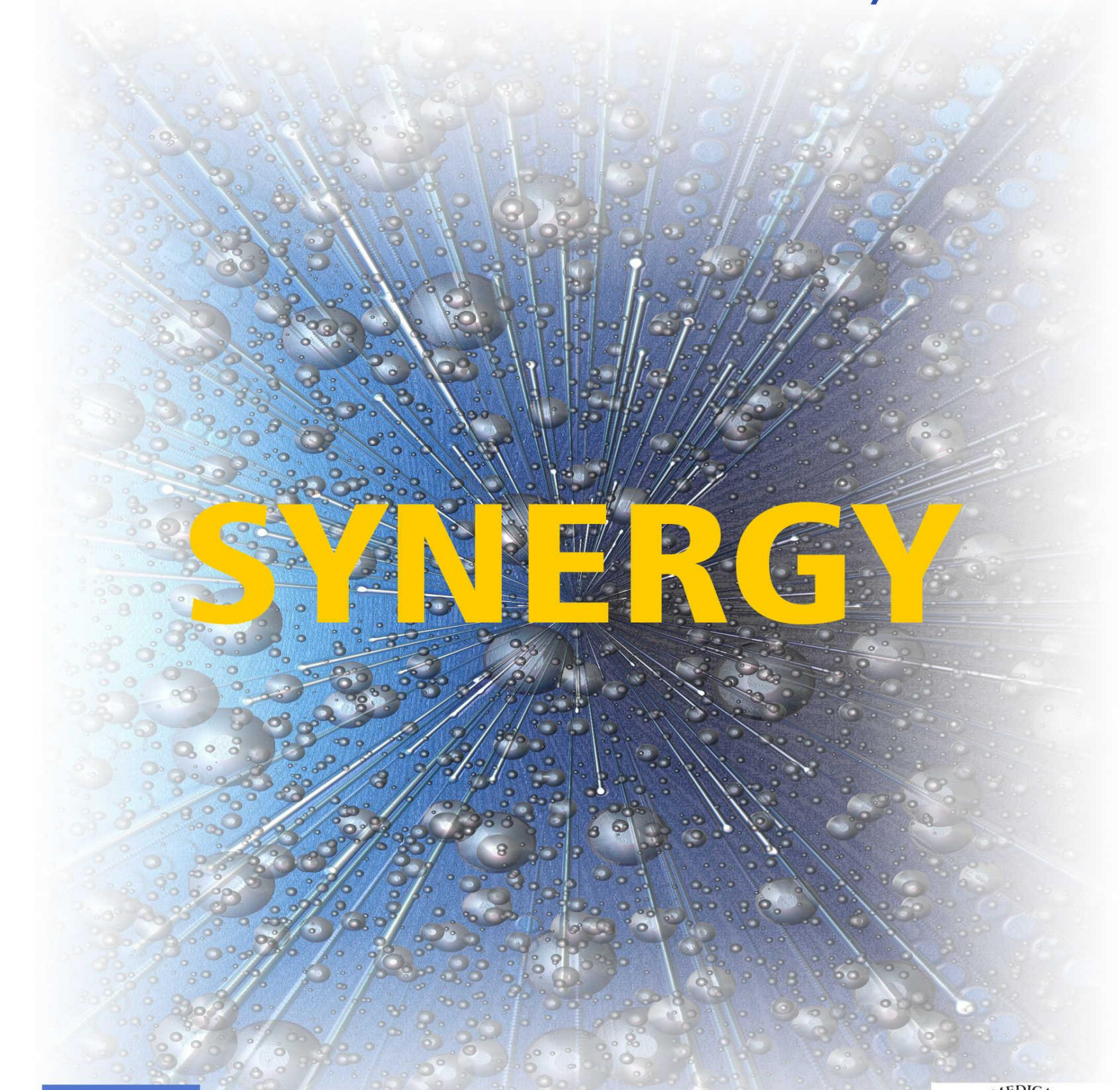
ACCORD

INTERDISCIPLINARY CONFERENCE ON DRUG SCIENCES

Warsaw
May 26-28 2022



WARSZAWSKI UNIWERSYTET MEDYCZNY

ABSTRACT BOOK

UNIVERSITAS MEDICA VARSOVIENSIS
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FACULTAS PHARMACEUTICA

INTERDISCIPLINARY CONFERENCE ON DRUG SCIENCES

Warsaw
May 26-28 2022

WYDARZENIE INFARMA 5214 CERTYFIKOWANE

Interdisciplinary Conference on Drug Sciences ACCORD 2022

Spełnia kryteria INFARMY

Warszawa, 26 maja 2022

ACCORD INTERDISCIPLINARY CONFERENCE ON DRUG SCIENCES

ABSTRACT BOOK

Warsaw
May 26-28 2022

ISBN 978-83-7637-586-1

Cover design and artwork
Paulina Dąbrowska-Dorożyńska

Welcome words

Dear Participants, Dear Friends,

It is our pleasure to invite you to the Accord – interdisciplinary conference on drug sciences which will take place from May 26 to 28, 2022, Faculty of Pharmacy, Medical University of Warsaw. We are excited to host this meeting.

The motto of the conference is "Synergy of interdisciplinary innovations". One of the key goals of the conference is to promote interactions between academic researchers, applied scientists and industry in order to develop innovative solutions for drug sciences. Your participation in this conference is pivotal to promoting the translation of scientific research into pharmaceutical applications. Please come and join us at the Accord Conference to share your ideas and discover our hospitality.

Joanna Kolmas, Ph.D., D.Sc.
Dean – Faculty of Pharmacy
The Medical University of Warsaw

The pharmaceutical industry belongs to the group of science-driven areas of economy together with other industrial branches i.e. aerospace, electronics, advanced optics, robotics, and advanced materials. This means that research is the main source of competitive advantages for pharmaceutical companies. At the same time, the cooperation between academia and the pharmaceutical industry remains not fully recognized. Nevertheless, this interface is an important area and brings the potential to emerge the positive synergies, which could be turned into fruitful collaborations and mutual projects.

The main problem in collaboration between different societies is the common language, which facilitates communication. The last uncertain years and especially the pandemic period have created a belief that there is an urgent need to create a space where representatives of two different worlds could meet to talk and get to know each other. That's how the idea of the ACCORD Conference occurred. The main theme of the conference is "SYNERGY", which is why we invited both leading scientists and industry leaders to share their achievements and experiences of working at the interface between academia and industry.

It is my deep belief that the Conference will contribute to closer cooperation between industry and academia.

Przemysław Dorożyński, Ph.D., D.Sc.
Chair
The Scientific Committee

I would like to welcome warmly all Participants of the Interdisciplinary Conference on Drug Sciences ACCORD 2022. The topic of the conference, 'Synergy of interdisciplinary innovations', underlines current challenges in the field of pharmacy not only in Poland but all over the world – the challenges that were dramatically emphasized by the coronavirus pandemic. After months of lockdowns and sanitary restrictions, it will be my great pleasure to host you in person in a vibrant Banacha Campus during the trees-blooming May. I hope that the atmosphere of the location together with notable lectures, fruitful discussions and opinion-forming panels organised during the Conference will significantly contribute to the development of the field. However, an in-person organized conference will provide also an opportunity to recognize our Capital City and to learn Polish hospitality. I would like to wish all Participants successful meetings and an enjoyable time at the Medical University of Warsaw.

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Piotr Luliński, Ph.D., D.Sc.
Chair
The Organizing Committee

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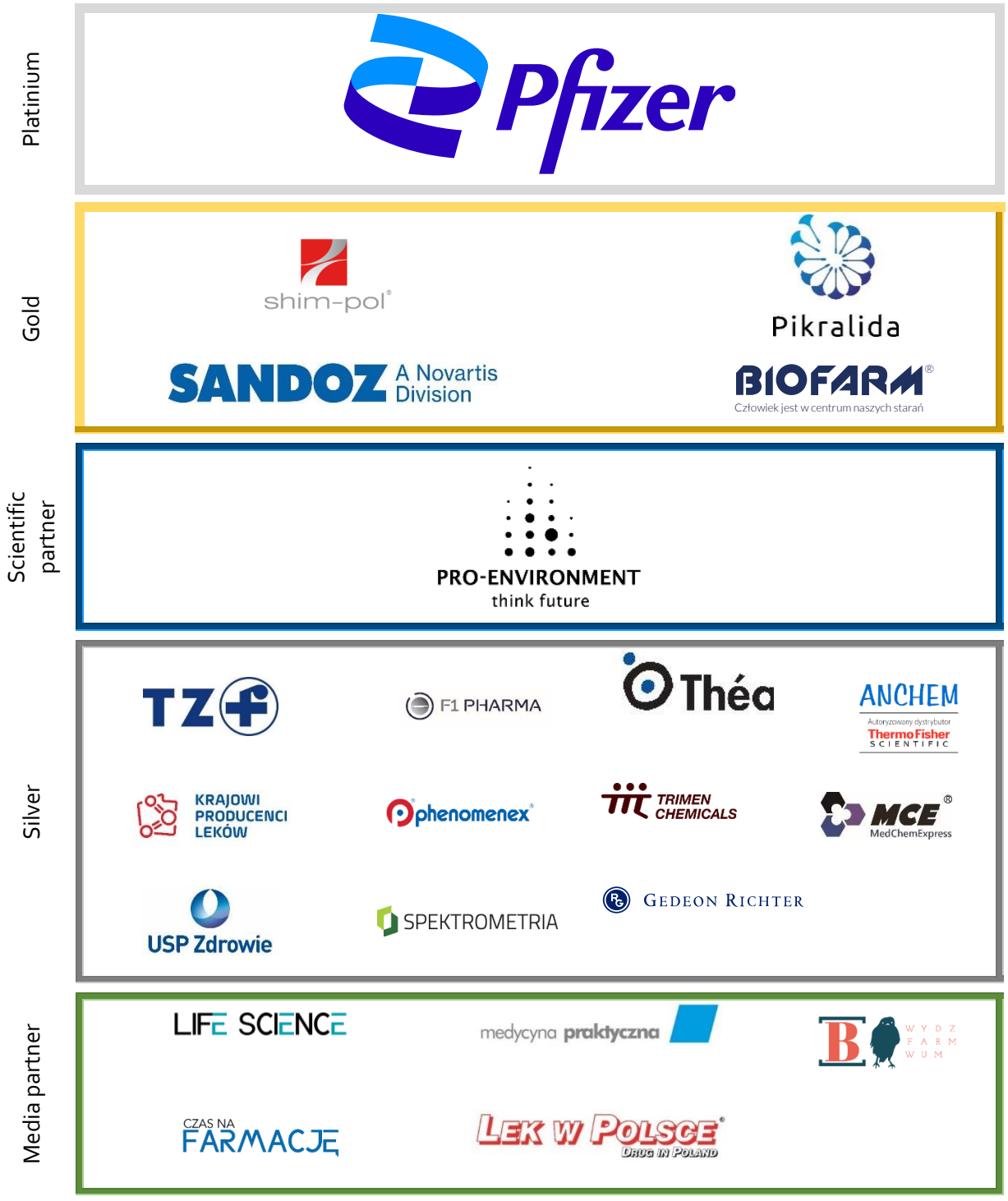

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6.	Prof. Marcin Drąg	Sanford Burnham Prebys Medical Discovery Institute, USA
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8.	Prof. Eniko Kallay	Medical University of Vienna, Austria
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15. Prof. Marcin Sobczak — Medical University of Warsaw
16. Prof. Jadwiga Turło — Medical University of Warsaw, Committee on Therapeutic and Pharmaceuticals Science PAN
17. Prof. Piotr Wroczyński — Medical University of Warsaw
18. Prof. Paweł Zajdel — Jagiellonian University Medical College, Cracow

Scientific Programme

IL	Invited Lecture
SC	Short Communication
SP	Short Presentation
SL	Sponsored Lecture
PPP	Printed Poster Presentations
PSP	Poster Spotlight Presentations
	Other activities

Thursday, May 26th, 2022

12:00	Registration & lunch	
Session 1 - chairpersons: E. Pindelska, P. Dorożyński		
13:30	Opening ceremony & Biniecki Awards presentation	
14:15	IL.1	Z. Derewenda *On the unique role of C-H….O hydrogen bonds in drug-protein interactions*
14:55	IL.2	M. Drąg *Activity profiling of viral proteases from SARS-CoV-2*
15:35	Coffee break	

Session 2 - chairpersons: M. Bujalska-Zadrożny, A. Kutner

16:00	IL.3	E. Kallay *The value of stereo-specific allosteric modulators in unravelling extracellular calcium-sensing receptor signalling*
16:30	IL.4	G.Brown *Retinoic acid receptor antagonists - targeting cancer stem cells*

17:00-19.00	Welcome Reception
17.15 - 18:15	Sponsored workshop *High Content Screening Imaging* and Reception (meeting at Reception Desk at 17.00)

Friday, May 27th, 2022

8:30		Registration & coffee

Session 3 - chairpersons: E. Pindelska, T. Pawiński

9:00	IL.5	D. Braga *The relevance of crystal forms in the pharmaceutical field - Damocle's sword or innovation tools*
9:30	IL.6	G. Stanisz *Metabolic MRI in cancer and neurological applications*
10:00	IL.7	R. Bucki *Shaping the antimicrobial action of gold nanoparticles*
10:30	IL.8	S. Bergan *Why patients respond differently to drugs - and ways to improve treatment*
11:00		Coffee break

Session 4 - chairpersons:
A. Kiss, S. Granica

11:30 IL.9 J. Rollinger
Harnessing complementarity for the discovery of drug leads from nature

12:00 IL.10 M. Melzig
Plant micro RNAs as active substances of plant extracts

12:30 SP.1 O. Kelber
Synergy of interdisciplinary innovations

Session 5 - chairpersons:
P. Zajdel, M. Pisklak

11:30 IL.11 L. Kaczmarek
Matrix metalloproteinase-9, MMP-9 in development of epilepsy as a novel drug target

12:00 SL.1 (Pfizer) J. Jemielity
Twelve years in twelve months - from inspiration to innovation

12:30 SL.2 (Shim-pol) M. Gawryś
HPLC method development according to Analytical Quality by Design using the Method Development Software

12:45 Lunch

Session 6 - chairpersons: P. Dorożyński, P. Zajdel

13:30 Discussion panel
M.Drąg, T.Grabowski, J. Jemielity, M.Winiarska, R.Kamiński, O.Plettenburg, K.Pyrć, L.Tajber
Synergy in drug development. Science - industry crosstalk. Is it feasible?

Poster Session 1 - chairpersons: J. Turło, S. Michorowska

15:00 PSP.01 - PSP.16 Poster Spotlight Presentations

16:00 PPP.001 - PPP.054 Printed Poster Presentations

16:00 Coffee break

Session 7 - chairpersons:
O. Demchuk,G. Nowicka

17:00 SL.3 (Biofarm) J.Pieczuro
Calcifediol - a new approach in the management of vitamin D deficiency

17:20 SC.1 P. Czeleń
The development of new isatin derivatives as promising competitive inhibitors of CDK-2 and GSK-3B

17:30 IL.12 V. Gubala
Implantable hydrogels, infused with vasoactive peptides attracts and trap aggressive glioblastoma cells

17:45 SC.2 K. Dziedzic
RVU330 Best-in-class dual A2A/A2B adenosine receptor antagonist

17.55 SL.4 (Dynamic Biosensors) K. Popova
Biophysical characterization of antibodies interacting with cells captured in microfluidic cell traps

Session 8 - chairpersons:
M. Czerwińska, M. Wrzosek

17:00 SL.5 (Sandoz) J. Feldman
Biosimilars biological drugs - from registration to use in everyday practice

17:20 SC.3 E. Juszczyk
Effect of food on human pharmacokinetics of CPL207280 - a new GPR40 agonist

17:30 SP.2 National Biniecki Awardee Lecture

17:45 SP.3 National Biniecki Awardee Lecture

20:00 Conference dinner at Sound Garden Hotel

Saturday, May 28th, 2022

8:30 Registration & coffee

Session 9 - chairpersons: M. Wróbel, M. Dawidowski

9:00 IL.13 K. Pyrć
You cannot squeeze blood out of a turnip: Antivirals during the pandemic

9:30 IL.14 O. Plettenburg
Drug discovery in Academia

10:00 IL.15 G. Popowicz
Opening the right locks with the right keys: How to efficiently employ structural biology for drug discovery purposes

10:30 IL.16 S. Polak
How science-fiction became science-reality - the fascinating story of clinical trials virtualization

11:00 Coffee break

Session 10 - chairpersons: W. Maruszak, J. Giebułtowicz

11:30 IL.17 L. Tajber
Anti-crystal engineering - a new catch phrase or an efficient approach to improve solubility of pharmaceutical molecules?

