus allowing for the design of potent enzyme inhibitors.

Methods: Multicomponent reactions (MCRs) constitute a powerful tool to create molecular diversity of drug-like compounds. Among them, Strecker reaction has been known to be versatile for amino-nitrile synthesis. Therefore, we decided to examine its use as a synthetic path to obtain biologically useful sulfoximine derivatives[2].

Results: The first goal was to find optimal conditions for the Strecker reaction. Next, we synthesized a diverse range of novel sulfoximine building blocks, using different sulfoximine or aldehydes and ketones as starting material. That leads us to the library of new sulfoximines nitriles and α-amino acids analogues that we hope will further enhance the usage of the sulfoximine group by medicinal chemists in drug discovery.

Conclusions: Strecker reaction of aldehydes, sulfoximines and trimethylsilyl cyanide is a robust and versatile tool to deliver new sulfoximine scaffold of potential use in medicinal chemistry and chemical biology.

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Abstract No. PPP.26

Novel Molecular Consortia of Cannabidiol with Selected Bioactive Carboxylic Acids

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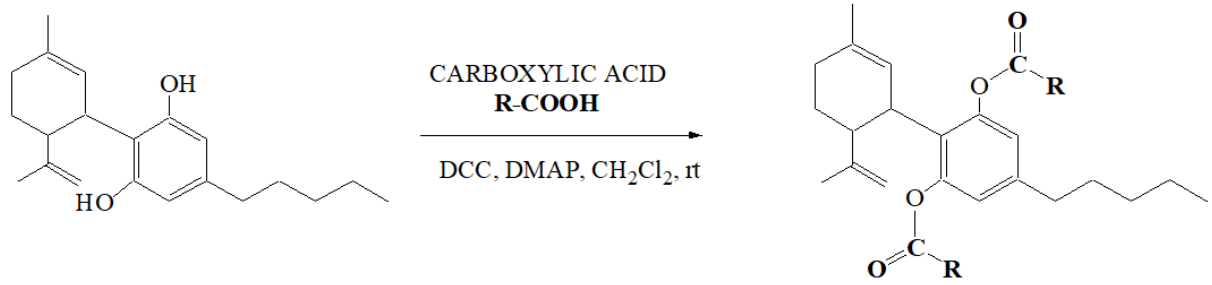
Objectives: Cannabidiol (CBD) is a natural non-psychoactive polyphenolic compound isolated from *Cannabis sativa* with a wide range of therapeutic activities, including antiviral, antioxidant, anticancer, anti-inflammatory and neuroprotective properties. The structure of CBD and the presence of reactive phenolic groups create the possibility of modifying its structure towards new ester derivatives using biologically active entities, particularly those containing carboxylic groups (Scheme 1). The concepts of structural hybridisation and molecular consortia creation are well established and have led to the approval of numerous interesting drugs.



Scheme 1. General synthetic concept of obtaining CBD consortia

Methods: CBD has been reacted with selected bioactive carboxylic acids, in the first step with derivatives of propionic acid such as ibuprofen, ketoprofen, naproxen and in the second step with derivatives of cinnamic acid such as ferulic acid, sinapic acid, caffeic acid under the mild conditions of the Steglich esterification. Novel compounds were isolated, purified and structurally characterised using MS, NMR and ATR-FTIR techniques. Some of the obtained compounds were subjected to preliminary biological assessment, including cytotoxicity and selected antiviral properties.

Results: In most cases, appropriate disubstituted CBD-carboxylic acid derivatives are obtained and isolated with the highest yield; however, monosubstituted derivatives have also been found. In order to obtain monoderivatives, it is necessary to optimise the reaction conditions in terms of the main reaction parameters.

Conclusions: Generation of bifunctional molecular consortia of CBD and carboxylic acid, particularly with carboxylic acids from anti-inflammatory and antioxidant groups, will most probably result in higher activity, e.g. antiviral and anti-inflammatory activity. Further studies on the activity of novel CBD molecular consortia should be continued.

Abstract No. PPP.27

Synthesis and cytotoxicity of new hybrid compounds of curcumin

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Objectives: Curcumin and oleanolic acid are natural compounds with high potential in medicinal chemistry. These compounds have been widely studied and structurally modified to improve their bioavailability and therapeutic value. New hybrid compounds were synthesized using curcumin- type compounds, oleanolic acid derivatives and popular non-steroidal anti-inflammatory drugs. Hybrid derivatives were obtained according to two types of modulation: directly – using the mutual reactivity of the functional groups and intermediately – using bifunctional intermolecular linkers (Scheme 1).

Scheme 1. General synthetic concept of obtaining curcumin hybrid compounds

Methods: The curcumin-type substrates (pyrazole, isoxazole, curcumin monocarbonyl analog) and oleanolic acid derivatives (oximes, methyl esters, oleanoyl hydrogen succinates) were obtained using known methods. In the first stage, according to the direct mode, curcumin and its analogues were subjected to a Steglich esterification reaction with the use of selected carboxylic compounds from the group of non-steroidal anti-inflammatory drugs (ibuprofen, naproxen). In the second stage, according to the intermediate model, monoester hybrid derivatives based on curcumin or its heterocyclic analogues and oleanolic acid with the succinic acid moiety were obtained. NMR and MS spectral data were used to confirm the structure of the obtained compounds. Cytotoxicity of obtained compounds against different cancer cell lines (MCF7, HeLaWT, HT-29) was evaluated and compared with the activity of curcumin. Additionally, sonodynamic effect of curcumin, triterpene oleanolic acid and their derivatives, on tongue cancer SCC-25 and hypopharyngeal FaDu cell lines, was investigated.

Results: Cytotoxicity of curcumin-triterpene hybrids against different cancer cell lines was evaluated. The IC50 value was in the range of 20.6–94.4 μM, in comparison to curcumin 15.6–57.2 μM. The highest sonotherapeutic activity was demonstrated by a linker mode curcumin pyrazole-oleanoyl hydrogen succinate hybrid.

Conclusions: The research results indicate the direction of designing potential new drugs, with an emphasis on specific modifications and combinations of two biologically active compounds to improve their effectiveness as selective anti-cancer drug. It is also suggested that oleanolic acid, curcumin, and their derivatives exhibit a high sonosensitizing potential in SDT therapy.

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Abstract No. PPP.28

Validated LC-MS/MS method for determination of low oral bioavailability of semaglutide in rat plasma: Application to a pharmacokinetic study

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Objectives: Semaglutide (SEM) is a glucagon-like peptide-1 (GLP-1) agonist available as oral and subcutaneous formulation and it is used in the treatment of overweight and obesity with or without type 2 diabetes. Unfortunately, oral administration of SEM is characterized by low bioavailability. The study aimed to develop a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for the determination of GLP-1 analogue in rat plasma after the application of an oral form. Validation of LC- MS/MS analysis included selectivity, the matrix effects, linearity, precision, accuracy, and stability. The validation parameters were assessed according to the respective EMA guidance.

Methods: SEM was extracted by liquid-liquid method and separated on Acquity™ Premier Peptide BSH C18, 130 Å, 1.7 µm (2.1 × 100 mm) with gradient elution and the mobile phase acetonitrile : water with 0.1% HCOOH. A liraglutide was used as an internal standard. Quantification of SEM was performed by the electrospray ionization tandem mass spectrometry in the MRM mode with positive atmospheric ionization at m/z 1029.3.

Results: The calibration curve of SEM showed good linearity in the range of 5.0 – 100 ng/mL with a correlation coefficient of 0.9942. The matrix does not affect the accuracy and precision of SEM determination. The limit of quantitation (LLOQ) was 5.0 ng/mL. Precision and accuracy were all within 3.62% at four concentration levels (including LLOQ). A total recovery of SEM and IS was beyond 90%. The mean plasma concentration-time profile after a single oral dose of 14 mg/kg administration to Wistar rats was determined.

Conclusions: Our investigation showed that the method can be applied to pharmacokinetic study samples for a given study and yield accurate and reliable results. The bioanalytical method was successfully validated and may be used for various research and other non-clinical and clinical studies.

Abstract No. PPP.29

Validated LC-MS method for determination of vemurafenib in human plasma: Application to a pharmacokinetic study in juvenile patients

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Objectives: Vemurafenib (VEM) is an inhibitor of BRAF serine-threonine kinase. It is used to treat patients with melanoma that has spread to other parts of the body or cannot be removed by surgery. It can only be used in patients whose cancer has a change (mutation) in the "BRAF" gene. The study was aimed to develop and validate a bioanalytical method to determine vemurafenib in human plasma after its administration in *BRAF*-positive juvenile patients with refractory histiocytosis.

Methods: The method was developed on the basis bioanalytical LC-MS method. Validation of LC-MS analysis included the selectivity, potential influence of ibuprofen (IBU) and paracetamol (PAR) on vemurafenib determination, matrix effects, linearity, precision, accuracy and stability tests. VEM was extracted by liquid-liquid method and separated on Zorbax SB-C18 100 x 3.0 mm, 3.5 µm connected to a guard column SecurityGuard C-18, 4 x 3 mm with isocratic elution and the mobile phase was 2mM ammonium acetate : acetonitrile. [$^{13}C_6$]-Vemurafenib (IS) was used as an internal standard. The detector was a single quadrupole setup with an electrospray ionization positive probe, operating in Single Ion Monitoring (SIM) mode at m/z 489.95.

Results: The calibration curve of VEM showed good linearity at a concentration range 1.00 – 100.00 µg /mL with a correlation coefficient of 0.9987. The limit of quantitation (LLOQ) was 1.00 ng/mL. The chromatograms of blank plasma samples and blank plasma samples spiked with IBU and PAR did not show any significant interference in the VEM and IS retention times. The matrix did not affect the accuracy and precision of VEM determination. The accuracy and precision were determined at four concentrations (3.00, 10.00, 45.00 and 80.00 µg/mL) and LLOQ. The upper limit of the 90% CI for precision at each concentration did not exceed 15%. The 90% CI for accuracy for each concentration ranged from 85-115%. The stability of VEM in biological material was tested at two concentration levels (3.00 and 80.00 µg/mL). For each concentration, the 90% CI for mean stability is within the 85-115% acceptance criteria for all validation tests performed.

Conclusions: The validation parameters were assessed according to the respective EMA guidance. All of the validation parameters met the acceptance criteria and the method was successfully applied in the pharmacokinetic study in juvenile patients.

Abstract No. PPP.30

The selectivity and specificity of the method for trametinib determination in human plasma: Application to pharmacokinetic studies in juvenile patients.

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Objectives: The aim of the study was to assess the selectivity of the method in human plasma and the specificity of the method towards co-medications for trametinib (TRAM) determination.

The determination of the TRAM concentration in human plasma was developed on the basis bioanalytical LC-MS/MS method and liquid-liquid extraction method. The reliable results of the pharmacokinetic studies are of critical importance to ensure the efficiency and safety of medicinal products. Therefore, the above bioanalytical method was validated in accordance with EMA guidelines. One of the most important validation parameters was the assessment of the selectivity and specificity of the method for TRAM determination in biological material. Selectivity is the ability of an analytical method to distinguish and measure the analyte in the presence of the potential interfering substances in the blank biological matrix. The specificity is the ability of a method to detect and distinguish the analyte from other substances. According to the non-commercial clinical trial protocol, samples collected from volunteers may contain vemurafenib (VEM). Additionally, remember that ibuprofen (IBU) and paracetamol (PAR) are commonly used painkillers. Therefore, during validation, it was necessary to check the potential impact of IBU, PAR and VEM on TRAM determination. The selectivity and specificity may influence method accuracy, precision and sensitivity. It is important to confirm the lack of influence of various factors on the reliability of the method.

Methods: The selectivity test was carried out using blank plasma from six different sources and haemolysed and hyperlipidaemic plasma. Evaluation of the potential influence of VEM, IBU, and PAR on the determination of TRAM was performed by assaying the blank plasma and quality control (QC) samples spiked with co-medications.

Results: Chromatograms of blank plasma from different sources and blank plasma samples spiked with VEM, IBU and PAR did not indicate any interferences at TRAM and IS retention times. Chromatograms of the accuracy of the method at 1.5 and 200.0 ng/mL of TRAM spiked with co-medications were in the range of 85-115%. No influence of VEM, IBU, PAR on the determination of TRAM was observed.

Conclusions: The assessment of the selectivity and specificity was performed according to the respective EMA guidance. The tests met the acceptance criteria. The method was successfully applied in the pharmacokinetic study.

Abstract No. PPP.31

Investigating the Impact of Sulforaphane on Triple-Negative Breast Cancer Using *In Vitro* and *In Vivo* Models: Insights into Hormetic Effects

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Objectives: Our research aimed to investigate how dietary doses of SFN impact the proliferation and migration of triple-negative breast cancer (TNBC) cells using *in vitro* and *in vivo* models. TNBC is a aggressive form of breast cancer with limited treatment options. Natural compounds have garnered significant attention for their potential to enhance cancer treatment effectiveness. Among these compounds, sulforaphane—a natural isothiocyanate—has emerged as a compound with hormetic properties, meaning it can have contrasting effects, either protecting cells or inducing toxicity, depending on its concentration.

Methods: In our experiments, we employed MDA-MB-231 cells and a murine TNBC model of Balb/c mice with implemented 4T1 cells. The safety and antitumor effectiveness of small dose of SFN was evaluated *in vivo*. An *in vitro* studies on a human TNBC 2D and 3D model was conducted to determine SFN mechanism of action - whether trough the cytotoxic effect or the inhibition on metastasis (i.e. proliferation or migration).

Results: The *in vivo* investigations revealed that treatment with sulforaphane led to a notable inhibition of tumor growth, with up to a 31% reduction observed. Additionally, we observed decreased cancer cell proliferation, reduced necrotic areas within the tumors, and changes in the types of immune cells present, suggesting a less aggressive tumor phenotype compared to untreated counterparts. Furthermore, sulforaphane treatment was associated with a decrease in the number of lung metastases.

In the *in vitro* studies, we found that sulforaphane effectively inhibited the migration of TNBC cells, particularly those derived from 3D spheroids, as opposed to cells cultured in a traditional 2D setting.

Conclusions: In conclusion, these findings shed light on the specific mechanisms through which sulforaphane impacts TNBC cells within their primary tumor environment. Our research underscores the potential of sulforaphane as a therapeutic agent for TNBC, highlighting its ability to modulate key aspects of tumor growth and metastasis.

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Abstract No. PPP.32

New thiolated derivatives of natural products - Coated Gold Nanoparticles. Synthesis, characterization and evaluation of its antioxidant and antitumor activities.

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Objectives: The field of nanotechnology related to targeted transport of therapeutic substances and their controlled release at the target site inside a living organism is developing very rapidly in medicine. Such systems can be successfully applied in anticancer therapy.

Methods: The aim of our first study was to investigate the properties of a new compounds thiodiosgenin (TDIOS) and thiogenistein (TGE) [1-2] and compare the properties of the new derivatives with their precursors: diosgenin and genistein - natural compounds with known antioxidant and anticancer properties. This study aimed to compare the therapeutic efficacy of new AuNPs-TDIOS and AuNPs-TGE conjugates with their precursors on human breast cancer (MCF-7 and MDA-MB-231) and prostate cancer (DU-145) cell lines, along with assessing their antioxidant activity on LPS-induced mice macrophages (RAW 264.7). Moreover, the safety of the new thio-derivatives was tested against the human epithelial line PNT-2. Additionally, the antioxidant activity of the new derivatives and their conjugates with AuNPs in vitro was determined on LPS-induced mice macrophages (RAW 264.7 line).

Results: Our synthetic approach was based on one hand on the ligand exchange of citrates to thiocompounds on gold nanoparticles on the other hand based on the attachment of GE and DIOS through an ester bond to the linker, which was 3-mercaptopropionic acid on gold nanoparticles. Preliminary in-vitro studies indicate that TDIOS and TGE exhibit higher cytotoxic activity towards human prostate and breast cancer cells and are safer for normal prostate epithelial cells (PNT-2) than diosgenin and genistein themselves. Additionally, it has been proved that the obtained conjugates are characterized by a higher cytotoxic activity towards cancer cells than TDIOS and TGE themselves. This behavior may be related to the different pH-dependent dissolution rates of the molecules. Studies have proven that TGE is released from AuNPs only at pH 5.5, characteristic of cancer cells. This effect supports the protection of healthy cells and the targeted action of the molecule only in cancer cells.

Conclusions: The new methods for the synthesis of conjugates has been developed and optimized for medical application. Significant new analytical methodologies were developed to characterize new conjugates.

Literature:

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Abstract No. PPP.33

Anticancer effect of 5-fluorouracil and sulforaphane combined treatment in *in vivo* model of triple-negative breast cancer

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Objectives: Triple negative breast cancer (TNBC) is one of the most aggressive breast cancer subtypes associated with poor patient prognosis. The main pharmacotherapeutic strategy is chemotherapy, e.g. 5-fluorouracil (5-FU), doxorubicine. Our previous studies in the TNBC *in vitro* model have shown that sulforaphane and 5-fluorouracil interact synergistically, resulting in a significant reduction in the number of live cells compared to either treatment alone. Based on these findings, the anticancer efficacy of SFN and 5-FU was evaluated using an in vivo model.

Methods: Female BALB/c mice were orthotopically injected with 4T1murine breast cancer cells. The mice were randomly divided into four groups: control group, 5-FU alone treatment group, SFN alone treatment group and combined treatment group. 5-FU (100 mg/kg m.c.) was administered intravenously. SFN (100 mg/kg m.c.) was administered intraperitoneally. Tumor growth inhibition (TGI) was quantified by comparing the mean tumour volume observed in treated mice with that observed in untreated control mice. Based on TGI , the type of interaction between SFN and 5-FU was calculated. The heart, liver, lungs and spleen were weighed at necropsy. A blinded macroscopic count for metastatic foci on the surface of lung tissue and a morphological analysis of blood were performed.

Results: Treatment with SFN alone slightly inhibited tumour growth (max. TGI was 19%); however, when combined with 5-fluorouracil (5-FU alone inhibited tumour growth by 37-54%), a synergistic effect was observed (experimental TGI was higher than that calculated hypothetically). The group receiving the combination therapy also had the lowest number of tumour lesions in the lungs of the mice. Importantly, this combination was shown to be non-toxic in animals.

Conclusions: Combined treatment can be considered a promising anti-cancer strategy for the treatment of highly aggressive and invasive triple-negative breast cancer.

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Abstract No. PPP.34

Multi-component biocomposites enriched with silver ions and ciprofloxacin for potential use in wound healing

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Objectives: The main goal was to develop a new, multifunctional chitosan-alginate biomaterial with antibacterial properties for potential use in wound healing. The designed materials were enriched with silver ions and served as a carrier for the antibiotic ciprofloxacin. Thanks to this, the synthesized biocomposite could gain a local antibacterial effect while minimizing side effects occurring in the systemic administration of antibiotics and most importantly, it may speed up the wound healing process.

Methods: The first step was to obtain polymer matrices with different weight ratios of alginate and chitosan. In the next stage, nanocrystalline hydroxyapatite doped with silver ions was synthesized with the general formula $Ca_{9,95}Ag_{0,05}(PO_4)_6(OH)_{1,95}$. Two different sources of silver ions were introduced into three types of polymer matrices: silver nanoparticles (with a declared concentration of 5 mg/ml) and previously obtained hydroxyapatite doped with silver ions. In total, six different biomaterials were obtained in which ciprofloxacin was incorporated.

Results: All composites were characterized using the following methods: FTIR mid-infrared spectroscopy, Scanning Electron Microscopy – SEM, Atomic Absorption Spectrometry - ASA and Powder Xray diffraction – PXRD. At the end the drug substance release profile was examined by liquid chromatography (HPLC). Preliminary *in vitro* biological tests were carried out (cytotoxicity tests and antibacterial properties were checked). All obtained biomaterials were characterized by a highly porous structure, and hydroxyapatite was effectively incorporated into the internal structure of the composite. The drug substance was successfully released from all composites, in most cases within the first 72 hours. Significant differences were noticed in the release of silver ions from individual composites depending on the source of silver ions used.

Conclusions: Drug release as well as silver ions release profiles vary slightly with the type of composite. Most of the obtained composites did not show cytotoxicity towards the 3T3 mouse fibroblast cell line, which proves the appropriate selection of silver ion concentrations. All materials showed antibacterial activity against *S. aureus* and *P. aeruginosa*. It seems that the use of chitosan-alginate materials with the addition of silver ions as well as a ciprofloxacin may be a promising combination in the wound healing process.

Abstract No. PPP.35

The stability studies of exosomes derived from lung cancer cells

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Objectives: The latest breakthroughs in nanomedicine have cleared the path for the development of targeted drug delivery systems. Nanoscale exosomes, present in various bodily fluids offer a novel mode of intercellular communication. Originating from membranes, they easily integrate with cells, serving as natural delivery vehicles while retaining their biological functionality and immunological compatibility. To ensure the viability of exosome for clinical purposes, quality assurance measures are imperative, especially during the shelf life. Therefore, the aim of our study was to identify optimal storage conditions for exosomes derived from lung cancer cells (A549).

Methods: A549 cells were expanded in T225 culturing flasks to generate exosomes. The media containing the exosomes were harvested and processed for exosomes isolation following a protocol. Subsequently, the exosomes were divided equally into groups and suspended in pure PBS, PBS with 5% DMSO, or 25 mM trehalose for storage at different temperatures (-80°C, -20°C, 4°C, and room temperature). The presence of exosomes in the harvested media was confirmed through Western Blot analysis and transmission electron microscopy. The stability of exosomes derived from A549 cells was assessed by analyzing size distribution and particle concentration using Nanoparticle Tracking Analysis. Furthermore, the preservation of the protein cargo in the stored exosomes was evaluated using the Bicinchoninic Acid Assay.

Results: In this study, we have identified that the addition of 25 mM trehalose to PBS buffer serves as an optimal cryoprotectant for preserving A549-derived exosomes stored at -80°C. Under these conditions, the exosomes maintain a constant concentration, size distribution, and stable levels of total protein cargos over time.

Conclusions: The research findings have demonstrated that the optimal preservation conditions for lung cancer-born exosomes involve the use of PBS buffer supplemented with 25mM trehalose as an effective cryoprotectant for storages at -80°C.

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Keywords: exosome, lung cancer cells, preservatives

Abstract No. PPP.36

Preparation and characterization of carmustine-α-cyclodextrin inclusion complexes for potential application in the treatment of high grade glioma

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Objectives: Carmustine (BCNU) is an antineoplastic agent, most commonly used in the treatment of brain tumors. Due to the fact that BCNU exhibits low solubility in water, short half-life, low stability, and its use can result in a variety of serious adverse effects, it is necessary to sought for its alternative and innovative delivery strategies. In order to overcome aforementioned difficulties, cyclodextrins (CDs) turn out to be beneficial, since complexing pharmacologically active substances in the CDs' cavities increases their stability, enhances solubility in aqueous solutions and reduces their toxicity. Therefore, the main objective of this study is to develop novel solid-state α-cyclodextrin (α-CD) inclusion complexes (ICs) of BCNU for potential application in the treatment of high-grade glioma.

Methods: The α-CD-BCNU ICs were prepared using 3 different techniques – co-grinding (M1), cryomilling (M2) and co-precipitation (M3) in equimolar ratios of α-CD and BCNU (Figure 1).

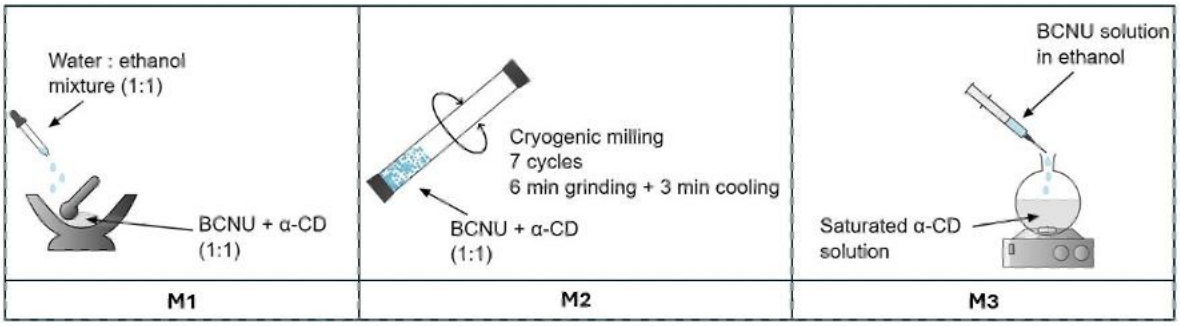

Figure 1. Schemes of the inclusion complexes preparing methods

So far, the obtained ICs were characterized with the use of ^{1}H NMR and FTIR spectroscopy, as well as HPLC studies to assess BCNU's encapsulation efficiency (*EE*). Furthermore, BCNU-α-CD ICs are going to be characterized with the application of UV-Vis spectrophotometry and differential scanning calorimetry (DSC) technique. Last but not least, the *in vitro* release study of BCNU will be performed in a near future.

Results: The current investigations show that the selected methods for α-CD-BCNU ICs development are effective and provide relatively high *EE* values (50,3%, 49,3%, and 77,8% for methods M1, M2 and M3, respectively). The FTIR spectra revealed changes in specific bands, indicating the formation of hydrogen bonds between α-CD and BCNU. This confirmed the effective encapsulation of BCNU. The ^{1}H NMR spectra provided information on the interactions between BCNU and α-CD, as well as the approximate orientation of BCNU within the host molecule.

Conclusions: The research conducted so far provided promising results for obtaining solid state α- CD- BCNU ICs. Nevertheless, the study needs to be extended in order to fully understand and confirm the structure of the synthesized ICs.

Abstract No. PPP.37

The use of sulfoximines in the Kabachnik-Fields and Mannich reactions

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Objectives: Despite the considerable interest of pharma industry in alternative drug modalities (like: peptides, ribonucleic acids and therapeutic antibodies). the 'classical' drug-discovery approaches initiated by screening of sets of small-molecular compounds for their activity against the specific disease still bring a large share of new drugs to the market.

Our research focuses on the technologies that give access to new, more 'sophisticated' chemical space and to the intensive research on novel functional groups that can improve the characteristics of the investigative agents. Within the project we are expanding yet largely unexplored chemical space of drug- like sulfoximine class and by widening scope of multicomponent reactions which are important tools in modern drug discovery, as they proceed with high atom economy and use simple, one-pot procedures, which makes them suitable for time- and cost-efficient tools for generating investigative new compounds for drug discovery. We present current results of optimization as well as scope and limitation of two important types of multicomponent reactions: Kabachnik-Fields and Mannich MCRs.

Methods: We used a large set of commercially available substrates: sulfoximines, carbonyl compounds, organophosphites and enol-analogues (generated mainly *in situ)* as well as carboxylic acids as efficient catalysts. The products were isolated using standard chromatography (normal and reverse-phase) methods and identified and characterized using LC-MS and NMR techniques.

Results: We completed the optimization of model Kabachnik-Fields and Mannich reactions. We implemented the developed protocols to synthesis diversity-oriented set of novel sulfoximine-based class of compounds. Broadening of the scope of reactions is still in progress.

Conclusions: Kabachnik-Fields and Mannich reactions are a robust and versatile tool to obtain new sulfoximine scaffold of potential use in medicinal chemistry and chemical biology.

Abstract No. PPP.38

Comparison of the modulatory effect of arctiin and its aglycone - arctigenin on the production of inflammatory mediators *in vitro* in macrophage cell model

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Objectives: Inflammation is a complex biological response implicated in various pathological conditions. A chronic inflammatory state has been identified to contribute to the development of non- communicable diseased, e.g. type II diabetes, atherosclerosis, neurodegenerative diseases, and their complications. Understanding the regulatory mechanisms of inflammation is crucial for developing therapeutic interventions.

Arctiin and arctigenin, bioactive compounds found in various plant materials, e.g., *Forsythia x intermedia* leaves and flowers, *Forsythia virdissima* flowers, and *Arctium lappa* L. seeds, have been previously associated with anti-inflammatory properties. However, a direct comparison of their modulatory effects on inflammatory mediators remains scarce. The study aimed to analyze the modulatory potential of two polyphenolic compounds from the lignan group: arctiin and arctigenin in a *Lipopolysaccharide* (LPS) stimulated THP-1-derived macrophages by assessing their impact on the gene expression profiles and the production of inflammatory mediators.

Methods: The cytotoxicity of the tested lignans was assessed using MTT test. Measurement of the content of individual inflammatory mediators (TNF-α, IL-6, MCP-1, IL-10) in the cell supernatants was performed using the ELISA tests. After a 6-hour incubation with the tested compounds (20 μM), LPS-stimulated macrophage cells were lysed to obtain material for mRNA isolation and reverse transcription. The PCR was performed using TaqMan assays. Results were presented with respect to LPS- control and positive control with dexamethasone (20 μM).