12:00 IL.18 J. Paszkowska
Biopredictive dissolution testing of oral medicines

12:30 IL.19 M. Alhnan
On demand manufacturing of oral dosage forms: a focus on multi-material and FDM 3D printing

13:00 Lunch

Poster Session 2 - chairpersons: M. Sobczak , M. Sobiech

14:00 PSP.17 - PSP.33
Poster Spotlight Presentation

15:00 PPP.055 - PPP.110
Printed Poster Presentations

Closing Session - chairpersons: P. Dorożyński, J. Kolmas

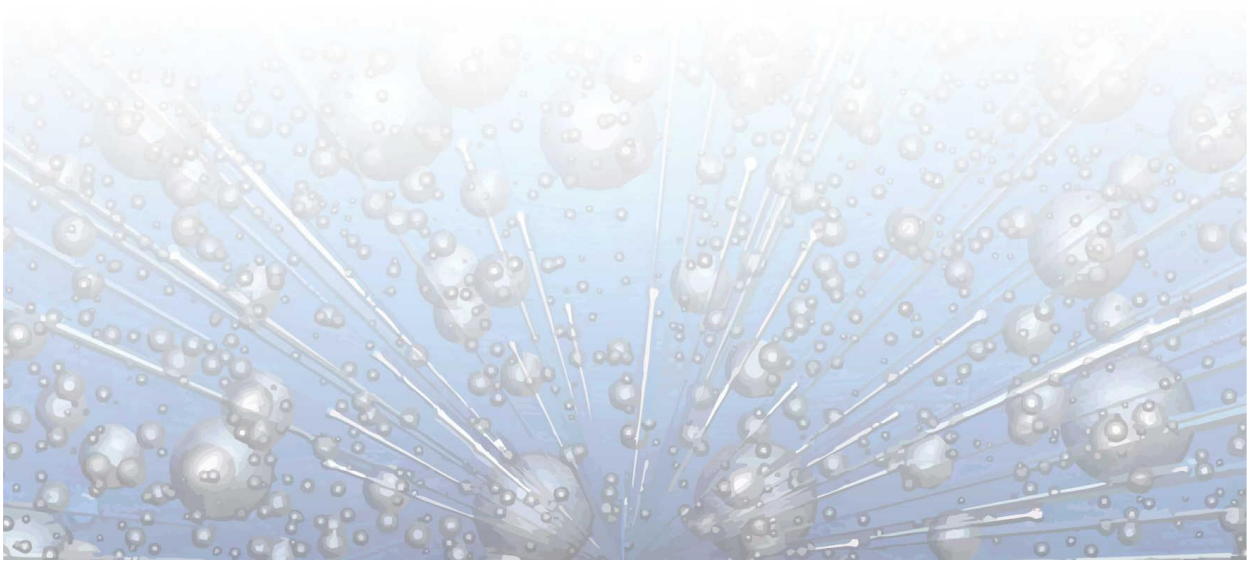
16:00 Closing ceremony and poster awards presentation

ACCORD INTERDISCIPLINARY CONFERENCE ON DRUG SCIENCES

Keynote Speakers

&

Panelists



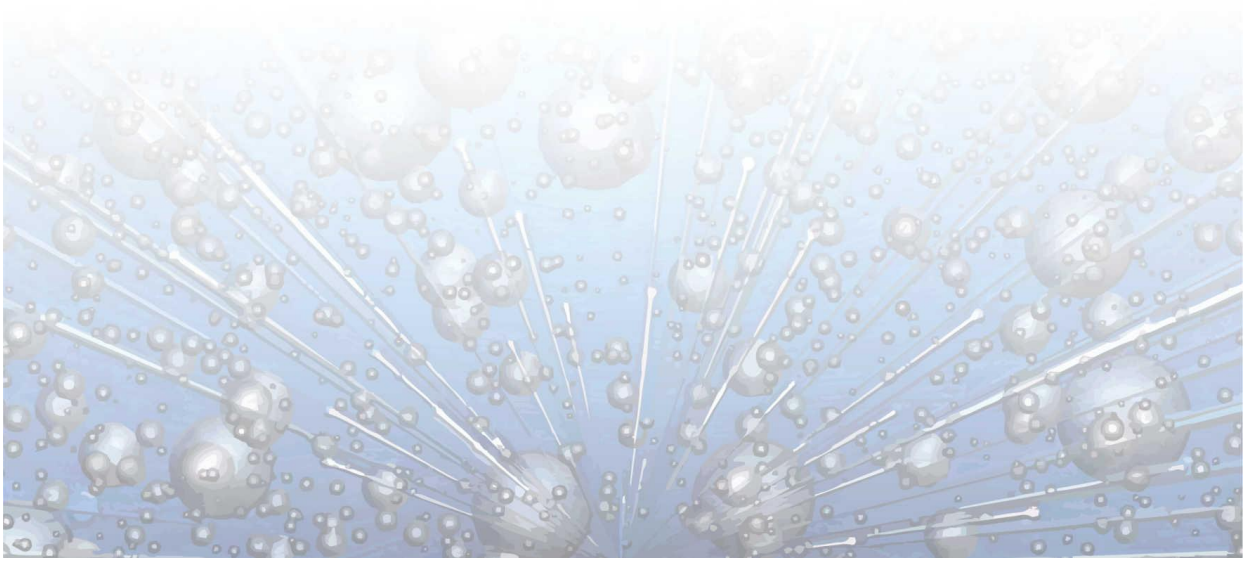
Zygmunt Derewenda

PhD, DSc, Professor; University of Virginia School of Medicine, Department of Molecular Physiology and Biological Physics

Dr. Derewenda has been trained as a macromolecular crystallographer and his filed on interest is protein structure and function, and protein-protein and protein-ligand interactions. He co-authored seminal papers about the mechanism of haemoglobin oxygenation and a number of pioneering structure determination of novel enzymes, such as lipases and esterases and select viral proteins He developed a new approach to protein and protein-ligand crystallization, used by many pharmaceutical companies. His current interest is in the molecular regulation of smooth muscle contraction.

Title of the lecture presented at the conference:

"On the unique role of C-H….O hydrogen bonds in drug-protein interactions".



Marcin Drąg

Professor; Department of Chemical Biology and Bioimaging, Faculty of Chemistry, Wroclaw University of Science and Technology, Poland and Tumor Microenvironment and Cancer Immunology Program, Sanford Burnham Prebys Medical Discovery Institute, La Jolla, CA, USA

Marcin Drąg was born in Świdnica (Poland) in 1975. He earned his M.Sc. degree from Department of Chemistry at University of Wroclaw in 1999. Next, he moved to Department of Chemistry at Wroclaw University of Science and Technology, where he earned his Ph.D. in organic and bioorganic chemistry working on new inhibitors of metallo- ad cysteine proteases under supervision of prof. Pawel Kafarski. His Ph.D. thesis was awarded the best thesis in organic chemistry by Polish Chemical Society and Sigma-Aldrich (2004). In 2003 he was appointed Assistant Professor at Wroclaw University of Science and Technology and shortly after (2004) adjunct position. In years 2005-2008 he conducted postdoctoral research at The Burnham Institute for Medical Research in La Jolla, CA (USA) in prof. Guy Salvesen laboratory. During post-doc he explored protease-driven pathways involved in apoptosis, infection, inflammation, and ubiquitin signaling. After coming back to Poland, in 2011 he received Doctor of Sciences Degree in chemistry (habilitation) for work on new types of combinatorial libraries to investigate proteolytic enzymes. In 2016 he received Professor title in chemistry from President of Poland. In 2019 prof. Drąg has received the 2019 Foundation for Polish Science Prize in the field of chemical and material sciences for developing a new technological platform for obtaining biologically active compounds, in particular proteolytic enzymes inhibitors.

Title of the lecture presented at the conference:

"Activity profiling of viral proteases from SARS-CoV-2".

Enikö Kállay

Ph.D., Assoc. Prof.; Department of Pathophysiology and Allergy Research, Center of Pathophysiology, Infectiology & Immunology, Medical University of Vienna, Austria

The goal of the Tumour Pathology Research Group is to understand and unveil cellular and molecular mechanisms that play a role in the prevention and pathogenesis of colorectal cancer and other chronic or malignant diseases. We are studying the role of vitamin D and dietary calcium in this process.

Our current major research interests include the examination of calcium-dependent anti-tumor molecular mechanisms; the role of the calcium-sensing receptor (CaSR) and its pharmacological modulators in colorectal carcinogenesis; identification of genetic and epigenetic mechanisms that regulate the action of calcium and vitamin D in tumourigenesis; investigation of the vitamin D system as a physiological defense against tumor progression; the impact of the vitamin D catabolizing enzyme 25 hydroxyvitamin D 24 Hydroxylase on colon tumourigenesis.

Title of the lecture presented at the conference:

"The value of stereo-specific allosteric modulators in unravelling extracellular calcium-sensing receptor signalling".

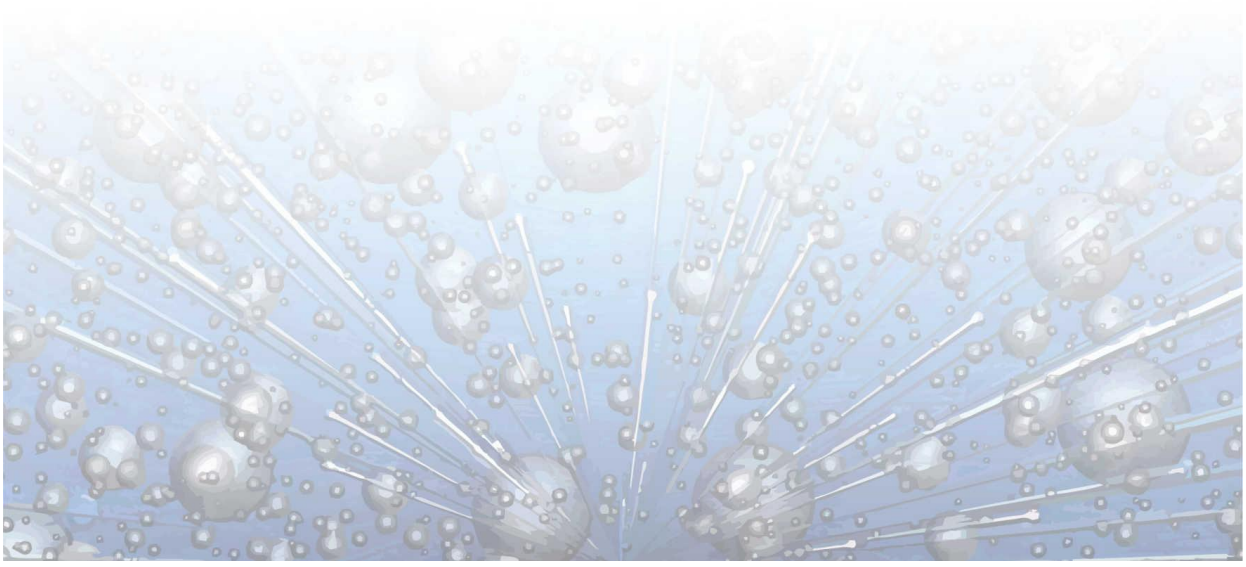
Geoffrey Brown

Ph.D., Associate Member Institute of Immunology and Immunotherapy; Institute of Clinical Sciences, College of Medical and Dental Sciences, University of Birmingham, UK

Geoffrey Brown received a BSc from Queen Elizabeth College, London and a PhD from University College (with Mel Greaves), London. Postdoctoral research was at the MRC Immunochemistry Unit (with Alan Williams and Rodney Porter) and the Nuffield Department of Clinical Medicine (in Sir David Weatherall's Department), Oxford, where he was also IBM Fellow, University of Oxford and Research Lecturer, Christ Church College. He is now Reader in Cellular Immunology, College of Medical and Dental Sciences, University of Birmingham. His research concerns the development of blood cells.

Title of the lecture presented at the conference:

"Killing cancer stem cells".



Dario Braga

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

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.

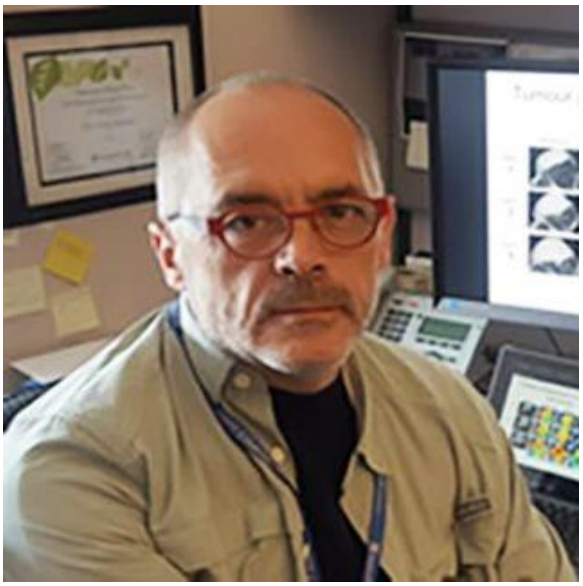
He is recipient of the Raffaello Nasini medal awarded by the Italian Society of Chemistry, the Federchimica prize, and the Gold Medal of the Italian Crystallographic Association. Dario Braga is currently Member of the Science Institute of the Academy of Science and President of the Institute of Advanced Studies of the University of Bologna.

Title of the lecture presented at the conference:

"The relevance of crystal forms in the pharmaceutical field – Damocles sword or innovation tools".

Greg Stanisz

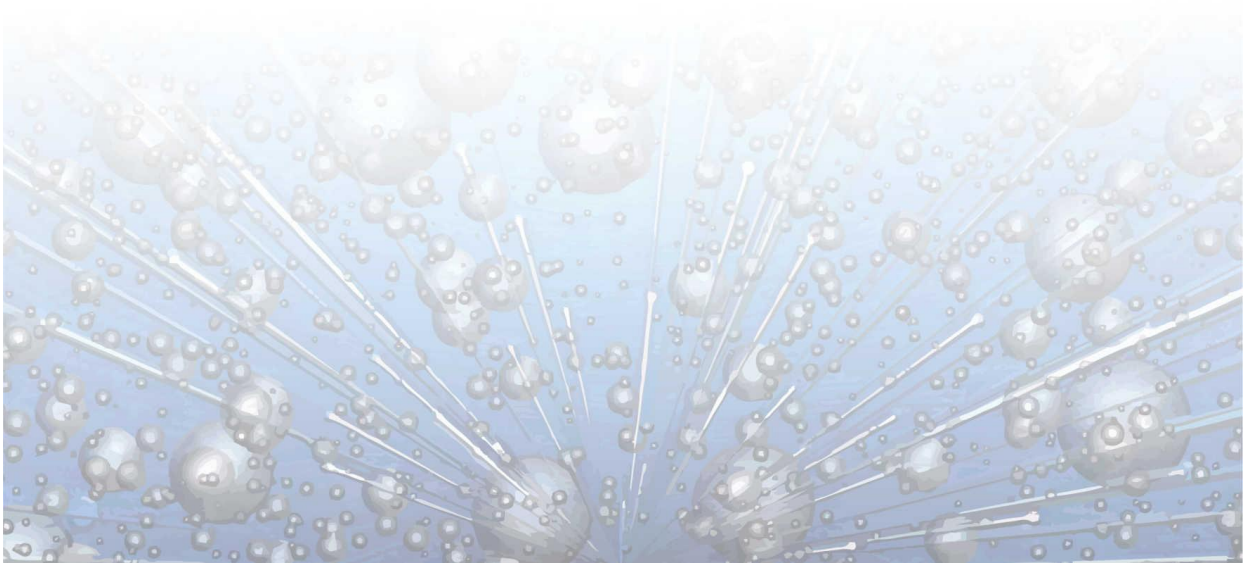
Professor, Sunnybrook Health Sciences Centre, Medical Biophysics, University of Toronto, Canada



Greg Stanisz, Ph.D., is a Senior Scientist at Sunnybrook Research Institute in Toronto, Canada, and a Professor at the Department of Medical Biophysics, University of Toronto. He received his Ph.D. in physics at the Jagellonian University, Cracow, Poland. The goal of his studies is to develop magnetic resonance imaging (MRI) methods to improve the specificity and sensitivity of non-invasive image methods in characterizing tissue pathology. He is using MRI to evaluate tissue microstructure and metabolism in a variety of different disorders. He is particularly interested in whether nuclear MR measurements can be used to evaluate the processes involved in central nervous system pathologies. He is also using MRI to monitor cancer therapies in preclinical models of cancer and patients. In particular, his group is interested in evaluating changes in tumor microstructure and metabolism and the processes of apoptosis using combined MRI and MR spectroscopy.

Title of the lecture presented at the conference:

"Metabolic MRI in cancer and neurological applications".



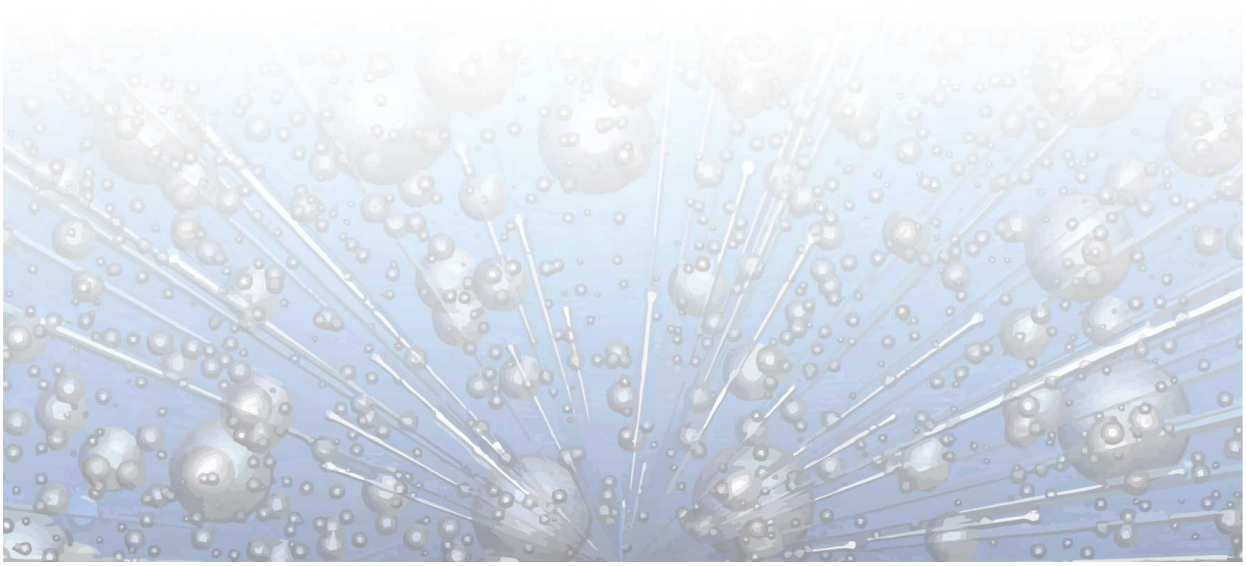
Robert Bucki

Professor; Head of the Department of Medical Microbiology and Nanobiomedical Engineering, Faculty of Medicine, Medical University of Bialystok, Poland

Robert Bucki is a professor of medicine at Medical University of Białystok where he studies aspects of lipid biology and the effects of polyelectrolytes on antibacterial agents. The areas of his scientific interests are: medical microbiology, nanomedicine, development of new antimicrobial molecules and nano-systems, rheological studies of animate matter. He received his M.D. and Ph.D. in physiology from the Medical University of Białystok and did postdoctoral work at the University Paris-Sud in Orsay, France and Institute for Medicine and Engineering at the University of Pennsylvania, USA.

Title of the lecture presented at the conference:

"Shaping the antimicrobial action of gold nanoparticles".



Stein Bergan

Professor II at the Department of Pharmacy Senior scientist at the section of Clinical Pharmacology; Oslo University Hospital, Rikshospitalet

His experience ranges from analytical work mainly with HPLC analysing narrow index drugs in clinical samples to current involvement in pharmacogenetics plus communication with the clinicians regarding application of TDM. He is also leader of a research group with focus on immunosuppressive therapy, statins, some cancer treatments plus aspects of pharmacogenetics. In relation to his professorship, he teaches students in pharmacy, mostly postgraduate, as well as continuing education courses for medical doctors. He has supervised nine PhD candidates and a number of master students. His list of publications now counts just above one hundred papers in peer-reviewed journals. For many years prof. Bergan has served as member of editorial boards, in particular for the journal Therapeutic Drug Monitoring. He is also a long-standing member of the IATDMCT and is currently chair of the Scientific Committee for Immunosuppressive Drugs in this society.

Title of the lecture presented at the conference:

"Why patients respond differently to drugs – and ways to improve treatment".

Judith Rollinger

Professor; Department of Pharmacognosy, University of Vienna, Austria

Dr. Judith M. Rollinger is a full professor in Pharmacognosy & Pharmaceutical Biology and Head of Phytochemistry & Biodiscovery Lab at the Department of Pharmaceutical Sciences, Faculty of Life Sciences, University of Vienna, Austria. The Lab is using interdisciplinary approaches to identify, analyze, and purify multi-component mixtures from plant, fungal, or marine sources focusing on the characterization of their bioactive secondary metabolites.