Results: Arctiin and its aglycone form - arctigenin reduce the inflammatory response induced by bacterial LPS, inhibiting gene expression and the production of pro-inflammatory mediators (TNF-α, IL-6, MCP-1). Interestingly, both lignans examined also initiated an increase in the secretion of anti-inflammatory IL-10, which may contribute to their immunomodulatory effect in immune system cell models.

Conclusions: Our findings provide insights into the distinct modulatory patterns exhibited by arctiin and arctigenin, shedding light on their potential differential mechanisms of action. The obtained results may constitute an introduction to further extended *in vitro* and *in vivo* analyses.

Abstract No. PPP.39

Novel volumetric microsampling device for methotrexate determination in capillary blood - LC-MS/MS method development and analytical validation

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Objectives: Methotrexate is an antimetabolite used in cancer chemotherapy (HDMTX, high dose of methotrexate) and rheumatoid arthritis (RA) treatment (LDMTX, low dose of methotrexate). MTX is a folic acid antagonist with a strong affinity for enzymes involved in the folic pathway. Due to high toxicity, therapeutic drug monitoring (TDM) is necessary, especially during HDMTX treatment. High- dose methotrexate (HDMTX) is administered as an intravenous bolus with a body surface area of > 500 mg/m2 . The Volumetric-Absorpitve Microsampling is an alternative strategy for body fluid collection, especially in TDM and adherence controlling.

Methods: For method optimization and validation, 10µL VAMS samplers (Mitra, Trajan, USA) were used. The various conditions of blood extraction from samples were used (drying time, extraction solvents, and supporting sonification). An analytical platform - Shimadzu 8050 with Nexera X2 (LC- MS/MS, liquid chromatography-tandem mass spectrometry) was used in the presented methodology with conditions adapted from the previously validated method for MTX determination in serum/plasma. The assay has been validated according to EMA (European Medicines Agency) guidelines.

Results: The blood extraction methodology was optimized - the acidified water was used with sonification. The optimal drying time was 2 hours. For appropriate sample purification, the LLE (liquid- liquid extraction) using hexane was used. The method was successfully validated in the calibration range: 0.01 – 25 µmol/L (R^2=0.988; CV%<15% for each QC). No matrix effect, as well as carryover was observed. The stability of the sample was 10 days, at room temperature, but the VAMS sample should be stored in the dark, due to the limited stability of MTX after exposure to bright light. No hematocrit effect and anticoagulant effect were observed.

Conclusions: A bioanalytical method for MTX determination in VAMS samples has been successfully developed and validated. Next, the method will be validated according to clinical utility—the introduction of a correlation formula between plasma and VAMS MTX levels should be introduced. Additionally, the possibility of MTX metabolites determination in capillary blood will be evaluated. The validated method may be applied to TDM of MTX during lower and higher doses of treatment.

Abstract No. PPP.40

New derivatives of coumarin as novel potential dual acetylcholinesterase- monoamine oxidases inhibitors against Alzheimer's disease

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Objectives: We investigated coumarin structures conjugated with 4,7-dimethyl-, 6- or 8-acetyl-, and phenylpiperazine *via* a flexible 3- and 4-carbon linker at the less explored position C-5 and C-7 as MTDLs (Multi-Target Directed Ligand) for the potential treatment of Alzheimer's disease (AD). Compounds were designed to incorporate coumarin structures known to show MAO-A and MAO-B (brain monoamine oxidases) inhibition and selected structural elements of Ensaculin a selective *h*AChE (acetylcholinesterase) inhibitor currently used in the pharmacological treatment of AD. The designed series of coumarin-piperazine derivatives were synthesized and tested *in vitro* to evaluate for their inhibitory activity agains AChE, BChE and MAO A/B enzymes.

Methods: The synthesis of compounds was carried out by reacting the bromoalkyl derivatives with appropriate arylpiperazine: 4-(2-methoxyphenyl)piperazine or 4-(3-methoxyphenyl)piperazine in acetonitrile and in the presence of potassium iodide and potassium carbonate. All compounds synthesized in this work were synthesized using a microwave reactor and were purified by column chromatography using silica gel. All compounds were characterized using ^{1}H NMR, ^{13}C NMR spectroscopy, and HRMS spectrometry. Inhibition of human recombinant AChE was determined by applying Ellman's spectrophotometric method. Inhibition of MAO A and B isoforms was determined by means of a reported spectrofluorimetric procedure. All obtained values were the means of three independent experiments and were expressed as % inhibition at 10 μM±SD.

Results: The obtained compounds belong to two groups of coumarin derivatives, which differ in the length of the linker between the coumarin and the piperazine ring. The presented arylpiperazinyl derivatives displayed varied hAChE inhibitory activity. The highest activity was found for compounds which were derivatives of 7-hydroxycoumarin and they showed also a good inhibitor profile against MAO-A. In the case of inhibitory activity against MAO-B the situation was completely different. The highest activity was found for derivatives of 5-hydroxycoumarin.

Conclusions: As expected, presented compounds showed dual acetylcholinesterase-monoamine oxidases inhibitors. Having picked the most potent derivatives, we are planning to extend the range of biological testing methods for them and synthesize similar compounds to be assessed.

Abstract No. PPP.41

The effectiveness of antimicrobial preservation for a liquid oral formulation drug product depending on the preservatives used, their concentration and the pH of the preparation

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Objectives: The subject of the study was to assess the efficacy of antimicrobial preservation for a liquid oral formulation in accordance with the requirements of the European Pharmacopoeia and the European Medicines Agency guidelines for medicinal products. An analysis of the influence of solution pH and the type of preservative (Methyl parahydroxybenzoate, Propyl parahydroxybenzoate) on the results of antimicrobial properties and the stability of the preservative was carried out. Additionally, an analysis of the influence of the concentration of the preservative (Methyl parahydroxybenzoate) on the obtained results of the efficacy of antimicrobial preservation was performed.

Methods: Tests were carried out on acidic and alkaline preparations to assess the effect of pH on the antimicrobial properties at the highest permissible level of parabens (SOL 1 - SOL 6 batches). Batches with different concentrations of Paraben M were prepared for acidic preparations to assess the antimicrobial effectiveness and select a safe and optimal amount (batches SOL 7 – SOL 11). The preparation is properly preserved if, under the test conditions, after a specified period of time and at the recommended temperatures, there is a significant decrease or no increase in the number of microorganisms in the inoculated preparation.

Results: Literature data on the lower stability of parabens (Parahydroxybenzoates), especially Paraben M, at alkaline pH have been confirmed. Parabens were stable in acidic formulations . Due to the significant decrease in the Paraben M content over time, batch SOL 4 was not subjected to further tests for the effectiveness of the antimicrobial preservation. .The results indicated that the acidic formulation needs preservatives to pass microbiological tests

Conclusions: The influence of pH on the stability of Parahydroxybenzoates was confirmed, and the influence of the concentration of Methyl parahydroxybenzoate on the obtained results of antimicrobial preservation efficacy was assessed.

Based on the results obtained and in order to ensure the patient's safety throughout the entire period of use of the medicinal product, it is recommended to choose an acidic formulation with Paraben M at a concentration of 0.1% as a preservative.

Abstract No. PPP.42

Development of a novel family of antifungal agents based on a quinone methide oxime framework

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Objectives: Fungal infections are a global problem and are consistently associated with high morbidity and mortality in immunocompromised patients. Here we propose the quinone methide oxime as a framework for development of a novel class of antifungal agents. From series testes quinone methide oximes showing antifungal activity against Candida albicans and non-albicans Candida, we chose one which appeared to be the most promising for further in-depth analysis. The molecular docking studies and quantitative quantumchemical calculations were performed to understand the mechanism of the biological interactions.

Methods: According to the docking model, interactions involving the aryl and the nitrile groups of quinone methide oximes within the active site of DYRK1A kinase facilitated the stabilization of the resultant complexes more then interaction of the hydroxyl group or it derivatives. The chemical synthesis furnished a series of quinone methide derivatives with altered functionality at the oxime hydroxide, aryl ring, and quinone moiety. The elucidation of stereo configuration of most active isomers was achieved applying the XRD analysis. Both in vitro and in silico investigations revealed that the synthesized compounds demonstrated notable inhibition of protein kinases, particularly exhibiting a robust affinity for the active site of DYRK1A kinase.

Results

The incorporation of an acyl group into compound phenylcyanomethylenequinone oxime resulted in a significant enhancement in the activity of syn- isomer of compound formed. Despite the positively passed safety test towards to the human calls, this acetylated derivative effectively suppresses the growth of C. albicans hyphae, eradicates mature biofilms, and demonstrates efficacy against several clinical fungal isolates, including those resistant to systemic drugs.

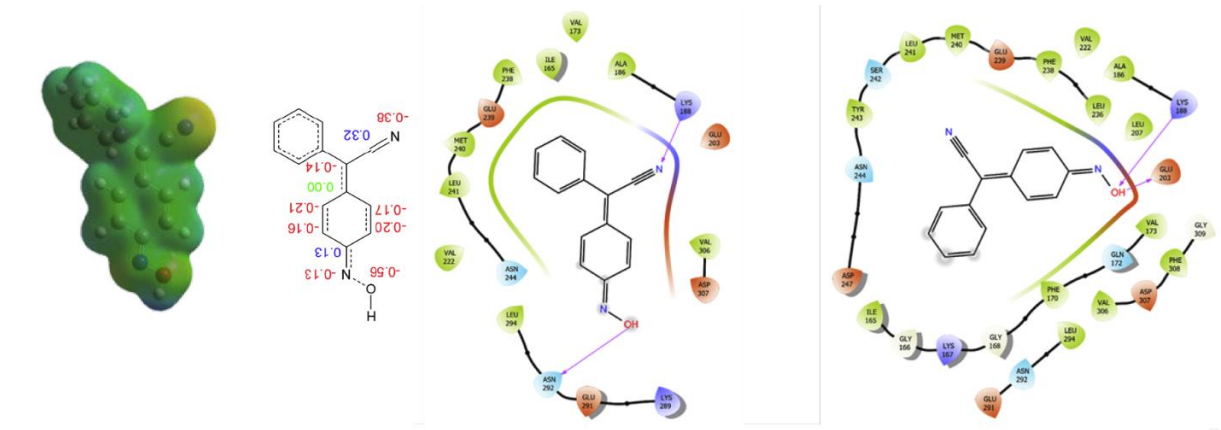

Figure 1. Presentation of electrostatic potential, natural atomic charges, and molecular docking into protein kinase DYRK1A.

Conclusions: Our research has revealed that some of the acquired compounds effectively hinder the growth of Candida albicans fungi. This characteristic positions them as promising contenders in combatting fungal infections, with enhanced antifungal properties, capacity to eradicate biofilms, and advantageous safety profile compared to normal human cells.

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Abstract No. PPP.43

Qualitative and quantitative analysis of cosmetical formulations containing bakuchiol

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Objectives: Bakuchiol is a meroterpene isolated from *Psolarea corylifolia*, plant used in traditional Chinese medicine. With its anti-aging and antioxidant properties it is described as retinoid analogue with different structure and thus its medical potential is constantly evaluated. As for now it is admitted to use in cosmetic (and 'cosmeceutical') products, however due to limited control of such products, the question of their quality is raised.

Methods: Four methods of both qualitative and quantitative analytical methods (spectroscopic - UV-Vis, qNMR with internal and external standard, chromatographic - HPLC) were applied to examine declared bakuchiol content in five cosmetic samples collected from market, varying in price and composition.

Results: All methods were compared and found usable in such analysis with spectroscopic methods being the most promising for future applications as they provided convergent results with HPLC in shorter time of analysis. Although the validation of presented methods is not complete, obtained results already allow to conclude quality falsification in one sample and quantity falsification in the other.

Conclusions: Selected methods will be used in future to further investigate the molecule and impact of different factors on its concentration and form allowing to better describe mechanism of its action.

Abstract No. PPP.44

Searching for guanylate kinase inhibitors from *Mycobacterium tuberculosis* - a new approach to the fight against tuberculosis

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Objectives: Tuberculosis (TB) is a formidable and persistent infectious ailment primarily afflicting the pulmonary system. A pivotal research approach in addressing TB involves the identification of novel targets and the development of more efficacious pharmacotherapeutic agents [1]. Nucleotide metabolizing enzymes play a crucial role in generating nucleotide triphosphates, essential for RNA and DNA synthesis, serve as a vital energy reservoir for cellular processes, and participate in crucial signaling cascades. Enzymes within metabolic pathways are indispensable for bacterial organisms' survival, proliferation, and propagation. Among these enzymes is guanylate kinase (EC 2.7.4.8, GK), which provides a reversible phosphate group transfer from ATP to GMP, yielding ADP and GDP [2]. Consequently, the pursuit of compounds capable of modulating the catalytic activity of GK from *Mycobacterium tuberculosis* (GK_{MT}) is judicious. The inhibition of this enzymatic entity holds promise as a prospective therapeutic avenue for tuberculosis.

Methods: The GK activity was analyzed for 10 min at 37°C using the reaction mixture (50 mM Tris-HCl pH 7.5, 2 mM $MgCl_2$, 1 mM ADP, ATP, GMP, and GDP). The concentrations of products were analyzed by the RP-HPLC method. A series of 28 compounds were tested: (1) quinone methide oximes which exhibit a diversified profile of properties and outstanding potential as new drug candidates [3] and (2) naphthoquinones which usually tend to have non-specific interactions with biological molecules [4]. IC_{50} values and the type of inhibition were determined for the four best compounds. Derivatives of quinone methide were obtained according to our previously described method [5] by condensation of appropriate arylacetonitriles and nitroarenes followed by functionalization of the hydroxy group. The naphthoquinones were obtained by direct functionalization (arylation and amination) of naphthoquinone or menadione [4].

Results: The tested compounds influenced the activity of GK_{MT} with different efficiency. The IC_{50} value for the best modulators was in the range of 50-200 μM.

Conclusions: The obtained results will allow the further design of a new generation of more efficient inhibitors against GK_{MT}.

The financial support from the Polish National Science Centre grant number 2019/33/B/NZ7/01608 is gratefully acknowledged.

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Abstract No. PPP.45

Antibacterial properties of PZ-1985, novel arylurea derivative of aryloxy(1- phenylpropyl) piperidine, against multidrug-resistant Gram - positive bacteria

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Objectives: The alarming increase in the resistance of bacteria to the currently used antibiotics necessitates the development of new effective antibacterial agents with alternative mode of action to combat multidrug-resistant priority pathogens such as methicillin-resistant and vancomycin-intermediate *S. aureus* strains (MRSA and VISA, respectively). To address these unmet medical needs, a series of novel arylurea derivatives of aryloxy(1-phenylpropyl)alicyclic diamines was designed, synthesized and biologically evaluated against clinically relevant Gram -positive bacterial, identifying PZ-1985 as a lead.

Methods: The synthesis was performed according to a multi-step procedures involving in-solution methods and sustainable mechanochemical approach. The antibacterial efficacy against selected multidrug resistant and biofilm producing pathogens was assessed using the broth microdilution method. The potential cytotoxicity was evaluated against mammalian cell lines in MTT assay. The metabolic stability was investigated *in vitro* in RLM assay. Bactericidal properties were determined by viable cell counts employing colony forming unit (CFU) plating experiments. The effect on bacterial membrane depolarization was tested using the BacLightTM Bacterial Membrane Potential Kit.

Results: PZ-1985 showed potent antibacterial activity against fatal drug-resistant strains including MRSA and VISA at low concentrations (MIC = 0.78–3.125 μg/mL) comparable to last resort antibiotics linezolid. It was also potent against biofilm-forming *S. aureus* and linezolid-resistant *S. epidermidis* strain. It exerted strong bactericidal properties against susceptible and drug-resistant Gram-positive bacteria. The metabolically stable **PZ-1985** displayed a six-fold selectivity index over the MCR-5 cell line without any cytotoxic effect toward BJ fibroblasts and horse red blood cells. It induced membrane depolarization of MRSA strain at concentration amounted to 2 × MIC values, suggesting a possible mechanism for its antibacterial activity.

Conclusions: The high antimicrobial activity of PZ-1985, along with its selectivity over mammalian cells proposes arylurea derivatives of aryloxy(1-phenylpropyl) alicyclic diamine as a new class of antibacterial agents against Gram-positive priority pathogens.

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Abstract No. PPP.46

Studies on the embryotoxic effect of quinoline yellow in zebrafish

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Objectives: Our daily diet often contains food additives present in numerous processed foods. Currently, the interest regarding their toxicity and negative effects on human health increases among researchers and national authorities. Quinoline yellow (QY), or E104, is a quinophthalone synthetic dye. The aim of the study was to examine the embryotoxic effects of QY with utilization of zebrafish as models.

Methods: Zebrafish embryos were treated with QY (5-2000 μg·mL^{-1}) for 96 hpf and morphological changes were subsequently investigated according to the OECD 236 protocol. The convolutional neural networks were applied to distinguish morphological changes in the QY-exposed and non-exposed embryos. The impulse neural networks and intermediate stage analysis were also used to make predictions of the final state and predicting dose-response relationships beyond the range of the exposure under the study. Computation studies on QY were performed based on QSAR to identify the major toxicological endpoints.

Results: Treatments of embryos with QY (500-2000 μg·mL^{-1}) led to pericardial edema, swollen yolk, blood stasis, lack of heart rate and coagulation. Dose-response effects for morphological changes and lethality of zebrafish embryos exposed to the QY were determined. A method for the image analysis in zebrafish dosed with and without QY was developed and applied for artificial intelligence studies. With superior accuracy, the experimental results have shown that the proposed model is a promising step towards a fully automated toxicity test of compounds. QSAR analysis of QY provides evidence of its toxicity and clastogenicity.

Conclusions: We established AI protocol for analysis of toxic endpoints in zebrafish embryos treated with QY. It was found that this food additive affected zebrafish embryonic development in a dose-dependent manner, causing extensive malformations followed by death. The study provides direct evidence for the developmental toxicity and teratogenic potential of quinoline yellow.

Keywords: artificial intelligence; toxicity; food additives

Abstract No. PPP.47

ONCOBREAST-TEST as a Promising Tool in Personalized Medicine

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Objectives: The study represents a simple, fast, and inexpensive diagnostic and therapeutic path in comprehensive care for patients with breast cancer. The developed and proposed diagnostic procedure and the sensitivity assessment of cancer cells obtained directly from the tumor ensure quick treatment and a fully personalized approach to the oncological patient.

The study aimed to assess the chemosensitivity of cells taken from a biopsy to chemotherapeutic drugs by selecting the optimal anticancer therapy.

Methods: Cells were obtained by core needle biopsy from patient No. 1 (female, 66 years old, NOS G3, ER 90, PR 5, Ki-67 70, HER2) and patient No. 2 (female, 80 years old, NOS G1, ER 100, PR 70, Ki-67 15, HER2). External markers (CD24 and CD44) and internal markers (Ki-67, cytokeratin, GATA3) used to verify cancer cells were determined by flow cytometry. The tumor cells were incubated with drugs used in patients with breast cancer according to the following schemes: doxorubicin + 4-hydroxycyclophosphamide (the active metabolite of cyclophosphamide); cisplatin; paclitaxel; paclitaxel + trastuzumab; docetaxel; docetaxel + trastuzumab. Cell morphology, viability, apoptosis, and proliferation were assayed after 24 hours of incubation. Additionally, LDH activity was measured in the culture medium.

Results: Both in the MTT test, microscopic evaluation, and the assessment of LDH activity, the highest anticancer activity was demonstrated by docetaxel + trastuzumab in patient No. 1 and paclitaxel with trastuzumab in patient No. 2.

Conclusions: Based on the research techniques proposed above, it is possible to select drugs to which cancer cells are most sensitive. The use of such a procedure will increase the effectiveness of chemotherapy, reduce side effects by eliminating ineffective drugs before using them, and thus protect the patient's health and shorten the treatment time, bringing multiple economic and social benefits.

Abstract No. PPP.48

New Phosphosilicate Materials with Remineralizing Properties for Potential Applications in Oral Surgery and Conservative Dentistry

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Objectives: In biomaterials engineering, the search is for materials with suitable physicochemical, biological, and mechanical properties for use in the form of bone substitutes and dental materials. A model compound, most closely resembling biological apatite, is hydroxyapatite (HA). HA is non-toxic, non-immunogenic, and biocompatible, but it exhibits low osteoinductivity. Recent studies show that calcium phosphosilicate (silicocarnotite (SiCar) with the formula $Ca_5(SiO_4)(PO_4)_2$), due to its high silicon content, may be a good material for inducing the formation of apatite at the implantation site. Furthermore, similar to HA, it can partially substitute ions, primarily Ca^{2+}, which may affect biological, mechanical and physicochemical properties. Our work aimed to synthesize and study the properties of new, ion-modified SiCar.

Methods: This study focused on the synthesis of SiCar enriched with Zn^{2+}, Mg^{2+}, or Sr^{2+} ions (additives). Synthesis was conducted using two methods: the dry and the wet method. The dry method was three- step, involving the synthesis in a high-temperature oven of $CaSiO_4$ enriched with additional ions and calcium orthophosphate. The substances obtained in the first and second steps were mixed in a 1 : 1 molar ratio in a mill, compacted, and heated at 1550°C for 2 hours, followed by 4 hours at 1400°C. The wet method involved the initial hydrolysis of tetraethyl silicate and the addition of a solution containing Ca^{2+} (and additives) and a solution containing PO_4^{3-}. The obtained precipitate was aged, filtered, and washed repeatedly with distilled water, then calcined at 1200°C. The obtained materials were analyzed by FT-IR, PXRD, Raman, and AAS methods. Preliminary cytotoxicity tests were also conducted.

Results: Materials obtained by the dry method contained additional phases, i.e. calcium oxide, and calcium pyrophosphate. The wet method allowed for more homogeneous phase materials with a yield of about 60-79%. The confirmation of the SiCar structure was performed by PXRD, FT-IR and Raman spectroscopy. Ion release analyses indicated the gradual and slow release of doped cations and silicates. Cytotoxicity test results varied and depended on the introduced ion and the method of synthesis.

Conclusions: The obtained materials, due to the content of silicon, calcium, and phosphorus ions (and any admixtures of magnesium, strontium, or zinc) and their gradual release over time, are promising materials for potential applications in implant dentistry (as coatings for implants). Optimization of solid- state synthesis conditions is necessary to obtain single-phase materials.

Abstract No. PPP.49

Assessment of potential morphine and disulfiram co-treatment toxicity – a consideration for chronic pain therapy

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Objectives: Opioids are the most potent drugs for pain management. Yet, the burden of analgesic tolerance and hyperalgesia may hinder their prolonged use. Recent studies report that disulfiram – a drug registered for alcohol use disorder, fully suppresses opioid tolerance as well as other side effects such as dependence and naloxone-precipitated withdrawal. Both clinical and preclinical studies show that disulfiram treatment may pose an increased risk of acute liver injury, but the potential toxicity of the disulfiram-morphine combination has not received any thorough scrutiny so far. Therefore, this study aims to determine the potential augmentation of disulfiram toxicity by morphine in terms of developmental and liver toxicity.

Methods: HepG2 cell viability following disulfiram, morphine alone or their combination was assayed in the MTT test after 24h and 7 days. Hatching rate and locomotor activity of *Danio rerio* larvae were determined following 24h and 5-day exposure. The levels of liver toxicity markers - AST, ALT, AP, GLDH, urea, albumins and total protein, were measured in rat serum with the ELISA assay after 21 days of morphine and disulfiram co-treatment. Analgesic tolerance was assessed in the Randall-Selitto test.

Results: Acute morphine and disulfiram co-treatment did not affect HepG2 cell viability. In fact, morphine protected against high-dose (30-100μM) disulfiram-induced decrease in cell proliferation. However, in a 7-day treatment regimen, a synergistic antiproliferative effect was seen in morphine- treated HepG2 cells additionally co-treated with 0.3-1μM of disulfiram. Neither disulfiram nor morphine affected *Danio rerio* larvae morphology or locomotor activity following acute exposure. However, a dose-dependent decrease in hatching rate was observed for disulfiram alone. A 5-day co- treatment also resulted in decreased hatching rate and swim bladder underdevelopment, but without any significant changes in larvae locomotor activity. In rats chronically co-treated with morphine and disulfiram serum liver toxicity markers were unchanged.

Conclusions: Disulfiram could serve as a promising future therapeutic option for counteracting morphine-induced tolerance and hyperalgesia. However, caution is advised in individuals with impaired liver function.

Abstract No. PPP.50

Electrochemistry coupled with High Resolution Mass Spectrometry to Study Nitroso- and Oxidation Products of Drugs with Hydrazone Group

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Objectives: The global crisis related to the contamination of medical products with carcinogenic nitrosamines and nitroso-APIs (NDSRIs) that has been ongoing since 2018 has driven analytics to seek new methods for predicting these impurities. Electrochemistry (EC) coupled with mass spectrometric (MS) techniques in off-line/online mode is a rapidly developing analytical tool in the field of predicting drug oxidation and degradation products and thus drug stability. Predicting drug degradation by Roxy reactors can help to identify new alarming impurities such as nitrosocompounds. Our research focused on impurities that can be formed in active pharmaceutical ingredients (API) during oxidation that have a hydrazone group in the structure.

Methods: The ROXy electrochemical reactor cell (EC) from Antec was used to generate oxidation API impurities. Measurements were made at different potentials, in three buffers with pH ranging from acidic, through neutral to basic. Identifications of the resulting electrochemical products were made using QTOF Maxis 4G (Bruker). Depending on the compound under study, the negative or positive ion mode of ESI MS was used.

Results: Four APIs with a hydrazone group were tested: rifampicin, nitrofural, furazidine and nitrofurantroin. Eight new oxidation products were identified in the drugs studied. Using fragmentation spectra from a high-resolution QTOF instrument, their structures were determined. For nitrofural, nitrofurantoin and rifampicin, oxidation products in the greatest amount are formed in an acidic environment. In the case of furazidine, oxidation occurred in each buffer, but the best results were obtained for neutral and alkaline buffers. Chemical oxidation (using hydrogen peroxide) was also compared with electrochemical oxidation in the area of stability of selected drugs.

Conclusions: In the present study, the main nitroso- and oxidation products of selected APIs at different redox potentials and environmental conditions were identified using electrospray ionization mass spectrometry. Environmental conditions under which selected drugs are not stable and degrade to impurities, including nitrosamines, have been established, which is important in predicting drug safety.

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Abstract No. PPP.51

A new series of coumarin derivatives as serotonin receptor agents - microwave synthesis and biological evaluation

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Objectives: Originating in plants, coumarins display wide range of activities and found application in the development of pharmaceuticals. Data presented in scientific papers indicate that hydroxycoumarins derivatives combined with an appropriate linker with arylpiperazine moiety may have an effect on the central nervous system.

The first step of the study was the synthesis of series of new oxiran-2-ylmethoxy coumarins. Then the first step products were converted to 2-hydroxy-3-(4-methoxyphenyl)piperazin-1-yl) propoxycoumarins. Biological activity studies were also performed for the 5-HT1A and 5-HT2A receptors affinity.

Methods: The microwave synthesis of a new series of compounds comprised O-alkylating of coumarins and synthesizing arylpiperazinyl derivatives. After purification via column chromatography, all newly synthesized compounds were subjected to in vitro evaluation of their functional activity for the 5-HT1A receptor with respect to 8-OH-DPAT and 5-HT2A receptor with respect to ketanserin.

Results: A series of new hydroxycoumarin derivatives were obtained. The structures of new compounds were established by ^{1}H and ^{13}C NMR spectroscopy and high-resolution mass spectrometry. Obtained compounds generally displayed varied selectivity for 5-HT1A and 5-HT2A receptor with respect to 8-OH-DPAT and ketanserin, a reference compounds.

Conclusions: As expected 2-hydroxy-3-(4-methoxyphenyl)piperazin-1-yl)propoxycoumarins showed 5-HT1A and 5-HT2A receptor activity. Having picked the most potent derivatives, we are planning to extend the range of biological testing methods for them and synthesize similar compounds to be assessed.

Abstract No. PPP.52

The novel LC-MS/MS method for mTOR inhibitors (sirolimus and everolimus) determination in human whole blood

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Objectives: Both sirolimus (rapamycin, SIR) and everolimus (EVE) are the mammalian target of rapamycin (mTOR) inhibitors widely used in transplantology, oncology, and neurology as well. The specific pharmacokinetics (PK) determines the necessity of those drugs concentration monitoring in clinical practice. Routinely, SIR and EVE are determined in EDTA-whole blood using immunochemical tests (IAs). The main disadvantage is cross-reactivity with the drug's metabolites, therefore the results are higher than in reference liquid-chromatography tandem-mass spectrometry technique (LC-MS/MS), characterized by high selectivity and sensitivity. The study aimed to develop a simultaneous method for SIR and EVE determination in a single analytical run using LC-MS/MS technique.