The major scientific interests include:

- unravelling nature's complexity to seek for novel drug leads from nature by using integrated chemoinformatic and chemometric approaches
- discovery of bioactive compounds from medicinal plants of ethnopharmacological sources by means of in silico techniques (molecular modelling, virtual screening) and phenotypic screenings using the model organism Caenorhabditis elegans.
- research on bioactive metabolites from diverse natural sources with special emphasis on natural leads against inflammatory processes, metabolic syndrome, and viral infections/influenza and potential cardiotoxic compounds in commonly consumed botanicals.

Professor Rollinger used to be a Member of Board Directors of the Society of Medicinal Plants and Natural Product Research (GA), Associate Editor of Frontiers in Pharmacology, Speciality Ethnopharmacology, Editor of Planta Medica, Vice President of the Society of Medicinal Plants and Natural Product Research (GA), Senator of the University of Vienna, and President of the Society of Medicinal Plants and Natural Product Research (GA).

Title of the lecture presented at the conference:

"Harnessing complementarity for the discovery of drug leads from nature".

Matthias Friedrich Melzig

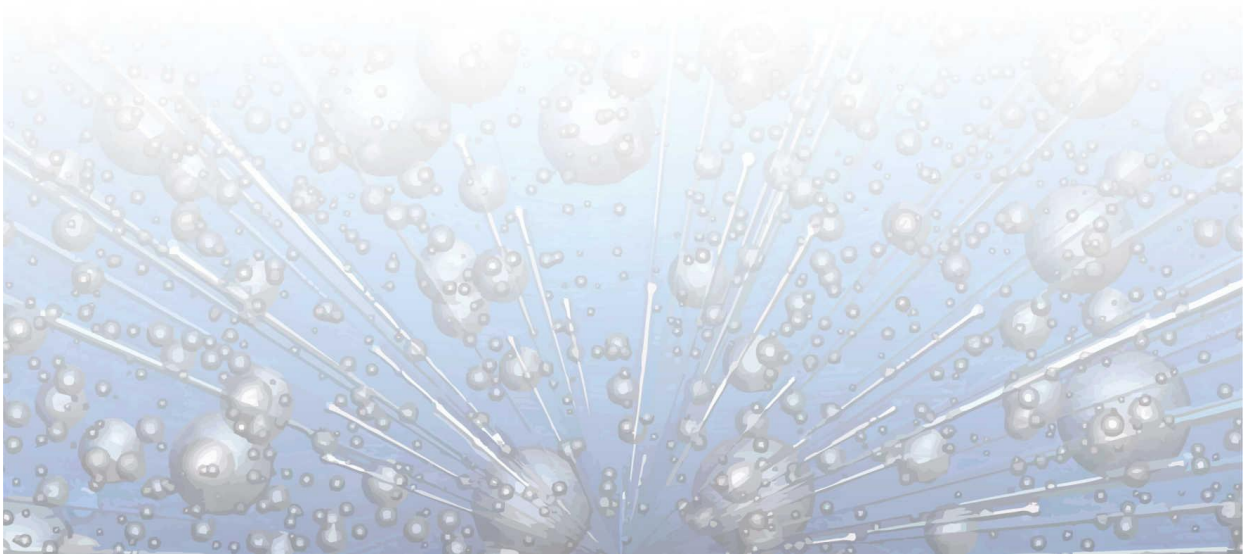
Prof. Dr. habil. Dr. h.c.; Freie Universitaet Berlin, Institute of Pharmacy, Berlin, Germany

Research topics are investigations in the biological and pharmacological activity of secondary products from plants using biochemical, cell and molecular biological methods. One focus was directed at inhibition of enzymes included in metabolism of neuropeptides or involved in immunological mechanisms, as well as plant-based inhibitors of enzymes within the digestion process. A second field of research was concerned with synergistic interactions between different natural products, e.g. saponins and toxic lectins regarding their toxicity, as well as components of essential oils and antibiotics regarding their antibacterial activity. Results of research are ap. 240 publications, 250 lectures and 3 patents.

The Faculty of Pharmacy of the Medical University Warsaw awarded me the honorary doctorate in 2018.

Title of the lecture presented at the conference:

"Plant microRNAs as active substances of plant extracts?"



Leszek Kaczmarek

Professor Head of the Department of Molecular and Cellular Neurobiology, The Marceli Nencki Institute of Experimental Biology of the Polish Academy of Sciences

Leszek Kaczmarek is a member of the Polish Academy of Sciences, European Molecular Biology Organization (EMBO) and Academia Europaea. His major research achievements include (i) discovery of c-Myc protein role in the regulation of the cell cycle; (ii) discovery of the learning-related gene (c fos) expression in the mammalian brain; (iii) revealing apoptotic component of excitotoxicity in the adult brain; (iv) discovery of a critical role of cyclin D2 in the adult brain neurogenesis; (v) discovery of the involvement of matrix metalloproteinases in synaptic plasticity, learning and memory, epileptogenesis, alcohol addiction and schizophrenia; (vi) defining the role of the central amygdala in appetitive learning and memory. He has published over 250 research papers, cited over 12 000 times. L. Kaczmarek was invited as a lecturer to more than 100 international and national meetings and over 300 times to talk on research seminars, workshops, etc.; he promoted over 40 PhDs and was either PI or coordinator on over 50 domestic and international grants. He was a postdoc at the Temple University (Philadelphia, USA), as well as visiting professor at the: University of Catania (Italy), McGill University (Montreal, Canada), UCLA (Los Angeles, USA), and the Institute of Photonic Sciences, ICFO, (Castelldefels, Spain). He served on numerous program and grant committees, editorial and advisory boards, as well as authorities of Polish and international scientific societies and organizations.

Kaczmarek studies focus on a question of brain-mind connection: how does the brain produce mind? Originally, in the middle of 80-ies, he and his colleagues have discovered that learning experience activates gene expression in the brain, and the very first such gene they have identified was found to encode a c-Fos protein, a transcriptional regulator. They have followed with revealing that genes encoding TIMP-1 (tissue inhibitor of matrix metalloproteinase) and MMP-9 (matrix metalloproteinase) are c-Fos targets in activated neurons. Then, they have shown that both TIMP-1 and MMP-9 may operate as an extracellular, synaptic system exerting control of efficacy and morphology of excitatory synapses.

Title of the lecture presented at the conference:

"Matrix metalloproteinase-9, MMP-9 in development of epilepsy as a novel drug target".

Vladimir Gubala

Ph.D.; Chemistry and Drug Delivery, Medway School of Pharmacy, University of Kent and Vital Signs Solutions, UK

Dr Vladimir Gubala obtained his B.Sc. and M.Sc. degrees in organic chemistry from the Slovak University of Technology, Bratislava, in 1999 and 2001 respectively. He then moved to the University of Puerto Rico, Rio Piedras, where in 2006 he received his Ph.D. in chemistry. His research focused on self-assembly of novel nanostructures and supramolecular chemistry. After a postdoctoral position at the University of Florida, he joined the research team at the Biomedical Diagnostics Institute (BDI) at Dublin City University (DCU) in 2007. He worked at BDI for nearly 5 years, managing a group of scientists working on novel surface chemistry approaches for the design of low cost diagnostics platforms. He started his appointment at the Medway School of Pharmacy in January 2012, where he developed his research, which now focuses on materials science, bio-organic and analytical chemistry and nanotechnology. His scientific interest in academia include the development of novel multimodal carriers for targeted drug delivery based on silica nanoparticles and the development of novel, implantable material for the treatment aggressive brain cancer. In 2015, he co-founded a diagnostics company called Vital signs Solutions Ltd. He employs a small group of scientist working on the next generation of point-of-care test to monitor and detect series of biomarkers, which is aimed to empower people to make informed choices about their lifestyle and prevent many conditions and diseases. In 2020, the company has developed a new, antibody-based Covid-19 finger prick test in collaboration with Biosure, The test is currently in clinical trials testing and it will be available on the market in early 2021. Vladimir has been a member of IUPAC since 2011, he is currently a secretary of Division VII – Chemistry and Human Health and a Chair of Interdivisional Subcommittee of Materials Chemistry ISMC).

Title of the lecture presented at the conference:

"Implantable hydrogels, infused with vasoactive peptides attracts and trap aggressive glioblastoma cells".

Krzysztof Pyrć

Professor; Laboratory of Virology of the Malopolska Centre of Biotechnology, Jagiellonian University, Cracow, Poland

Virologist. Graduated from Jagiellonian University (M.Sc.), University of Amsterdam (Ph.D.), and University of Lodz (habilitation).

In January 2019 received the title of Professor in the field of biological sciences. Head of the Laboratory of Virology at the Malopolska Centre of Biotechnology, Jagiellonian University. A certified project manager. Leader of Virogenetics research group, supervisor, and promoter of students and Ph.D. students. Authored over 100 publications in journals as Nature Medicine, Science Translational Medicine, PNAS, PLoS Pathogens, Journal of Virology, and Science Signaling, which have been cited nearly four thousand times in the world literature. Reviewer and expert in science funding institutions in Poland and abroad (Horizon2020, ERC, FNP, NCN, and others).

Author of several patent applications and patents, on the basis of which 2 spin-off companies have already been established (Acatavir and StartIt Vet). Manager and coordinator of numerous research grants, including under Horizon2020, IMI2, European Cooperation in Science and Technology, NCN, FNP, NCBIR, and MNiSW, as well as grants funded by commercial entities. Member of the advisory team of the Minister of Science and Higher Education for activities related to the prevention, counteraction, and eradication of COVID-19. Deputy chairman of the advisory team (COVID-19) to the President of the Polish Academy of Sciences, member of the scientific board of the initiative Science against Pandemic. Member of the Medical Council to the Prime Minister. Member of the Healthcare Council to the President of the Republic of Poland. Expert of the Agency for Health Technology Assessment and Tarification – AOTMiT in the field of SARS-CoV-2 therapy and diagnostics.

Title of the lecture presented at the conference:

"You cannot squeeze blood out of a turnip: Antivirals during the pandemic"

Oliver Plettenburg

Professor; Institute of Medicinal Chemistry, Helmholtz Zentrum München GmbH, German Research Center for Environmental Health

Oliver Plettenburg is Director of the Institute of Medicinal Chemistry at the Helmholtz Center for Environmental Health in Munich and Professor for Medicinal Chemistry at Leibniz Universität Hannover. Previously he held various positions within the pharmaceutical industry, last as Head of Biosensors & Chemical Probes in Sanofi's Diabetes Division. The group's main responsibility was to support evaluation of validity of novel targets, provide tools to visualize pathologically relevant processes, to develop new methods to quantify important biomarkers and to explore new treatment options at the drug-device interface.

Oliver was with Sanofi for 14 years. Before joining the Diabetes Division he worked as a project leader in several medicinal chemistry projects in the area of Diabetes and Cardiovascular Diseases and was deeply involved in the Chemical Biology approach within Aventis.

After receiving his PhD in organic chemistry, he joined The Scripps Research Institute as a postdoctoral fellow, working in the group of Chi-Huey Wong on the total synthesis of glycosyl sphingosides.

Title of the lecture presented at the conference:

"Drug discovery in Academia".

Grzegorz Popowicz

Ph.D.; Institute of Structural Biology, Helmholtz Zentrum München GmbH, German Research Center for Environmental Health

Dr. Popowicz's initial education was Medicinal Physics (MSc). Later, he focused on structural biology in the group of Professor Robert Huber (Nobel Prize in Chemistry 1988) at the Max Planck Institute for Biochemistry, Martinsried. He completed his doctorate in 2006 and continued research as a postdoctoral fellow under supervision of prof. Huber. He used X-ray crystallography, NMR and computational techniques to solve structures and explain function of proteins critical for health and linked to disease. His hallmark works include solving a structure of Insulin-like Growth Factor (IGF) Binding Proteins in complex with IGF and its receptors, the first published structure of MDMX oncoprotein and the development of peptidic and small molecule MDMX inhibitors. In 2012, he joined the Institute of Structural Biology at Helmholtz Zentrum München as a Senior Scientist. He organized a Structure- and Fragment-based Drug Discovery facility that integrated with HMGU drug discovery activities. He later became a group leader with a team focusing on structure-based drug discovery.

Title of the lecture presented at the conference:

"Opening the right locks with the right keys: How to efficiently employ structural biology for drug discovery purposes".

Sebastian Polak

Professor; Laboratory of Pharmacoepidemiology and Pharmacoeconomics, Pharmacy Department, Medical College, Jagiellonian University, Cracow, Poland and Certara UK

Dr. Sebastian Polak holds a tenured position at the Faculty of Pharmacy Jagiellonian University Medical College, Cracow, Poland (Professor in Biopharmacy) where he leads a multidisciplinary team of scientists and engineers working on applying various modelling and simulation approaches in drug development. Dr. Polak is also a Senior Scientific Advisor in Certara UK, part of an international Certara company. He led the development of the cardiac safety modelling and simulation system based on the biophysically detailed models of human cardiac myocytes operating at the population level (CSS – Cardiac Safety Simulator). Dr. Polak joined Simcyp (now Certara UK) in 2007 where he is a member of the Modelling & Simulation group involved in further development and improvement of the absorption and bioavailability model of the Simcyp Population-based Simulator. Among others, he leads dermal absorption module development. He has published over 80 peer-reviewed articles and has given invited presentations at national and international scientific meetings. He earned an MSc in pharmacy from the Faculty of Pharmacy at Jagiellonian University where he also received his PhD and DSc (biopharmacy).

Title of the lecture presented at the conference:

"How science-fiction became science-reality – the fascinating story of clinical trials virtualization".

Lidia Tajber

PhD/Associate Professor in Pharmaceutics and Pharmaceutical Technology; Trinity College Dublin, Ireland

Lidia's research programme concentrates on the fundamental understanding of molecular interactions governing the properties and performance of pharmaceutical substances at molecular, supramolecular and formulation levels:

- pharmaceutical ionic liquids (anti-crystal engineering)
- multicomponentpharmaceutical systems: salts, cocrystals and amorphous materials
- rational preformulation studies leading to quality solid-state formulations.

Lidia's work has been funded by Science Foundation Ireland, Enterprise Ireland, Irish Research Council as well as the European Commission. She is involved in the Synthesis and Solid State Pharmaceuticals Centre, a H2020 RISE network "ORBIS" (Open Research Biopharmaceutical Internships Support)and is a management committee member of COST Action CA18112 (Mechanochemistry for Sustainable Industry). She has published around 100 high quality peer-review papers and contributed to numerous international conference presentations. She is currently serving as Director of Research in the School of pharmacy and Pharmaceutical Sciences, Trinity College Dublin, Ireland.

Title of the lecture presented at the conference:

"Anti-crystal engineering – a new catch phrase or an efficient approach to improve solubility of pharmaceutical molecules?"

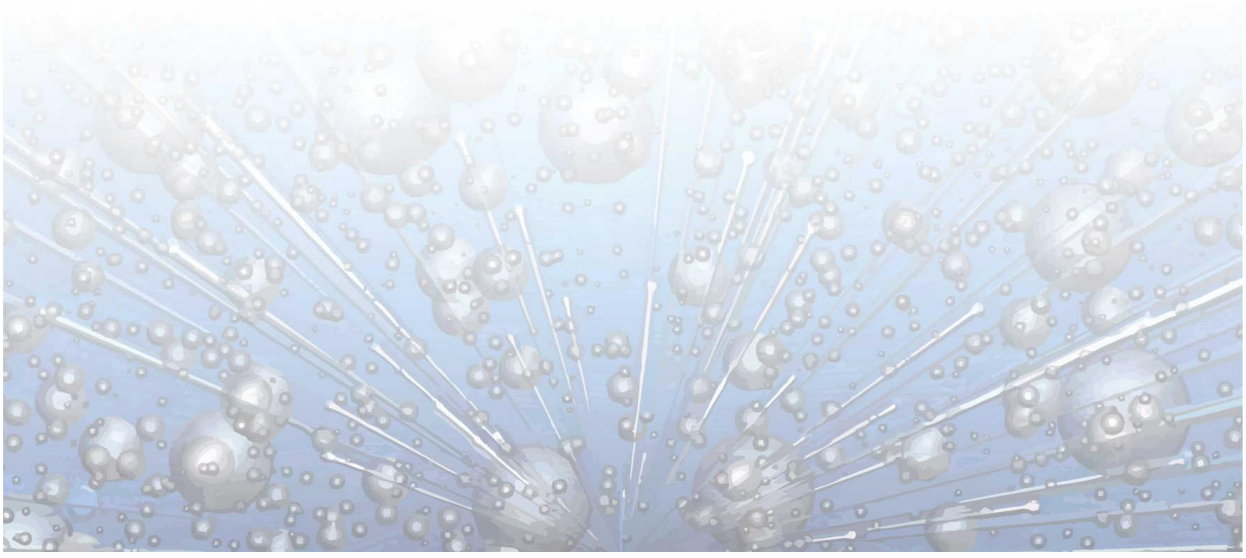
Grzegorz Garbacz

Ph.D., D.Sc.; Physiolution GmbH, Physiolution Polska

Grzegorz Garbacz was born in 1980 in Nysa, Poland. In the years 1999-2004, he studied Pharmacy at the Medical University of Wroclaw in Poland. In the year 2004, he joined the group of Prof. Werner Weitschies and started his work on the construction and optimization of bio-relevant test models for simulation of mechanic parameters of the gastro-intestinal passage of solid oral dosage forms. Working on this highly interesting topic he graduated from his studies in Pharmaceutical Sciences in 2005, Ph.D. in 2010, and habilitation (D.Sc.) in 2019. In the year 2009, he co-founded the pharmaceutical company Physiolution GmbH and in 2017 Physiolution Polska. His research is focused on the development of test devices capable of simulating the GI transit conditions, predictive dissolution testing of solid oral dosage forms, the development of analytical methods and test procedures as well as novel drug delivery devices.

Title of the lecture presented at the conference:

"Biopredictive dissolution testing of oral medicines".



Mohamed Albed Alhnan

Ph. D.; Institute of Pharmaceutical Medicine, King's College, London, UK

Mohamed A Alhnan is a Senior Lecturer in Pharmaceutical Medicine in the School of Cancer & Pharmaceutical Sciences. Mohamed's research focuses on applying the latest advances in material science and electronics in the pharmaceutical field. This research is an endeavour to bridge the gap between drug delivery and pharmaceutical technology on one hand and new design and production methods such as 3D printing on the other hand. The goal of this effort is to break current boundaries of oral drug delivery systems and formulation design and to link pharmacy to an increasingly smart and digitally connected environment of this age. This effort can enable the digitalisation of dosage form design and prototyping, innovation of new drug delivery systems that are responsive to biosensors and environmental changes as well as improving the personalisation of dosage forms.

His fundamental research has led to several world firsts; first example of using pharmaceutical grade polymers in FDM 3D printing, first 3D printed tablets to meet the US and British Pharmacopoeias for delayed release products, and first examples of 3D printing of liquid capsule. He introduced and patented the innovative concept of tablets of complex architecture as a solution for fast disintegration and dissolution. Mohamed is working with industrial and clinical partners on the manufacturing of 3D printed dosage forms. He is also collaborating with two global manufacturer of pharmaceutical coating on the scale-up and commercialising a next generation of coating solution of nutraceutical products.

Title of the lecture presented at the conference:

"Fused Deposition Modelling 3D printing: A Potential Point-of-Care Manufacturing Method for Personalised Dosage Forms".

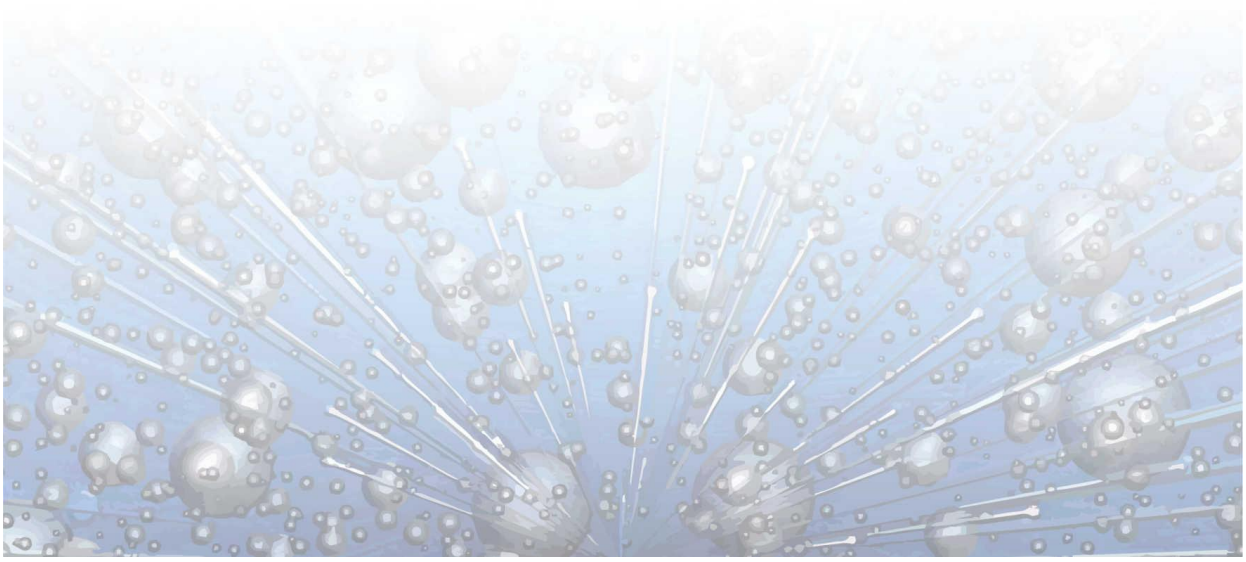
Olaf Kelber

Dr. rer. nat..; Bayer Phytomedicines Supply & Development Center Steigerwald Arzneimittelwerk GmbH, Bayer Consumer Health, Darmstadt, Germany

My field is all aspects of natural products of research, starting from analytical concepts, pharmacology and toxicology up to clinical research. In my function at the Phytomedicines Supply and Development Center of Bayer Consumer Health, as a board member of scientific societies (GA, www.gaonline.org and GPT, www.phytotherapy.org), and in expert groups, I engage for the international scientific exchange in the field of natural product research.

Title of the lecture presented at the conference:

"Synergy of interdisciplinary innovations".



Jacek Jemielity

Professor, Laboratory of Bioorganic Chemistry, Center for New Technologies, Warsaw, Poland

Prof. Jacek Jemielity is the head of the Laboratory of Bioorganic Chemistry at the Center for New Technologies. He received his PhD from the Department of Chemistry, the University of Warsaw in 2002, and in the same year, he started working as an assistant professor in the Division of Biophysics, IFD, Faculty of Physics, University of Warsaw. In 2020 he became a professor of chemistry. He is engaged in research on the synthesis, properties, and applications of chemically modified nucleotides. He develops methods to synthesize biologically important nucleotides, creates tools to modify nucleic acids useful in studies of genetic information expression and medical applications. He developed a method to obtain mRNA with properties necessary for therapeutic applications. His inventions are used in several clinical trials on cancer immunotherapy. He is the author of over 130 scientific publications in peer-reviewed scientific journals (including the most prestigious journals in the field such as Nucleic Acids Research, J. Am. Chem. Soc., Angewandte Chemie Int. Ed, RNA, Organic Lett., Chemical Science, Molecular Cell, Nature Structural & Molecular Biology, Nature Communications and others). His papers have been cited more than 2160 times. He has co-authored 6 patents protecting his inventions almost worldwide and several patent applications. One of these technologies involving mRNA modification is used in clinical trials for cancer vaccines by companies such as BioNTech, Sanofi, Roche, Pfizer. He is a scholarship holder of the "Polityka weekly" (2008) and winner of the President of Poland's Economic Award in the "research + development" category (2017), he was nominated in the competition organized by the European Patent Office "European Inventor Award" 2018 in the category "Research". In his career collaboration with talented young people fascinated by scientific research has always been very important. He is the co-founder and president of ExploRNA Therapeutics, a UW spin off company focused on developing mRNA modification technologies and developing innovative mRNA-based therapies.

Marta Winiarska

Managing Director of the Polish Union of Innovative Medical Biotech Companies BioInMed

Marta Winiarska – lawyer, manager, public relations and public affairs expert in healthcare area. A graduate of the law faculty at the Lazarski University (specialization in the field of intellectual property protection), as well as postgraduate studies in management at the University of Warsaw and the Warsaw School of Economics. She also completed postgraduate studies at Public Relations Academy.

For many years she is associated with economic and healthcare issues. At the beginning of her professional career, she was a journalist of the Polish Radio Trójka. In the following years, a spokesman, advisor, head of economic and healthcare departments in PR agencies.

She cooperated closely with the Ministry of Development, the Ministry of Foreign Affairs and the Ministry of the Environment on projects aimed at the acceleration of start-ups and innovative SMEs and the international expansion of Polish entrepreneurs, including to Great Britain, China, Russia, Germany or the Czech Republic. She co-organized the Polish American Innovation Week in California in 2014.

For almost 5 years, she was responsible for public affairs and public relations stategy in The Employers' Union of Innovative Pharmaceutical Companies INFARMA, representing the organization, inter alia, in Parliament, at the Social Dialogue Council, at industry conferences, in the media and in EFPIA.

From October 1, 2021, she took the position of the managing director of the Polish Union of Innovative Medical Biotech Companies BioInMed. During the first General Assembly, she was elected president of BioInMed.

Rafał Kamiński

Ph.D., Angelini Pharma, Italy

Dr Rafal Kaminski has broad international experience in the discovery and development of innovative small-molecule drugs. After obtaining a medical degree and a doctorate in pharmacology at the Medical University of Lublin (Poland), he completed his post-doctoral training in the Netherlands (Radboud University) and USA (National Institutes of Health), as well as obtained the Diploma in Pharmaceutical Medicine (ULB, Belgium). He joined the pharmaceutical industry more than 15 years ago and worked at UCB Pharma (Belgium) and Roche (Switzerland). Dr Kaminski served as Chief Scientific Officer and Board Member of Molecure Therapeutics; a leading Polish biotech company listed on Warsaw Stock Exchange. At Molecure he was responsible for an integrated R&D organization, spanning from discovery to clinical development and was a key contributor to the licensing deal signed with Galapagos NV, which had the largest value on the Polish biotech scene at the time. Currently, Dr Kaminski is Chief Scientific Officer and Head of R&D at Angelini Pharma (Italy). He is also an Adjunct Scientist at the Department of Medicinal Chemistry, Faculty of Pharmacy, Jagiellonian University Medical College. Three of his drug discovery projects successfully completed Phase I/II studies and one drug candidate entered Phase III clinical development. He also has experience in coordination and oversight of drug discovery projects performed in collaboration with academic partners. He was a member of scientific advisory boards and expert committees (FP7 UE; Wellcome Trust). Dr Kaminski co-authored more than 100 scientific papers (including Nature group journals) and his publications were cited more than 4000 times. He is also an inventor in 7 patent applications.

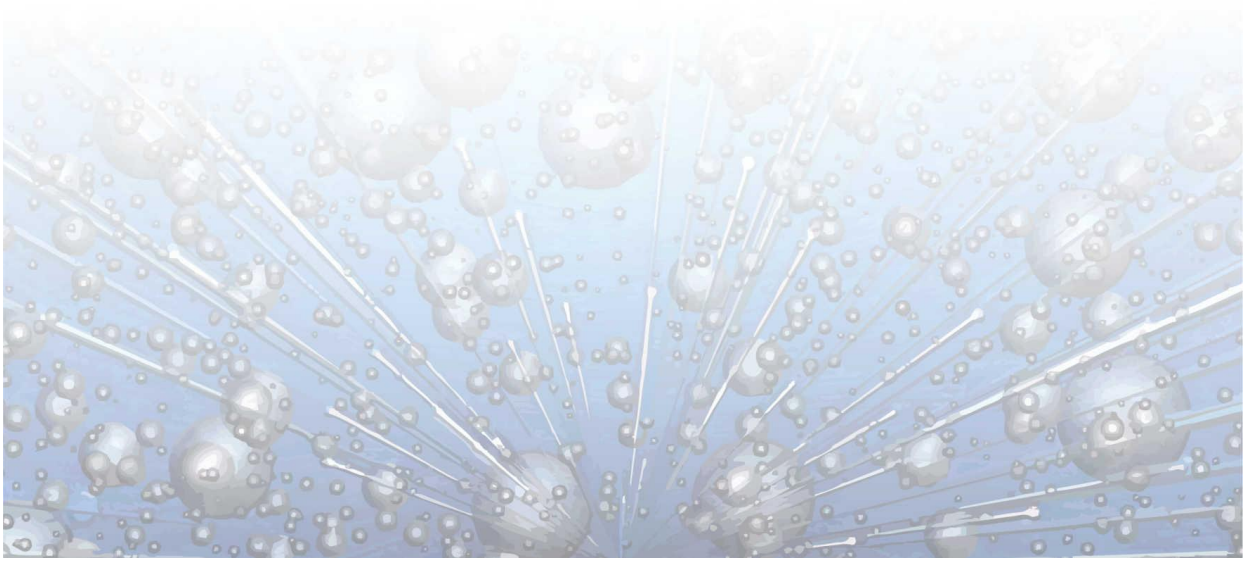
Tomasz Grabowski

Professor, Ph.D., D.Sc., Polpharma Biologics SA, Gdańsk, Poland

Tomasz Grabowski, Ph.D., D.Sc., professor and a veterinary medicine doctor, specialist in pharmacology and bioanalytics. In 2000, shortly after completing his doctoral studies and a short job as an assistant professor, he took the position of a senior assistant at the Herbapol S.A. Experimental Laboratory. in Wroclaw, Poland. In the years 2004-2011 he worked at Ravimed company as the Head of the Pharmacokinetic Research Center FILAB. In this unit, he managed the implementation of pharmacokinetic research projects from the early preclinical phase to the clinical trial phase, in the system of good laboratory practice. Since 2011, an employee of Polpharma Biologics SA with headquarters in the Gdańsk Science and Technology Park. Currently employed as the Head of Pre-clinical Research of Polpharma Biologics SA. Member of the editorial board of the European Journal of Drug Metabolism and Pharmacokinetics and the International Journal of Pharmacokinetics, author of over 100 original scientific publications and an e-book on pharmacokinetics. For twenty years, he has been continuing his scientific career as an employee, exclusively in the pharmaceutical industry.

ACCORD INTERDISCIPLINARY CONFERENCE ON DRUG SCIENCES

Abstracts of invited lectures



Abstract No. IL.01

On the unique role of C-H….O hydrogen bonds in drug-protein interactions

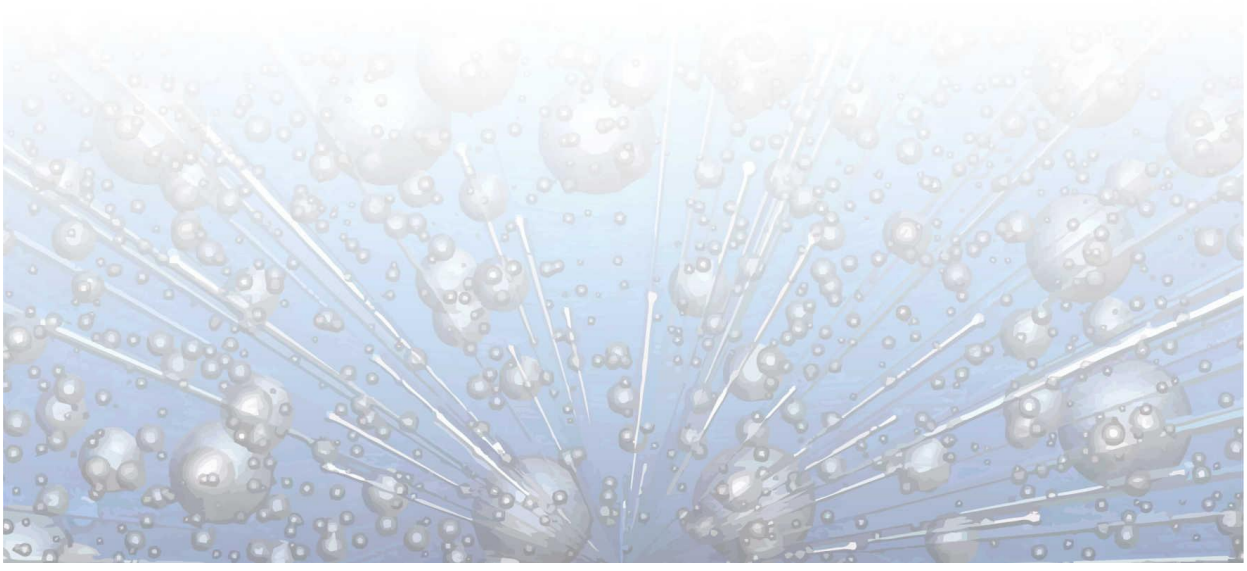
Zygmunt Derewenda[1]

[1] Molecular Physiology and Biological Physics, University of Virginia, United States

E-mail address of presenting author: zsd4n@virginia.edu

Hydrogen bonds (H-bonds) are critically important in protein structure and protein-drug interations, and are responsible for specificity and directionality of interactions. Typically H-bonds are considered between electronegative atoms, such as O or N, one of which is protonated, as in a hydroxyl or amide groups, which then act as donors. However, there is also a class of weaker H-bonds, in which various CH groups can act as donors. C-H…O hydrogen bonds constitute a unique class of cohesive interactions. Their properties are similar to those of canonical H-bonds, although their energy is significantly lower, typically in the 0.5 – 2.5 kcal/mol range. Polarized C-H groups, such as those adjacent to electronegative groups, or within aromatic moieties, are particularly strong donors. C-H…O bonds are ubiquitous in nucleic acids and in proteins, notably stabilizing the β-sheet secondary structure. They have also been observed in numerous protein-ligand interactions. Here, we analysed crystal structures, deposited in the Protein Data Bank, of complexes of FDA-approved protein kinase inhibitors with cognate kinases, to assess the possible role of $C\text{-}H^{inhibitor}$ …$O^{protein}$ hydrogen bonds. The conserved hinge motif of protein kinases with two solvent exposed carbonyl groups and one exposed backbone amide, is well known to be involved in canonical H-bonding with inhibitors. We now find that in virtually all complexes where the inhibitor interacts with the hinge backbone, at least one of the hinge carbonyl groups accepts an H-bond from a C-H inhibitor group, which is either aromatic or adjacent to an electronegative group. These observations are important for design of hinge-binding scaffolds of novel kinase inhibitors for therapeutic use.

Derewenda, Z., Hawro, I., Derewenda U. IUBMB Life 2020 pp 1233-1242



Abstract No. IL.02

Activity profiling of viral proteases from SARS-CoV-2

Marcin Drąg[1]

[1] Department of Chemical Biology and Bioimaging, Wroclaw University of Science and Technology, Poland

E-mail address of presenting author: marcin.drag@pwr.edu.pl

Diseases such as cancer, diabetes or viral and bacterial infections are one of the main causes of human mortality, regardless of age and origin. Perfect methods of treatment have still not been found. Much hope is placed on research into the origin of a disease, usually involving tens or hundreds of biological macromolecules called enzymes. From this point of view, one of the most important groups of enzymes are proteases, the increased or decreased level of which allows for rapid clinical diagnosis using specific markers, and also gives the opportunity for rational, rapid research on drug discovery based on protease activity. An excellent reason for research on proteases are commercially available anti-cancer, anti-diabetic and anti-viral HIV drugs that rely on the inhibition of protease activity. Unfortunately, these drugs can only be used for a limited number of diseases, and many other proteases (around 650 proteases have been described in humans to date) involved in various disorders in humans and other living organisms require further research.

Proteases are key players in the development of viral diseases. Recent studies show that proteases operate in a network that involves the activity of many different proteolytic enzymes at the same time. Given the fact that more and more proteases are actively involved in viral diseases, there is an urgent need to develop new chemical tools that, thanks to the activity of enzymes, can be used for their precise monitoring or the search for drug candidates. Moreover, in order to detect the active form of the protease, one should use chemical tools called activity-based probes. The lecture will present modern techniques of creating tools for the study of viral proteases.

Acknowledgement: This work was supported by the Medical Research Agency in Poland through its Own Project (grant 2020/ABM/SARS/1), by the National Science Center grant UMO-2020/01/0/NZ1/00063, and the "TEAM/2017-4/32" project, which is carried out within the TEAM program of the Foundation for Polish Science, co-financed by the European Union under the European Regional Development Fund.

1. Drag & Salvesen, Nature Reviews Drug Discovery **2010**
2. Kasperkiewicz et al. Proc. Natl. Acad. Sci. U S A. **2014**
3. Rut et al. Antiviral Research, **2020**
4. Rut et al, Nature Chemical Biology, **2020**
5. Rut et al. Chemical Science, **2020**
6. Rut et al., Science Advances, **2020**
7. Patchett et al., Cell Reports, **2021**

Abstract No. IL.03

The value of stereo-specific allosteric modulators in unravelling extracellular calcium-sensing receptor signalling

Martin Schepelmann[1], Enikoe Kallay[2]

[1] Center for Pathophysiology, Infectiology & Immunology, Institute of Pathophysiology and Allergy Research, Medical University of Vienna, Austria
[2] Medical University of Vienna, Austria

E-mail address of presenting author: enikoe.kallay@meduniwien.ac.at

The main role of the extracellular calcium-sensing receptor (CaSR), a C-class G protein-coupled receptor, is to monitor and regulate serum calcium (Ca^{2+}) concentration. Besides this central function, the CaSR also regulates other physiological and pathophysiological processes such as inflammation, airway constriction, water transport, cardiovascular effects, neuronal development and function, and enteroendocrine hormone secretion, *etc.* in a tissue-dependent manner. The CaSR has several different orthosteric and allosteric ligands and modulators.