Methods: The analytical method has been based on LC-MS/MS platform (LC system Nexera 2 coupled with Shimadzu 8050 mass detector). The samples (from patients or spiked) were prepared using precipitation by ACN and zinc sulphate mixture, after that the amount of supernatant was mixed with MTBE (methyl tertbuthyl ether). The dryness of the MTBE phase was reconstituted with a water/methanol mixture and injected into LC-MS/MS system. The method has been validated in accordance with EMA (European Medicines Agency) guidelines. A validated and optimized method has been applied in clinical practice - the 50 whole blood samples from patients treated with SIR were analyzed. No samples with EVE were analyzed.

Results: The LC–MS/MS method was successfully developed and validated according to EMA guidelines. No matrix effect and carry over were observed. The stability at room temperature was 5 days, while in 4^{O}C- 2 weeks. The optimized sample preparation protocol allowed for 35µL of whole blood use only.

Conclusions: Presented study confirmed, that an optimized and validated method could be introduced for routine TDM of mTOR inhibitors. Due to the short run time, method is relatively productive with 20 sample analyses per hour.

Abstract No. PPP.53

Effect of magnesium ion concentration contained in the composition of preservative fluid on renal function based on studies of biochemical indices in homogenates of isolated porcine grafts

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Objectives: Preservation fluids are medical devices designed to preserve organs in the peri- transplantation period. Depending on the procedures performed, they are used to maintain flow through the organ undergoing mechanical perfusion or to flush blood from the graft and its storage. Their function is to protect organs from the harmful effects of low temperature, minimize ischemic damage, and neutralize the action of oxygen free radicals. The aim of this study was to analyze the effect of magnesium ion concentration, as a component of the Biolasol model fluid, on renal function.

Methods: Pilot studies were carried out in an isolated porcine kidney model Polish "Large White", with the approval of the II Local Ethics Committee Krakow; number 1046/2013. Biolasol liquid (FZNP "Biochefa", Poland) was modified with the addition of Mg^{2+} at a dose of 2.5 mg/l and 5 mg/l. Three study groups were formed: Biolasol (A, n=10), Biolasol+Mg^{2+}/2.5 mg/l (B, n=10), Biolasol+Mg^{2+}/5.0 mg/l (C, n=10). Kidneys were flushed and stored by static method in hypothermia for 48h. After reperfusion, homogenates were collected. Samples were homogenized in chilled 0.1M phosphate buffer (pH=7) and centrifuged. Biochemical indices, i.e. creatinine and protein, were analyzed in the resulting supernatants.

Results: It was found that in the kidney homogenates collected, creatinine concentration was lower by 15% in group B *vs* control group A ($p<0.05$) and by 20% in group C *vs* control group A ($p<0.05$). Protein concentration decreased by 30% in group B with respect to control group A ($p<0.05$) and by 50% in group C *vs* control group A ($p<0.05$).

Conclusions: Supplementation of Biolasol fluid composition with magnesium ions at 2.5 mg/l and 5.0 mg/l had a beneficial effect on renal function.

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Keywords: magnesium, kidney, preservation solution

Abstract No. PPP.54

Structural and spectroscopic properties of the selected azoles with antifungal activity – experimental and theoretical studies

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Objectives: Econazole, sulconazole, itraconazole, ketoconazole, posaconazole, voriconazole, fluconazole, isoconazole, bifonazole, ravuconazole and isavuconazole are hetarenes with proven antifungal activity. To the best of our knowledge, there are limited studies regarding spectral analysis of mentioned azoles. The main purpose of our investigation was to demonstrate what basis sets or method applied for spectra predictions would produce the most consistent results with the experimental data.

Methods: The initial structures of optimized rotamers of the analytes were taken from the crystallographic base CCDC, or taken from the PubChem database. They were initially optimized (Gaussian 16 A.03 program) using DFT formalism, namely: a) B3LYP/6-31G(d,p), b) CAM- B3LYP/6- 31G(d,p), c) B3LYP/6-311+G(d,p), d) PBE1PBE/6-31G(d,p), e) PW6B95D3/6-31G(d,p), f) M06L/6-31G(d,p), g) M062X/6-31G(d,p), h) APF/6-31G(d,p) and i) APFD/6-31G(d,p) approaches in the gaseous phase (IR and UV spectrum calculations) or by applying the CPCM model (UV and NMR spectrum calculations). For UV-vis calculations we applied using TD-DFT method, CPCM solvation model, and 14 solvents: n-hexane, carbon tetrachloride, toluene, chloroform, chlorobenzene, tetrahydrofuran, n- octanol, acetone, methanol, ethanol, acetonitrile, *N,N*-dimethylformamide, dimethylsulfoxide and water. The NMR shift for the TMS reference proton (H_{ref}) was calculated by the a–i approaches in DMSO or $CHCl_3$ at 293 K using the gauge-including atomic orbital (GIAO) method.

Results: The paper compares the experimental FT-IR, UV-vis and ^{1}H NMR spectra of azoles with the DFT calculations using different functionals. The results were compared with previously reported data related to their analogue (i.e. posaconazole). The analysis of calculated IR spectra with use functionals shows good accordance with the experimental IR spectrum. The best compatibility between the experimental and theoretical UV spectra was observed with the use of DFT formalism too. The HOMO-LUMO orbitals are discussed. The calculated ^{1}H NMR spectrum shows that the DFT formalism give an accurate description of the chemical shifts.

Conclusions: The results show that the DFT methodology seems to be a potentially useful tool for prediction IR, UV-vis and ^{1}H NMR properties of biologically active conazoles. We wish to investigate this standpoint further in the near future.

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Abstract No. PPP.55

Application of Hot Melt Extrusion technology in the development of innovative formulation to control hypertension and prevent the occurrence of cardiovascular diseases

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Objectives: The project's purpose was to improve the bioavailability of the hypertensive drug substance by using the Hot Melt Extrusion (HME) technique and developing an innovative combined preparation dedicated to the treatment of hypertension.

According to the United Nations Organization, the world population is aging – it is predicted that by 2050, there will be 426 million people over the age of 80 (three times as many as in 2019). Demographic changes are imposing a transformation of the healthcare system, which will need to focus more on patients struggling with chronic diseases, including hypertension. The treatment of hypertension, in most cases, requires polytherapy. The combination of two or more active substances in one drug form (Fixed-Dose Combination (FDC)) allows for reducing the number of tablets administrated, which is especially important for elderly patients struggling with more chronic diseases. Additionally, the application of suitable pharmaceutical technologies in the development of FDC can enhance bioavailability, potentially allowing for dose reduction and thus mitigating adverse effects.

Methods: For the development of a Fixed-Dose Combination (FDC) for the treatment of hypertension, we utilized the Hot Melt Extrusion (HME) technique. This process aimed to achieve an amorphous solid dispersion of the hypertensive drug substance. HME is a solvent-free technique that involves applying high temperature and pressure to melt a mixture of the active pharmaceutical ingredient (API) with a thermoplastic polymer, thereby enhancing the bioavailability and solubility of the drug.

Results: Due to the low melting temperature of the API, it was necessary to implement a solution enabling continuous processing at reduced temperatures, thus protecting the substance against thermal degradation and ensuring the appropriate release profile of the active ingredient.

Conclusions: The conducted research and development work enabled the obtaining of an amorphous extrudate. The extrudate is characterized by an appropriate impurities profile and stability.

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Abstract No. PPP.56

Evaluation of food effect in a Phase 1 Clinical Trial for an Investigational Medicinal Product, PKL-021, administered orally to healthy subjects

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Objectives: The study aimed to determine the pharmacokinetic profile of single and multiple doses of PKL—021 and evaluate the food effect on the PK profile of a single dose in healthy adult volunteers (men and women). The safety and tolerability of the investigational medicinal product (IMP) were assessed as a secondary objective.

Methods: In the case of drugs taken orally, food is known to affect drug absorption by delaying gastric emptying time, altering gastrointestinal pH, stimulating bile flow, increasing splanchnic blood flow, or physically interacting with drugs. In this clinical trial, the effect of high fat, high calory breakfast on PKL- 021 administration was tested in a PK study of 200 mg single dose of the compound. Two tablets of IMP 100 mg PKL-021 were administered with 200 mL of water to all subjects under fasting (Period 1) and fed conditions (Period 2). Then, blood collections were performed before the administration of the study medication and at 0.25, 0.50, 1.00, 1.50, 2.00, 3.00, 4.00, 6.00, 8.00, 10.00, 12.00, 15.00, 24.00, 36.00, 48.00 and 72.00 hours after the drug administration to determine concentrations of PKL-021 and its metabolite – PKL-024. PK parameters in fed (T_{FA}) and fasted (T_{FE}) conditions were obtained using non- compartmental analysis in Phoenix® WinNonlin® (version 8.4). The 90 % confidence intervals for the T_{FE}/T_{FA} ratio of the Least Squares (LS)-means for PKL-021 and PKL-024 based on ln-transformed data of $AUC_{(0-t)}$, $AUC_{(0-\infty)}$, and C_{max} were used to assess the food-effect.

Results: To evaluate the food effect, acceptance criteria of bioequivalence studies were adopted. According to bioequivalence rules, no food effect is observed when the 90% confidence intervals for the T_{FE}/T_{FA} ratio of the Least Squares-means for PKL-021 and PKL-024 based on ln-transformed data of $AUC_{(0-t)}$, $AUC_{(0-\infty)}$, and C_{max} is within 80%–125%. The clinical data showed 90% confidence limits from 73% to 112% for PKL-021 and from 44% to 110% for PKL-024, for the PK parameters expressing the drug exposure.

Conclusions: In the study, the means for $AUC_{(0-t)}$, $AUC_{(0-\infty)}$, and C_{max} of PKL-021 and its metabolite were not found within the acceptance limit; thus, the effect of food cannot be completely ruled out.

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Abstract No. PPP.57

Application of Untargeted Analysis to Detect Markers of Oxidative Changes in AIO

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Objectives: Parenteral nutrition (PN) is a life-saving intervention for patients where oral or enteral nutrition cannot be achieved or is not acceptable. All-in-one admixtures (AIO) provide safe, effective and lowrisk PN for a wide range of indications. Nevertheless, in recent years, there has been an increasing focus on the progressing lipid peroxidation in AIO formulations, the byproducts of which may adversely impact the patient. Researchers are continually exploring methods to minimize peroxidation during the storage of AIO. However, a challenge persists in the insufficient number of reliable markers capable of accurately determining the extent of peroxidation. Specifically, the method for determining one of the most frequently used markers, dialdehyde malonate, lacks specificity. Therefore, new markers are needed to assess the oxidative stability of AIO admixtures.

The aim of the study was to identify new potential markers of oxidative stability of AIO components using untargeted approach.

Methods: Three types of parenteral nutrition solutions with different lipid emulsions were examined. The admixtures were stored in the dark at room temperature for 24 hours to replicate infusion conditions. The analysis of samples collected before and after storage were performed by high-performance liquid chromatography coupled with mass spectrometry (LC-MS) using untargeted approach. The statistical evaluation was performed using MetaboAnalyst.

Results: The analysis showed that both hydrophilic and hydrophobic degradation products were formed during the storage of AIO. Their structure was proposed based on the fragmentation spectrum. The abundance of degradation products depended on the type of lipid emulsion.

Conclusions: The new lipid peroxidation markers identified in this study may be applied as indicators of lipid peroxidation. However, it is essential to establish quantitative analytical methods and validate them.

Abstract No. PPP.58

Bacterial CamA methyltransferase as a novel drug target: elucidating enzymatic catalysis using a computational approach

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Objectives: Modern medicine is confronted with a significant challenge posed by bacterial infections, further exacerbated by increasing antibiotic resistance among bacteria. Consequently, there is a growing need to develop new drugs that target novel molecular targets to effectively combat these infections. Among the most noteworthy challenges faced by modern medicine are *Clostridioides difficile* infections. One contemporary strategy in developing drugs against this bacteria is the design of inhibitors targeting bacterial methyltransferases. Specifically, in the case of *C. difficile*, the focus is on inhibiting the CamA methyltransferase responsible for spore formation and biofilm development. This enzyme catalyses the methyl group transfer from the cofactor S-adenosylmethionine (SAM) to adenine in the recognised DNA sequence. Inhibiting the activity of this enzyme weakens bacterial defense mechanisms, potentially enhancing the effectiveness of antibiotics. The utilization of CamA inhibitors has the potential to reduce the treatment duration for infections and lower the recurrence rate as well. A deeper understanding of the biological function of CamA methyltransferase at the molecular level may lead to the development of new innovative therapies to selectively inhibit this enzyme.

Methods: Molecular dynamics (MD) simulations were used to sample the conformational space of the enzyme. Selected conformations of the enzyme from MD simulations were used to determine the barrier for the methyl transfer using quantum mechanical (QM) calculations. The resulting models were verified by comparison with available experimental data.

Results: Through MD simulations, it was identified that the methylated adenine binding pocket consists of the amino acid residues ASN165, PRO166, and TYR168. These residues not only recognize the reaction substrate but also orient it to accept the methyl group. Using QM calculations, it was shown that the protein exhibits an active conformation in MD simulations and the reaction barriers obtained for the different reaction pathways are comparable to the experimental barrier.

Conclusions: Using an approach combining MD simulations and QM calculations, it was possible to obtain active conformations of the CamA methyltransferase and to propose a mechanism for the reaction catalysed by this enzyme.

Abstract No. PPP.59

Comparative Analysis of Antazoline Metabolites: *In Vivo versus* Electrochemical Detection

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Objectives: Identifying drug metabolites is essential for understanding pharmaceutical fate, toxicity, mode of action, and interactions. Electrochemistry (EC) offers a rapid and cost-effective method to simulate metabolic redox processes *in vitro*, eliminating the need for metabolite isolation from complex biological mixtures. Numerous studies have highlighted EC's efficacy in generating metabolites of various drugs, including cardiovascular and immunosuppressive agents. However, while EC is valuable, its ability to mimic *in vivo* metabolism differs across pharmaceuticals, requiring individualized experimental assessment. Antazoline (ANT), an effective antiarrhythmic drug for atrial fibrillation, lacks commercially available metabolites. Thus, this study aimed to compare ANT metabolites detected *in vivo* with those detected using EC.

Methods: The electrochemical conversion of ANT was conducted using the Roxy™ RC system (Antec, The Netherlands) with a Flexcell containing a boron-doped diamond electrode. Subsequently, for further characterization of the EC products, LC-MS with Orbitrap Focus was employed. The EC results were then compared with the results obtained from *in vivo* experiments involving patients who had undergone ANT administration.

Results: While the predominant metabolites identified *in vivo* were M1 (MW189), M2 (MW281), M3 (MW457), M4 (MW441), M5 (MW457b), M6 (MW487), M7 (MW375), and M8 (MW455), those detected using ROXY were E1 (MW275) and E2 (MW251), along with M1 (MW189) and M2 (MW281). However, the common metabolites detected by both methods are M1 and M2, which are the principal ANT metabolites in the human body.

Conclusions: This study demonstrates the feasibility of using EC to generate metabolites of antazoline (ANT) and compares them with those identified *in vivo*, indicating the potential of electrochemical methods for metabolite analysis in pharmaceutical research

Abstract No. PPP.60

Effect of food on pharmacokinetics of drug candidate CPL409116 and its M3 metabolite in healthy volunteers

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Objectives: Rheumatoid arthritis (RA) is a complex autoimmune disease with diverse clinical manifestations and intricate pathogenesis involving genetic and environmental factors. CPL409116 is a novel Janus kinase inhibitor, developed by Celon Pharma. It is targeting Janus family kinases (JAKs) and Rho-associated kinases (ROCKs) to inhibit the activation of inflammatory cell pathways. Phase I clinical trials primarily aim to assess the safety of a drug candidate in humans. However, secondary objectives include evaluating pharmacokinetics (PK) and determining the impact of food on bioavailability. Food- drug interactions can complicate oral drug development due to various physicochemical, physiological, and formulation-dependent mechanisms that may influence pharmacodynamics and PK. The aim of the study was to assess effect of high-fat high-calorie meal on PK of CPL40916 and its metabolite (M3).

Methods: A Phase I, one-center, dose-escalation, safety, and pharmacokinetics study, encompassed three parts: Part A (single-dose, open-label), Part A additional (crossover) to evaluate food effect on pharmacokinetics, and Part B (multiple-dose, randomized, double-blind). The food effect was studied in healthy males and females at a dose 120 mg of CPL409116 administered orally. CPL409116 and M3 metabolite concentrations in plasma were measured using LC-MS/MS method validated according to EMA guideline and in compliance with Good Laboratory Practice. The 90% CI of geometric mean ratios for fed vs. fasting conditions were calculated using ANOVA models for various log-transformed C_{max} and $AUC_{(0\text{-}inf)}$ with the conditions and subject fixed effects. A non-parametric Wilcoxon ($p < 0.05$) test was used to assess the T_{max} difference between the two conditions.

Results: CPL409116 bioavailability is statistically significantly increased by food intake – geometric means of C_{max} and $AUC_{(0\text{-}inf)}$ are significantly greater in fed than in fasting conditions. Food delays the median T_{max} of CPL409116 ($p = 0.095$, non-significant) and M3 ($p = 0.033$, significant). C_{max} and $AUC_{(0\text{-}inf)}$ of M3 are higher in fed conditions. The half-life ($T_{1/2}$) is significantly lower for both: CPL409116 and M3.

Conclusions: Food intake seems to modify the PK of CPL409116 and its metabolite M3. Clinical relevance of this finding will be assessed during further studies.

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Abstract No. PPP.61

Development of Novel Steroidal Androgen Receptor Antagonists

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Objectives: Second-generation androgen receptor (AR) antagonists currently used in the treatment of prostate cancer also lead to resistance over time, which is associated with poor prognosis. This study aimed to develop steroidal androgen receptor antagonists with improved selectivity and potency to bind, inhibit, and degrade AR and its variants.

Methods: Ligand-receptor docking and molecular dynamics simulations were performed, using ADFR and MGLTools scripts for ligand preparation. The androgen receptor structure (3l3x) was prepared using the AutodockTools package. Two promising compounds were synthesized and subjected to preliminary biological activity studies.

Results: Molecular docking revealed that synthesized DHEA derivatives modified with pyrrolopyridine and pyrrolopyrimidine scaffolds formed hydrogen bonds with Thr877 which have not been reported for either galeterone or second-generation non-steroidal antiandrogens showing stacking interactions of pyrazole with W742. One of the pyrrolopyrimidine nitrogen atoms acted as a hydrogen bond acceptor while the nitrogen of the amine group of the pyrrolopyridine acted as a hydrogen bond donor. The formation of this hydrogen bond results in a lower binding energy compared to other known steroidal antiandrogens. Biological activity studies confirmed the superior antiproliferative effects of these compounds compared to the abiraterone analogue, galaterone.

Conclusions: Docking experiments together with the results of biological studies confirm that such modification affects the binding of the synthesized compounds to the androgen receptor (AR), which can consequently, enhance its inhibition potential.

Abstract No. PPP.62

Physicochemical, theoretical, and structural studies on a novel compound with nootropic activity – Noopept

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Objectives: The aim of the study was firstly to develop a validated method for Noopept characterization in pharmaceutical products and to verify whether it is a substance suitable for API purposes. Secondly, we made an effort to investigate the chemical properties of Noopept thoroughly. We also wanted to document potentially different solvatomorphs of Noopept which could influence the chemical properties of this molecule. Our study aimed to achieve a deep understanding of noopept molecule behavior like stability, phase transitions, and structure.

Methods: Potentially amorphic solid noopept was recrystallized from many different solvents. Received crystals were studied using scanning electron microscopy (SEM), Fourier-transform infrared spectroscopy (FTIR), differential scanning calorimetry (DSC), thermogravimetric analysis (TGA), powder X-ray diffraction (PXRD), single crystal X-ray diffraction (SCXRD) and solid-state nuclear magnetic resonance (ss-NMR). The obtained results were used for further investigation using methods of computational chemistry.

Results: High-quality crystals of Noopept were obtained. All analytical methods made it possible to receive valuable chemical data about this compound. The unit cell of noopept crystals was successfully obtained and mapped. In our study, no other polymorphic structures were observed.

Conclusions: Noopept is a highly crystalline, room temperature stable white solid. Despite the nature of peptides, crystals obtained during recrystallization were well-developed and enabled all analytical methods to be involved. Noopept seems to occur only in one polymorphic form. This makes it easy to handle and use in future pharmaceutical products.

Abstract No. PPP.63

Determination of esketamine and its three metabolites in animal plasma – evaluation of specificity

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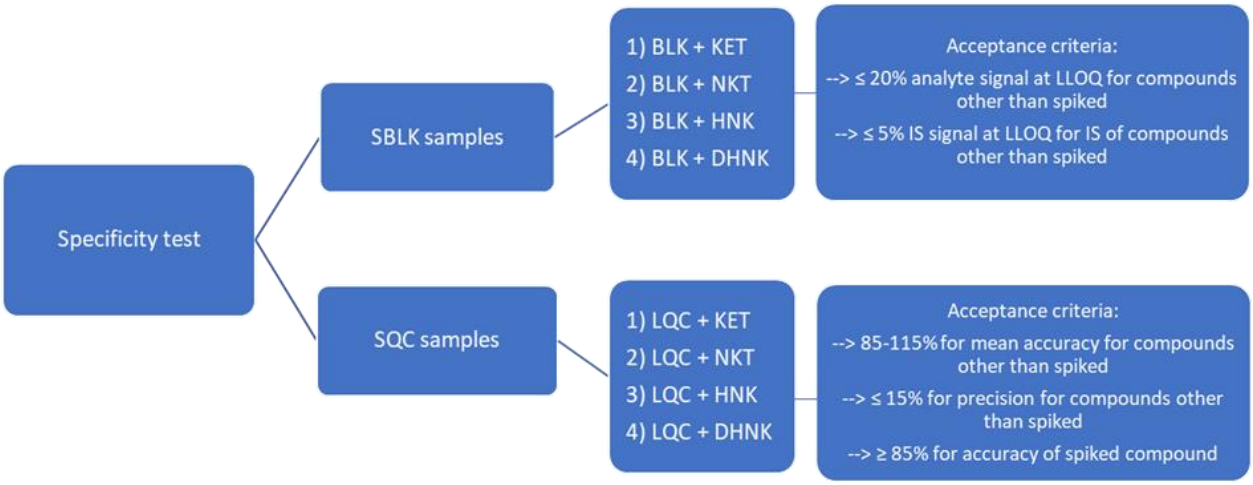
Objectives: Esketamine is used as dissociative anesthetic and antidepressant. The main metabolic pathway of esketamine (KET) produces: hydroxynorketamine (HNK), norketamine (NKT) and dehydronorketamine (DHNK). Developing a drug for new administration route (inhalation [1]) requires conducting toxicokinetic study. Thus, a new bioanalytical method to simultaneously determine esketamine and its selected metabolites in dog and rat plasma was developed. The aim of the study was to design and evaluate specificity test according to the ICH M10 guideline [2]. The guideline recommend testing specificity only in blank samples. However, in case of determination of four analytes, it was necessary to evaluate cross-influence between them.

Methods: High performance liquid chromatograph (1290 Infinity II, Agilent Technologies) coupled to tandem mass spectrometer (Triple Quad 6460, Agilent Technologies) was used for separation and detection. Electrospray ionization in positive mode, Multiple Reaction Monitoring, was applied. Samples were prepared in 96-well plates, using the protein precipitation, with acetonitrile containing a mixture of isotopically labeled standards. For each species, specificity test was conducted in blank samples (SBLK) and QC samples (SQC), each spiked with a single analyte according to the graph below:



Results: Specificity test in SBLK and SQC samples met acceptance criteria for all analytes except DHNK. Mean accuracy ranged: KET 99.9-104.1%, NKT 97.9-113.0%, DHNK 97.8-188.4% and HNK 95.2-114.5%. Mean precision was: KET ≤ 2.57%, NKT ≤ 2.89%, DHNK ≤ 6.84% and HNK ≤ 5.24%. Lack of specificity for DHNK was attributed to contamination of NKT solution.

Conclusions: Proposed experimental design enabled to confirm specificity of the method for simultaneous determination of several analytes. The new bioanalytical method enables quantification of KET (5–2000 ng/mL), NKT (5–2000 ng/mL) and HNK (5–2000 ng/mL). Determination of DHNK required further research on its stability in animal plasma.

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Abstract No. PPP.64

Evaluation of disintegration time and spreading ability of the vaginal tablets

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Objectives: Vaginal tablets (VT) are intended to have an appropriate disintegration time and spreadability to obtain a therapeutic effect. The main aim of the study was to assess the disintegration and spreadability of model VT.

Methods: A and B series of the VT with different content of gelling agent X (0.5% or 5.0%) were studied. The disintegration and spreadability of the VT was studied in the vaginal fluid simulant at 37 °C following Owen and Katz [1]. Disintegration tests were performed with a newly developed system in a standard volume of medium. The device was equipped with cameras and allowed measurement of the three tablets per single vessel. Spreadability of the VT was determined during the "sandwich test", presented in Fig. 1. First, the tablet was incubated in 4 mL of medium up to 120 min. and then it was subjected to mechanical stress (300 g for 1 min.).

Fig. 1. "Sandwich test" device.

Results: The tablets' length was calculated using the photos made during the disintegration test. The length of A series tablets increased during the test, especially in the first 5 min, while the length of B series tablets was almost constant. The spreadability of studied VT was evaluated based on the surface covered by the tablet after incubation in medium and application of a load. Series A tablets were characterized by higher spreadability than series B tablets. Tablets with 0.5% of ingredient X showed fast medium sorption and uniform hydration of the tablet core. Poor medium sorption was observed in series B. The presence of thick gel layer inhibited the hydration of the tablet core resulting in poor spreadability and slow disintegration.

Conclusions: The methods enable the determination of the effect of ingredient X on tablets disintegration and their spreading ability. Hydration uniformity and/or depth of the medium penetration can be investigated by visual inspection of the intact tablets and tablets after mechanical stress. Higher content of ingredient X had an adverse effect on tablet disintegration and spreadability. Series B was characterized by lower medium sorption than series A.

References:

[1] Owen D. H., Katz D. F. A Vaginal Fluid Simulant. Contraception 1999; 59: 91-95

Abstract No. PPP.65

Novel device for check on the disintegration of solid dosage forms

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Objectives: One of the key parameters in assessing the quality of solid dosage forms (SDFs) is their disintegration in various media. It is important to both visually investigate the disintegration process and accurately determine the disintegration time (DT). Therefore, drug disintegration time points should be recorded as reproducibly as possible. The aim of this work was to design a new system enabling visual assessment of the disintegration of SDFs and determining their DT.

Methods: The newly developed system enables disintegration testing of various SDFs up to three samples per vessel. The system can be configured in two sets. The first one is designed for SDFs fully immersed during the disintegration test (Fig. 1). This set also has movable perforated discs. The discs in upper position are above the medium level and allow visual assessment of the disintegration process. The second set is used for DSFs that are not fully immersed and have only surface contact with the medium (e.g. vaginal tablets). It is equipped with covers ensuring the appropriate humidity. The perforated discs have dimensions consistent with the recommendations of the European Pharmacopoeia for testing the disintegration of vaginal tablets. Moreover, both sets have cameras enabling visual recording of the disintegration process.

Fig. 1. Disintegration system.

Results: The results of the disintegration test consist of photos that can be taken every minute. The cameras have a fixed position so the formulations can be easily compared with each other. Furthermore, it allows visual inspection of the disintegration process (e.g. swelling, erosion, fast/low medium sorption) and accurate determination of drugs' DT. SDFs dimensions (length, width) can also be calculated based on photos.

Conclusions: Novel device for check on disintegration of SDFs was developed.Its main advantage is the versatility, as it can be used to study and record the spontaneous disintegration process of various SDFs including vaginal tablets. The results can be presented graphically, e.g. changes in SDFs length as a function of time, based on the calculation of SDFs dimensions using cameras and image processing performed in dedicated software scripts.

Abstract No. PPP.66

Green-friendly synthesis of 1,3,5-triazine derivatives using a sonochemical protocol

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Objectives: The 1,3,5-triazine core is a prominent motif in various chemical compounds, exhibiting potential bio-activity. It is commonly used in fields of industry such as organic synthesis, pharmaceutical chemistry, cosmetic chemistry or photochemistry. S-triazine in the role of main core it has the influence on increasing the activity of them as anticancer, antibacterial, antiviral etc. agents. However, these types of compounds are obtained through a 3 step synthesis method, where the starting substrate is cyanuric chloride. The most unecological and uneconomical stage of production of compounds derived from s- triazines is the third stage of synthesis, especially in the case of production on a larger scale. The synthesis methods presented so far involve carrying out the reaction at high temperatures for hours, which requires the supply of large amounts of energy but also the use of significant amounts of water for cooling. Additionally, the processes described so far use huge amounts of environmentally harmful and expensive solvents, which after the reaction require removal and disposal. Due to the growing interest and use in industry of s-triazine derivatives and the currently used methods of producing, which do not meet the principles of green chemistry, our team decided to focus on developing a more ecological and economical synthesis method

Methods: By closely monitoring the third stage of s-triazine derivative synthesis, we developed an efficient ultrasonic method following green chemistry principles, using water as the solvent. The research was conducted in two stages: the first stage involved selecting the best synthetic conditions for the model reaction between 2-phenetylamine and N2-(2-(1H-indol-3-yl)ethyl)-6-chloro-1,3,5-triazine- 2,4- diamine. The second stage was assessing the utility of the developed method by synthesizing a library of compounds containing tryptamine, aniline or benzylamine scaffolds.