The majority of the CaSR ligands interact with other molecules (receptors, channels) as well. Therefore, it is very difficult to determine whether a certain effect of a CaSR ligand was indeed mediated *via* the CaSR or *via* other routes, in especially in non-calciotropic organs, where the role of the CaSR is not clear-cut, and the expression of the receptor is low.

The role of the CaSR in the intestine is not yet clear. While its function in water and ion secretion is well documented, its effect on regulating intestinal inflammatory processes is controversial. By exploiting that the *R*-enantiomers of both the positive CaSR-modulator NPS-568 and the negative modulator NPS-2143 are many times more selective for the CaSR than their corresponding *S*-configured mirror molecules, we are investigating which pathways are indeed mediated by the CaSR, rather than unspecific activity of the drugs on other targets. We have used the colon cancer cell line HT29, transfected either with the CaSR ($HT\text{-}29^{CaSR\text{-}GFP}$) or with the empty vector ($HT29^{GFP}$), and assessed the role of the CaSR in regulating the expression of the inflammation marker interleukin 8 (IL-8). Using both the *R*- and *S*-enantiomers of both modulators, we could prove that in this cell model, the activation of the CaSR by Ca^{2+} or NPS R-568 increased the expression of the inflammation marker IL-8. This suggests a pro-inflammatory role for the CaSR in the colon, substantiating our previous finding that positive allosteric CaSR modulators are associated with intestinal pro-inflammatory effects *in vivo*.

Our research has shown the importance of stereo-specific allosteric modulators in clarifying controversies in the signalling of the CaSR and possibly, also other receptors for which such enantiomer-pairs exist.

Abstract No. IL.04

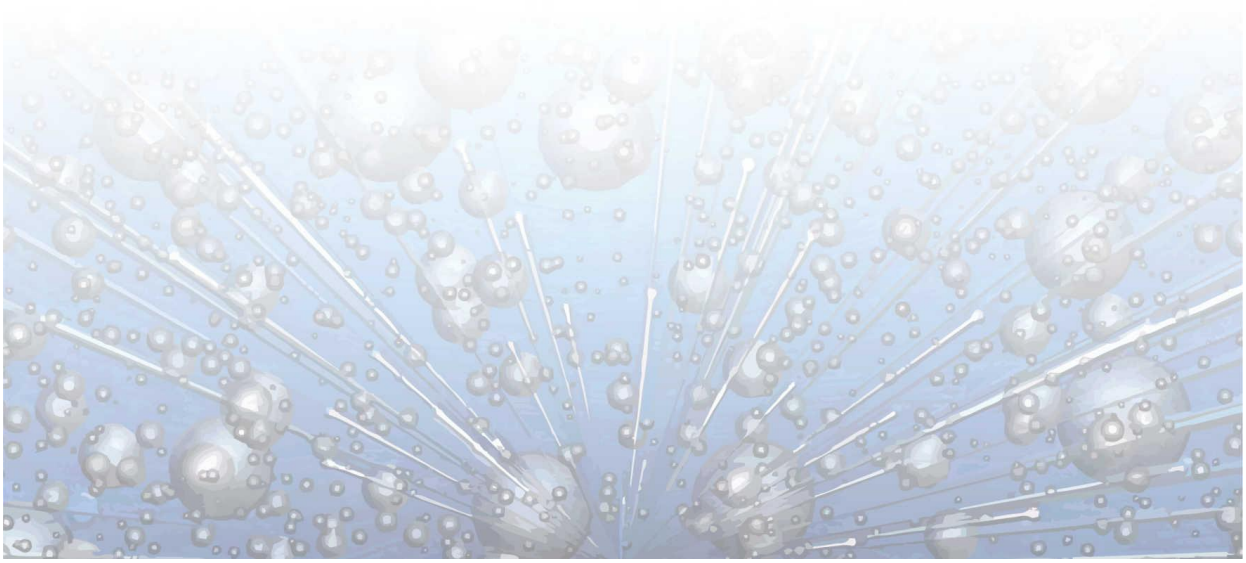
Killing cancer stem cells

Geoffrey Brown[1]

[1] Institute of Clinical Sciences, University of Birmingham, United Kingdom

E-mail address of presenting author: g.brown@bham.ac.uk

There is an urgent need to develop new treatments for cancer that eliminate cancer stem cells (CSC). These cells sustain a cancer and are also largely responsible for disease relapse and metastasis. Conventional chemotherapeutics and radiotherapy are often highly effective against the bulk cells of a cancer, which are proliferating, but they spare cancer stem cells. And, therapeutics that specifically target cancer stem cells may provide a *bone fide* cure for cancer. There are two rationales to targeting the retinoic acid receptor (RAR) g. First, RARg is expressed selectively within primitive cells, for example, hematopoietic stem cells and their immediate offspring. Secondly, RARγ is a putative oncogene for a number of cancers. Some AML patients' cells harbour RARγ fusion proteins, RARγ is often over-expressed in colorectal and renal cancer, RARγ promotes the growth of hepatocellular cancer xenografts in mice, and prostate cancer cells depend on RARγ activation for their survival. The use of a synthetic retinoid to antagonize RARγ caused necroptosis of prostate cancer CSC-like cells. Antagonizing all RARs resulted in necroptosis of breast cancer CSC-like cells and the CSCs that give rise to neurosphere-like structures from paediatric patients' primitive neuroectodermal tumours and astrocytoma. Achieving cancer-selectively is a longstanding paradigm to the development of new treatments, and normal prostate epithelial cells were 50% less sensitive to the pan-RAR antagonist than primary prostate cancer cells, and fibroblasts and blood mononuclear cells were insensitive. The RARγ and pan antagonist are promising new cancer therapeutics.



Abstract No. IL.05

The relevance of crystal forms in the pharmaceutical field – Damocles sword or innovation tools

Dario Braga[1]

[1] Chemistry G. Ciamician, University of Bologna, Italy

E-mail address of presenting author: dario.braga@unibo.it

The quest for, hence the identification and characterization of polymorphs, solvates and co-crystals of an active molecule is one of the most active and challenging research area of modern solid state chemistry. The effort is by no means purely theoretical or academic. In the pharmaceutical field in particular, the existence of multiple crystal forms of an active pharmaceutical ingredient (API) is relevant for the selection of the solid material to carry through the various stages of drug development as well as for the choice of dosage and of excipients suitable for drug development and marketing. This is because the physico-chemical properties of the solid API (solubility, dissolution rate, thermal stability, processability etc.) may depend, sometimes dramatically, on the crystal form with important implications both in terms of the drug's ultimate efficacy and in terms of the protection of intellectual property rights and patenting strategies. For these reasons, polymorph screening is required from the beginning of the life of a new API long before entering trial stage and should be part of the quality control process once the API is on the market to ensure persistence of pharmaceutical properties as shown in the scheme. These activities strongly calls upon the expertise of solid state chemists, crystal engineers and crystallographers.

Crystal polymorphism has been known and studied since the early days of crystallography but it is only in the recent past that it has emerged as a strategic research area for all human activities involving the use of molecular crystalline materials (pharmaceuticals, nutraceuticals, fertilizers, pigments, high energy materials, etc.).

Although the unexpected appearance of a new crystal form of a known active principle is often a threat for an API on the market, it is also true that the urge for a careful pre-screening and form selection is a potent stimulus for research in various areas and provides opportunities for innovation and new discoveries, especially in the burgeoning subfield of molecular co-crystals.

See for example

[1] Braga, D.; Grepioni, F.; Gavezzotti, A.; Bernstein, J. *Cryst. Growth Des.* **2017**, *17*, 933–939
[2] Wouters, J.; Quéré, L., *Pharmaceutical salts and co-crystals*. Royal Society of Chemistry: 2011.

Abstract No. IL.06

Metabolic MRI in cancer and neurological applications

Greg Stanisz[1]

[1] Medical Biophysics, University of Toronto, Canada

E-mail address of presenting author: stanisz@sri.utoronto.ca

The role of non-invasive imaging role in managing cancer patients has increased beyond localization of solid tumour pathology and it nowencompasses image-guided therapy, monitoring treatment, and identification of the tumour grade, allowing for more precise and effective management of cancer patients. Currently, in the standard of care, response assessment is performed by evaluating changes in linear tumour dimensions; however, it may take weeks to months before significant changes occurs, by which time the therapeutic window is often lost. Assessing tumour response early after the treatment enables to identify responders from non-responders and may allow for a change of treatment. We suggest that saturation transfer MRI (ST-MR), a novel MRI technique, permits the robust evaluation of tumour micro-environment including regions with high metabolic activity and is capable of detecting early cell death (apoptosis) following treatment. We have recently applied these techniques in patients with brain metastasis undergoing stereotactic radiosurgery (SRS) and glioblastoma multiforme (GBM). To date we have scanned ~500 patients with High Grade Glioma (HGG) and brain metastases (BM).

1.In HGG, we observed that ST-MRI can identify which patients will progress as early as two weeks into their 6-week treatment.

2.Changes in tumour metabolism (CEST) allow for separation of responders from non-responders one week post-treatment of BM. qMRI can predict the amount of tumour shrinkage in BM at least one month before treatment.

3.CEST allows the assessment of treatment-induced side-effects of radiation treatment and permits distinguishing between tumour progression and radiation-induced necrosis

4.Even before treatment begins, several ST metrics can assess tumour aggressiveness and predict patient response.

We have also demonstrated a great potential in assessing tumour and NAWM environment in Low Grade Gliomas and its ability to predict further damage. Similar techniques could be also used in detecting metabolic changes in the brain as a result of neurological disorders. ST-MRI has been successfully used in probing glucose metabolism in Alzheimer Disease and in modulation of GABA/Glutamate cycle in animal models of anxiety disorders using probiotics. It may be also possible to probe metabolites associated with the serotonin pathway non-invasively. In summary, metabolic MRI (such as ST-MRI) may serve as an non-invasive tool to monitor the effects of treatment for various disorders.

Abstract No. IL.07

Shaping the antimicrobial action of gold nanoparticles

Robert Bucki[1]

[1] Department of Medical Microbiology and Nanobiomedical Engineering, Medical University of Białystok, 15-222 Mickiewicza 2C, Poland

E-mail address of presenting author: buckirobert@gmail.com

Objective: Microbial drug resistance is a global health problem that requires immediate action. Based on the available epidemiological data, it is estimated that in 2050 the death rate from infections caused by bacteria resistant strains will exceed the number of deaths from cancers. At the same time, it will increase the costs of hospitalization, which will significantly burden the health care system. The latest data show that nanotechnology can help make progress in the development of new antibiotics effective against resistant strains of bacteria. Nanoparticles, including those made of metals, can be effectively used as antimicrobial agents alone or as carriers for other biologically active molecules, including ceragenins (CSAs), which are synthetic analogs of natural antimicrobial peptides. Importantly, the therapeutic efficacy of such nanoformulations, their toxicity profile or pharmacokinetic parameters can be effectively modulated by appropriate shaping of the nanoparticles with respect to their size, morphology or surface chemical nature.

Material and methods: A variety of gold nanoparticles with potent antibacterial activity in the shapes of rods, peanuts, stars, and spherical-like, porous ones, were synthesized and thoroughly tested against clinical strains of *Pseudomonas aeruginosa, Staphylococcus aureus*, and uropathogenic *Escherichia coli*. In addition, non-spherical nanosystems based on synthesized nanoparticles and ceragenins were prepared and tested against drug-resistant pathogens for the purpose of assessment of gold nanoparticles-assisted modulation of bactericidal activity of CSAs.

Results: The developed non-spherical nanoparticles are more effective in combating the tested microorganisms than spherical gold nanoforms. Notably, their antimicrobial spectrum includes bacteria with various drug resistance mechanisms. Additionally, attachment of ceragenins to the surface of gold nanoparticles effectively increases their antimicrobial activity against drug-resistant pathogens, without the tendency to induce drug resistance.

Conclusions: This study fulfills the need for microbiological studies of non-spherical gold nanoparticles in order to develop antimicrobial agents targeting multi-drug resistant bacteria that cause life-threatening infections.

Keywords: microbial drug resistance, nanotechnology, ceragenins

Abstract No. IL.08

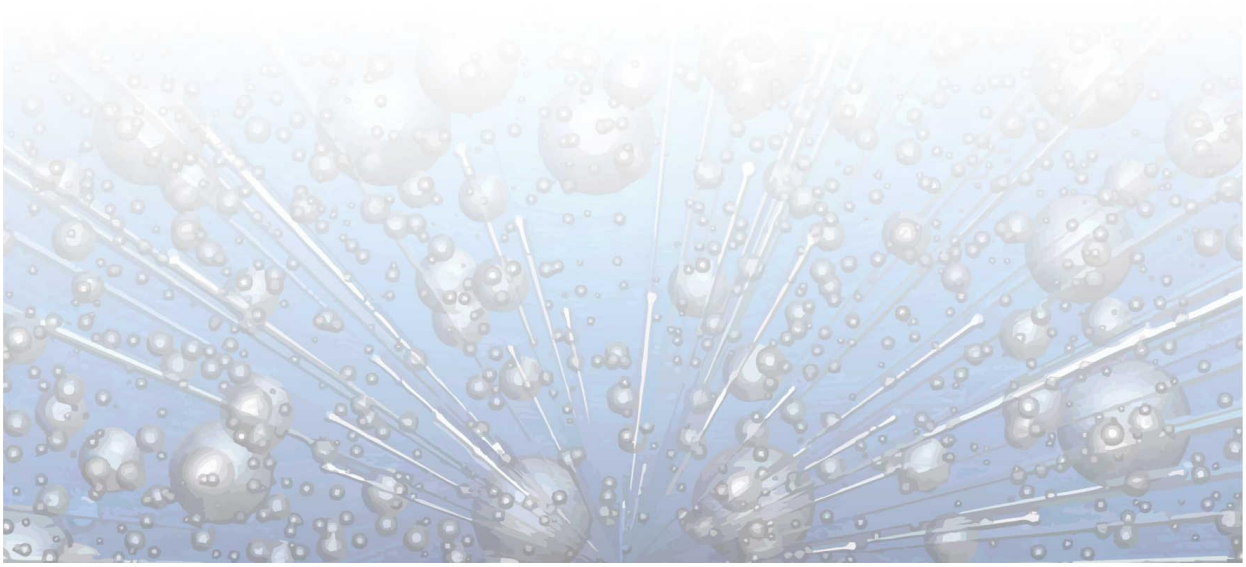
Why patients respond differently to drugs – and ways to improve treatment

Stein Bergan[1]

[1] Oslo University Hospital, Norway

E-mail address of presenting author: stein.bergan@farmasi.uio.no

The response to a given drug may be variable between patients, even when the dose is the same. For some drugs there is a small difference between the dose that is less effective and a dose which gives serious adverse effects. As the response to the drug is crucial, these drugs are referred to as narrow therapeutic index drugs. For many of these drugs, tools have been developed to individualize or personalize selection of the appropriate drug and adjust the dose on an individual basis; i.e. Therapeutic Drug Monitoring (TDM). This means that the concentration of the drug is measured in blood or plasma, and the dose is adjusted in order to obtain the defined optimum. One explanation for the variability in response may be that many drugs are metabolized by enzymes that show variable activity between patients. This could be caused by variants in the genes that encode these enzymes. In such cases the identification of the gene variants can be performed before treatment to provide a better prediction of the optimal dose for each patient. Examples of drugs for which such methods are applied in clinical practice are traditional small molecular drugs like tacrolimus in organ transplantation, thiopurines in the treatment of inflammatory bowel disease and busulfan in the conditioning before stem cell transplantation. For several of the so-called biologicals, i.e. larger molecules that are monoclonal antibodies, the TDM approach is also established or being investigated.



Abstract No. IL.09

Harnessing complementarity for the discovery of drug leads from nature

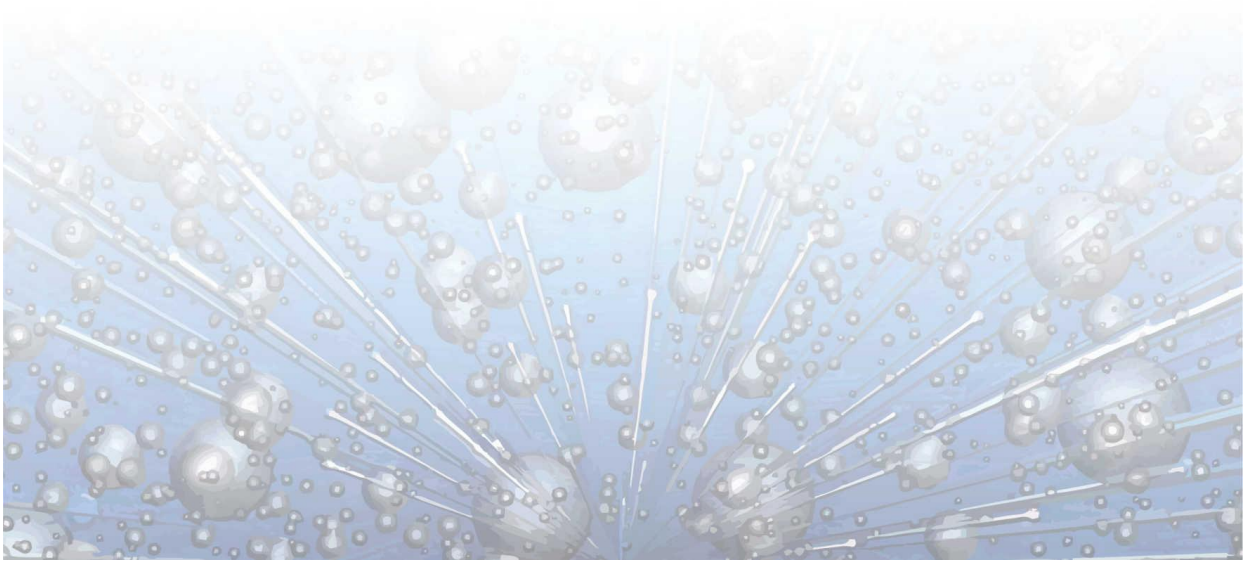
Judith Rollinger[1]

[1] University of Vienna, Department of Pharmaceutical Sciences, Pharmacognosy, Austria

E-mail address of presenting author: judith.rollinger@univie.ac.at

Nature has historically proven to be the best chemist on earth and to ultimately protect its host by the synthesis of a potent chemical arsenal. This is reflected in the chemical complexity of an extract's metabolite profile. Many of these extracts derived from different medicinal plants have been used since ages as herbal remedies. Today, the information from traditional medicine on the one side and the tremendous advances in natural product technologies as well as computational sciences on the other hand offer new possibilities in the identification of novel drug leads.