Results: Synthesis of model reaction can be performed using various "green solvents" like ethylene glycol, glicerin, ion liquids, methanol however the best results (yield more than 95 %) was obtained when water was used as a solvent. In term of tested based, the best results (yield more than 95 %) were obtained when sodium carbonate or potassium carbonate were applied.

Conclusions: Based on the developed method, it is possible to obtain products with up to 75% yield. The use of ultrasound allowed for a 12-fold reduction in the synthesis time. Moreover according to DOZNTM2.0 tool - developed method is 13-times more eco-friendly than conventional heating.

Abstract No. PPP.67

New multicomponent crystals of prasterone - structural and pharmaceutical studies

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Objectives: Prasterone (DHEA) is the steroid drug indicated for the treatment of moderate to severe dyspareunia associated with menopausal vulvar and vaginal atrophy [1]. It is classified as class II drugs in the Biopharmaceutical Classification System (BCS), which characterized by low solubility and high permeability. The bioavailability of DHEA is severely limited by its poor solubility (23 µg/mL) [2]. This is important factor that determines the bioavailability of the administered drug orally, thus affecting the effectiveness of therapy. Therefore, it is justified to search for and research new salts or cocrystals of DHEA. In this study we present a method of a synthesis of new multicomponent crystals of DHEA and their structural and pharmaceutical studies.

Methods: The liquid assisted milling method has been used successfully to obtain new multicomponent crystals of DHEA. Fourier transformed infrared spectroscopy (FT-IR) and powder X-ray diffraction (PXRD) were used to provide information about the formation of new crystal forms. Structural studies were supplemented by solubility and dissolution tests.

Results: The PXRD patterns and FT-IR spectra of DHEA crystals are clearly different from those of the parent drug. Changes in the positions and intensities of the peaks in the PXRD patterns indicate that these were not ordinary physical mixtures. New multi-component crystals were successfully formed. The water solubility and dissolution rate of DHEA were measured using high-performance liquid chromatography.

Conclusions: The tests carried out show that the newly obtained multicomponent DHEA crystals showed higher solubility and dissolution rate than DHEA. Increasing the solubility of DHEA allows you to significantly reduce the dose of DEHA, which will positively affect the patient's safety during DHEA therapy and reduce treatment costs.

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Abstract No. PPP.68

Exploring the influence of buffers on physiochemical properties and *in vitro* efficacy of lipid nanoparticles loaded with mRNA

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Objectives: The stability of lipid nanoparticles (LNPs) carrying mRNA is crucial for the success of mRNA- based therapeutics, therefore the choice of neutralization or storage buffer plays a critical role in the formulation and stability of lipid nanoparticles (LNPs) loaded with mRNA. The type of buffer can influence various physicochemical properties and *in vitro* efficacy of the LNPs. Literature data suggest also that LNPs may require different excipients depending on the lipid composition.

In this work, we investigated the characteristics of LNPs-mRNA prepared and stored in different buffers, commonly used in cell culture and molecular biology experiments. We carried out short-term stability test and evaluated the LNPs properties after storage in +4°C, −20°C and −80°C. All buffers used for storage were supplemented by sucrose as cryoprotectant.

Methods:

Lipid nanoparticle synthesis: LNPs were prepared by microfluidic mixing using a NanoAssemblr Ignite instrument (Precision Nanosystems).

Physicochemical properties: Particle size and polydispersity index (PDI) were measured using Zetasizer Ultra (Malvern). For encapsulation efficiency and mRNA concentration RiboGreen assay was used.

In vitro transfection method: Transfection with the LNP containing luciferase as model mRNA was performed using HEK293 cells. The read-out was performed 24hours after transfection. Naked mRNA and lipofectamine were used as controls.

Results: We evaluated LNPs before and after short-term storage at +4°C, −20°C and −80°C in respective buffers. The comparison of LNP size, PDI and encapsulation efficiency showed differences dependent on the buffer type. *In vitro* experiments also identified the variable rates of endosomal escape and transfection efficiency.

Conclusions: Our results indicate that the choice of neutralization or storage buffer can significantly influence the formation, properties, and stability of LNPs. There are still additional studies required to fully understand the impact of the composition, pH values and storage conditions on LNPs properties and nucleic acid delivery.

Abstract No. PPP.69

New furazidine salt – synthesis, structural and pharmaceutical studies

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Objectives: Furazidine (FU) is a nitrofuran derivative and has antibacterial activity with bacteriostatic action against both gram positive and gram negative bacteria [1]. Mostly is used in the treatment of urinary tract infections [2]. FU is poorly water-soluble drug. The aim of conducted research was to obtain novel crystal forms of FU which exhibit enhanced solubility. Due to ongoing patent procedures, we are unable to disclose any information about the counterion used.

Methods: New FU salt was synthesized and characterized by ^{13}C, ^{1}H NMR spectroscopy, infrared and Raman spectroscopy and powder X-ray diffraction (PXRD) studies. Physicochemical studies were completed with solubility tests and stability. Dissolution profiles of FU salt at distilled water were studied using LC-MS analysis.

Results: Thanks to the conducted research, it was possible to confirm the structure of the new FU salt and characterize it spectroscopically. Investigated new FU salt exhibited higher solubility than pure FU and is stable after a year of storage.

Conclusions: The preliminary results of these studies indicate the application nature of the new FU salt, therefore research on it will be continued. It should be remembered that in the case of oral drugs, excellent water solubility and good stability are desirable.

References:

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Abstract No. PPP.70

The effect of knock-out the gene encoding prolidase in MCF7 breast cancer cells (MCF7ko-pepd)

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Objectives: Prolidase (PEPD) is an enzyme that catalyzes the hydrolysis of imidodi- and imidotri- peptides containing C-terminal proline (Pro) or hydroxyproline (HyPro). The enzymatic PEPD activity, as a factor determining intracellular levels of Pro and HyPro, contributes to the regulation of key cellular processes in cancer-survival, metastasis, oxidative stress, hypoxia - characteristic in the tumor progression. PEPD regulates the energy state of the cell, and, depending on its intracellular level, the processes of cell death - apoptosis and autophagy. Our research has also shown that prolidase, independently of its catalytic activity, is able to interact with p53, providing a molecular mechanism for impairing p53-dependent apoptosis induction, which may be a reason for the cell resistance to chemotherapy. The aim of this studies was to understand the metabolic changes occurring in breast cancer cells with different level of prolidase expression and to investigate the effect of *PEPD* knock-out on the apoptosis.

Methods: MCF7 and MCF7 overexpressed PEPD ($MCF7^{PEPD}$) cells were conducted. Three nucleotide sequences were designed for *E. coli* plasmids. Plasmids were multiplicated and DNA was isolated. MCF7 (p6) cells were transfected using the CRISPR-Cas9 method. Selection was carried out using Puromycin. In the three cell types, prolidase activity was assessed by colorimetric assay, protein expression and translocation were estimated using fluorescent microscopy. The evaluation of protein expression was confirmed by Western Blot.

Results: Knock-out PEPD caused cell death within 72h. Cells after the selection step showed no proliferative potential. Obvious morphological differences were observed compared to MCF7. Death does not allow the obtain of a stable cell line. Supplementation of the medium with amino acids (products of PEPD activity) and glutamine, no improvement in cell survival was observed. In transfected cells, not undergoing selection, indicates a decrease enzyme activity. Reduced PEPD and p53 expression were observed in $MCF7^{KO\text{-}PEPD}$.

Conclusions: Overexpression of PEPD causes resistant to chemotherapy, on the other hand knock-out PEPD results in MCF7 cell death which provides the promising perspectives for gene therapy of breast cancer. However, the traditional procedure of the CRISPR/Cas9 method makes it impossible, to obtain a stable, uniform cell line, prompting the use of a different transfection system (Tet On/Off where CRISPR/Cas9 is under the control of Doxycycline) that will allow us to obtain results unambiguously to evaluate the role of prolidase in the metabolism of MCF7 cells.

Abstract No. PPP.71

Molecular dynamics study of unique androgen receptor deformation induced by novel pyrrole-containing steroidal antagonists

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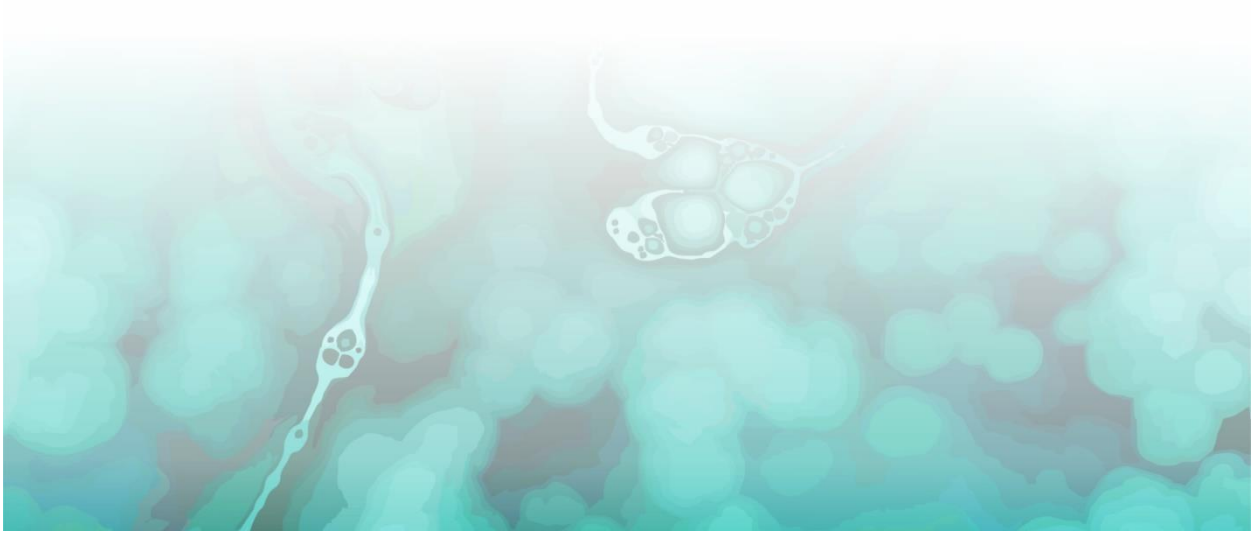
Objectives: The aim of this study was to use molecular dynamics to elucidate the mechanism behind antagonism of androgen receptor when pyrrole containing steroidal antiandrogens are present in its ligand binding domain.

Methods: VINA was used to obtain ligand poses inside 3L3X and 2PIV androgen receptor structures. The most promising positions were used for molecular dynamics simulations with the protein. Protein was described with AMBER99SB-ILDN force-field and ligands with GAFF force-field, utilising acpype package. The complexes were immersed with TIP3P water molecules and systems were heated to 300 K using modified Berendsen thermostat. The MD simulations were conducted over 30ns using GROMACS 2024.1 package.

Results: MD simulation analysis has explained biological study results and structure-activity relationship. It was established that pyridine's nitrogen atom was in a suitable position to form hydrogen bonds over a water molecule with Leu707. Due to this interaction, the pyrrol ring could enter the space of Arg-752 and from a bond with Gly-683. This deformed the surface of AR LBD considerably and is thought to disrupt AR dimerization and thus stop prostate cell proliferation. This has not been spotted for neither enzalutamide nor galeterone. While the conformation was not stable, other compounds, with substituted pyrrole ring, are proposed that could enhance the stability of this particular AR-ligand conformation.

Conclusions: This study details an alternative androgen receptor antagonist design that promises a unique antagonist mechanism of action not seen for currently employed second generation AR antagonists. A new path for their further modification is also suggested.



Abstract No. PPP.72

Pleurotus ostreatus as a novel strategy for mianserin and mycophenolic acid elimination in wastewater treatment

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Objectives: Conventional wastewater treatment plants' inefficiency in removing pharmaceutical active compounds (PhACs) leads to their presence in aquatic environments, posing risks to organisms. Innovative approaches for PhAC removal, such as using white-rot fungi (WRF), are crucial to minimize environmental pollution. Although extensive research on WRF's pollutant removal capabilities exists, further investigations are needed to fully adapt the process for practical implementation.

Methods: This study aims to validate WRF *Pleurotus ostreatus*, for eliminating eight PhACs in municipal wastewater containing corn steep liquor (CSL) as a carbon and nitrogen source. The cultures were conducted in batch mode. The PhAC residue in the medium was quantified by HPLC-MS analysis. Additional assessment of laccase activity used the spectrophotometric method

Results: A significant increase in *P. ostreatus* biomass in wastewater was achieved with a minor addition of CSL (i.e. 0.53% *w/v*). The highest removal efficiencies were observed for mycophenolic acid (MPA) and mianserin (100% and 73.4%, respectively). Notably, even in unenriched wastewater, *P. ostreatus* demonstrated laccase secretion.

Conclusions: *P. ostreatus* shows promise in removing PhACs like MPA and mianserin, especially with minor CSL supplementation. This underscores its potential for effective PhAC removal in wastewater treatment, contributing to environmentally responsible pharmaceutical waste management efforts.

Abstract No. PPP.73

Synthesis and physicochemical characterization of new mesoporous silica material/Hydroxyapatite composite for potential use as a drug delivery system

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Objectives: Hydroxyapatite have been playing an important role in biomaterial engineering for years, as they are being used in many branches of medicine: reconstruction and repair surgery, implantology, otorhinolaryngology, conservative dentistry, dental surgery and injection and oral DDS. They serve as bone replacement material, coatings of metal implants, components of composite biomaterials, bone and dental cements [1].

Mesoporous silica material has emerged as a promising drug vehicle, primarily due to their unique mesopores structure that while preserving a level of chemical stability, surface functionality and biocompatibility. It ensures the controlled release of a variety of drug molecules, so they are very useful for bone tissue regeneration. Composites containing these two biomaterials are expected to have better properties, so in this study they were synthesized [2]. As a result, molecular sieve/ hydroxyapatite composites based on six different molecular sieves were obtained: MCM-41, MCM-48, SBA-15, SBA-16, KIT-6 and KCC-1. Tree of these, SBA-16, KIT-6 and KCC-1, were used for the first time for this type of composite.

Methods: Composites were synthesized with six silica mesoporous materials: MCM-41, MCM-48, SBA- 15, SBA-16, KIT-6 and KCC-1) previously synthesized by wet methods. In the next step of the composite synthesis, the hydroxyapatite was also deposited on the surface of the resulting molecular sieves using a wet method. The obtained products were examined using various analytical methods: powder X-ray diffractometry (PXRD), infrared spectroscopy (IR) and transmission electron microscopy (TEM).

Results: The obtained TEM photos of the synthesized sieves confirm the assumed structure and pore size. TEM photos taken of the composites show their surface covered with crystalline hydroxyapatite nanocrystals.The results of the PXRD method confirm the synthesis of the five molecular sieves and obtaining hydroxyapatite on their surface. The results obtained by TEM and PXRD were confirmed by FT-IR and Raman methods.

Conclusions: Six molecular sieve/hydroxyapatite composites were successfully synthesised, three of which have not yet been described in the literature: SBA-16/HA, KIT-6/HA and KCC-1/HA. Future studies are planned to investigate the release of model drug from the materials obtained.

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Abstract No. PPP.74

A Suitable Size Exclusion Column for Efficient Adeno-Associated Virus Aggregate Analysis

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Objectives: To support the continued advancement of Adeno-Associated Viruses (AAVs) for gene therapy, fit for purpose analytical liquid chromatographic (LC) methods are needed to analyze Critical Quality Attributes (CQAs), such as aggregation. Various non-LC based analytical methods exist for aggregate analysis (DLS, AUC and Flow Cytometry) and are commonly utilized in the development of the manufacturing process. These methods have limited accuracy and precision and require extensive method development or re-qualification whenever the matrix of the sample is changed. Complementary or orthogonal analytical methods are advantageous to have, particularly during the early phases of process or analytical development, since the aggregation of the biological target is not fully known and therefore multiple methods may be used to verify the CQA to increase the confidence in steering critical decisions.

Analytical LC methods can be designed to achieve reproducible accuracy and precision. Biozen™ dSEC- 7, a chromatographic SEC column, is described and tested for various AAV serotype aggregates. Its capabilities are demonstrated and several applications discussed.

Methods: AAV 1 to 9 and rh10 were purchased from Virovek (Houston, TX). For AAV8-CMV-GFP five samples of different concentrations were prepared in 0.2µm filtered 1X PBS with 0.001% Pluronics F68 into HPLC vials. For serotype AAV studies on the BioZen dSEC-7 column, separate dilutions of 4x1011vg/mL were prepared and centrifuged at 10000rcf for 5 min prior to injection on the LC system.

Results: Loading and linearity performance studies including chromatogram of AAV8-CMV-GFP on a Biozen dSEC-7 column of 5 different loads and AAV8-CMV-GFP load (vg) vs FLD 280/340nm monomer peak area. Recovery performance on various AAV serotypes including zoomed-in chromatograms of AAV serotypes on a Biozen dSEC-7 column demonstrating consistent recovery of AAV monomer and impurities across tested serotypes.

Conclusions: The analytical capabilities of the Biozen dSEC-7 column designed for AAVs was demonstrated. Reproducible separations and recoveries for various AAV serotypes 1 to 9 and rh10 are shown.

Due to the reproducible analytical performance of this column, it may be used in stability studies to determine the purity and quantity of the monomer left in the solution and it may also be used to drive the development of other analytical methods. It also enables the determination of the critical quality attributes in drug substances and drug products.

Abstract No. PPP.75

Advancing Inhibitor Specificity for DYRK1A and DYRK1B: A Targeted Approach

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Objectives: DYRK1A and DYRK1B, closely related kinases in the DYRK family with over 85% similarity in their kinase domains. Both paraloquesg play distinct roles in human health: DYRK1A is crucial for brain development, cognitive performance, and glucose regulation and its dysfunction is associated with intellectual disabilities, Down syndrome, and diabetes, while DYRK1B primarily influences cell cycle control and cell division, implicated in cancer, metabolic syndrome, and nonalcoholic fatty liver disease. The potential compensatory interaction, marked by increased DYRK1B levels upon DYRK1A silencing, underscores their interconnected roles and their potential as therapeutic targets. This study aims to advance targeted drug design by determining the crystal structures of both kinases with the inhibitor AZ191, performing comparative analyses to understand their interaction differences, and conducting biochemical and cellular assays to evaluate AZ191's efficacy, providing insights crucial for the development of selective inhibitors.

Methods: We conducted structural analyses by obtaining the crystal structures of DYRK1B and DYRK1A with AZ191. These structures provided a basis for comparative analysis. The efficacy of AZ191 was evaluated through a series of biochemical and cellular assays, including an ADP-Glo Kinase Assay for kinetic parameter determination, ITC, Thermal Shift assay and NFAT translocation assays.

Results: The crystal structures of DYRK1A and DYRK1B in complex with AZ191 were successfully determined, revealing the atomic-level binding modes of the inhibitor. Biochemical assays confirmed the efficacy of AZ191, showing significant stabilization of the protein-inhibitor complex and effective inhibition of kinase activity in cellular models. Comparative analysis highlighted distinct interactions contributing to the specificity of inhibitor binding between DYRK1A and DYRK1B.

Conclusions: The study successfully elucidated the structural basis for the interaction of DYRK1A and DYRK1B with the inhibitor AZ191, addressing the previous challenge of lacking a DYRK1B crystal structure. The findings allow for the informed design of more selective inhibitors, potentially leading to therapeutic applications for diseases associated with DYRK1A and DYRK1B dysfunctions. The assays employed confirmed the inhibitor's efficacy, supporting its further development and optimization.

Abstract No. PPP.76

New multifunctional carrier for local delivery of ipriflavone.

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Objectives: The project aims to synthesize calcium phosphate materials enriched with strontium and silicon ions. Subsequently, ipriflavone, whose solubility was increased by complexation with cyclodextrins, was introduced into the obtained 3D shapes.

Methods: In this project, hydroxyapatites enriched with strontium ions with antiresorptive properties and silicon with osteogenic properties were synthesized. The substrates for the synthesis of hydroxyapatite were $Ca(NO_3)\cdot 4H_2O$, $(NH_4)HPO_4$, and $Si(CH_3COO)_4$ and $Sr(NO_3)_2$. The resulting material was micronized and dispersed in an aqueous solution with the addition of one of three dispersants: CTAB, TWEEN, and PVA. Subsequently, polyurethane and collagen sponges were soaked in the resulting suspension, dried and calcined at 1050°C. The obtained 3D shapes were examined by optical microscopy and SEM.

In parallel, the complexation of ipriflavone with four cyclodextrins (α, β, γ and hydroxypropyl-β-CD) was carried out by different methods at different molar ratios. Structures of the resulting inclusion complexes were modelled at the DFT level and the theoretical results were compared with the experimental results of UV/VIS, FT-IR and NMR analysis.

The obtained shapes were soaked in an aqueous solution containing inclusion complexes.

Results: The efficiency of matrix formation depended on the dispersant used. Collagen sponges showed significantly higher functionality than polyurethane sponges. The resulting porous shapes had pore sizes of 1 nm or larger, which was examined by SEM.

The resulting complexes showed increased solubility in water and PBS solution of pH = 7.4. The efficiency of the ipriflavone complexation process depended on both the type of cyclodextrin and the method used.

Conclusions: The obtained complexes provide increased bioavailability of ipriflavone, which, thanks to the created shapes enriched with strontium and orthosilicate ions, can be directly introduced into the bone where it will effectively reach therapeutic concentrations. Subsequent studies will be based on testing the cytotoxicity and biocompatibility of the obtained materials in vitro on two cell lines: mouse fibroblasts and human osteosarcoma cells.

Abstract No. PPP.77

Occurrence of Antidepressants in the Sediments Vistula River near the Effluent Point from WWTP in the Warsaw Agglomeration Area

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Objectives: The presence of pharmaceutical residues (PhACs) in the environment raises global concerns, particularly in aquatic ecosystems where treated wastewater serves as a major source. Of particular concern is the occurrence of antidepressants, which even at low concentrations, can adversely affect aquatic life by disrupting nervous system homeostasis in both vertebrates and invertebrates. Due to their physicochemical properties, antidepressants bind to sediments, from where they continue to be released into the water, forming a reservoir of PhACs in the environment. Currently, there is limited data on the presence of antidepressants in sediments of Eastern Europe. Thus, this study aimed to assess the occurrence of 21 antidepressant pharmaceuticals at specific points along the Vistula River, the main river in Poland.

Methods: The sediments were collected from the Vistula River in the Warsaw agglomeration area, both upstream and at various distances from the Czajka wastewater treatment plant outfall. PhACs were extracted using the QuEChERS method and analyzed using liquid chromatography coupled with mass spectrometry.

Results: The highest concentration of antidepressants was detected near the discharge point of the Czajka wastewater treatment plant. All tested compounds were found there except for maprotiline, mianserin, and mirtazapine. Some of the analyzed antidepressants were also present in sediments upstream of the Czajka outfall.

Conclusions: Sediments collected from the Vistula River in the Warsaw agglomeration contain antidepressants. Further research is needed to determine the impact of the presence of these compounds on the ecosystem.

Abstract No. PPP.78

Assessment of selected quality aspects of falsified medicinal products used in the treatment of thyroid diseases

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Objectives: The purpose of this study was to qualitatively analyze the composition and identify contaminants in adulterated thyroid hormone medicinal products, as well as to quantitatively analyze the pharmacologically active substance declared by the manufacturer and selected impurities - pharmacologically active substances identified in the tested products.

Methods: The study used 10 medicinal products from two liquidated illegal drug factories in Poland, containing liothyronine and levothyroxine. The use of liquid chromatography coupled with mass spectrometry enabled the analysis of tested samples of counterfeit medicinal products for the presence and content of pharmacologically active substances and other potential contaminants.

Results: The presence of the declared active substances liothyronine and levothyroxine was confirmed in all the tested falsified medicinal products. However, all analyzed products contained an incorrect dose of the declared active substance - in the case of levothyroxine too small, and in the case of liothyronine too large. Other pharmacologically active substances not declared on the package (including stanozolol, methandienone, clomiphene, tamoxifen, letrozole, furosemide, allopurinol), thyroid hormone breakdown products and other substances (e.g., cocaine, salicylic acid) were also identified in the examined material.

Conclusions: The analysis results confirm that the tested products are of low quality and may pose a threat to the health and life of those who use them.

Abstract No. PPP.79

Using the Cambridge Structural Database in Drug Sciences

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Objectives: Structural databases such as the Cambridge Structural Database (CSD) contain a wealth of information that can be used to derive new knowledge. This poster will demonstrate how this small molecule structural data can be used for pharmaceutical applications.

Methods: Following a vision that the collective use of data would lead to new knowledge and generate new insights Dr Olga Kennard established the CSD in 1965, a step which transformed modern structural science. Today over 1.25 million structures are shared through this resource and the data in the CSD provide the fuel that drives scientific discovery across diverse areas of research. CSD Software can be used to aid pharmaceutical investigation from drug discovery, to investigation of structural stability, to study of particle properties. This software includes functionality for ligand validation, protein ligand docking, using GOLD and components that include sophisticated analysis and prediction of intermolecular interactions, and crystal packing.

Results: Knowledge derived from the structural data contained in the database has underpinned fundamental chemical discoveries and played a key role in designing new materials from drugs to pigments and beyond.

Conclusions: This poster will focus on how this wealth of data can be used in drug sciences and will showcase some of the approaches that are embedded in the pharmaceutical industry and beyond.

Abstract No. PPP.80

Antibacterial effect of application of silver nanopartigles (agnps) and cefiderocol

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Objectives: Cefiderocol (Fetroja®) is an injectable siderophore cephalosporin. Its mechanism of action involves the use of active bacterial channels transporting iron and penetrating the periplasmic space of bacteria. The drug binds to penicillin binding proteins (PBPs), inhibiting the synthesis peptidoglycan cell wall of bacteria, which leads to cell lysis and death. In the presented research, work was undertaken to increase the effectiveness of cephalosporin by adding nanosilver.

Methods: Cefiderocol (Fetroja®) Shionogi inc. All other materials were in analytical grade.

AgNPs synthesis

Silver nanoparticles were produced using chemical reduction method. In this method, a solution containing 2 mM $AgNO_3$ was mixed with solution of ascorbic acid (20 mM) as a reducing agent in a glass vessel (volumetric ratio 1:1). All reagents were freshly prepared before AgNPs synthesis. After reagents mixing, the colorless solution turned light yellow (t = 2 min) and yellow (t = 10 min). The color of the solution and registered LSPR (localized surface plasmon resonance) with a maximum at 420 nm confirm the presence of silver nanoparticles. For further studies, selected samples containing AgNPs were mixed with CEF solution in a 1:1 volumetric ratio. Then the prepared mixture (1 mL) was dispersed on filter paper. All samples were protected from light and heat.

Microbiological analysis

The examination of silver nanoparticles and their combinations with Cefiderocol was performed using the disk diffusion method on Mueller-Hinton Agar against the reference strains *Escherichia coli* ATCC 25922 and *Acinetobacter baumannii* ATCC 19606. The strains were suspended in 0.85% saline solution and plated in the culture medium, and then the discs were placed. Samples with the addition of pure silver (Ag(I) ions), nanosilver, Cefiderocol and Cefiderocol with the addition of silver nanoparticles were tested. The plates were incubated at 37°C for 24 hours, after this time the inhibition zones were measured.

Results: Based on the conducted research, the antibacterial effect of Cefiderocol was confirmed. Moreover, it has been shown that the addition of silver in its pure form does not affect the disappearance of bacteria. In turn, the addition of silver in the form of nanoparticles doubles the effect of the antibiotic. This effect also depends on the application time of nanosilver particles, and therefore on the degree of its growth and/or aggregation.

Conclusions: The antimicrobial activity of AgNPs was successfully combined with antibacterial activity of Cefiderocol. It opens new perspective for effective treatment of infections.