Here, I will present and compare some recently developed strategies, which can be used to overcome gaps between the knowledge from traditional medicine and the use of big data analysis harnessing the tremendous advances in cheminformatics, in analytical, spectral and separation techniques. This ranges from virtual screening for hit identification, machine learning tools to a recently in-house developed ^{1}H NMR-MS-based biochemometric approach. The effectiveness, strengths and limitations of the presented strategies will be demonstrated on most recent application examples for the identification of novel antiviral, antimicrobial and anti-inflammatory natural lead structures.



Abstract No. IL.10

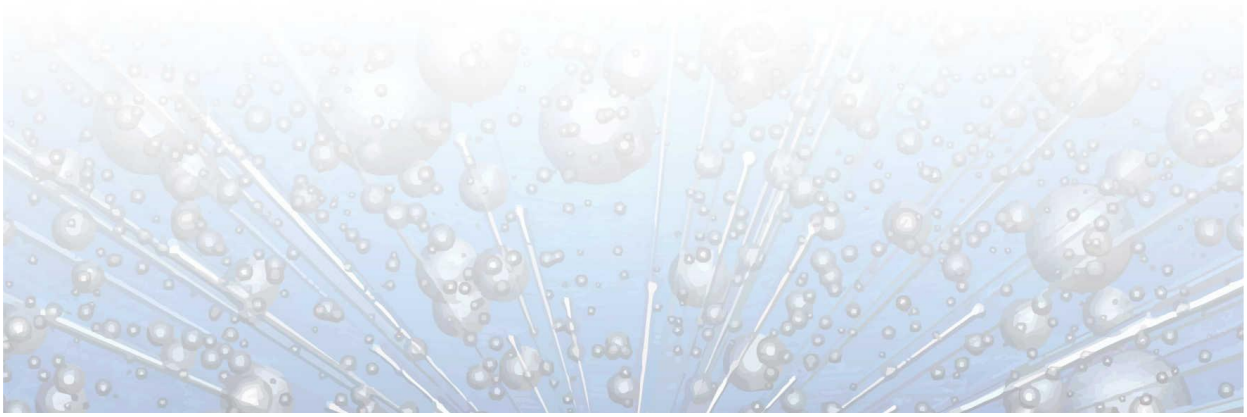
Plant microRNAs as active substances of plant extracts?

Matthias Melzig[1]

[1] Institute of Pharmacy, Freie Universitaet Berlin, Germany

E-mail address of presenting author: matthias.melzig@fu-berlin.de

Since the 19th century, the basis for assessing the therapeutic value of a medicinal plant has been its constituent spectrum of secondary metabolites, such as alkaloids, phenols, tannins, terpenes, etc. These substances have evolved as a result of intensive interaction between plants and their environment resulting in a broad spectrum of biological activity. Since the end of the 20th century, the question has increasingly arisen as to whether there are not further plant products that give plants an evolutionary advantage in confronting microorganisms and animals. Functional studies *in vitro* and *in vivo* demonstrated that plant micro RNA in food can regulate the expression of target genes in mammals and indicate the evidence of cross-kingdom regulation by microRNA. There is no reason why plant-derived microRNA should not be involved in the pharmacological action of a medicinal plant. To prove this thesis, microRNA was isolated from mistletoe, *Viscum album* L., characterized and investigated in in vitro experiments with regard to antitumor activity. A total of 699 conserved microRNAs and 1373 novel miRNAs have been identified and human target genes were predicted for 29 most genuine novel microRNA candidates. Among all the identified human target genes especially two corresponding pathways were highly related with reports on the therapeutic efficacy of mistletoe extracts: cancer including transcriptional mis-regulation and cardiovascular diseases. Six novel miRNAs with the most abundant expression were selected for investigation of their biological effects in a panel of cancer cell lines. Val-miR218 strongly inhibited osteosarcoma cell proliferation, induced cell apoptosis and arrested cells at G0/G1 phase. Further studies have shown that the microRNA is packaged in plant extracellular nanovesicles called exosomes that can be isolated from whole plant extracts. In summary, it can be concluded from these studies that mistletoe contains microRNA packaged in extracellular vesicles that induces specific antitumor activity in vitro. We see mistletoe as an example for other medicinal plants, which, in addition to their secondary metabolites, also contain microRNA, which should be included in pharmacological studies as a further class of natural compounds from plants.



Abstract No. IL.11

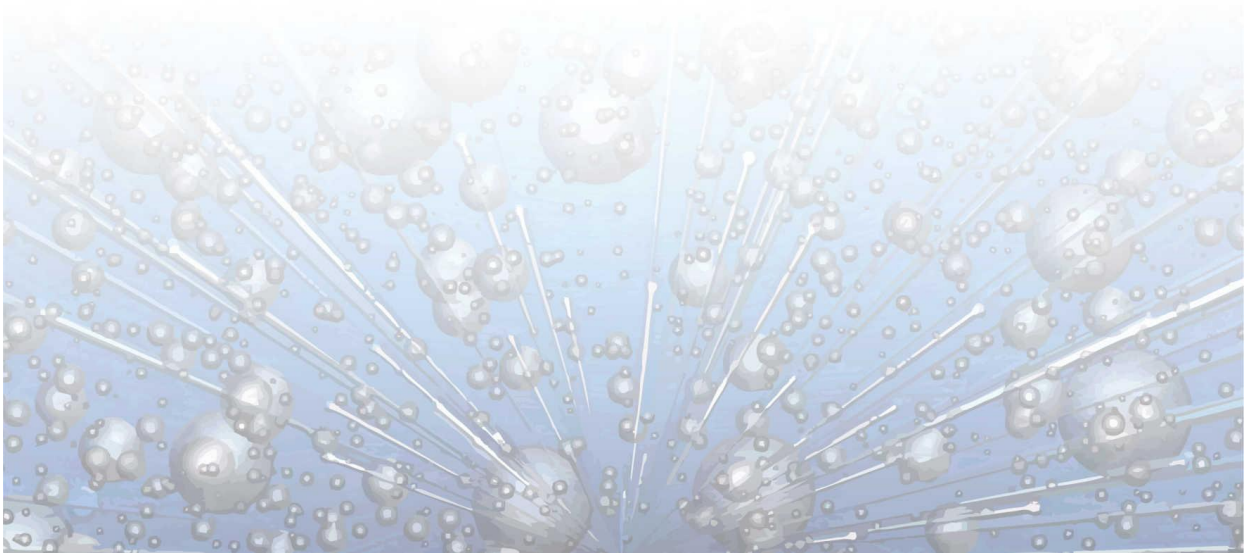
Matrix metalloproteinase-9, MMP-9 in development of epilepsy as a novel drug target

Leszek Kaczmarek[1]

[1] BRAINCITY, Laboratory of Neurobiology, Nencki Institute, Poland

E-mail address of presenting author: l.kaczmarek@nencki.edu.pl

Epileptogenesis is the process responsible for converting normal brain into an epileptic one. It may be triggered by an event such as, e.g., either brain injury or stroke or status epilepticus (SE). The main mechanisms apparently responsible include neuroinflammation and blood-brain barrier (BBB) disruption, pathologic neuronal networks' reorganisation and aberrant synaptic plasticity. Accumulating evidence from animal models and epileptic patients strongly suggest that matrix metalloproteinase 9 (MMP-9) is potentially one of the key executors of the processes of epileptogenesis. In particular, by affecting synaptic plasticity MMP-9 is suggested to enable epileptic remodelling of the brain circuitry. Our studies have provided genetic evidence that MMP-9 contributes critically to epileptogenesis and that MMP-9 inhibitors can indeed be potential drug targets to treat the disease.



Abstract No. IL.12

Implantable hydrogels, infused with vasoactive peptides attracts and trap aggressive glioblastoma cells

Paraskevi M. Kasapidou[1], Emmanuel Laillet de Montulle[2], Kleouforo-Paul Dembele[2], Alexandre Mutel[2], Laurence Desrues[2], Vladimir Gubala[3], Helene Castel[4]

[1] University of Cambridge, United Kingdom
[2] INSERM Rouen, Normandie Universite, France
[3] Medway School of Pharmacy, University of Kent, United Kingdom
[4] Institute for Research and Innovation in Biomedicine, Normandie Universite, France

E-mail address of presenting author: vgubala@gmail.com

Glioblastoma multiforme (GBM) is the most deadly and aggressive malignant brain tumour of the central nervous system in adults. These tumours show a high proliferation rate, variability in tumour histopathology and diffusely infiltrate adjacent brain tissue, rending glioblastoma a very challenging cancer to treat. Current conventional methods of treatment include surgical resection combined with adjuvant radiotherapy or chemotherapy aim to increase the patient life expectancy. However, tumour recurrences are inevitable with a median survival of approximately 14 months after diagnosis.

The key challenge in successful glioblastoma treatment lies in destroying the cancer cells that invade the brain tissue and exist in the brain parenchyma after the removal of the primary tumour bed. Therefore, we here propose a unique design of hyaluronic acid-based hydrogel, an artificial 3D-scaffold containing a soluble chemoattractant to attract residual glioma cells and chemotherapeutic agents that can mimic the tumour microenvironment. The central hypothesis is that this new matrix can attract and destroy residual GBM cells in a less invasive and more efficient way compared to the currently available methods.

In this talk, I will be presenting relevant data on the preparation and full characterisation of the physico-chemical properties of biocompatible hydrogels that were prepared at different crosslinking densities, e.g. low and high density, by crosslinking hyaluronic acid with various concentrations of adipic acid dihydrazide (i.e. HA-ADH hydrogels). I will also discuss the details of our *in vitro* findings, which showed that HA–ADH hydrogels could be used as potential injectable depots in the tumour resection cavity for attracting via a small chemokine peptide UII and tackling residual glioma cells. In the present work, U87MG cells demonstrated migratory behaviour, being able to invade the hydrogel network and to migrate in response to the chemoattractant hUII. In addition, hydrogels loaded with doxorubicin demonstrated significant cytotoxicity leading to less than 80% U87MG cell viability after 48 hours when compared to the control sample. This work provides new advances into a promising approach to pursue therapeutic strategies with a non-invasive method for the surrounding neural tissues, which can be readily translated *in vivo* for the treatment of one of the most devastating brain tumours.

Abstract No. IL.13

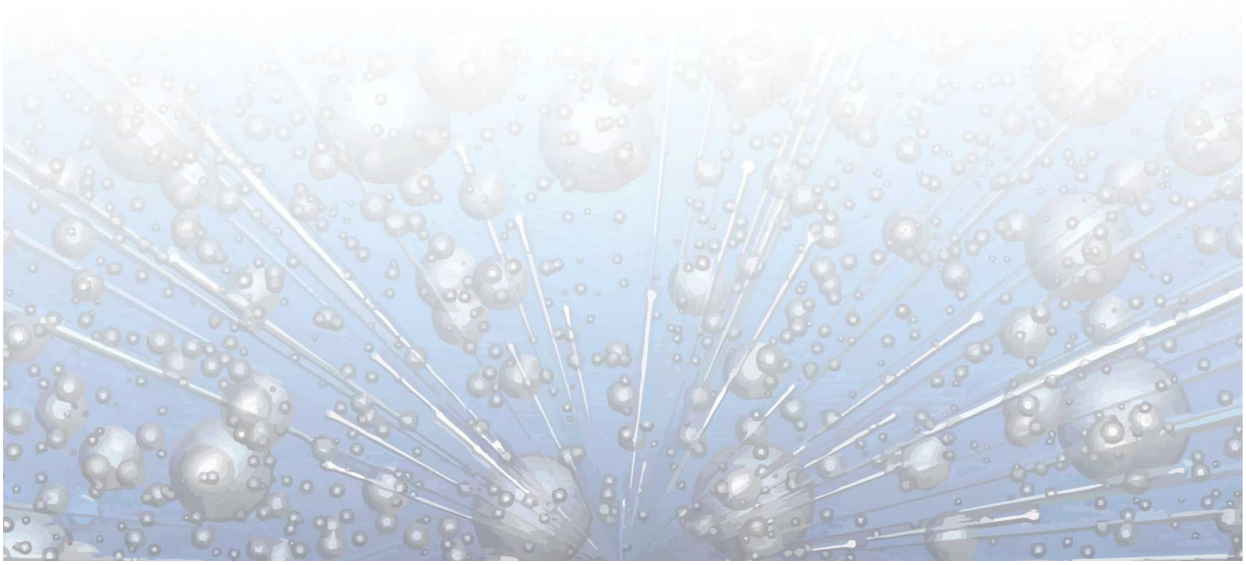
You cannot squeeze blood out of a turnip: Antivirals during the pandemic

Krzysztof Pyrć[1]

[1] Laboratory of Virology of the Malopolska Centre of Biotechnology, Jagiellonian University, Cracow,, Poland

E-mail address of presenting author: k.a.pyrc@uj.edu.pl

The COVID-19 pandemic caused by SARS-CoV-2 has been devastating socially and economically. Despite an unprecedented research effort and available vaccines, effective therapies were unavailable during the first two years of the pandemic. However, during this period some 'wonder drugs' gained substantial media coverage, as premature successes were announced. Here, we will discuss the problems we have faced with the drug repurposing and design and present our research line, which already yielded several active compounds and drugs used in the clinic.



Abstract No. IL.14

Drug discovery in Academia

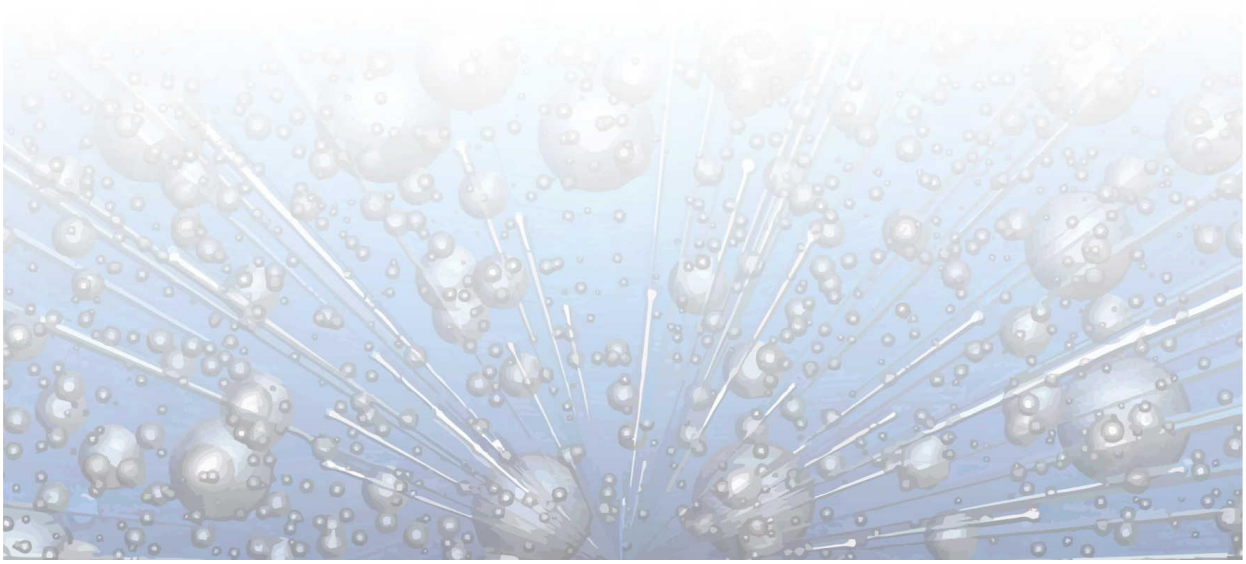
Oliver Plettenburg[1]

[1] Institute of Medicinal Chemistry, Helmholtz Munich, Germany

E-mail address of presenting author: oliver.plettenburg@helmholtz-muenchen.de

Despite all efforts and scientific advances, success rates in current drug development are still unsustainably low. Some of the challenges of current drug discovery efforts and potential ways forward will be presented.

A key requirement for drug discovery is the development of highly selective modulators displaying favorable physicochemical properties. I will discuss a case study in the kinase field, showcasing multidimensional drug discovery. In a second aspect of the talk the development of chemical probes will be discussed, which can not only enable the discovery of new biology, but also facilitate clinical development of new drugs. Besides this more conventional medicinal chemistry approach for the discovery and advancement of new compounds, academia is a perfect playground for probing conceptionally new ideas. I will conclude the talk by presenting a selected example for smart drug delivery.



Abstract No. IL.15

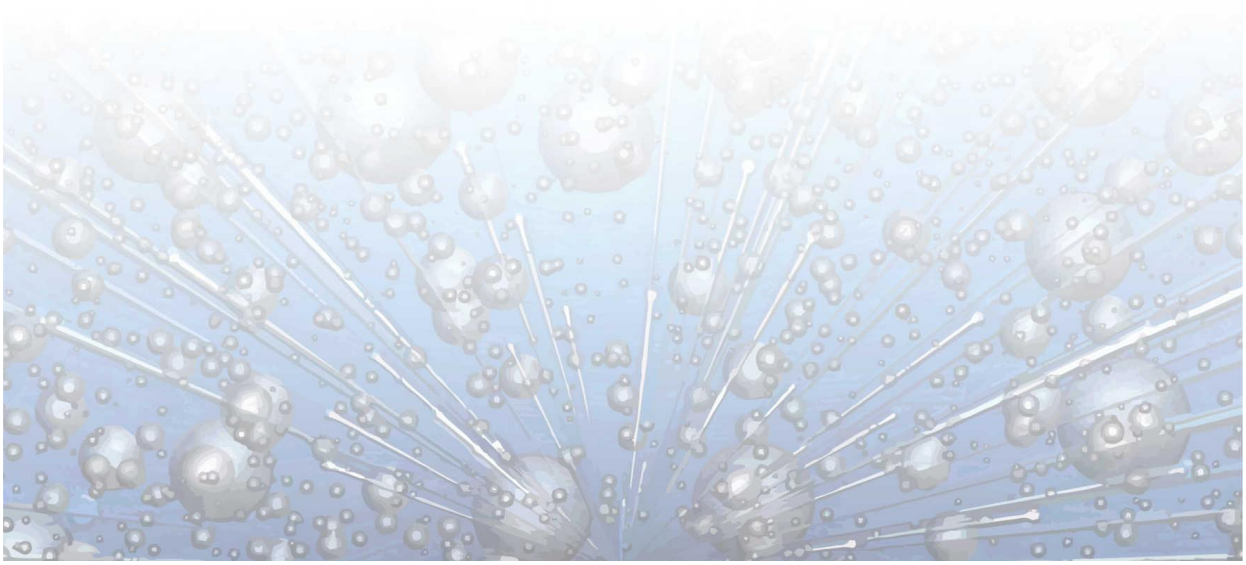
Opening the right locks with the right keys: How to efficiently employ structural biology for drug discovery purposes

Grzegorz Popowicz[1]

[1] Institute of Structural Biology, Helmholtz Zentrum München GmbH, German Research Center for Environmental Health, Germany

E-mail address of presenting author: grzegorz.popowicz@helmholtz-muenchen.de

The amount of structural biology data is growing nearly exponentially. The revolution brought by high-throughput crystallography and, more recently, cryo-electron microscopy, allows us to "see" atomic details of important biological systems. Yet, translation of structural data into new drug molecules is a difficult process. One of the most interesting examples is generating an inhibitor of PEX14-PEX5 interaction as an anti-parasitic drug. This interaction malfunction causes metabolic catastrophe within trypanosoma protists. We used massive amount of structural data to build a small molecule able to exert therapeutic action in vivo. Yet, the process of translating of structural information into a lead development molecule has taught us a lot about our limited understanding of drug-target interaction. Here, we were able to successfully employ Artificial Intelligence to help us understand and design new molecules.