Abstract No. PPP.81

Testing the biological activity of exopolysaccharides of the medicinal mushroom *Lentinula edodes* (shiitake mushroom)

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Objectives: Polysaccharides of mushroom origin have universal multidirectional structures with pharmacological activities, including anticancer, immunomodulatory, antioxidant, antibacterial, antifungal, antiviral, hypoglycemic, hypolipidemic, hypotensive and hepatoprotective effects. Mycelial cultures of fungi have the ability to secrete exopolysaccharide species (EPS) into the culture medium, often with a structure different from isolates from mycelial biomass, which also exhibit biological activity. The aim of the research was to use the mycelium cultures of the fungus *Lentinula edodes* grown on liquid with additional selenium compounds, which were designed to release EPS fractions into the bases and to have a protective effect of these substances.

Materials and Methods: To achieve the assumed goals, the experimental part was carried out in several stages:

1. Biosynthesis and purification of selenated EPS from the culture medium and mycelial biomass:

2. Tests of the biological activity of the EPS fraction:

• Testing the impact of selenium incorporation on the immunomodulatory activity of the EPS fraction, including the impact on the proliferation of T and B lymphocytes (PBMC test)

• Testing the cytotoxicity of polysaccharide fractions on HUVEC and HeLa cell lines

• Investigation of the antioxidant activity of polysaccharide fractions by examining their protective effect on cells against exogenous oxidative stress

Results: The isolated EPS fractions showed immunosuppressive effects. EPS fractions in a cytotoxicity test (MTT) showed a selective increase in the survival of normal cells (HUVEC), without affecting cancer cells (HeLa). It also showed high antioxidant activity.

The study results showed that the EPS fraction had an inhibitory effect on T cell proliferation in the OKT-3 assay, without having any effect on B cells as suggested by the *S. aureus* post-stimulation (SAC) test results.

Conclusions: As a result of the biosynthesis, new macromolecules were isolated - EPS fractions, enriched in selenium. Research results confirmed that the post-culture medium is a source of biologically active EPS.

Due to the potential use of EPS derived from the mycelium of *L. edodes* as new immunosuppressive drugs in transplantology and autoimmune diseases, further research on the mechanisms of action and pharmacokinetic properties of these compounds is planned.

Abstract No. PPP.82

The synthesis and activity of 8-substituited coumarin peptidomimetics against mdr bacteria

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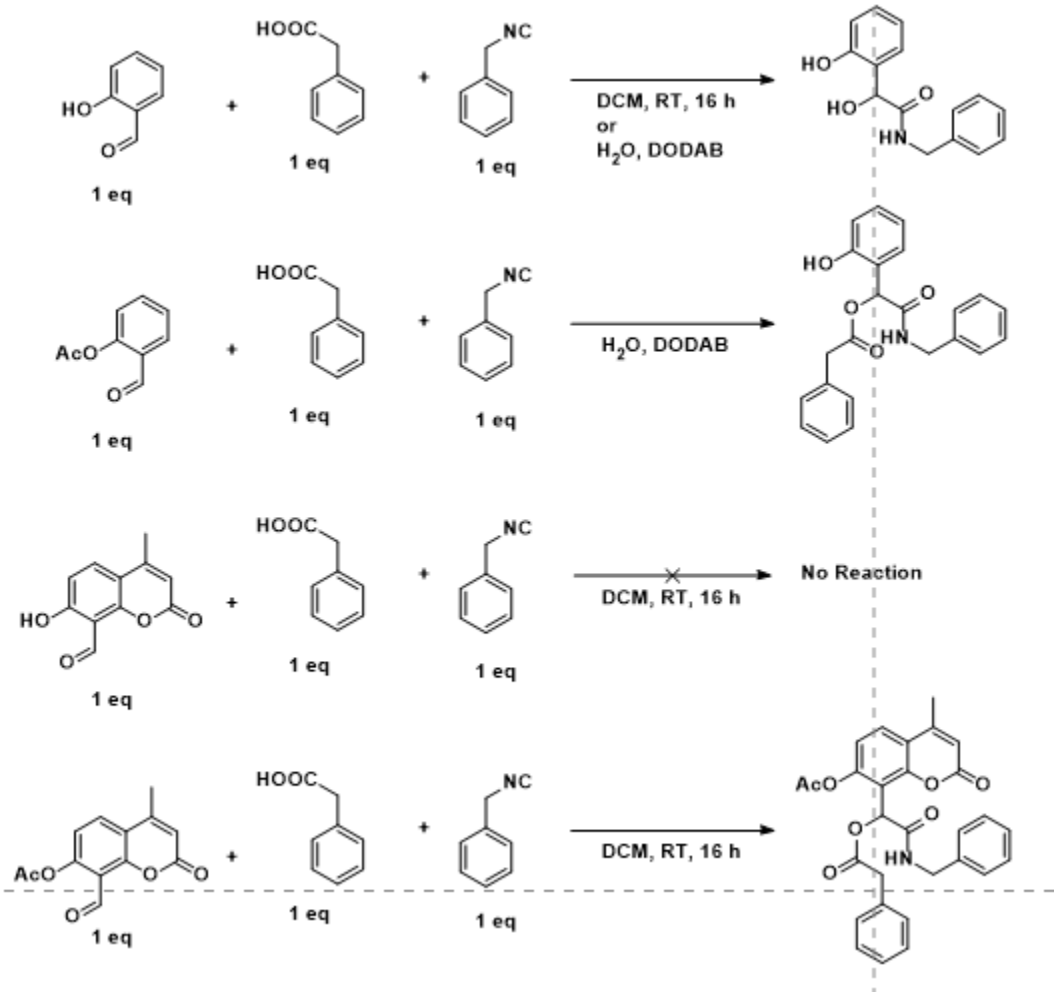
Objectives: Antibiotic resistance is a growing global health issue, causing 1.27 million deaths in 2019. Researchers are exploring the potential of coumarin derivatives, Particularly we have focused on underrated C8 position of coumarin, for antimicrobial activity. The study aims to design and synthesize coumarin-based peptidomimetics, which disrupt bacterial cell membranes and may be a solution to antibiotic resistance. Combining coumarin with peptidomimetics could be a viable approach to curb antibiotic emergence. The investigation will evaluate bacterial responses and explore clinical implications.

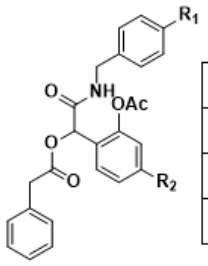
Results: Our focus initially centered on efficiently synthesizing Peptidomimetics from salisaldehyde, which was crucial for developing the methodology for synthesizing peptidomimetics from 7-hydroxy-8-formyl coumarin moiety. Notably, in the Passerini reaction, salisaldehyde used as a substrate yielded a two-component product. Upon protection of hydroxy group of salisaldehyde by acyl group, the reaction proceeds to a three-component product. Initial experiments performed in water- DODAB solution according to previously established condition were successful. However, when attempting the Passerini reaction of coumarin in water with DODAB, we encountered challenges. Consequently, we transitioned to dichloromethane as a solvent.

We extended this strategy to synthesis of coumarin-peptidomimetic by using 7-hydroxy-8-formyl-coumarin known for their antimicrobial activity. Following the salisaldehyde approach, we synthesized nine different peptidomimetics using three different isocyanides, maintaining phenyl acetic acid as the constant acid moiety in the Passerini reaction.

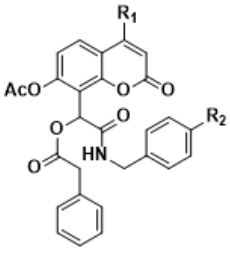
The impact of the 16 compounds under scrutiny, was evaluated within bacterial cells using a published methodology. Through the determination of MIC and MBC, MIC values spanned from 0.25 to 4.5 µM, while MBC values ranged from 1 to 8 (+/−0.5) µM for E. coli (K12, R2, R3, R4), A. baumannii, P. aeruginosa, Enterobacter and staphylococcus.

Notably, the lactone moiety of coumarin plays a pivotal role in biological activity, as evidenced by compounds No. 1 and 15. Analysis of MIC values indicates that compound no. 10 and 11 shows lower potency compared to other peptidomimetics. Compounds No. 1, 2, 3, 4, and 9 demonstrate a broad spectrum of activity.

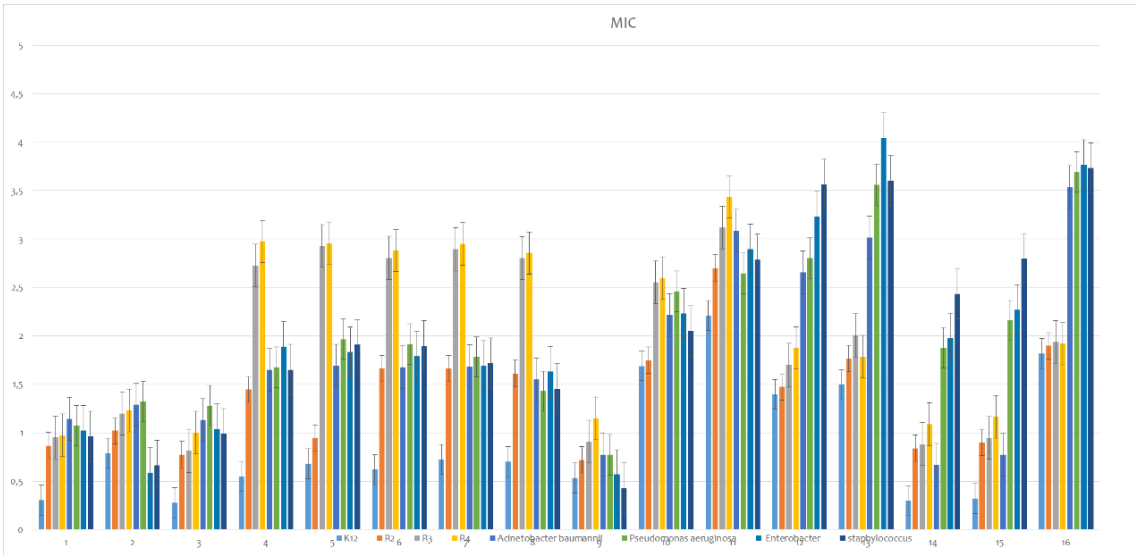




Entry	R_1	R_2	Entry	R_1	R_2
12	OMe	Br	15	H	H
13	H	Br	16	F	Br
14	OMe	H			



Entry	R_1	R_2	Entry	R_1	R_2
1	CH_3	H	5	H	F
2	CH_3	OCH_3	6	CH_3	F
3	H	H	7	CF_3	H
4	H	OCH_3	8	CF_3	F
			9	CF_3	OCH_3



Conclusions: Our study unveils coumarin's C8 antimicrobial potential, synthesizing potent compounds and evaluating efficacy against various pathogens.

Acknowledgement: National Science Center Poland project **OPUS No. 2019/33/B/ST4/01118** and **COST action EURESTOP CA21145**

Abstract No. PPP.83

Determination of purity of active substances for new drugs using new DSC technology

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Objectives: The main goal of the conducted measurements was to determine the purity of active substances used for drug manufacturing using differential scanning calorimetry (DSC). This allowed testing a new type of DSC apparatus design made by Linseis that instead of a classical two-crucible setup uses a ceramic chip with a single crucible

Materials and Methods: Measurements were conducted using Linseis Chip-DSC 10 differential scanning calorimeter with a unique ceramic sensor, which already incorporates an in-build reference side. The experiment was done in crimped aluminum crucibles in the air. The heating rate was set to 20 K/min. Each measurement was performed three times using each time a newly weighed sample.

Results: Three following measurements of the same selected active substance were conducted. In each measurement, two peaks are visible, which are related to the melting points of both measured substance and impurities. Melting of the substance of interest occurs at about 172,9°C and reaches its maximum at about 177,8°C. Enthalpy of the process, so information about the quantity of energy released during melting, was determined to be about -115 mJ/mg. The second, smaller peak from the impurities, can be observed at a lower temperature value. Three consecutively performed measurements show highly similar results, proofing measurement repeatability and efficiency of the chip design.

Conclusions: The DSC method can be used for the qualitative analysis of products and indicates the level of their purity. The examined chip design allows high heating and cooling rates without an external chiller, which leads to more experiments that can be performed within a working day. It also significantly reduces the costs of the setup, and in case of damage or contamination of the measuring part, it can be easily replaced by a user at a low cost.

The DSC method is incomparable for the thermal analysis of active compounds of new drugs, indicates the level of their purity and number of impurities.

Abstracts of Poster Spotlight Presentations

Abstract No. PSP.01

Development of highly potent tyrosinase inhibitor through computational and wet lab approaches

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Objectives: The metalloenzyme tyrosinase is a binuclear copper containing enzyme catalyzes L-tyrosine to L-3,4-dihydroxyphenylalanine (L-DOPA) and further oxidation of (L-DOPA) to dopaquinone which then transformed through several reactions into brown to black melanin. Tyrosinase is also responsible for the production of neuromelanins. The abnormal production of melanin results in Melasma, post-inflammatory hyperpigmentation (PIH), Parkinson's and other neurodegenerative diseases.

Methods: Previous reports confirmed that presence of hydroxy substituted phenyl ring is considered to be important in tyrosinase inhibitory activity. The natural phenolic antioxidants have been selected to design and synthesize their derivatives as tyrosinase inhibitors. The computational and wet lab approaches have been utilized to get highly potent tyrosinase inhibitors. The drug development strategies through enzyme based assay particularly tyrosinase inhibitors have been established. The designed and synthesized compounds inhibitory effects on mushroom tyrosinase have been evaluated and kinetic mechanism of the potent derivatives was also determined. The docking studies of synthesized analogues have also been performed against tyrosinase protein to compare the binding affinities with IC_{50} values.

Results: We are able to find out a derivative which is thousand times more active than the standard tyrosinase inhibitor (Kojic acid). The cytotoxicity and cell based tyrosinase inhibition studies of highly potent derivatives have also been performed. These derivatives also exhibited better tyrosinase inhibitory activity in murine skin melanoma (B16F10) cells.

Conclusions: The highly potent derivatives obtained may act as drug candidates after clinical trials.

Abstract No. PSP.02

Nucleation kinetics of Ascorbic acid in aqueous solvents

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Objectives: L-Ascorbic acid is an essential compound with many applications in the pharmaceutical and food industries. Crystallization is an essential separation and purification step to produce ascorbic acid. Furthermore, the solvent employed for crystallization plays a fundamental role in determining the nucleation kinetics which significantly affects final crystal attributes. Hence, it becomes crucial to understand the nucleation kinetics to optimize the crystallization process.

Methods: The present work studies the effect of adding alcohol (ethanol/isopropanol) as a co-solvent in water on the nucleation kinetics by the isothermal method. Induction time was measured more than 80 times at five different supersaturations (*S*) using *Technobis Crystallization Systems' Crystal16 V3* instrument. The experimentally measured induction time data points determine the probability of forming a nucleus in that timeframe (Fig. 1).

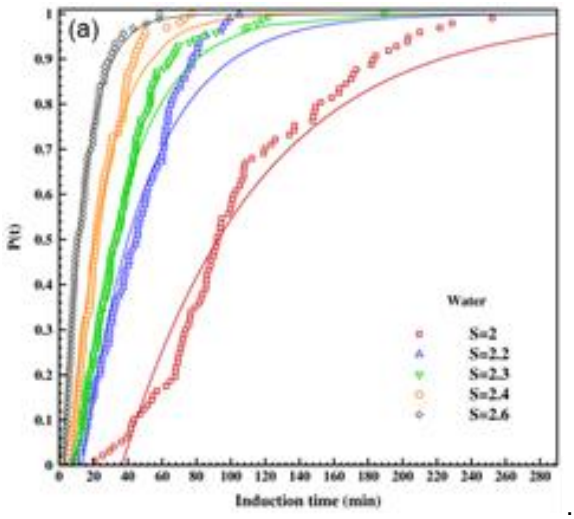

Figure 1: Induction time probability distribution in water

Nucleation kinetics is defined by classical nucleation theory, which relates the nucleation rate with the supersaturation, represented by the equation below.

$$J = ASexp\left(\frac{-B}{ln^2 S}\right)$$

Where *A* and *B* are kinetic and thermodynamic nucleation parameters, respectively.

Additionally cooling crystallization experiments were performed using *Crystalline V2* instrument to visualize the effect of solvent system on crystal habit.

Results: With the addition of alcohol in water, the kinetic parameter (*A*) decreases, indicating less nucleation site whereas the thermodynamic factor (*B*) increases, implying a high activation energy for nucleation (Table 1). Furthermore, adding alcohol in water during cooling crystallization experiments transforms the crystal habit from cubic to crystals with prominent growth along one crystallographic axis resulting in lengthened prism-shaped crystals in water-alcohol binary solvent systems.

Table 1: Nucleation kinetic parameters

Solvent	A	B	R^2
Water	2662.48	1.589	0.986
0.6EtOH+0.4W	1981.20	2.425	0.856
0.6iPrOH+0.4W	1092.94	2.633	0.847

Conclusions: Ascorbic acid, being a polar molecule, has a high affinity with polar solvents such as water. The addition of alcohol reduces the polarity of the solvent mixture resulting in lower solubility in water- alcohol solvent systems, and subsequently affecting the nucleation kinetics and crystal habit.

Abstract No. PSP.03

Statin-induced myalgia - a metabolomics study

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Objectives: Statins are one of the most commonly prescribed medicines worldwide. Its main purpose of use is to lower low-density lipoprotein (LDL) levels. However, besides the main results, many statin users also report side effects. The prevalence of these side effects differs between the source of information. The healthcare practitioners indicate a quite high risk of statin-induced side effects, but these data are not confirmed with randomized trials. The most common undesirable effect is connected with muscle (broad spectrum from myalgia and myopathy to rhabdomyolysis). Many statin users abort therapy when side effects appear, which significantly increases the risk of cardiovascular incidences (statins *via* controlling LDL levels, also decrease this risk). The main purpose of the presented research is an application of metabolomics for the explanation of molecular mechanisms connected with statin- induced myalgia.

Methods: Plasma samples were collected from 80 patients. 62 of them were treated with simvastatin (23 reported myalgia, and 39 did not report any side effects). The rest of them (18 samples) were control group. All samples were analyzed with a metabolomics procedure based on liquid chromatography combined with mass spectrometry as an analytical technique.

Results: There was not detected a significant difference between the metabolic profile of statin-treated patients with and without side effects. However, part of the metabolites that differ between statin users and the control group are specific to the selected study group (with or without myalgia).

Conclusions: Observed symptoms may not be directly connected with statin treatment. The obtained results support the thesis presented in randomized trials and meta-analysis reports that the prevalence of myalgia incidence is not as high as healthcare practitioners suggest.

This study is funded by a grant from the National Science Centre, Poland (2020/37/N/NZ4/03903).

Abstract No. PSP.04

Exploring Levan@CBD biocomposite sponges as cutting-edge, antibacterial wound dressing materials

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Objectives: Wound healing is a complex process; therefore, new dressings are frequently required to facilitate it. This study focuses on obtaining and characterizing porous bacterial levan-based sponges containing cannabis oil (Lev@CBDs) to promote wound healing potentially. The primary active substance in cannabis oil is the non-psychoactive cannabidiol, which has many beneficial properties.

Methods: The obtained materials were characterized by several different techniques and methods, including Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), and thermogravimetric analysis (TGA). Their mechanical properties, antioxidant and anti-inflammatory effects, biodegradation rate, and swelling properties were also examined. The obtained Lev@CBD materials were evaluated regarding their interaction with proteins, human serum albumin, and fibrinogen. Moreover, the antibacterial properties were checked against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The cytotoxicity of the obtained materials was tested on two cell lines: mouse and human fibroblasts.

Results: The sponges exhibited a suitable swelling ratio, proper water vapor transmission rate, sufficient thermal stability, desired mechanical properties, and good antioxidant and anti-inflammatory properties. The obtained Lev@CBD materials were evaluated in terms of their interaction with proteins, human serum albumin, and fibrinogen, of which fibrinogen revealed the highest binding effect. Moreover, the obtained biomaterials exhibited antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*, as well as being non-hemolytic material as indicated by hemolysis tests. Furthermore, the sponges were non-toxic and compatible with L929 mouse fibroblasts and HDF cells.

Conclusions: The biomaterials consisting of levan sponges enriched with cannabis oil are expected to be suitable wound dressing due to their highly effective characteristics. Specifically, a competent group of advantages (porous structure, good mechanical, antioxidant, anti-inflammatory, and antimicrobial properties, a high swelling ratio in different pH, and a biodegradation profile) renders the sponges an appropriate solution for treating damaged tissue. *In vitro*, biocompatibility studies confirmed that the prepared sponges were non-toxic toward L929 and HDF cells. Moreover, the obtained biomaterials can interact with essential proteins in wound healing. These results showed that prepared sponges enriched with cannabis oil might have significant potential for applications in wound healing, tissue engineering, and cell culture.

Abstract No. PSP.05

Optimisation of Quinoxaline Derivatives Synthesis Using Novel Green Analytical Technique of *In situ* FTIR/Microwave Irradiation Coupled System

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Objectives: In response to the growing demand for ecologically conscious and efficient analytical methodologies, there has been a surge in the development of real-time techniques for monitoring chemical reactions. Among these, *in situ* Fourier Transform Infrared spectroscopy (FTIR) has emerged as a powerful tool for non-invasive monitoring and characterising molecular changes. Microwave (MW) chemistry has been recognised for accelerating chemical transformations and providing access to more ecologically responsible conditions. Based on these advantages, our research aims to integrate a multifunctional reactor supporting MW irradiation with an ATR/FTIR spectrometer. This innovative coupling of synthetic and analytical tools offers a comprehensive platform for studying chemical reactions. Our research aims to optimise the synthesis of selected N-heterocycles using this novel analytical approach.

Methods: Multifunctional reactor UWave-1000 by SINEO capable of MW irradiation coupled with an ATR/FTIR Nicolet iS50 Tri-Detector Gold Flex Spectrometer equipped with a fiber optic probe, which connects those two devices were employed, making it a non-invasive tool for analysis. The synthesis of 2,3-diphenylquinoxaline was conducted using 1,2-phenylenediamine and benzil as precursors in various solvent-catalyst systems. MW power levels were varied to assess their impact on reaction kinetics. Comparative studies with conventional reflux methods were conducted to evaluate the integrated system.

Results: *In situ* FTIR enabled the direct visualisation of the finish of the reaction and purity of final products, providing insights into reaction pathways. The integration of MW accelerated reaction kinetics, leading to shorter reaction times than conventional methods. Statistical analysis of the results highlighted optimal reaction conditions, demonstrating the efficacy of the integrated approach in enhancing reaction efficiency.

Conclusions: Integrating *in situ* FTIR with MW advances green chemistry. Our study demonstrates the utility of this approach in optimising the synthesis of 2,3-diphenylquinoxaline and potentially other N- heterocycles. This integrated system holds promise for developing efficient and environmentally friendly processes by providing real-time insights into reaction mechanisms and facilitating reaction optimisation.

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Abstract No. PSP.06

Preparation and characterization of poly(amidoamine) dendrimer/camptothecin complex loaded biodegradable nanosystems for potential application in the treatment of non-small cell lung cancer

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Objectives: Camptothecin (CPT) is a cytotoxic alkaloid, that inhibits cancer cell proliferation by interfering with the DNA topoisomerase I. Its use, however, is limited due to poor water solubility, low biocompatibility, significant systemic toxicity, and last but not least, the instability of the lactone ring, what results in a formation of pharmacologically inactive carboxylic form of CPT. In order to overcome aforementioned difficulties, a novel approach utilizing poly(amidoamine dendrimer) (PAMAM) and biodegradable polyesters has been applied. The primary objective of this study was to provide controlled and sustained release of the CPT while also preserving the stability of the pharmacologically active lactone form of this drug.

Methods: In the first stage of this research, the PAMAM-CPT complex and biodegradable polyester matrices, based on *L*-lactide, ε-caprolactone and glycolide were synthesized and comprehensively characterized using many spectroscopic and chromatographic methods. Additionally, hydrolytic degradation study was conducted to assess pH value changes. The synthesized PAMAM-CPT complex and polymeric matrices were combined to create innovative nanosystem, utilizing the coacervation method. The obtained products were characterized with the use of transmission electron microscopy (TEM) and dynamic light scattering (DLS). Furthermore, an *in vitro* release study was carried out in order to determine the release kinetics of CPT.

Results: The results of the NMR examination proved effective encapsulation of CPT molecules within the cavities of PAMAM 4.0 dendrimer. The polymeric matrices have been shown to degrade hydrolytically, resulting in a noticeable reduction of microenvironmental pH. The obtained nanosystems, created with the use of PAMAM-CPT complex and biodegradable polymeric matrices, were spherical in shape and their size did not exceeded 350 nm. *In vitro* release study revealed effective drug release while maintaining the pharmacologically active form of CPT.

Conclusions: Based on the findings, it can be concluded that the developed nanosystems might constitute a potential formulation for the targeted anticancer therapy. Nevertheless, the research is still ongoing in our laboratory, as biological examination utilizing cancer cell lines is necessary to determine the effectiveness of the obtained nanosystem in the treatment of non-small cell lung cancer.

Abstract No. PSP.07

The safety and efficacy of mycophenolate mofetil in patients with idiopathic inflammatory myopathies - pilot study

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Objectives: Idiopathic Inflammatory Myopathies (IIM) are the largest group of myopathies in adults. Patients with IIM have increasing difficulty with daily tasks requiring the use of proximal muscles. Mycophenolate mofetil (MMF), a prodrug of mycophenolic acid (MPA) can be effectively used in management of IIM and may be suitable alternative to the conventional immunosuppressive agents. The aim of the study is to evaluate the association between MPA trough level (Co) and disease activity in adult patients with IIM.

Methods: A prospective study was performed on a group of patients, who were on MMF therapy for a minimum period of 3 months. The study included 11 patients with a male/female ratio 5:6. The mean age and body weight was 47.8 years and 70.6 kg respectively. MMF was used orally 2g BID, at a mean dose of 31.1 mg/kg. MPA concentrations were measured using a validated HPLC method developed in our laboratory. Active disease was defined as at least one of the activity domains: muscle activity, worsening weakness or creatinine kinase (≥190 U/L), myositis disease activity assessment tool and in particular cases skin activity.

Results: Mean MPA Co was 1.9 µg/mL (range: 0.38-4.37 µg/mL) , and only 4/11 Co were above the 1.5 µg/mL threshold. No difference in Co for MPA was found between patients active and inactive disease (0.94 vs 0.98, p=0.7). Also, no correlation has been noted between MMF dose and clinical outcome using spearman`s rank (r=0.06, p=0.87).

Conclusions: Neither in case of MPA Co, nor MMF dose any correlation with disease activity was found in idiopathic inflammatory myopathies. More prospective studies are still needed to assess usefulness of MPA monitoring during treatment patients suffering from IIM, as well as to control the impact of increasing MMF dose in IIM patients to improving clinical outcome.

Abstract No. PSP.08

Exploring new classes of sulfoximine derivatives of potential biological activity

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Objectives: Sulfoximines constitute a chemical group that is of increasing interest in drug discovery. This moiety is considered a bioisosteric to e.g.sulfone, sulfonamide, secondary amine, hydroxyl, and carbonyls. It has interesting reactivity and desired pharmaco(chemical) properties, such as high aqueous solubility and stability(1). Consequently, sulfoximines are considered promising leads and first compounds bearing this functional group are currently in clinical trials(1). Considering the desired properties of sulfoximines and the rich possibility of exploring novel chemistries around them, we investigate their possible use in obtaining biologically interesting derivatives through multicomponent reactions (MCRs), particularly the UGI reaction(2). We focus on the optimization of reaction conditions and obtaining compound libraries of sulfoximine derivatives to demonstrate the usefulness of the applied synthetic methodology in medicinal chemistry and chemical biology. The results obtained may provide useful for the pharmaceutical industry and researchers in the field of drug discovery.

Methods: The Ugi reaction was conducted in 1 mmol scale. Each Ugi product was separated and purified using preparative chromatography in automatic and manual methods. Characterisation of the each product was performed using spectroscopic techniques (NMR, HRMS, LC/MS).

Results: The Ugi reaction was successfully used to obtain sulfoximine derivatives that could have potential applications as building blocks in drug discovery. In this research, we obtained a significant change in yields, between 13% and 96%. Lower yields between 13% and 38% were observed using ketones as starting materials instead of aldehydes (11% and 96%), besides longer reaction conditions.

Conclusions: This result shows the possibility of obtaining different sulfoximines derivatives using the Ugi multicomponent reaction, and it is a good starting point for construction of libraries of small- molecular scaffolds and peptidomimetics synthesis.

References:

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Abstract No. PSP.09

The Anti-Inflammatory Activity of the *Aronia melanocarpa* Leaves and Flowers

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Objectives: *Aronia melanocarpa* berries are a valuable component of a healthy diet. However, there have been only a few studies on the composition and potential applications of chokeberry leaves and flowers. As by-products of aronia cultivation, leaves are abundant and serve as inexpensive raw material. During spring and summer, the leaves appear green, while in autumn, they turn red and become decorative. Chokeberry leaves and flowers may exhibit interesting biological effects due to their content of phenolic compounds and high antioxidant activity. Among these phenolic components are flavonoids, hydroxycinnamic acids, and proanthocyanidins.

The aim of our study was to investigate the anti-inflammatory activity of extracts from chokeberry flowers, green leaves, and red leaves. We tested the hypothesis regarding the potential antiatherosclerotic effect of aronia extracts using human umbilical vein endothelial cells (HUVECs). Our studies involved measuring the expression of adhesion molecules (ICAM-1 and VCAM-1), IL-6, and MCP- 1 in endothelial cells.

Methods: The surface expression of the intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) in HUVECs was determined by flow cytometry. The mRNA levels of VCAM-1, ICAM-1, IL-6 and MCP-1 were assessed with the quantitative reverse transcription polymerase chain reaction in real-time (qRT-PCR). The content of chlorogenic and neochlorogenic acids, anthocyanins, flavonoids, and carbohydrates in the extracts was determined with HPLC-DAD/RI.

Results: Chokeberry extracts from flowers, green and red leaves were subjected to the *in vitro* study on HUVECs. All extracts reduced TNF-α induced surface expression of VCAM-1 and showed anti- inflammatory properties. The qRT-PCR method revealed that extracts from flowers most effectively repressed ICAM-1 genes than extracts from green and red leaves.

Conclusions: Aronia leaves and flowers may have therapeutic potential in the prevention of atherosclerosis and other pathological events involving leucocyte adhesion. They can be considered as a promising component of functional food.

Abstract No. PSP.10

DILOC2 – developing of innovative drug for obesity and diabetes type 2

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Objectives: The rapid development of urbanization, and accelerated socio-economic growth favored an improved living standard but a more stressful, sedentary lifestyle and unhealthy dieting habits. Especially in the last two decades, obesity and type 2 diabetes have become a global pandemic threatening people's life. World Obesity Atlas 2022 predicted, that by 2030, 20% of women and 14% of men, or over 1 billion people, will be living with obesity globally. These alarming statistics indicate the need to develop effective drugs to reduce this problem. Research conducted in recent years indicates that the key to effective therapy will be coordinated stimulation of incretin receptors - GLP-1R and GIPR, and the glucagon receptor (GCGR). The aim of DILOC2 project was to design a potential drug candidate with an innovative triagonistic mechanism of action, to fully exploit the incretin effect in the treatment of patients with obesity.

Methods: The in-depth analysis of literature and patents resulted in total 2018 sequences collected from 44 publications and 26 patent applications describing various triagonists published up to 09.2023. They were stored with the proper activity values in a dedicated database application and used to package of bioinformatic analyses (distance matrix, violin and scatter plot analysis). The results allowed for the design of new triagonistic structures, which were synthesized and tested *in vitro* in FMP activity tests (Fluorescence membrane potential assays).

Results: Bioinformatics analysis allowed for the determination of the most matching amino acids for each position in the sequence and the relationships between them, as well as the optimal sites of chemical substitution, which contributed to the design and synthesis of more than 100 modified peptides. 20 potential triagonists were selected in *in vitro* activity tests. The most active compounds had activity similar to the reference incretin drugs.

Conclusions: The developed triagonists showed the effectiveness in *in vitro* assays, providing a solid foundation for their further optimization, which in the future may improve the quality of life of millions of patients including those suffering from obesity and diabetes.

Abstract No. PSP.11

Electrostatic charging of powder blends with amlodipine besylate and rosuvastatin calcium salt

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Objectives: The aim of the research was to assess the initial electrostatic charge and the potential for its generation during technological processes for pure APIs, excipients and their mixtures. Contact of the powder with various surfaces during manufacturing may cause the formation of electrostatic charges, at the same time increasing the tendency to separation and adhesion to surfaces, and thus affecting the quality of the final product. The selection of appropriate excipients can balance the charge accumulation, improving the properties of the powder.

Methods: Four substances were used in this study, amlodipine besilate (AML), rosuvastatin calcium salt (RSV), microcrystalline cellulose (MCC) and pregelatinized starch.

Electrostatic charge measurements were performed using a JCI 178 meter (Chilworth Technology Ltd, UK), equipped with a Faraday bucket. The initial electrostatic charge was measured by directly transferring material from the bulk packaging to the Faraday bucket. In order to determine the charge accumulation, the sample was placed in a stainless steel bowl, which was stirred in a circular motion for 60 s, and then the accumulated electrostatic charge was measured.

Results: The initial and accumulated charge was measured for pure APIs and their mixtures with MCC or starch in a weight ratio of 1:1. The API:exciepient mixtures are processed in individual technological stages and are used as a material for encapsulation, therefore it is extremely important to balance the electrostatic charge that arises, which could lead to problems related to the uniformity of the content of active substances. AML and MCC have the greatest potential to generate charge.

The selection of excipients was justified because they generate the opposite charge to the tested APIs. Starch showed the best electrostatic charge balancing properties in binary mixtures of API:excipient. While mixtures with MCC ultimately generated about a 5-fold greater difference in electrostatic charge compared to mixtures with starch.

Conclusions: The tests carried out for APIs and selected excipients showed a difference in the initial and accumulated charge, which allowed the selection of the optimal API:excipient mixture for manufacturing process. The proposed procedure allows to avoid many problems at further stages of product development, which could be related to the potential for segregation and the uniformity of the content of API in the final drug product.

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Abstract No. PSP.12

Peculiarities of the pharmaceutical analysis of the Clarithromycin substance by the method of high-performance liquid chromatography

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Objectives: The actual task of this experimental study is to adapt the conditions of chromatography using the method of high-performance liquid chromatography (HPLC) for determining accompanying impurities of Clarithromycin substance taking into account the selection and properties of the stationary phase (chromatographic columns), the parameters of the mobile phase to obtain the separation of the peaks of accompanying impurities of Clarithromycin within the limits allowed by the State Pharmacopoeia of Ukraine (SPU), evaluation of the obtained results.

Methods: Samples of Clarithromycin substance, pharmacopoeial standard sample of SPU - Clarithromycin, HPLC with UV detection (Agilent 1260 Infinity II chromatograph with UV detector), method of computer analysis using software OpenLab CDS; reagents with purity for HPLC (acetonitrile *P*, phosphoric acid *P*, potassium dihydrogen phosphate *P*, water for chromatography; mobile phase *A* (a solution of 4.6 g/l of potassium dihydrogen phosphate *P*, the pH of which is adjusted to 4.4 with diluted phosphoric acid *P*), mobile phase B (acetonitrile *P*).

Results: We selected the optimal conditions for the separation of accompanying impurities, taking into account their separation. As a result of the conducted research, it was established that the Clarithromycin substance contains identified accompanying impurities in its composition. The total content of specified accompanying impurities does not exceed 3%, which is acceptable for the use of antibiotics in the production of medicinal products (fig.1).

When conducting research, we used a longer HPLC column with a modified C18 phase, which provided better separation of peaks. Changing the time of chromatography and time parameters of the gradient, as well as its percentage content (within the limits allowed by SPU) had a positive effect on the shape of the peaks and their separation.

Conclusions: According to the results of the HPLC study of Clarithromycin substances, it was established that a longer HPLC column with a modified C18 phase provided better peak separation; the mobile phase was adapted and the optimal conditions for the gradient were selected (within the limits allowed by the DFU), which had a positive effect on the shape of the peaks and their separation.

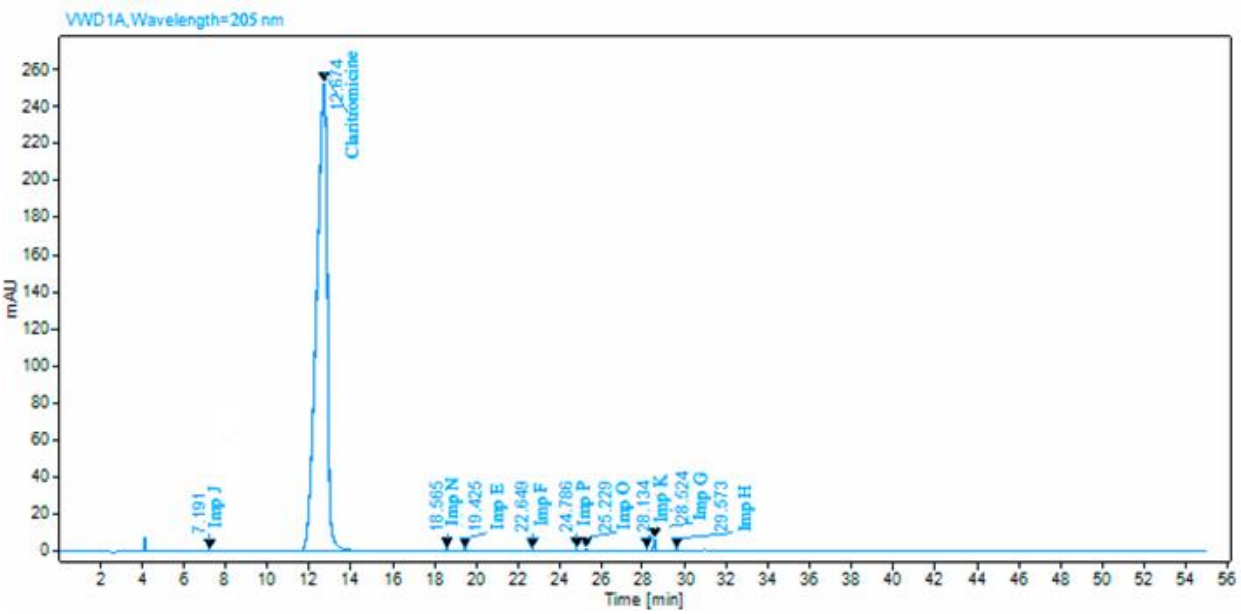

Figure 1. Chromatogram of Clarithromycin sample (Rt=12.674 min); identified impurities: J (Rt=7.191 min), N (Rt=18.565 min), E (Rt=19.425 min), F (Rt=122.649 min), P (Rt=24.786 min), O (Rt=25.229 min), K (Rt=28.134 min), G (Rt=28.524 min), H (Rt=28.524 min).

Abstract No. PSP.13

Amorphous polymer-phospholipid dispersions as a way to co-deliver curcumin and piperine

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Objectives: Curcumin and piperine are plant compounds known for their health-promoting properties. Moreover, there are reports concerning the synergistic effect of co-administration of these compounds. Their pro-healthy potential is limited by their low solubility. To overcome this drawback, amorphous polymer-phospholipid dispersions were manufactured.

Methods: Hot-melt extrusion technology was used to obtain curcumin-piperine amorphous polymer- phospholipid dispersions. As a polymeric carrier, PVP K25 was employed. Due to the high glass transition temperature of the polymer, the addition of a plasticizer was necessary. The amorphous state of the prepared matrices was confirmed by X-ray powder diffraction, whereas differential scanning calorimetry provided information on the miscibility of active-polymer-phospholipid dispersions. Further, we proceeded with biopharmaceutical properties assessment, such as solubility and permeability studies, using the Parallel Artificial Membrane Permeability Assay (PAMPA) *in vitro* model.

Results: The prepared dispersions showed an amorphous character and good miscibility of the system components. In solubility tests, we observed a considerable improvement in solubility. The most effective system allowed us to improve the solubility of curcumin 25593 times in phosphate buffer pH 6.8 and 17009 times in HCl pH 1.2; piperine's solubility increased 447 times and 209 times, respectively. Moreover, the same system stimulated improved permeability of curcumin 1734 times and 2644 times in the gastrointestinal tract and blood-brain barrier models, respectively. For piperine, it was 413 times and 310 times in the same models.

Conclusions: In this work, we demonstrated the approach that makes hot-melt extrusion a viable manufacturing method for polymer-phospholipid dispersions. The developed systems showed an amorphous character, resulting in improved solubility and permeability. The presented technological solution, combining polymer and phospholipid as poorly soluble compounds carriers, stands out as a promising solubility-enabling formulation.

Abstract No. PSP.14

Development of efficient system for human adenylate kinase 4 (AK4) production and evaluation of the inhibitory potency of suramin and its derivatives against AK4

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Objectives: The critical driver of tumorigenesis is mitochondria-centered energy metabolism. Mitochondrial adenylate kinase 4 (AK4) is GTP/ATP:AMP phosphotransferase regulating the nucleotide homeostasis and energy metabolism, which has emerged as a novel positive regulator of tumor growth and metastasis. Notably, AK4 was found to regulate cancer cell proliferation, apoptosis, epithelial-mesenchymal transition, and anti-cancer drug resistance [1]. However, no AK4 inhibitors have been found so far. The currently available strategies to develop therapeutic interventions to target AK4 are challenging and expensive RNA interference or genetic engineering. Discovery of AK4 inhibitors and development of safe pharmacological approaches could vastly accelerate basic and pre-clinical research on the AK4 therapeutic potential. Against this background, we developed a new expression system for human AK4 production and screened a collection of commercially available suramin derivatives (symmetric polysulfonated polyaromatic urea compounds), which are known to inhibit nucleotide receptors and nucleic acid-binding enzymes [2].

Methods: Human AK4 was produced as a recombinant His-tagged enzyme in the bacterial pET-26b(+)-BL21-CodonPlus(DE3) expression system and purified using IMAC. Suramin and its six commercially available derivatives (NF023, NF110, NF157, NF279, NF449, NF546) were tested against AK4 in the presence of ATP or GTP as phosphate donor, using RP-HPLC. Molecular docking using the SwissDock Server was applied to predict possible binding sites of tested compounds to AK4.

Results: The employed production system resulted in obtaining active and stable AK4 of high purity. Some of the tested compounds inhibited AK4 with micromolar range of IC_{50} values, and the type of a phosphate donor did not have a significant effect on the inhibition yield. Molecular docking studies suggest ligand interactions with amino acid residues of the LID domain, which is crucial for the catalytic efficiency by covering the substrate binding sites. Surprisingly, AK1, which may act as a negative regulator of tumorigenesis, was inhibited by some of the tested compounds with comparable or higher efficiency as compared to AK4.

Conclusions: The developed production system can be used for obtaining high quantities of human AK4 required for inhibitor/drug discovery. Further studies are needed to develop a new generation of symmetric polysulfonated polyaromatic urea compounds or identify completely new scaffolds with higher selectivity and inhibitor potency against AK4.

Abstract No. PSP.15

Effect of epigallocatechin gallate and vitamin D on the methylation status of human uterine fibroid cells

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Objectives: Uterine fibroids (UFs) are the most common benign gynecologic tumors in reproductive-age women. UF pathogenesis invokes the genetic modifications of a single myometrial stem cell (MyoSC) into a tumor-initiating cell (MyoF) that sustains clonal tumor growth. The effects of vitamin D (VD) and epigallocatechin gallate (EGCG), a polyphenol of green tea, on the growth inhibition of UFs have been demonstrated. However, the exact molecular mechanisms and the role of epigenetic regulation such as methylation, are not fully understood. The study aimed to recognize the effect of EGCG and/or VD on the level of global DNA and histone methylation and DNA methylation enzymes: DNA methyltransferases (DNMTs).

Methods: Freshly collected human fibroid and adjacent myometrial samples were obtained *via* the Biorepository at the University of Chicago (IRB protocol No. 20-1414) from Black women (22–55 years old) undergoing hysterectomy for symptomatic UFs (for MyoF cells, n=3) or vaginal prolapse (for MyoN cells, n=3). The tissues were digested with buffer containing collagenase Type IV (Worthington, USA), and DNase I (Sigma-Aldrich, USA). Cells were grown in DMEM/F12 with 10% FBS and 1% antibiotic. MyoF cells were treated with 100 uM of EGCG (PhyProof) and/or 100 nM of VD (Sigma, USA) against vehicle for 24 h. EpiQuick Global Tri-methyl histone H3-K4 Quantification kit and MethylFlash DNA methylation (relative quantification of 5-mC%) kit (Epigentec, USA) were used to recognized methylation changes. Gene expression of DNMT1, 3a and 3b with GAPDH as endogenous control was determined with specific primers, SybrGreen chemistry and CFX Thermocycler (BioRad, USA).

Results: We found over three times more trimethylated H3K4 in MyoF compared to MyoN (p=0,021). Administration of EGCG and/or VD resulted in a significant decrease in the amount of trimethylated H3K4. A higher level of 5-mC (%) was found in MyoF compared to MyoN (0,607± 0,422% vs 0,746± 0,217%). Treatment of MyoF with EGCG alone and in combination with VD significantly increases the level of global DNA methylation (1,309%; p=0,015 and 2,121%; p=0,038; respectively). No significant changes in gene expression of DNMTs have been found after treatment with EGCG and/or VD+ EGCG.

Conclusions: EGCG and VD may act on epigenetic regulators as their administration significantly affected H3K4 trimethylation and global DNA methylation levels in tumor-initiating cells (MyoF).

Abstract No. PSP.16

Sonidegib bioisosteres: activity and ADME properties

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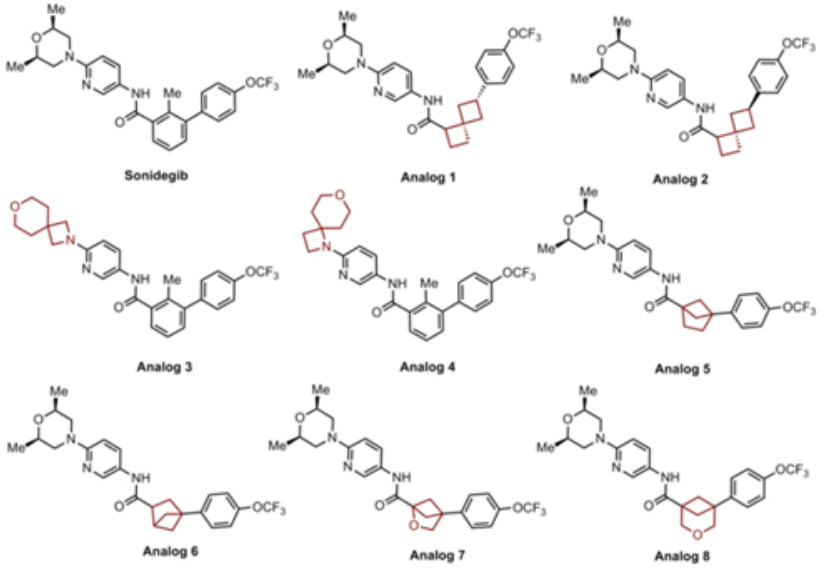
Objectives: Sonidegib is a patented drug used for skin cancer therapy, which is characterized by low solubility and high logD.

Methods: We synthesized spiro[3.3]heptane, 7-oxa-azaspiro[3.5]nonane, bicyclo[2.1.1]hexane and oxabicyclo[2.1.1]hexane analogs of Sonidegib (Fig. 1). ADME parameters and inhibition of the Hedgehog signalling pathway in the Gli-Luc NIH3T3 reporter cell line were tested for all analogs.



Results: Replacing the meta-substituted phenyl ring in Sonidegib structure with spiro[3.3]heptane (analogs 1 and 2) has virtually no effect on logD, plasma protein binding and solubility in PBS. At the same time, the metabolic stability of both analogs was lower and differed significantly between trans- and cis- isomers. Spiro[3.3]heptane analogs were less active inhibitors of Hedgehog signaling pathway but more cytotoxic on NIH3T3 cells compared to Sonidegib. Replacement of the 2,6-dimethylmorpholine with 7-oxa-2-azaspiro[3.5]nonane and 7-oxa-1-azaspiro[3.5]nonane (analogs 3 and 4) resulted in slightly increased solubility but decreased metabolic stability (for analog 4 only). Both analogs were less cytotoxic on NIH3T3 cells and analog 3 retained nanomolar inhibition of the Hedgehog signaling. Analogs 5-8 were less active but more cytotoxic (5, 6, 8) compared to Sonidegib. ADME parameters do not differ significantly, only analog 8 exhibited slightly higher solubility.

Conclusions: Thus, these structures can be useful structural elements in drug discovery projects

Abstract No. PSP.17

Establishing the Ischemia-Reperfusion Model in Rats for Chronic Renal Pathology Investigation

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Objectives: Unilateral ischemia-reperfusion injury (UIRI) with contralateral nephrectomy (Nx) is a valuable model for acute and chronic renal injury research. Rats, due to their size and tolerance to the pathology, are the preferred lab animals for this model. However, while the model is well-established in mice (Skrypnyk NI, et al. J Vis Exp. 2013;(78):50495), there are no established protocols for its implementation in rats

Methods: Young male SD rats were utilized in this study. UIRI was induced with occlusion times of 35, 40, and 45 minutes, followed by Nx on the 10th day post-UIRI. Animals were observed for up to 28 days post-UIRI. Serum creatinine and urea levels were monitored on days 1, 12, 17, 21, and 28. Kidney morphology was evaluated via Hematoxylin and Eosin staining in a semi-quantitative manner, while renal fibrosis was assessed using Masson's trichrome staining by measuring the area occupied with connective tissue, on day 28.

Results: Rats exhibited good tolerance to UIRI and subsequent Nx, with mortality rates not exceeding 20% regardless of occlusion time. Serum creatinine levels increased 1.5-2 times on day 1 post-UIRI, followed by a 5-6 fold increase post-Nx, gradually returning to baseline levels. Urea levels showed a more significant and prolonged increase, albeit returning to baseline by the study's end (Figure 1). While the observed changes showed consistency across all UIRI-Nx groups, they did not exhibit a strict correlation with occlusion time. However, kidney injury severity and fibrosis development were notably influenced by occlusion duration, with longer occlusion periods resulting in more profound injury and increased replacement of renal tissue with fibrotic tissue (Figure 1).

Conclusions: We successfully established a rat model for kidney ischemia-reperfusion injury, defining occlusion timeframes for various research objectives. For assessing kidney function post-treatment, occlusion times of 35-40 minutes are adequate, while longer occlusion times are recommended for studying fibrosis development

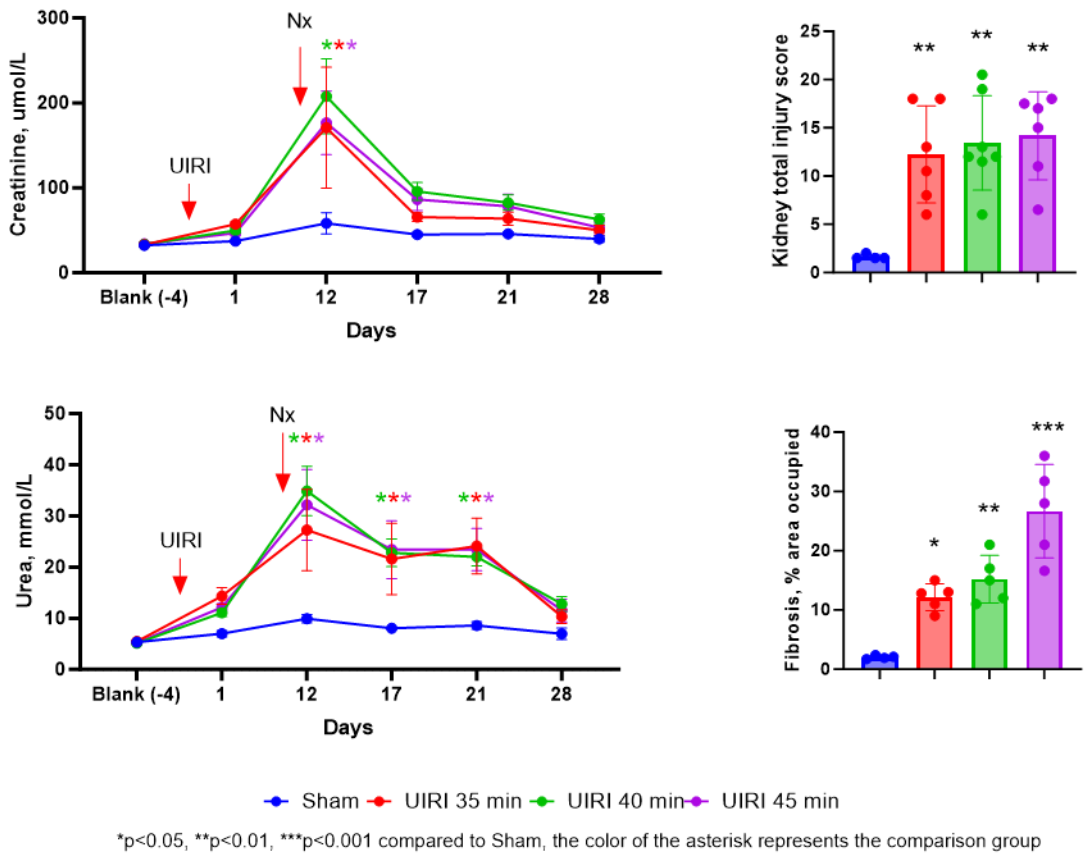

Figure 1. Serum Creatinine and Urea levels, kidney total injury and fibrosis-occupied area in UIRI-Nx rats

Abstract No. PSP.18

Theoretical analysis of the mechanism of selective action of vitamin D receptor agonists with anticancer activity

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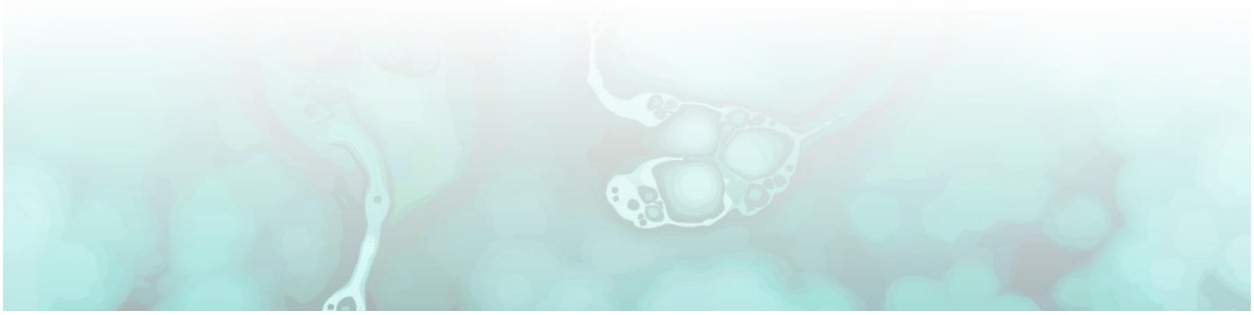
Objectives: In our study, we theoretically analyzed for the first time, a group of vitamin D_2 analogs with high biological activity, to understand the mechanism of their interaction with the VDR receptor. The study aimed to determine, using molecular modelling techniques, how selected analogs of $1\alpha,25(OH)_2D_2$ interact with the VDR receptor, specifically what conformation adopts the ligand in the receptor's binding pocket, how these ligands affect the helix H12 of VDR responsible for the transcriptional mechanism, and what kind of bonds these analogs form with the amino acid residues of VDR. Analogs that exhibited significant differentiation and antiproliferative activity were selected for this study. *In vivo* toxicity confirmed their reduced, compared to $1\alpha,25(OH)_2D_3$, effect on increasing blood calcium level.

Methods: The crystallographic structure of the ligand-binding domain (LBD) of the VDR was extracted from the RCSB PDB database. The molecular docking procedure of selected vitamin D agonists to the VDR and molecular dynamics simulations were carried out in BIOVIA's Discovery Studio software.

Results: At the molecular level, the selected vitamin D analogs were localized to the central part of the VDR binding pocket. Based on simulations, it was noted that apparent differences in positioning occur in the side chain region. An important influence on the interaction of the ligand with the VDR LBD is the presence or absence of the C-19 methylene group. All the studied compounds form characteristic hydrogen bonds with the amino acid residues of the receptor, however, there are differences in the length and strength of these bonds. The differences are also in the number of hydrophobic interactions of the ligands with VDR amino acid residues, mainly within the helix H12 responsible for VDR transcriptional activity.

Conclusions: In our analysis of the molecular interactions of synthetic agonists with the VDR LBD active pocket, conducted for the first time, a correlation was observed between the interaction of vitamin D analogs with the VDR and biological activity. The methodology we developed allows us to assess effectively the mode of interaction of agonists with the VDR and can be successfully applied in future work on optimizing the structures of synthetic vitamin D analogs to reach the required profile of biological activity.



Abstract No. PSP.19

Interactions of agonists and antagonists with the retinoid receptor RARγ studied by molecular modeling

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Objectives: In our study, we first analyzed a series of selected agonists and antagonists of the RARg based on the structures of ligand-receptor complexes obtained by molecular docking and molecular dynamics simulations. We determined the affinity of these ligands for RARg and found out which conformation they adopt in the receptor's binding pocket, and how they affect the structure of the RARg key helix H12. A preliminary theoretical analysis of the relationship between the interaction of selected agonists and antagonists with the receptor RARg and their biological activity was carried out, and the potentially most active compound was selected for further study.