Abstract No. IL.16

How science-fiction became science-reality – the fascinating story of clinical trials virtualization

Sebastian Polak[1]

[1] Jagiellonian University, Poland

E-mail address of presenting author: sebastian.polak@uj.edu.pl

Model-informed Drug Discovery and Development (MIDD or MID3) is a concept of mathematical model-based integration of all accessible information in helping with decision making at each stage of drug discovery and development, including post-marketing phase [PMID: 33205426]. Its application includes not only more advanced drugs kinetics prediction (Physiologically Based Pharmacokinetic - PBPK - models) but also catching up pharmacodynamics assessment (Quantitative Systems Pharmacology - QSP - models) [PMID: 34404115]. The concept of PBPK modeling can be traced back to the mid of 20th century when Teorell reported a set of rate equations to simulate the ADME processes of drugs in the body [URN: urn:nbn:se:uu:diva-172102; URN: urn:nbn:se:uu:diva-172103]. Since then, PBPK, QSP specifically and MID3 in general evolved from being academic considerations to become a suggested decision supporting tool at the regulatory level [PMID: 30653670]. PBPK models reached the level where they are used to replace and waive clinical trials, what was proved by hundreds of cases [PMID: 33205429; PMID: 33460193]. The areas of application include New Drug Applications focusing mostly on DDI assessment and prediction of pharmacokinetics modification in special populations. During the 10 years from 2008 to 2018, a total of 24 NDA submissions included the use of PBPK absorption modeling and simulations for biopharmaceutics-related assessment [PMID: 33619657]. PBPK model was also used to waive PD endpoint BE clinical trial for locally acting complex generics [PMID: 33547863]. More recently PBPK models has been applied to therapy optimization and precision dosing, where patients receive individually tailored therapy to maximize effect and minimize the risk of potential adverse drug effects by exploring the 'virtual twin' concept [PMID: 33035445]. Due to significantly higher complexity mathematical mechanistic models' application in the PD area is delayed as compared to PK. Nevertheless, recent advancement effected in the use of QSP models for drugs R&D projects de-risking, where utilization of biophysically realistic models of neurophysiology are used i.e., to select best combination of drugs for Alzheimer disease treatment [PMID: 34966890]. COVID-19 pandemic raised the need for optimal use of vaccines and their dosing optimization. Quantitative systems pharmacology (QSP) model of immune system is perfectly placed to help in a quantitative manner [PMID: 34331834].

Abstract No. IL.17

Anti-crystal engineering – a new catch phrase or an efficient approach to improve solubility of pharmaceutical molecules?

Lidia Tajber[1]

[1] Trinity College Dublin, Ireland

E-mail address of presenting author: ltajber@tcd.ie

Objectives: This work focuses on the assessment of a new paradigm in pharmaceuticals sciences, which is anti-crystal engineering and its application to the synthesis of multicomponent systems, such as ionic liquids and eutectic systems, aimed at improving solubility of poorly soluble drugs.

Materials and Methods: The main synthetic method was mechanochemistry, which is a process in which mechanical motions/energy control chemical and physical transformations. Following the production, the systems were characterised using a panel of physiochemical and biopharmaceutical techniques.

Results: Marketed medicines comprise crystalline drugs due to the convenience of production using organic solvent-based crystallisation processes. However, while physically stable, crystalline materials cannot achieve their full potential in term of solubility due to the high lattice energies arising from the strong bonds keeping the molecules together. For drug molecules to be active in the body they need to escape from the crystal lattice, thus crystallinity prevents the drugs from achieving their full therapeutic potential. Consequently, the anti-crystal engineering approach is used to identify and intentionally avoid crystallisation of interacting/reacting components, by preventing the formation of robust synthons building the crystalline scaffolds. It is also preferred that the excipient molecules interacting with the drug are bulky and sterically demanding, with delocalised charge and a minimal number of hydrogen bond donors and acceptors. This work will explore the advantages and disadvantages of anti-crystal engineering in relation to forming multicomponent pharmaceutical materials.

Conclusions: Anti-crystal engineering of multicomponent pharmaceutical materials may become a viable method of improving solubility of poorly performing drugs, however it is of a paramount importance to understand the fundamentals of their synthesis, crystallisation and manufacturability.

Acknowledgments: Science Foundation Ireland (grants 15/CDA/3602 and 12/RC/2275_P2), the European Cooperation on Science and Technology (COST) Action CA18112 "Mechanochemistry for Sustainable Industry".

Keywords: anti-crystal engineering, mechanochemistry, multicomponent pharmaceutical materials

Abstract No. IL.18

Biopredictive dissolution testing of oral medicines

Jadwiga Paszkowska[1], Grzegorz Garbacz[1]

[1] Physiolution Polska, Wrocław, Poland , Physiolution GmbH, Greifswald, Germany, Poland

E-mail address of presenting author: g.garbacz@physiolution.pl

Introduction: The presentation will focus on the pragmatic question: what are the truly "biopredictive" conditions for testing of oral medicines? The key issues of realistic simulation of GI transit will be presented.

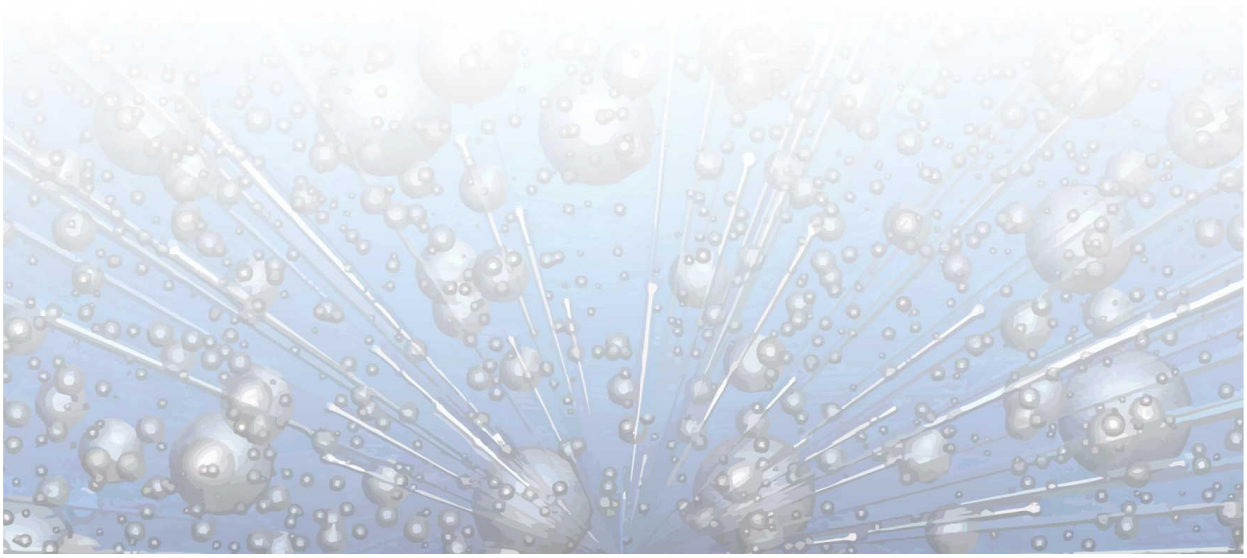
Materials and Methods: The development of biopredictive dissolution tests aims at realistic simulation of mechanical and chemical conditions of the GIT. It can be successfully performed using simple, abstract dissolution models such as the biorelevant dissolution stress tester, or its later modifications Advanced Modular Platform. Although the models do not reflect the anatomy of the GI tract, they can successfully simulate individual physiological factors via working principles and parametrization [1].

Results: Variability of physiological factors relevant to the dissolution performance of oral medicines, such as pH pressure and temperature, will be discussed in the context of literature and experimental data. Clinically relevant examples of biopredictive dissolution tests will be presented and discussed.

Conclusion: The use of the biopredictive methods supports the rational, physiology driven development of oral medicines and enables the identification of undesired drug delivery performance at the preclinical stage. Consequently, it increases the reliability and biopharmaceutical quality of oral drugs, the effectiveness of the pharmacotherapy and it significantly reduces the dosage forms related side effects and therapy failures.

References:

1. Vinarov Z, Adv Drug Deliv Rev. 2021 Apr;171:289-331.



Abstract No. IL.19

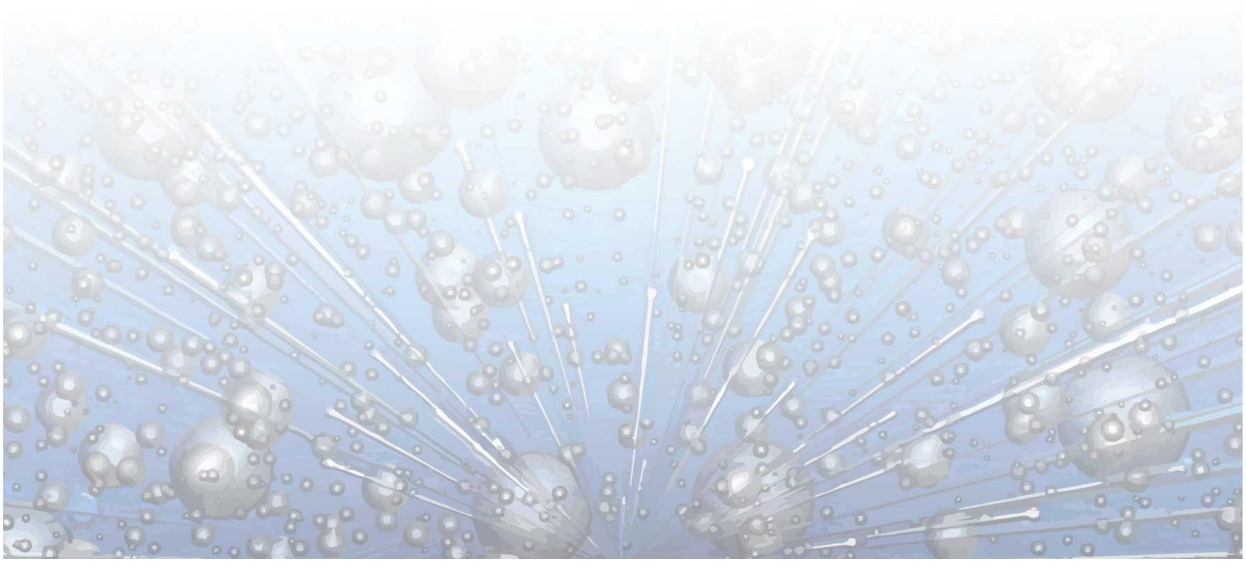
Fused Deposition Modelling 3D printing: A Potential Point-of-Care Manufacturing Method for Personalised Dosage Forms

Mohamed Albed Alhnan[1]

[1] King s College London, United Kingdom

E-mail address of presenting author: alhnan@kcl.ac.uk

3D printing is an innovative manufacturing technology with great potential to revolutionise solid dosage forms. Novel features of 3D printing technology confer advantage over conventional solid dosage form manufacturing technologies, including rapid prototyping and an unparalleled capability to fabricate complex geometries with spatially separated conformations. Such a novel technology could transform the pharmaceutical industry, enabling the production of highly personalised dosage forms with well-defined release profiles. In presentation, the current state of the art of using fused deposition modelling (FDM) 3D printing for point-of-care manufacturing of personalised dosage form with a focus on paediatric applications. Finally, the presentation also covers the design opportunities alongside the technical and regulatory challenges that these rapidly evolving technologies present.



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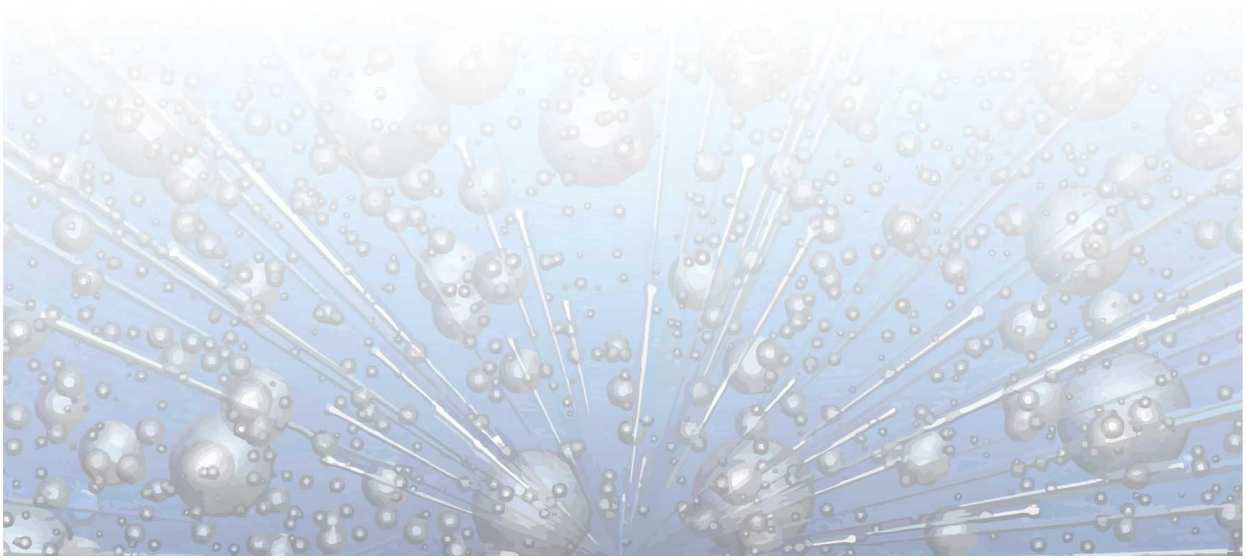


Abstract No. PPP.001

Stilbenes in wood industry waste materials - study on composition of conifers branch wood

Andrzej Patyra[1], Iga Herczyńska[1], Anna Kiss[1]

[1] Department of Pharmacognosy and Molecular Basis of Phytotherapy, Medical University of Warsaw, Poland

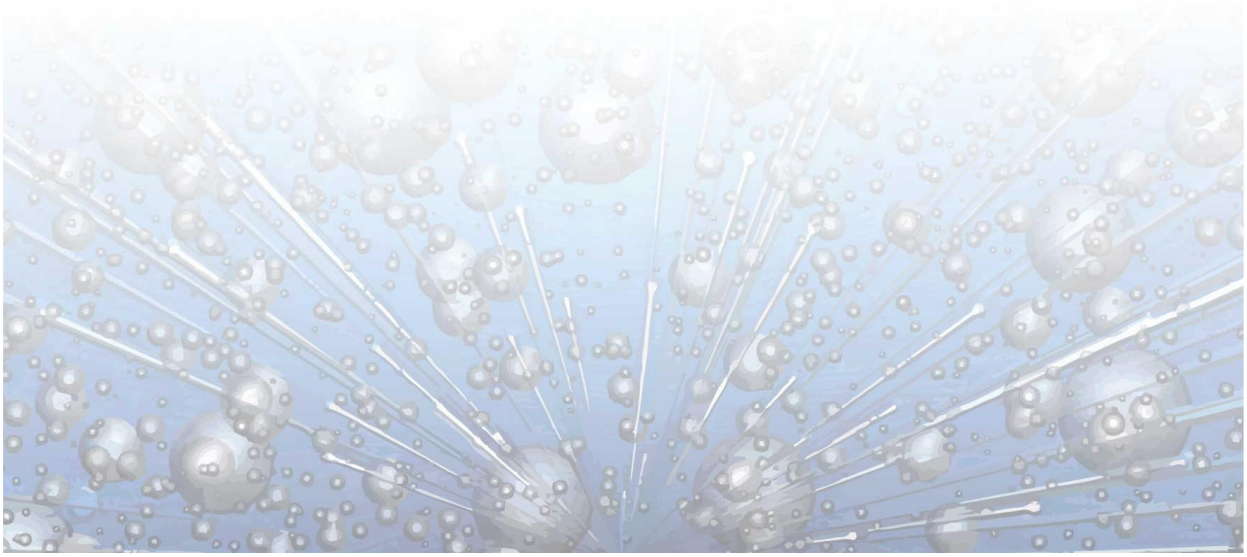
E-mail address of presenting author: andrzej.patyra@wum.edu.pl

Objectives: The aim of the study was to analyze branch wood of Pine family species growing in Poland in search of a new source of potentially active compounds from the group of stilbenes and thus find an application for wood industry waste materials.

Materials and Methods: Plant material was collected from botanical gardens of Polish Academy of Sciences in Powsin, Poland and University of Warsaw, Warsaw, Poland. Methanolic extracts from branch wood of each species were prepared and analyzed using the High-Pressure Liquid Chromatography Diode Array Detector Electrospray Ionization Tandem Mass Spectrometry method (HPLC-DAD-ESI-MS/MS). Through this analysis stilbenes were identified.

Results: Branch wood methanolic extracts were phytochemically profiled. Norway spruce (*Picea abies* (L.) H.Karst.) branch wood was the most abundant source of stilbenes, with astringin and isoxapontin established as dominant constituents.

Conclusions: Wood industry waste materials, such as Norway spruce branch wood can be a potent source of stilbenes.



Abstract No. PPP.002

Effect of selected derivatives of 4-nitrophenoxyacetic acid hydrazide on the early development stages of Danio rerio

Paweł Kozyra[1], Kamil Mańko[2], Anna Serefko[2], Aleksandra Szopa[2], Ewa Poleszak[2], Monika Pitucha[1]

[1] Independent Radiopharmacy Unit, Faculty of Pharmacy, Medical University of Lublin, Poland
[2] Chair and Department of Applied and Social Pharmacy, Laboratory of Preclinical Testing, Faculty of Pharmacy, Medical University of Lublin, Poland

E-mail address of presenting author: pawekoz@interia.pl

Introduction: Thiosemicarbazide derivatives are of great interest to the medical community due to their various activities, including antimicrobial, anthelmintic, analgesic, and anticancer activity. A key step in the drug design process is the study of potential toxicity. World literature reports that only less than 10% of compounds undergo clinical trials, and almost half of them drop out due to unexpected toxicity. Therefore, it is crucial to study toxicity already in the early stages of designing a potential drug, which allows for optimization of the drug design process and reduction of costs.