Methods: The crystallographic structure of the ligand-binding domain of the RARg was extracted from the RCSB PDB. Molecular docking and molecular dynamics simulations were carried out in Discovery Studio software from BIOVIA.

Results: All ligands penetrate the hydrophilic region at the entrance to the receptor's binding pocket and the hydrophobic region at the bottom of the pocket. All compounds formed hydrogen bonds with amino acid residues (including Ala234, Met272, Ala397) specific to the receptor RARg. Differences were observed in the lengths of hydrophobic bonds formed by ligands with the H12 helix of the RARg, responsible for its transcriptional activity.

Conclusions: The analysis of the interactions of agonists and antagonists with the RARg was conducted for the first time. Based on visual analysis of ligand-receptor complexes, correlations between the interaction of agonists and antagonists on the RARg and their biological activity were observed. The structural data on the factors promoting strong binding of the tested compounds to the receptor in cell lines are important in optimizing the structure of new compounds aiming to the desired profile of biological activity.

Abstract No. PSP.20

Mesoporous Matrices as a Promising New Generation of Carriers for Multipolymorphic Active Pharmaceutical Ingredient Aripiprazole

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Objectives: The application of nanotechnology, particularly incorporating active pharmaceutical ingredients (APIs) into porous membranes composed of nano-sized pores made of nontoxic materials, represents one of the known methods for enhancing the properties (e.g., poor solubility and low bioavailability) of currently available APIs. Following this line of thought in our work, the antipsychotic API aripiprazole (APZ) was infiltrated into anodic aluminum oxide, native silica, and silanized silica mesoporous matrices (pore diameters 8–10 nm) and compared to amorphous and crystalline bulk API.

Methods: We considered crystallization kinetics, molecular dynamics, and physical stability. Calorimetric, dielectric, and X-ray studies were conducted. Additionally, solubility studies were performed in a 0.1 M HCl solution (pH 1, sampling points 15, 30, 60, 120,180 min.).

Results: We showed the advantages of incorporating systems compared to bulk substances in solubility studies. We found that, unlike the crystalline and amorphous bulk samples, all confined systems exhibited a logarithmic increase in API dissolution over time, close to a prolonged release effect, without any signs of precipitation. Moreover, calorimetric data, supported by temperature-dependent X-ray diffraction measurements, revealed that in the bulk system, the recrystallization of polymorph III, followed by conversion to a mixture of forms IV and I, was evident, whereas in the case of confined samples, polymorphic forms I and III of APZ were produced upon heating of the molten API at varying rates. Importantly, the two-step crystallization observed in thermograms obtained for the API infiltrated into native silica templates may suggest crystal formation by both interfacial and core molecules. Furthermore, dielectric studies led us to conclude that there was no evidence of crystallization of spatially restricted API during one month of storage at 298 K.

Conclusions: We demonstrate that mesoporous matrices appear to be promising candidates as carriers for unstable amorphous APIs, such as APZ. In addition to protecting them against crystallization, they can provide the desired prolonged release effect, potentially leading to increased drug concentration in the bloodstream and thus higher bioavailability. The application of porous membranes as a novel generation of drug carriers might offer unique perspectives for the further development of drugs characterized by prolonged release.

Abstract No. PSP.21

Efficient method of (s)-nicotine synthesis through the stages of myosmine reduction and enantiomeric separation of nornicotine

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Objectives: (*S*)-Nicotine and analogues modulate nicotinic acetylcholine receptors, showing promise in CNS disorder treatment like Parkinson's disease, Alzheimer's disease, and Tourette's syndrome. Furthermore, nicotine replacement therapy provides a safer alternative to cigarettes, with lower nicotine levels and devoid of harmful chemicals commonly found in cigarettes. Novel synthesis methods for nicotine and its derivatives are of great importance.

Methods: The chemical synthesis of (*S*)-nicotine was conducted using a four-step synthesis including reduction of myosmine, enantiomeric separation of nornicotine, and subsequent methylation of the corresponding enantiomer to yield (*S*)-nicotine. The key stage, enantiomeric separation of nornicotine, was carried out using chiral acids. The structures of obtained compounds were confirmed by NMR and XRD techniques.

Results: The synthesis of optically (S)-nicotine was carried out as presented in **Figure 1**.

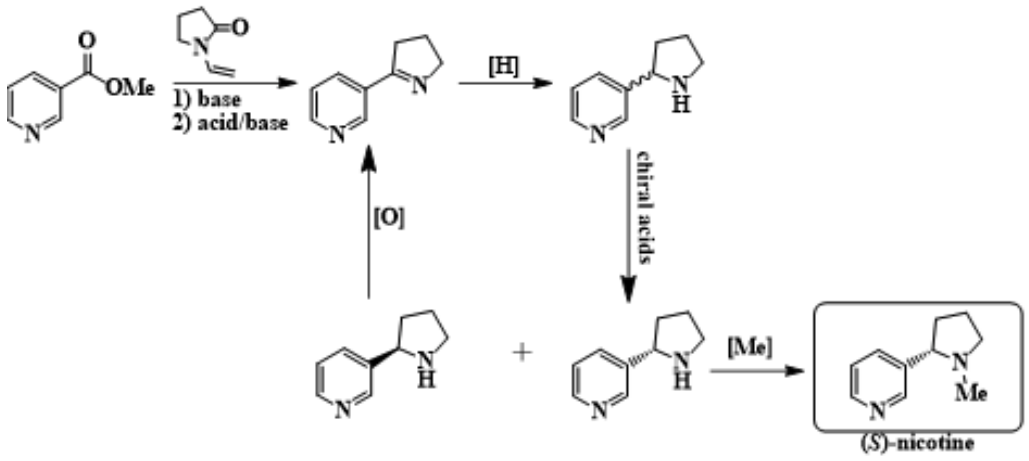

Figure 1. Synthesis of (*S*)-nicotine synthesis via myosmine reduction and enantiomeric separation of nornicotin.

Myosmine reduction was explored via electrochemical and chemical routes. In both synthetic routes, yielding up to 90% pure nornicotine. Enantiomeric separation of nornicotine involved formation of diastereomeric salt with *N*-acyl chiral amino acids, especially *N*-lauroyl-D-alanine in a mixture of MTBE with chloroform, provided favorable results. Excess of (*S*)-enantiomer was more then 91%. The process involved salt preparation, nornicotine isolation, and acid regeneration. Our procedure showed high efficiency, easy regeneration of reagents (e.g. chiral acid) and solvents (tert-butyl methyl ether, chloroform), and low production costs. Importantly, this procedure is suitable for industrial-scale production.

Conclusions: Our research has shown the efficient synthesis method for (*S*)-nicotine, involving myosmine reduction, enantiomeric separation of nornicotine, and subsequent methylation of the corresponding enantiomer to yield target (*S*)-nicotine.

Abstract No. PSP.22

Application of ReactIR™ and EasyViewer™ probes for the analysis of reaction kinetics during the scale-up process of tadalafil

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Objectives: The relocation of API production to the European market is necessary to ensure the continuity of drug supply. Consequently, chemists face significant challenges in API production, particularly during the scale-up processes.

The aim of the project was to pioneer a technological breakthrough in the synthesis of tadalafil, based on a predefined synthetic pathway.

Methods: As part of our strategies, we employed ReactIR™, an in situ process FTIR spectroscopy and EasyViewer™, an inline particle size analysis tool, to streamline pharmaceutical manufacturing processes, showcasing the potential for optimization. The validity of utilizing ReactIR™ was confirmed through verification of the liquid phase composition and comparison of obtained data with HPLC analysis results.

Results: During a reaction that occurred immediately upon reagent addition, ReactIR™ allowed for the tracking of its progress through continuous monitoring. Meanwhile, EasyViewer™ played a significant role in confirming the completion of the crystallization process.

Conclusions: Ultimately, the combined implementation of ReactIR™ and EasyViewer™ resulted in a significant reduction in reaction time, effectively reducing the time required for three out of four synthesis steps in tadalafil production.

The project POIR.01.01.01-00-0072/21-00 "Development of an innovative pilot line and its validation under real conditions using synthesis of a model active pharmaceutical ingredient" was co-financed by the European Union under the Smart Growth Operational Program 2014 – 2020, Priority Axis I "Support for R&D activities of enterprises", subaction 1.1.1. Industrial research and developmental activities conducted by enterprises.

Abstract No. PSP.23

Influence of lactose particle size distribution on the performance of inhaled drug product

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Objectives: The study aimed to assess how various lactose PSD (particle size distribution) influences DPI (dry powder inhaler) product performance through *in-vitro* assessment. The selected PSD parameters were tested to directly influence the mass of the active substance collected on stages 1-7 of the next- generation impactor (NGI). The impact of these parameters was verified using linear regression. Subsequently, the correlation coefficient between parameter with the greatest impact and mass of API (active pharmaceutical ingredient) on each stage was determined.

Methods: Active Pharmaceutical Ingredient – Industriale Chimica supplier; Assay – 100,3%; total impurities – 0,1%

Lactose monohydrate was used as a carrier in five formulations, each with varying d50 values:19.8; 37.5; 41.6; 44.2, and 74.45.

Magnesium stearate from Peter Greven Gmbh & Co. KG, constituting 0,15% of the overall formulation, was used as a force control agent.

All ingredients were mixed in two steps using a turbo rapid high-shear blender (TRV, GEA). The first step involved mixing of lactose with magnesium stearate, while the second one embraced API addition. The blends were conditioned at room temperature for 24 hours. Encapsulation was performed on Drum Lab encapsulation machine (Harro Höfliger, Germany) using HPMC size 3 capsules (Qualicaps Europe). The aerodynamic properties of the product were analyzed using NGI (Copley Scientific Limited, UK). Statistical parameters were determined using MS Excel.

Results: Different API masses were collected on the particular impactor stages. The linear regression model showed that the greatest impact on substance deposition on impactor stages had the d50 value of lactose. The percentage of lactose fraction smaller than 10um influenced API deposition to the lower extent. The D50 of lactose had the greatest impact on the mass on stage 2 (R^2=0,88), and the smallest impact on the mass on stage 3 (R^2=0,08). A significant correlation between the d50 of lactose and the mass of API on the impactor was observed for stages S2-S6.

Conclusions

1. The grade of lactose significantly influences product performance *in vitro*.

2. The results of linear regression analysis suggest that the d50 value of lactose is a better parameter for describing its effect on product performance than the fraction of lactose particles <10µm.

3. Higher d50 lactose values enables reduced API mass deposition on stage 2 while increasing deposition on stages S4-S6.

Abstract No. PSP.24

Synthesis of new marinoquinoline derivatives with potential antimalarial activity and determination of their molecular target

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Objectives: The objective of this work is to synthesize marinoquinoline derivatives and evaluate them against *P. falciparum*. The mode of action of marinoquinolines is not known, therefore, another objective of the project is identify the target of these compounds.

Methods: For the synthesis of marinoquinolines, a synthetic route involving 9 to 10 steps was used, starting from pyrrole and 4-methoxy-2-nitroaniline. These substances were synthesized, purified and properly characterized by spectroscopic and spectrometric methods. After synthesis, these derivatives were evaluated *in vitro* against *P. falciparum*.

Results: Malaria is a disease caused by species of the parasite *Plasmodium* spp, which is transmitted to humans through the bite of the female *Anopheles* mosquito when infected with *Plasmodium*. According to the World Health Organization, in 2020 alone, malaria caused the deaths of 627,000 people. Currently, there are several drugs available on the market for the treatment of malaria, however, the parasites are gaining resistance to them, thus making the treatment ineffective. Furthermore, the only currently available vaccine (RTS,S/AS01) is not effective against all parasite species. Therefore, the development of new drugs capable of efficiently combating this disease is urgent. In this context of new drug discovery, a class of compounds called marinoquinolines (MQs) has been gaining prominence due to their inhibitory activities against *Plasmodium*. Based on the structure of **MQ 1** (**Figure 1**), previously synthesized by professor Correia's group, it was decided to make new modifications to the indole portion of this substance, aiming to carry out a structure-activity relationship (SAR) study for this class of substances. Until now, 24 new marinoquinolines have been synthesized, of which 18 were sent for bioassays for antimalarial activity, who showed that 83% of them were active against *P. falciparum* 3D7, with IC_{50} values ranging from 0.55 to 5.9 µM.

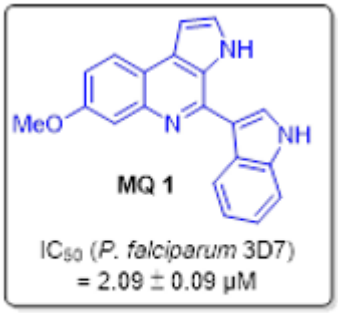

Figure 1. Structure of **MQ 1**.

Conclusions: Marinoquinolines show promise for the development of new drugs, and until now good results are being obtained. Among the 18 marinoquinolines evaluated, 15 were active against *P. falciparum* 3D7, with IC_{50} values ranging from 0.55 to 5.9 µM. The molecular target of marinoquinolines has not yet been identified, and new future studies will be carried out to achieve this objective.

Acknowledgements: We thank Sao Paulo Research Foundation (FAPESP) for the Research Internship Abroad Grant (BEPE) granted to Wellington da Silva, grant 2023/03295-3, São Paulo Research Foundation (FAPESP).

Abstract No. PSP.25

Strain makes a difference – study on inflammation associated colorectal cancer in mice

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Objectives: Most of the preclinical studies on colorectal cancer are conducted in young mice, questioning their relevance for modeling a disease of the elderly. Furthermore, different mouse strains show differences in susceptibility as cancer models. Therefore, we studied the impact of age, strain and vitamin D supplementation on inflammation-associated colorectal cancer (CAC) development in young (3 months old) and old mice (18 months old) of two different mouse strains (BALB/c and C57BL/6) receiving diets containing either high or low levels of vitamin D/calcium.

Methods: We used the Azoxymethane (AOM) /Dextrane-Sulphate-Sodium (DSS) model of chemically induced CAC. Mice received vitamin D and calcium deficient diet (100 IU vitamin D and 0.05% Ca^{2+}) or a diet rich in vitamin D and calcium (2,500 IU vitamin D and 1.5% Ca^{2+}). We assessed mucosal remodeling, dysplasia score, inflammation level, immune cell infiltration (CD3 and CD20), and cell proliferation (KI67) in colon sections. Gene expression was determined by RNAseq and validated by RT-qPCR.

Results: C57BL/6 mice were much more sensitive to the AOM/DSS treatment than Balb/C. Young C57BL/6 mice developed more tumors per mouse and their cumulative tumor-severity score was significantly higher ($p<0.001$) than that of the Balb/C mice. Changes in proliferation and immune cell infiltration were also more pronounced in young mice. CYP2R1 gene, crucial for the synthesis of the most abundant form of vitamin D in the circulation and precursor of an active form of vitamin D, was significantly higher expressed in both young and old C57/Bl6 mice than in BALB/c mice. IL6 gene expression was reduced in the descending colon of AOM/DSS treated C57/Bl6 mice receiving diet with high vitamin D and calcium. IL6 plasma-levels were reduced in young mice from both strains receiving diet with high vitamin D and calcium. In old mice the effect was observed only in C57BL/6 mice. RNASeq data suggested that the biggest discriminator among groups was the strain.

Conclusions: Mouse strain is an important factor that significantly affects the outcome of chemically induced CAC. Even the effect of age is dependent on the different strains. Nevertheless, based on our current results, diets high in vitamin D and calcium could be promising approach for prevention of CAC.

Abstract No. PSP.26

Development of the sustainable ester to alcohol reduction in the synthesis of a key intermediate for PI3Kδ inhibitor (CPL302415) by Ru-catalyzed hydrogenation and $LiAlH_4$ reduction in batch and flow.

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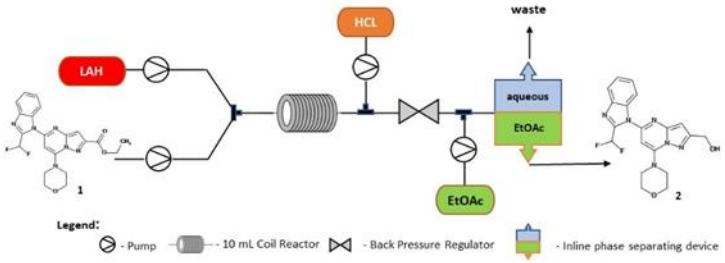
Objectives: The aim of this work was to develop the catalytic or stoichiometric sustainable reduction of ester function in 1 to the corresponding primary alcohol 2 in continuous flow process as key intermediate in the synthesis of CPL302415, which is our new promising PI3Kδ inhibitor. The CPL302415 is now under evaluation for the treatment of Systemic Lupus Erythematosus.



Methods: The batch hydrogenation was performed in Büchi stainless steel autoclave. Flow experiments with H_2 were performed using a Vapourtec R2C+ with one gas-liquid reactor (15 mL, id = 1 mm) and two stainless still reactors (10 mL, id = 1 mm) while the reduction with $LiALH_4$ test were done on Vapourtec easy-Medchem with one standard PFA tubular reactor (10 mL, id=1 mm) respectively. The DoE study and statistical analysis were performed using the design of experiment tools of STATISTICA software (v.13.3). The experimental data were fitted using multiple linear regression. The main and interaction effects were generated based on multivariate ANOVA. The statistical significance level was set up to 0.05. The goodness of fit of the models was expressed in regression coefficient R^2.

Results: The flow reduction with $LiAlH_4$ resulted 83 % yield with productivity (11.3 g/h) and can be done on commercially available Vapourtec easy-Medchem System whereas the catalytic batch reduction let us reach up to 98% yield of the desired alcohol 2. In addition we also calculated green metrics for each of applied synthesis for example in flow reduction the space time yield was increased almost 180-fold in comparison to batch procedure.

Conclusions: Independently we developed two highly effective methods for reduction of our ester 1 (the CPL302415 precursor) to alcohol 2. The first one by batch high pressure hydrogenation in the presence of Ru-catalysts and the second by continuous flow reduction with $LiAlH_4$. We compared the efficiency and selectivity of various reduction methods (catalytic with stoichiometric both in flow and batch mode).

Acknowledgements: This work was supported by National Centre for Research and Development (Poland) POIR.01.02.00-00-0085/18-00.

Abstract No. PSP.27

Formulation development strategy of lipid nanoparticles (LNPs) for intravenous administration of mRNA

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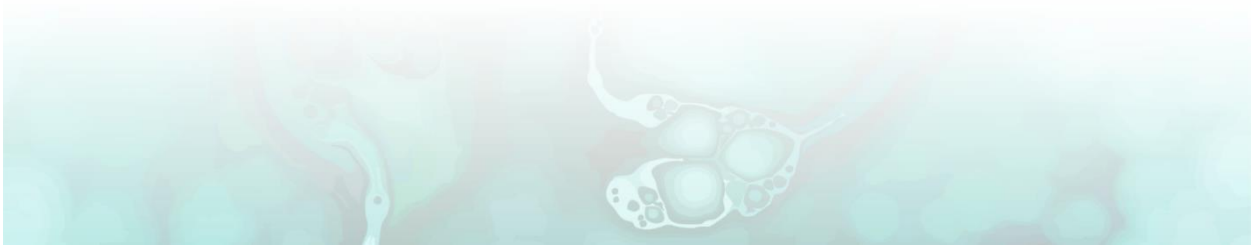
Objectives: The aim of this work was to develop a liquid dosage form for intravenous (IV) administration of mRNA to treat alpha-1 antitrypsin (AATD) deficiency. Nucleic acid was administered in the form of lipid nanoparticles (LNPs), whose role was to protect mRNA, improve cellular uptake and increase endosomal escape to cytoplasm, where it can be translated into a protein. Here we present our strategy to formulation development of LNPs containing mRNA for IV administration.

Methods: LNPs were manufactured via microfluidics. Particle size (PS) and polydispersity index (PDI) were measured by DLS. Encapsulation efficiency (EE) and mRNA concentration were assessed by Ribogreen assay. *In vitro* efficiency assay (IVE) of the LNPs was performed using HEK293 cells. The read- out was performed after 24h. Naked mRNA and lipofectamine were used as controls. In *in vivo* study, three mice were taken to test each composition. Each mouse received LNP formulation containing 1µg of luciferase-coding mRNA via IV injection. The luminescence intensity was measured at the 4^{th}, 24^{th}, 48^{th}, 72^{nd}, and 144^{th} h of the experiment.

Results: AATD deficiency requires mRNA delivery to the liver. The first step of formulation development was therefore screening of ionizable lipids and selection of one that selectively targets the liver. It embraced both *in vitro* (PS, PDI, EE, IVE) and *in vivo* studies. The second step was to find a proper lipid composition (a sterol, a PEG-lipid and a phospholipid) and their contents to obtain well-shaped LNPs of appropriate PS, PDI and EE values. For this purpose, *Design of Experiment* approach was applied. Next, downstream process was optimized, e.g. buffer concentration, type and cryoprotectant level. Finally, the selected compositions were submitted into stability program in various storage conditions (+4°C, -20°C, -80°C). Formulations of best performance upon time in terms of PS, PDI, EE and IVE will be tested again on animals to verify their efficiency *in vivo*, or to detect any possible changes in its *in vivo* performance after storage.

Conclusions: Here we present our strategy to formulation development of LNP composition for IV administration of mRNA encoding AATD. The presented approach embraced both *in vitro* and *in vivo* tests of various compositions as well as systematic experimental work and decision making in order to reveal the best formulation able to deliver mRNA to the liver of patients suffering from AATD deficiency.



Abstract No. PSP.28

Selection of optimal concentration of Doxycycline hyclate in a composition of Topical Foam Aerosol

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Objectives: Doxycycline hyclate, a synthetic derivative of tetracycline, serves as a broad-spectrum antibiotic, retaining its efficacy even after more than 40 years of clinical use [1]. Aerosol dosage forms offer several advantages over other pharmaceutical forms, including ease of use, purity, and cost- effectiveness in delivering potent efficacy [2].

Methods: Our research aims to justify the optimal concentration of doxycycline hyclate. The concentration of doxycycline in our model sample ranged from 0.25% to 1%, with a 2-fold increase in concentration. We introduced doxycycline into the concentrated solution in the form of a suspension with polyethylene glycol.

Results: The results of studies on the antimicrobial activity of the model sample are presented in Table 1.

Table 1. Growth inhibition zones of test microorganisms (n=5; P 95%).

Concentration of doxycycline, %	Diameter of growth inhibition zone of the test strain (mm) 10^7 CFU/ml in the upper layer of the culture medium	
	E. coli (SCA)	*S. aureus (SCA)*
0,25	19.1	23.3
	19.4	23.6
	18.9	24.3
	19.6	23.9
	19.3	24.2
X ±ΔX	19.26±0.75	23.86±0.52
0,5	20.1	24
	20.7	24.6
	20.9	25
	21.3	24.3
	21.5	24.7
X ±ΔX	20.9±0.68	24.5±0.46
1	24.1	28
	23.7	28.6
	23.9	28.3
	23.3	27.9
	23.5	28.2
X ±ΔX	23.7±0.88	28.2±0.34

Conclusions: The analysis of the data indicates that increasing the concentration of doxycycline from 0.25% to 1% results in a gradual increase in the zones of growth inhibition for the test cultures. At a doxycycline concentration of 0.25%, the inhibition zone diameters were measured at 19,26 mm ± 0.75 for *E. coli* and 23,86 mm ± 0.52 for *S. aureus*. A twofold increasing in concentration of doxycycline (from 0.25% to 0.5%) led to a 1.1-fold increase in the zones of growth inhibition for E. coli and a 1.03-fold increase for *S. aureus*.

Further increasing the doxycycline concentration from 0.5% to 1% resulted in enhanced antimicrobial activity, with inhibition zone diameters increasing from 20.9 mm ± 0.68 to 23.7 mm ± 0.88 for *E. coli* and from 24.5 mm ± 0.46 to 28.2 mm ± 0.34 for *S. aureus*. The zones of inhibition around the wells increased by 1.13 and 1.18 times for *E. coli* and *S. aureus*, respectively. Therefore, it is advisable to select a concentration of 1% doxycycline in the composition of the model sample.

References:

[1] Saliy, O., Popova, M., Tarasenko, H., & Getalo, O. (2024). Development strategy of novel drug formulations for the delivery of doxycycline in the treatment of wounds of various etiologies. In European Journal of Pharmaceutical Sciences (Vol. 195, p. 106636). Elsevier BV. https://doi.org/10.1016/j.ejps.2023.106636

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Abstract No. PSP.29

Quinolone chemical modifications and influence on its physicochemical and biological properties

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Objectives: The subject of this research was the chemical modification of the well-known and currently used active pharmaceutical ingredients that are quinolones and fluoroquinolones. These substances are used to treat bacterial infections of the urinary, nervous, gastrointestinal systems, in particular. As a result of their low solubility in water and the developing resistance of microorganisms to their action, their modification with ammonium ionic liquids was approached.

Methods: The first stage of the research involved the synthesis of ionic liquids and new substances based on quinolone derivatives and ammonium ionic liquids of the active pharmaceutical ingredient-ionic liquid (API-IL) and API sodium salts (NaAPI). Calorimetric studies were carried out to determine the phase transitions of the pure substances. Then, the solubilities of the substances were determined using the dynamic method in the widest possible range of molar fractions in the form of phase diagrams in binary systems {solid (1) + liquid (2)}. The obtained data were subjected to correlation with thermodynamic models based on the local concentration theory. Biological tests were carried out on the newly synthesized substances. Minimum inhibitory concentrations (MIC) of the substance were determined to establish whether the formation of API-IL alters the activity of the substance against *Escherichia coli ATCC 8739*, *Pseudomonas aeruginosa ATCC 9027* and *Staphylococcus aureus ATCC 6538*. Cytotoxicity tests were also carried out on the *Vero* cell line.

Results: A significantly higher solubility was exhibited by API-IL relative to pure APIs, as expected. The addition of ionic liquids slightly affected the cell viability of the *Vero* line. At higher concentrations (200 µM), it can be seen that substances containing an ionic liquid cation with a longer carbon chain show higher cytotoxicity. Chemical modification did not affect MIC values.

Conclusions: The work approached represents basic research on API-IL using quinolone derivatives and ammonium ionic liquids as examples. This is new issue that requires further research at the intersection of scientific fields such as pharmacy, chemistry and biology. Additions of ionic liquids to therapeutic substances show potential for modifying interactions with pathogens and improving the adverse pharmacokinetic parameters of active pharmaceutical ingredients.

Abstract No. PSP.30

EMDR+ to support PTSD treatment

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Objectives: We assessed in people suffering from the effects of trauma the feasibility, safety, acceptance, and efficacy of EMDR enriched with sound stimulation (by administering neutral sounds synchronized with the guided bilateral alternating stimulation of the gaze) and musical reward (musical listening based on the patients' predisposition and personal tastes). Feasibility, quantified by the number of patients who completed the treatment, was excellent as this was the case in 12 out of the 12 enrolled people with psychological trauma. Safety and acceptance, assessed by self-compiled questionnaires, were excellent, with an absence of side effects and high satisfaction. Efficacy, quantified by the number of EMDR treatment sessions required to reach the optimal scores on the Subjective Units of Disturbance (SUD) and Validity of Cognition (VOC) scales typical of EMDR protocols, revealed an average duration of 8.5 (SD 1.2) sessions, which is well below the 12 sessions considered a standard EMDR treatment duration. EMDR+ appears to be a relevant personalization of EMDR, particularly in music-sensitive people, consolidating the therapeutic alliance through a multisensory communicative bond for trauma treatment.

Methods: Adult people of both sexes enrolled (6 females, 6 males, 48.4 ± 15.3 years old) who had developed at least six months of continuous symptoms related to psychic trauma (Person suffering by Psychic Trauma and stress-related disorders, PwPT). Inclusion: -Diagnosis of PwPT as assessed by professionals in the healthcare system applying criteria for 'Disorders specifically associated with stress' as determined by WHO ICD-11, 6B.

To describe the dynamics of the therapeutic process, we calculated the change between the VOC-SUD (t1) and the VOC-SUD (to) in the session when each phase was concluded. The single subject values of these variations were subjected to an ANOVA model with the within-subject factor Phase (Anamnesis, Preparation,..., Revaluation) to examine whether there was a significant Phase effect indicating that the clinical state improved more in specific phases. Finally, we compared the Cohen's d coefficient with the known thresholds. Statistical analysis was performed by SPSS v28.0.

Results

SUD Cohen's d = (4.68 – 7.31)⁄0.43 = –6.1

VOC Cohen's d = (4.29 – 2.84)⁄0.67 = 2.0.

Conclusions: We observed signs of EMDR+ being an effective therapeutic tool in the treatment process, with short treatment duration and both SUD and VOC changes revealing effect sizes well above the threshold indicated for large efficacies.

Abstract No. PSP.31

The safety and efficacy of mycophenolate mofetil in patients with idiopathic inflammatory myopathies - pilot study

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Objectives: Idiopathic Inflammatory Myopathies (IIM) are the largest group of myopathies in adults. Patients with IIM have increasing difficulty with daily tasks requiring the use of proximal muscles. Mycophenolate mofetil (MMF), a prodrug of mycophenolic acid (MPA) can be effectively used in management of IIM and may be suitable alternative to the conventional immunosuppressive agents. The aim of the study is to evaluate the association between MPA trough level (Co) and disease activity in adult patients with IIM.

Methods: A prospective study was performed on a group of patients, who were on MMF therapy for a minimum period of 3 months. The study included 11 patients with a male/female ratio 5:6. The mean age and body weight was 47.8 years and 70.6 kg respectively. MMF was used orally 2g BID, at a mean dose of 31.1 mg/kg. MPA concentrations were measured using a validated HPLC method developed in our laboratory. Active disease was defined as at least one of the activity domains: muscle activity, worsening weakness or creatinine kinase (≥190 U/L), myositis disease activity assessment tool and in particular cases skin activity.

Results: Mean MPA Co was 1.9 µg/mL (range: 0.38-4.37 µg/mL) , and only 4/11 Co were above the 1.5 µg/mL threshold. No difference in Co for MPA was found between patients active and inactive disease (0.94 vs 0.98, p=0.7). Also, no correlation has been noted between MMF dose and clinical outcome using spearman`s rank (r=0.06, p=0.87).

Conclusions: Neither in case of MPA Co, nor MMF dose any correlation with disease activity was found in idiopathic inflammatory myopathies. More prospective studies are still needed to assess usefulness of MPA monitoring during treatment patients suffering from IIM, as well as to control the impact of increasing MMF dose in IIM patients to improving clinical outcome.

Abstracts of Sponsored Lectures

Abstract No. SL.01

Exploration of MMP inhibition in neurological disorders: results of phase I, single and multiple dose studies of oral PKL-021 in healthy volunteers

Magdalena Tyszkiewicz[1], Anna Krause[1], Magdalena Kania[1], Hanna Kierońska[1], Joanna Lipner[1], Marzena Maciejewska[1], Marta Magdycz[1], Marek Masnyk[1], Lukasz Mucha[1], Anna Pałubicka[1], Iwona Pawłowska-Kapusta[1], Sylwia Piasecka[1], Stanisław Pikul[1], Katarzyna Rogut[1], Katarzyna Sidoryk[1]

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Objectives: The primary objective of the Phase 1 clinical study was to determine the pharmacokinetic (PK) profile of single and multiple doses of PKL-021 and to evaluate the effect of food on the PK profile of a single dose in healthy volunteers. The secondary objective was to evaluate the effect of food on the PK profile of a single dose of PKL-021 in healthy volunteers.

Methods: Matrix metalloproteinases (MMPs), a protein family of zinc (Zn^{2+})-containing endopeptidases, are pivotal agents that are involved in various biological and pathological processes in the central nervous system (CNS). Abnormal regulation of MMPs plays a complex role in CNS disorders, contributing to neuroinflammation, neurodegeneration, and neurovascular dysfunction. Targeting MMPs pharmacologically represents a potential therapeutic strategy for mitigating CNS pathology.

Currently, more and more research groups are involved in research on MMP inhibitors, both in the context of understanding the mechanisms of action of MMPs and developing new drugs with therapeutic potential in various diseases in which excessive MMP activity plays an important role.In our studies, PKL-021 – a small-molecule, very potent MMP-9 inhibitor penetrating the blood-brain barrier (BBB)—was selected as a drug candidate. The project covers, among other things, the development of the active substance and investigational medicinal product manufacturing technology, analysis of the MMP-9 inhibitor's therapeutic potential in animal models, and carrying out the preclinical and Phase I clinical studies.

Results: In total, twelve healthy volunteers were included into the Phase I study and dosed. All subjects that finished at least one study period and provided any evaluable data for PKL-021 under fasting or fed conditions were included in the pharmacokinetic and statistical analysis. Pharmacokinetic parameters of active component has been calculated from plasma concentrations determined by validated achiral HPLC/MS/MS method for PKL-021.The safety was evaluated in all subjects who received the medication.

Conclusions: PKL-021 was well tolerated in healthy volunteers as a single or multiple oral dose administered under fasting and fed conditions. No SAE occurred during the whole course of the Study.

Acknowledgment: Project no. POIR.01.01.01-00-0235/20-00 „Utilisation of a matrix metalloprotease inhibitor to develop an innovative therapeutic method that prevents the development of post-traumatic and post-stroke epilepsy", was co-financed by the European Union.

Abstract No. SL.02

Radicalize Your Mass Spectrometer to Solve Unanswered Questions: Identifying Double-Bond-Positions of Phospholipids in Mouse Liver by Using Simultaneous Positive/Negative Ion Switching Analysis of LCM

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Objectives: The OAD-TOF system is a new technology for Q-TOF LCMS that realizes OAD (Oxygen Attachment Dissociation), which is Shimadzu's proprietary fragmentation technology. It allows the analysis of the position of carbon-carbon double bonds in lipids and other organic compounds. Combined with the LCMS-9050, which boasts world-class high mass accuracy and fast polarity switching, it enables the creation of innovative applications. It can measure fragment ions that cannot be obtained by conventional collision-induced dissociation (CID), where ions are fragmented by collision with an inert gas such as argon or nitrogen. The irradiating oxygen radicals specifically oxidize/dissociate double bonds between carbons, which is useful for structure estimation of organic compounds such as lipids. The technique can be applied to monovalent ions and negative ions, which have been difficult to fragment using radical reactions by electrons and anions, and provides completely new structural information.

Methods: All experiments were performed using an ESI Q-TOF LCMS-9050 (Shimadzu, Kyoto, Japan). OH and O radicals generated by a compact microwave-driven radical source was introduced into the collision cell (Q2) through the quartz tube to obtain OAD-MS/MS spectrum. Shimadzu Nexera LC system was used for the separation of the lipids in the extracts of mouse liver.

Results: Two peaks at m/z = 782.5695 [M+H]+ from positive ion analysis mode and at 826.5604 [M+HCOO]- from negative ion analysis mode were detected from a mouse liver sample. Carbon chain lengths and the number of double-bonds were estimated from CID-based negative ion analysis, and double-bond positions were identified with OAD-based positive ion analysis. A combination of CID/OAD and positive/negative switching analysis by Q-TOF was used to identify lipids in mouse liver. Three compounds were found from two peaks detected at the same m/z.

Conclusions: By using the combination of OAD and pos/neg switching analysis, the chemical structure of lipids in a mouse liver were identified. It was proved that alpha-linolenic acid and gamma-linolenic acid are able to be distinguished by OAD.

Abstract of Short Presentation

Abstract No. SP.01

New active and selective phosphoinositide 3-kinase δ inhibitor the CPL302415 - transfer into the flow synthesis.

Anna Maj[1], Stanisław Michałek[1], Lidia Gurba-Bryśkiewicz[1], Wioleta Maruszak[1], Mariola Stypik[1], Marcin Zagozda[1], Krzysztof Dubiel[1], Maciej Wieczorek[1]

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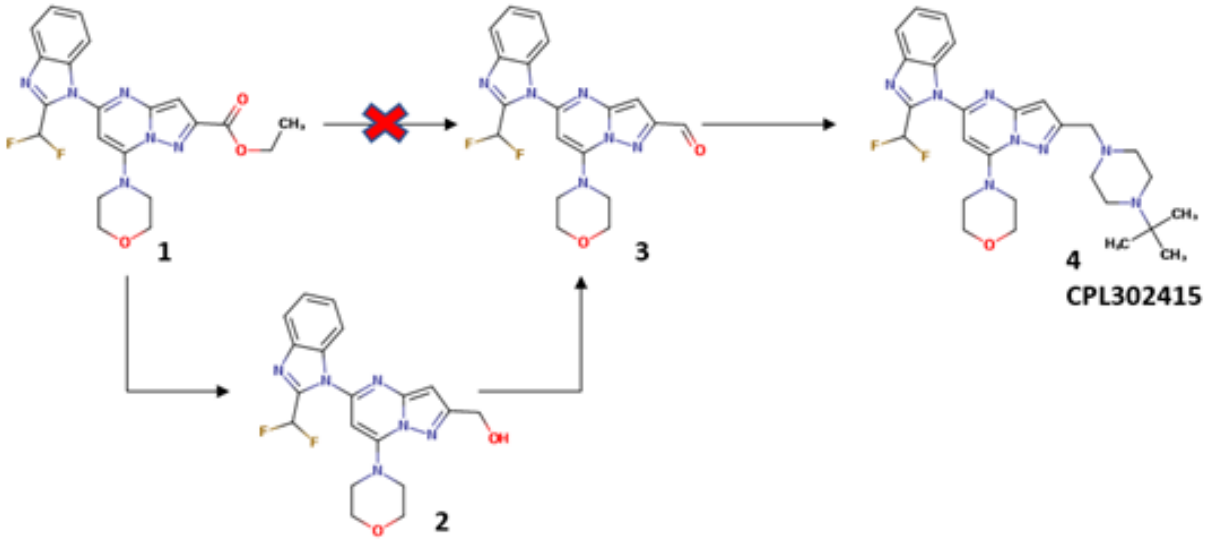
Objectives: CPL302415 is a selective inhibitor of PIK3δ, currently in the preclinical research of inflammatory and autoimmune diseases including asthma or systemic lupus erythematosus. Herein, we will present the synthesis of CPL302415 with the emphasis on transfer the batch procedure into the flow process. The use of Design of Experiment (DoE) enabled to determine critical process parameters and their interactions, as well reduce the number of experiments, accelerate of work, and reduce the amount of produced waste.



Methods: The batch hydrogenation was performed in Büchi stainless steel autoclave. Flow experiments with H_2 were performed using a Vapourtec R2C+ with one gas-liquid reactor (15 mL, id = 1 mm) and two stainless still reactors (10 mL, id = 1 mm) while the reduction with $LiALH_4$ and oxidations test were done on Vapourtec easy-Medchem with one or four standard PFA tubular reactors (10 mL each, id=1 mm) respectively. The DoE study and statistical analysis were performed using the design of experiment tools of STATISTICA software (v.13.3). The experimental data were fitted using multiple linear regression. The main and interaction effects were generated based on multivariate ANOVA. The statistical significance level was set up to 0.05. The goodness of fit of the models was expressed in regression coefficient R^2.

Results: For the flow synthesis of our CPL302415 we developed and optimized highly effective (83 % yield), productive (11.3 g/h) and chemoselective (91.1 %) continuous flow $LiAlH_4$ ester reduction of 1 in mild conditions. Independently we also establish new very effective flow oxidation procedure of alcohol 2 into the corresponding aldehyde 3 (84 % yield).

Conclusions: We developed a new PI3Kδ inhibitor and successfully transferred two important synthetic steps from batch process to flow synthesis. We compared the efficiency, selectivity and ecological indicators of different applied reduction and oxidation methods.

Acknowledgements: This work was supported by National Centre for Research and Development (Poland) POIR.01.02.00-00-0085/18-00.

Keywords: Flow synthesis, Ester reduction, Aerobic oxidation of alcohols, Design of Experiment (DOE), API synthesis, PIK3*δ* inhibitor.

Abstract No. SP.02

Recent progress in the synthesis of ferroptosis inhibitors and their biological assessment

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Objectives: Ferroptosis is non-apoptotic cell death characterized by (1) peroxidation of polyunsaturated fatty acids (PUFAs) in phospholipid membranes, (2) redox-active iron metabolism, and (3) the loss of GPX4 reducing capacity. Ferroptosis plays an important regulatory role in the occurrence and development of neurodegenerative diseases, tissue and organ injury, and inflammatory diseases. Various medicinal chemistry strategies aimed at halting ferroptosis have been identified, including the use of iron chelators, radical trapping antioxidants (RTAs), deuterated PUFAs, as well as targeting enzymatic entities, namely lipoxygenases, ACSL4, GPX4, and FSP1.

Methods: The research groups of the Augustyns and Vanden Berghe focused on the development of RTAs. Over the last few years, based on extensive structure-activity relationship analysis, several generations of new RTAs have been synthesized and evaluated for their potency *in vitro* in ML162-induced ferroptosis in HT1080 cells and *in vivo* in various animal disease models, namely acute liver and kidney injuries, andrelapsing–remitting multiple sclerosis.

Results: UAMC-3203 has proven to be life-saving against multiorgan dysfunction and acute liver injury, potent in acute kidney injury, and it has also reduced demyelination and delayed relapse in a multiple sclerosis model. Two novel series of RTAs were designed to improve membrane permeability. The majority of novel analogues maintained high ferroptosis inhibitory potency. Moreover, compounds exhibiting drug-like physicochemical and biochemical properties are currently being screened in *Drosophila* and mouse models.

Conclusions: Evolving understanding and the development of novel ferroptosis inhibitors offer promising prospects for advancing therapeutic strategies across various pathological conditions. Extensive pharmacokinetic studies, alongside toxicology and safety pharmacology assessments, are pivotal for comprehending the interaction of inhibitors with the body to exclude side effects due to off-ferroptotic interactions, optimizing dosing protocols, and ensuring their safety profile prior to clinical application.

This project has received funding from the European Union's Horizon 2020 Research and Innovation Programme under the Marie Skłodowska-Curie Grant Agreement N°101065370 (NeuroFerro).

Abstract No. SP.03

Platinum(II)-peptide nucleic acid conjugates as antibacterials

Rosa Maria Dell Acqua[1], Monika Wojciechowska[1], Francesco Fagnani[2], Daniele Marinotto[3], Joanna Trylska[1], Alessia Colombo[2], Silvia Cauteruccio[2]

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Objectives: The aim is to develop novel bioorganometallic platinum(II)-peptide nucleic acid (PNA) conjugates that can be employed as dual-activity antibacterial agents. These systems combine the antisense properties of PNAs with the photodynamic properties of a Pt(II) complex, which serves as the photosensitizer capable of generating cytotoxic singlet oxygen under light excitation.

Methods: The PNA and innovative bioorganometallic Pt(II)-PNA conjugates (*Figure 1*) were obtained through solid-phase synthesis and their photophysical properties were examined. UV-monitored melting profiles of the PNA and PNA conjugates with complementary DNA and RNA were obtained. The Pt(II) complex was also conjugated to the antibacterial PNA sequence to target mRNA of the *acpP* gene encoding the essential acyl carrier protein in *E. coli*.

Results: The characterization of the novel Pt(II)-PNA conjugates revealed that the photophysical features of the Pt(II) complex were enhanced through conjugation with PNA, without compromising the recognition properties of the PNA. The anti-*acpP* PNA and the corresponding control scrambled PNA sequences linked to the Pt(II) complex were synthesized. The antibacterial properties of these unique compounds have been tested to understand their potential in acting as antibacterials (*Figure 1*).

Conclusions: The potential of Pt(II)-PNA conjugates to help fight bacterial infections has emerged and the use of these novel systems as antibacterial agents opens up new possibilities for the treatment of infectious diseases, which represent one of the biggest threats to global health.

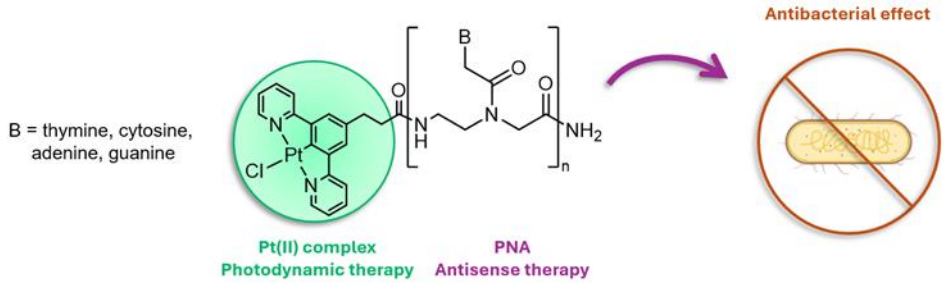

Figure 1: PNA conjugated to luminescent Pt(II) complex - potential antibacterial agent.

Abstract No. SP.04

Anti-PD-L1 Macrocyclic Peptide pAC65: Comparable Potency to Monoclonal Antibodies with Potential for Oral Administration

Ismael Rodriguez[1], Justyna Kocik-Krol[1], Lukasz Skalniak[1], Bogdan Musielak[1], Aneta Wisniewska[1], Agnieszka Ciesiołkiewicz[2], Łukasz Berlicki[2], Jacek Plewka[1], Przemyslaw Grudnik[3], Malgorzata Stec[4], Maciej Siedlar[4], Tad Holak[1], Katarzyna Magiera-Mularz[1]

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[2] Chemistry, Wrocław University of Science and Technology, Poland
[3] Malopolska Centre of Biotechnology, Jagiellonian University, Poland
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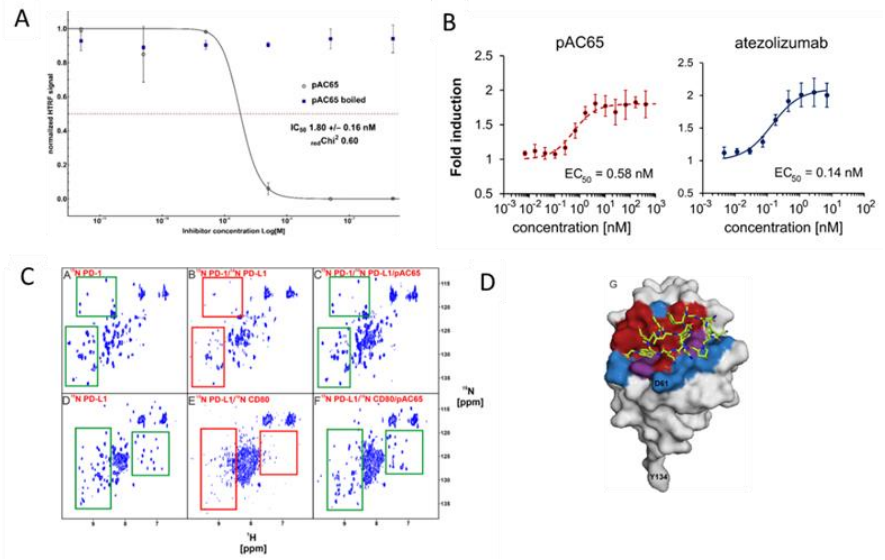
Objectives: Recent advances in cancer immunotherapy have predominantly relied on monoclonal antibodies (mAbs) targeting the PD-1/PD-L1 pathway. This study introduces pAC65, a macrocyclic peptide designed as a non-antibody inhibitor of PD-L1, aiming to achieve comparable potency to mAbs while exploring its suitability for oral administration.

Methods: The synthesis and structural characterization of pAC65 involved binding assays, such as AIDA- NMR experiment, and co-crystallography of the complex to elucidate its interaction with PD-L1 at the molecular level. The peptide's efficacy in disrupting PD-1/PD-L1 and PD-L1/CD80 complexes was assessed using biochemical assays, providing a comprehensive understanding of its mechanism of action.

Results: Our investigation reveals that pAC65 possesses remarkable potency in disrupting PD-1/PD-L1 complex, demonstrating an IC_{50} of 1.80 ± 0.16 nM and EC_{50} of 0.58 nM, on par with FDA-approved monoclonal antibodies. We observed that the addition of pAC65 disrupted formation of PD-1/PD-L1 as well as PD-L1/CD80 complexes using AIDA-NMR approach. We also elucidated crucial interactions of pAC65 to PD-L1 and how they overlay with the PD-1/PD-L1 interface perfectly.



Conclusions: The macrocyclic peptide pAC65 emerges as a highly promising PD-L1 inhibitor, surpassing conventional small-molecule inhibitors in terms of specificity and potency. Notably, its potential for oral administration presents a significant advantage over mAbs, potentially improving patient compliance and treatment accessibility, while still being substantially cheaper to produce than mAbs. Further preclinical studies are warranted to explore pAC65's therapeutic potential and safety profile in diverse cancer models.

Reference:

Rodriguez, I. *et al*. Structural and biological characterization of pAC65, a macrocyclic peptide that blocks PD-L1 with equivalent potency to the FDA-approved antibodies. *Mol Cancer* 22, 150 (2023). https://doi.org/10.1186/s12943-023-01853-4

Abstract No. SP.05

Automated microfluidics-assisted bioprinting of double-emulsion droplets for the culture of spheroids

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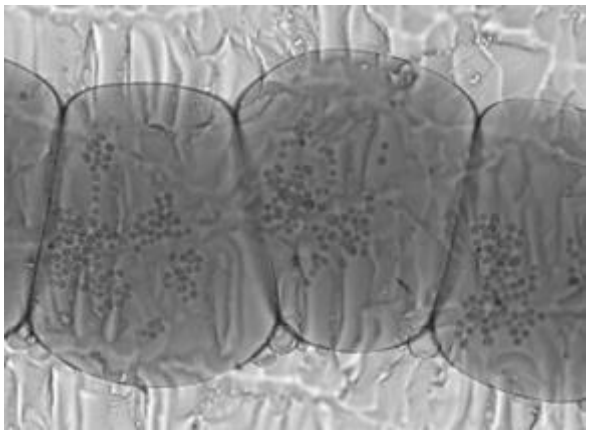
Objectives: Microfluidic droplet-based bioprinting offers several advantages over conventional extrusion-based bioprinting methods such as high-precision control over the contents of each droplet including cells, molecules, drugs and bioinks; ease of their compartmentalization and high repeatability.

In this work we present an automated setup capable of printing single-file chains of double emulsion aqueous core-droplets onto substrates under external aqueous media. This method was submited for a patent application EP23461682.

Materials: Double emulsion droplets are generated using an aqueous solution of GELMA 6% w/v + 0.2% w/v photoinitiator (LAP) as the inner phase, NOVEC 7500 + 3 % PFPE-PEG-PFPE surfactant as the shell/intermediate phase and distilled water as the external phase. The substrate is a glass slide treated with a fluorophilic coating NOVEC 1720. 769P renal cancer cells were used. The microfluidic chip is displaced using a 3D printer Gate 3Novatica.

Methods: GelMA droplets are encapsulated in NOVEC 7500 using a microfluidic T-junction micromilled in a polycarbonate chip. The generated droplets are then directed towards a substrate through a 25G needle immersed in an external aqueous media. The needle-substrate is precisely adjusted to <200 µm to allow immediate deposition of the droplets at the substrate via wetting by the oil phase while leaving enough space for the droplets to remain stable upon extrusion.

Results: We show that the GelMA droplets can be printed onto a substrate in the form of a line of liquid 'cores' encapsulated by a thin oil 'shell' under external aqueous media. The presence of the surfactant- rich oil phase not only prevents coalescence of the droplets but also leads to adhesive capillary forces between them which stabilizes the printed lines. In figure below, a line containing 769P renal cancer cells after ~4 h after is shown after being crosslinked.



Conclusions: In this present a method to print single- file chains of double emulsion droplets. As a proof-of-concept we printed droplets containing cells. In the future, we expect to improve the biomaterial formulation to allow for faster cell aggregation and spheroid formation. The next step will be to perform drug screening on those spheroids.

Abstract No. SP.06

The impact of plant compounds with anticancer properties on liposomal doxorubicin in triple negative breast cancer oncological therapy

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Objectives: The purpose of the research was to develop effective liposomal preparations containing liposomal doxorubicin (DOX) in combination with compounds of plant with anticancer properties. The obtained liposomal formulas were analyzed from the point of view of their effectiveness in triple negative breast cancer (TNBC).

Methods: Ultrasonic homogenizations method was used to obtain liposomal systems containing DOX and one/two plant compounds. For measuring the size, the stability and degree of polydispersity of nanoparticles dynamic light scattering (DLS) was used. Fluorescence spectroscopy was used to analyze the DOX release profile from liposomes at different pH values. In order to determine the effectiveness of the obtaines liposomal formulations, the two cell survival tests have been released - the MTT test (absorbance measurement) - 2D cell culture and the PrestoBlue test (fluorescence measurement) - 3D cell culture. A representative cell line of the most aggressive type of breast cancer - triple negative breast cancer (MDA-MB-231) was used to assess tumor cell survival. Confocal microscopy was used to determine the effect on the size of the spheroids (3D culture).

Results: The obtained sizes of liposomes allow to use them as drug delivery systems. The addition of natural compounds resulted in the enhancement of DOX activity and reduction of cell survival of the MDA-MB-231 line. Analysis of the DOX release profile and the effect of the addition of plant compounds confirmed the release of higher amounts of cytostatic under acidic pH conditions, corresponding to the microenvironment of the tumor, compared to neutral pH, reflecting blood pH.

Conclusions: Liposomes with combinations of doxorubicin and natural, plant compounds have shown enhanced efficacy against cancer cells, giving the possibility of their use in the treatment of triple negative breast cancer.

Keywords: liposomal doxorubicin, triple negative breast cancer, chemotherapy

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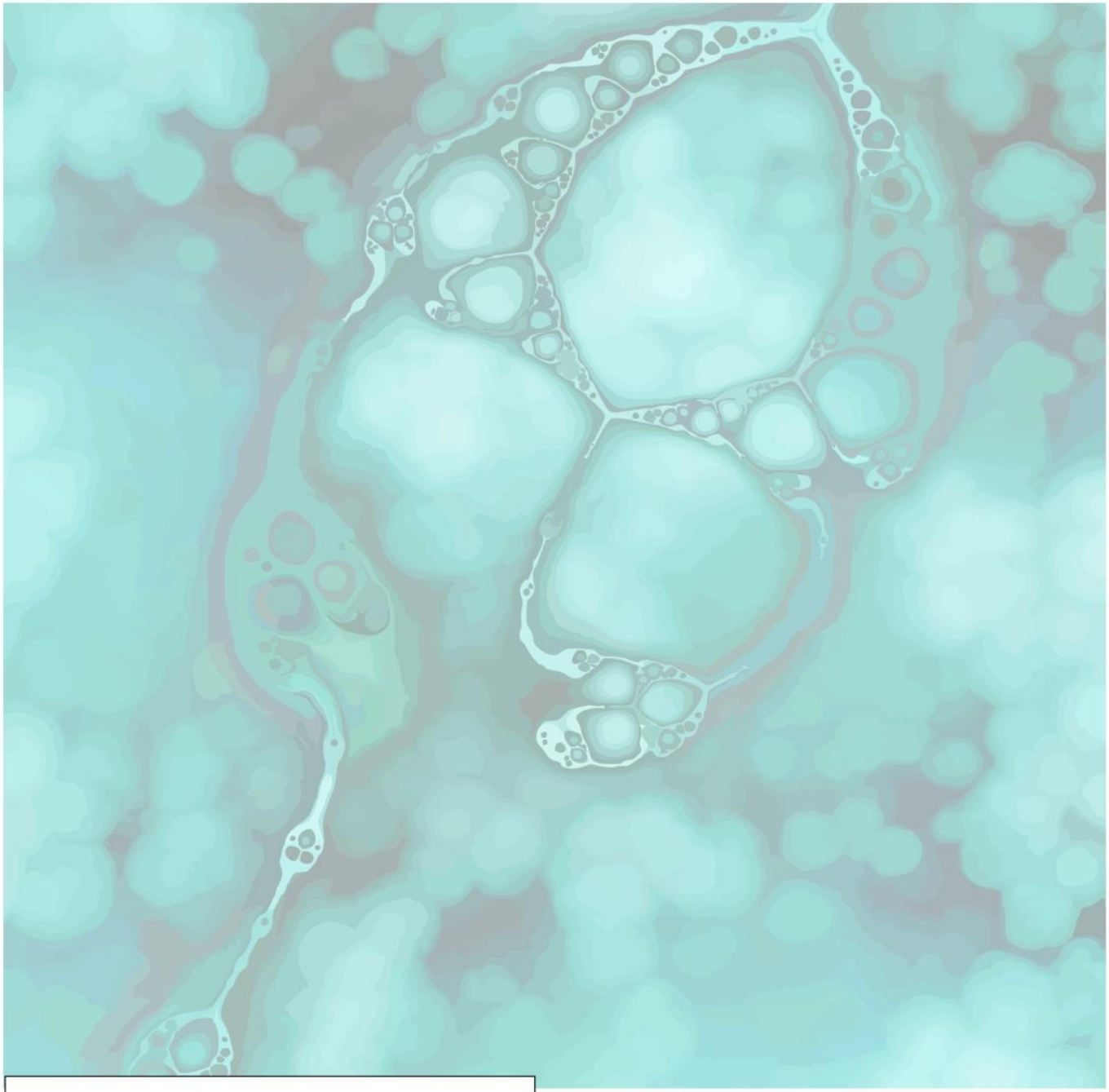

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