Objectives: The study aimed to evaluate the effect of three thiosemicarbazide derivatives with the 4nitrophenoxyacetic group on the early development stages of Danio rerio.

Materials and methods: The fish embryo acute toxicity test was carried out in healthy, freshly fertilized Zebrafish eggs according to the published guidelines (OECD, test no. 236). At least 5 different concentrations of 3 various 4-nitrophenoxyacetic acid hydrazide thiosemicarbazide derivatives (i.e., RP3, RP4, RP6 compounds) were assessed.

Results: The obtained results demonstrated relatively high toxicity of RP3 at concentrations above 500 µmol/ml, i.e. high mortality of embryos and larvae, numerous deformities, developmental defects, and cardiotoxicity were observed. RP3 was safe for the tested subjects only at concentrations below 0.1 µmol/ml. RP4 at the tested doses (200-0.1 µmol/ml) did not significantly induce mortality in developing Zebrafish, but the larvae treated with the 200 and 100 µmol/ml solutions were deformed. As for RP6, its tested doses (100-0.05 µmol/ml) were not toxic.

Conclusions: RP6 has the safest profile amongst the 3 tested 4-nitrophenoxyacetic acid hydrazide thiosemicarbazide derivatives. However, the obtained results require confirmation in further experiments in higher organisms, such as mice or rats.

Keywords: thiosemicarbazide, Danio rerio, toxicity

Abstract No. PPP.003

MM-129 as novel potential anticancer agent against colorectal cancer

Justyna Hermanowicz[1], Iwona Kwiatkowska[1], Arkadiusz Surażyński[2], Dariusz Pawlak[1]

[1] Department of Pharmacodynamics, Medical University of Bialystok, Poland
[2] Department of Medicinal Chemistry, Medical University of Bialystok, Poland

E-mail address of presenting author: justyna.hermanowicz@umb.edu.pl

Objectives: Colorectal cancer is the third most common cancer type occurring in the world and its effective therapy is still being sought. MM-129 a new pyrazolo[4,3-e]tetrazolo[1,5-b][1,2,4] triazine sulfonamide was evaluated against human colon cancer in vitro and in a zebrafish cancer model.

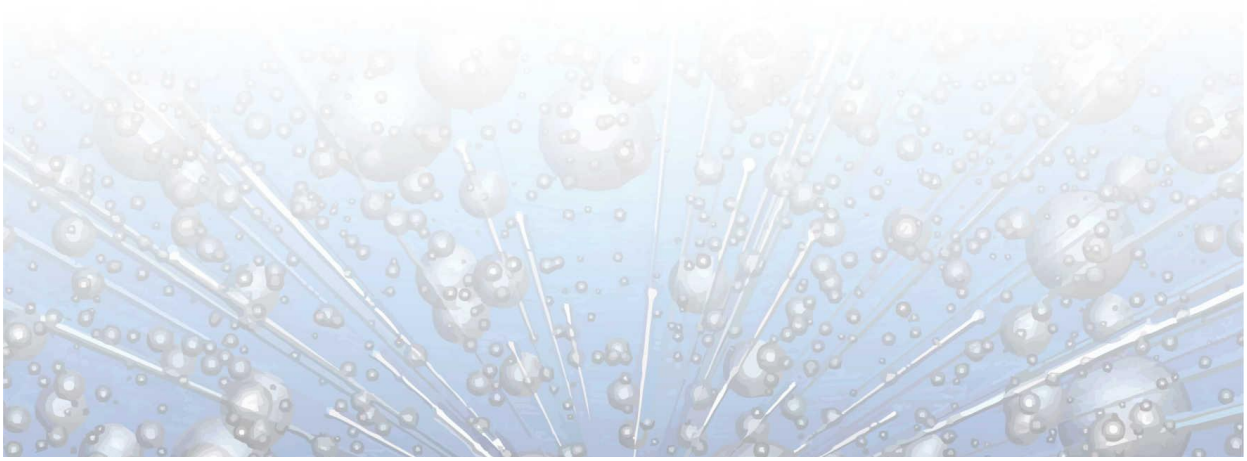
Materials and Methods: DLD-1 and HT-29 colon adenocarcinoma cells were cultured in a medium with MM-129, roscovitine, and 5-fluorouracil. Cell viability of was assessed with MTT assay. Zebrafish xenografts have been established and treated with MM-129, 5-fluorouracil and a combination of these drugs for 48 hours. To investigate the mechanisms mediating the anticancer effects of MM-129, the expression and level of Bruton's tyrosine kinase (BTK) was determined by RT-PCR, Western blot and confocal microscopy.

Results: Our results show that new synthesized compound effectively inhibits cell survival in BTK-dependent mechanism. Its effectiveness is much higher at a relatively low dose as compared with the standard chemotherapy used for colorectal cancer, i.e. 5-fluorouracil. We also found that MM-129 effectively inhibits tumor development in zebrafish embryo xenograft model, where it showed a markedly synergistic anticancer effect when used in combination with 5-FU.

Conclusions: Our results suggest that MM-129 may be a promising candidate for further evaluation as a chemotherapeutic agent against colorectal cancer.

Acknowledgment: This research was funded by National Science Center, Poland grant number 2018/31/B/NZ7/00875.

Keywords: 1,2,4- triazine derivative, zebrafish, colon cancer



Abstract No. PPP.004

Indol-4-yl pyrazolo[1,5-a]pyrimidine derivatives: highly active and selective inhibitors of PI3Kδ – potential candidates for treatment of chronic obstructive pulmonary disease and asthma

Stanisław Michałek[1], Mariola Stypik[1], Marcin Zagozda[2], Nina Orłowska[1], Martyna Banach[2], Urszula Kędzierska[2], Beata M. Zygmunt[2], Kamila Gala[2], Maciej Dziachan[2], Barbara Dymek[2], Daria Zdżalik-Bielecka[2], Paweł Gunerka[2], Paweł Turowski[2], Zbigniew Ochal[3], Krzysztof Dubiel[2], Jerzy Pieczykolan[2], Maciej Wieczorek[2]

[1] Celon Pharma S.A.; Warsaw University of Technology, Poland
[2] Celon Pharma S.A., Poland
[3] Warsaw University of Technology , Poland

E-mail address of presenting author: stanislaw.michalek@celonpharma.com

Objectives: Development of new PI3K inhibitors for treatment of asthma and COPD. PIK3δ (phosphoinositide 3-kinase), the member of the class I PI3K family, plays crucial role in differentiation, proliferation, migration and survival of immune cells. Many PI3K inhibitors are on the market and many more in the preclinical development. Most of these inhibitors are used in cancer therapies, but only few have been approved as drugs in treatment of respiratory diseases. In this study, we developed indol-4 yl-pyrazolo[1,5-*a*]pyrimidine derivatives with inhibition activity values in the nanomolar range and with high selectivity against PI3Kδ.

Materials and Methods: The possible binding site of compounds to the kinase was examined using Auto-DockVina program (PDB: 2WXP) IC50 was determined using ADP-Glo Kinase Assay™ (Promega). Assessment of metabolic phase I stability in mouse (CD-1™) and human microsomes (Thermo-Fisher Scientific) was performed on 96-well non-binding plates (Greiner). Kinetic solubility was determined by shake-flask protocol, sample concentrations was determined by UHPLC-UV/Vis.

Results: The most potent compound, **CPL302-253** (IC_{50}=2.8nmol), was chosen a candidate for pre-clinical studies and the biological results obtained were published by *Gunerka et al.*[1]

Conclusions: The most selective compounds turned out to be those in which the R^2 substituent was *tert*-butylpiperazine. Given this the presence of the lipophilic *tert*-butyl system probably causes a very strong interaction with the tryptophan plate (Trp-760) present in the delta isoform. Further modifications of R^1 will enable treatment of inflammatory diseases associated with PI3K activity.

Acknowledgement: This work was supported by The National Centre for Research and Development (POIR.01.01.01-00-1341/15).

Keywords: PI3K; asthma, COPD

References:

1) Gunerka P., Gala K., Banach M., Dominowski J., Hucz-Kalitowska J., Mulewski K., et al. Preclinical characterization of CPL302-253, a selective inhibitor of PI3Kδ, as the candidate for the inhalatory treatment and prevention of Asthma, *PLoS ONE*, (2020), DOI:10.1371/journal.pone.0236159

Abstract No. PPP.005

CPL302415 - pyrazolo[1,5-a]pyrimidine derivative as a novel, very active and selective PI3Kδ inhibitor, highly potent drug candidate for SLE and other inflammatory and autoimmune diseases.

Mariola Stypik[1], Stanisław Michałek[1], Marcin Zagozda[2], Nina Orłowska[1], Martyna Banach[2], Urszula Kędzierska[2], Beata M. Zygmunt[2], Kamila Gala[2], Maciej Dziachan[2], Daria Zdżalik-Bielecka[2], Barbara Dymek[2], Paweł Gunerka[2], Paweł Turowski[2], Zbigniew Ochal[3], Krzysztof Dubiel[2], Jerzy Pieczykolan[2], Maciej Wieczorek[2]

[1] Celon Pharma S.A.; Warsaw University of Technology, Poland
[2] Celon Pharma S.A., Poland
[3] Warsaw University of Technology, Poland

E-mail address of presenting author: mariola.stypik@celonpharma.com

Objectives: PI3K (Phosphoinositide 3-kinase) is the family of lipid kinases that participate in many key cellular processes like proliferation, growing up, migration, cytokines production, and apoptosis. Inhibition of PI3K (most of the first class which contains four subunits: *α*, *β*, *γ*, *δ*) is considered to be very attractive as a promising therapeutic strategy mechanism for the treatment of many diseases like SLE (Systemic Lupus Erythematosus), MS (Multiple Sclerosis) and other inflammatory and autoimmune diseases. In this work, we present a series of new, active and selective small molecule inhibitors based on the structure of appropriately substituted heterocycle as it is 5-(2-difluoromethylobenzimidazo-1-yl)pyrazolo[1,5-*a*]pyrimidine.

Materials and Methods: Docking procedure of the structures in the PI3Kdelta protein (PDB: 2WXP) was prepared using Auto-DockVina program. All compounds were synthesized in multistep synthesis. The experiments were carried out using ADP-Glo Kinase Assay kit (Promega). Assessment of metabolic phase I stability in mouse (CD-1) and human microsomes (Thermo-Fisher Scientific) was performed on 96-well non-binding plates (Greiner).

Results: We synthesized a library of benzimidazole derivatives that can be very potent inhibitors of kinase activity of PI3K*δ* (PI3K*δ* IC_{50} = 0.02 – 1.07 μM). Then we developed the process of lead compounds synthesis to select the preclinical candidate for SLE.

Conclusions: Molecular modeling studies explain the interaction of active structures in the PI3K*δ* ATP binding site. Pyrazolo[1,5-*a*]pyrimidines with morpholine at position 7, 2(difluoromethyl)benzimidazole at position 5 and N-*tert*-butylamine as amine (CPL302415) proved to be the most potent structure with good activity (IC_{50} = 18 nM), selectivity (PI3K*α*/PI3K*δ* = 79; PI3Kγ/PI3K*δ* = 939) and promising other parameters. For that reason, that compound was selected as a clinical trial candidate and then subjected to further biological and physicochemical studies.

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

Keywords: PI3K*δ* inhibitors, inflammatory therapy, 5-benzimidazole-pyrazolo[1,5-*a*]pyrimidine

Abstract No. PPP.006

Platelet-rich plasma counteracts interleukin-1 induced inhibition of collagen biosynthesis through recovery of impaired β1-integrin signaling and prolidase activity in human skin fibroblasts

Arkadiusz Surażyński[1], Tomasz Guszczyn[2], Adam Kazberuk[1], Magda Chalecka[1]

[1] Department of Medicinal Chemistry, Medical University of Bialystok, Poland
[2] Department of Pediatric Orthopedics and Traumatology, Medical University of Bialystok, Poland

E-mail address of presenting author: arkadiusz.surazynski@umb.edu.pl

Objectives: Although inflammation is the first step in wound healing, it contributes to down-regulation of collagen biosynthesis delaying the process of tissue regeneration.

Purpose: The study was conducted to evaluate the effects of platelet-rich plasma (PRP) on interleukin-1 (IL-1)–dependent inhibition of collagen synthesis, prolidase activity, and β1-integrin signaling in cultured human skin fibroblasts.

Materials and Methods: Platelet Rich Plasma (PRP) was obtain using the SmartPReP®2 Autologous Platelet Concentrate+ System. Collagen biosynthesis and prolidase activity were measured in confluent human skin fibroblast cultures with IL-1, PRP, HA, and mixtures of IL-1 with PRP or HA. Immunofluorescence analysis were performed to evaluate expression of β1 integrin receptor, COX-2 and NF-κB protein.

Results: Incubation of fibroblasts with 2% PRP for 24 h contributed to ~5-fold increase in collagen biosynthesis and a significant increase in the expression of β1-integrin receptor and prolidase activity, compared to the control cells. In the cells treated with interleukin-1 (IL-1), collagen biosynthesis, β1-integrin receptor expression, and prolidase activity were decreased in a dose-dependent manner, while cyclooxygenase-2 (COX-2) and nuclear factor-κB (NF-κB) expressions were significantly increased. All these processes were recovered to control values by PRP or hyaluronic acid (HA); however, PRP was found to act more effectively than HA. It was found that PRP counteracted IL-1-dependent inhibition of collagen synthesis through recovery of β1-integrin receptor expression and prolidase activity and down-regulation of COX-2 and NF-κB expressions in cultured fibroblasts.

Conclusions: The data suggest that PRP evoke anti-inflammatory activity that is desirable in tissue regeneration.

Keywords: PRP, collagen, COX-2

Abstract No. PPP.007

The role of oxidative stress in prolidase-dependent chemotherapy resistance of breast cancer cells MCF7

Magda Chalecka[1], Adam Kazberuk[1], Arkadiusz Surażyński[1]

[1] Department of Medicinal Chemistry, Medical University of Bialystok, Poland

E-mail address of presenting author: magda.chalecka@umb.edu.pl

Objectives: Tumor resistance to chemotherapy is a cause of treatment failure. One of the molecular reasons for this phenomenon may be the level of intracellular prolidase (PEPD). This enzyme is a multifunctional protein that has the ability to bind and inactivate p53 function, thereby blocking the induction of apoptosis by chemotherapeutics. On the other hand, the PEPD-p53 complex can dissociate under oxidative stress. The aim of this study was to investigate the effect of oxidative stress on the activation of p53-dependent apoptosis in breast cancer cells with different PEPD expression levels.

Materials and Methods: MCF7 breast cancer cell (wild type), MCF7 breast cancer cell with prolidase overexpression ($MCF7^{PL}$), and zebrafish model were used. Doxorubicin (a direct activator of p53) was used to induce apoptosis in both cell lines models. Tert-butyl was used to induce oxidative stress, and ascorbic acid was used as an antioxidant. Cell apoptosis, mitochondrial potential was determined by Nucleo-Counter NC-3000, DNA biosynthesis was determined by the radiometric method. Prolidase activity was determined by the colorimetric method. Expression and translocation of selected proteins were evaluated by fluorescence microscopy and Western Immunoblot.

Results: Doxorubicin induced apoptosis in the MCF7 cell line, and the zebrafish embryo xenograft model in a dose-dependent manner, whereas this effect was observed at a much lower level in $MCF7^{PL}$ cells. However, induction of oxidative stress in test models (by t-BHP treatment), resulted in increased apoptosis, especially in $MCF7^{PL}$ cells. The addition of an antioxidant (vitamin C) reversed the adverse effects of t-BHP. The molecular mechanism of apoptosis induction under the conditions and cell models tested was associated with increased expression and translocation to the cell nucleus of p53 and increased expression of active forms of caspases 9 and 7. This was accompanied by decreased in DNA biosynthesis and mitochondrial potential.

Conclusions: Overexpression of prolidase in MCF7 cells ($MCF7^{PL}$) counteracts p53-dependent apoptosis induced by doxorubicin. However, induction of oxidative stress increased apoptosis, especially in $MCF7^{PL}$ cells, which was counteracted by antioxidants. Thus, combination therapy: a drug that initiates p53-dependent apoptosis with an inducer of oxidative stress may be a potential therapy for tumors with prolidase overexpression.

Keywords: prolidase, chemotherapy resistance, cancer

Abstract No. PPP.008

Bioactive basis of antitumor activity of novel transition metal complexes with berenil and nitroimidazole

Robert Czarnomysy[1], Dominika Radomska[1], Anna Muszynska[1], Olga Szewczyk[1], Anna Bielawska[2], Krzysztof Bielawski[1]

[1] Department of Synthesis and Technology of Drugs, Medical University of Bialystok, Poland
[2] Department of Biotechnology, Medical University of Bialystok, Poland

E-mail address of presenting author: robert.czarnomysy@umb.edu.pl

Objectives: Despite the development of thousands of anticancer structures, cancer is still a major cause of death worldwide. Therefore, there is a constant search for new compounds with potential anticancer effects. One class of compounds with high hopes as potential anticancer therapeutics are transition metal complexes with berenil and nitroimidazole. According to our studies, platinum(II) complexes with berenil have comparable or greater anti-tumor activity compared to cisplatin with less drug toxicity (Czarnomysy et al. Int J Mol Sci. 2021;22(11):5581.1). Therefore, the our goal of this study was synthesis and evaluation of anticancer activities of novel transition metal (Au, Pd, Pt) complexes with berenil and nitroimidazole moiety in MCF-7 and MDA-MB-231 human breast cancer cells.

Materials and Methods: The cytotoxic activity of the novel complexes was examined using the MTT method of Carmichael. Evaluation of apoptosis induction was done with the Annexin V/propidium iodide assay. Moreover, using the flow cytometer, the effects of the test compounds on mitochondrial potential change were assessed. Anti-cancer activity has been confirmed on zebrafish embryo xenograft model.

Results: The results showed higher cytotoxicity of tested compounds in comparison with cisplatin in MCF-7 and MDA-MB-231 human breast cancer cells. The complexes in which the central atom was palladium or platinum appeared to be the compounds of highest cytotoxicity. In relation to human normal breast epithelial cell MCF-10A the novel metal complexes were characterized with lower cytotoxicity than in case of examined MCF-7 and MDA-MB-231 neoplastic cells. Additionally our experiments revealed that these complexes inhibited the proliferation of breast cancer cells by increasing the number of apoptotic cells. In addition, we have observed that these complexes selectively concentrate in tumor cell mitochondria which may explain the increased proapoptotic activity of these compounds. We have further confirmed the effectiveness of the synthesized compounds on zebrafish embryo xenograft model, too.

Conclusions: : Results of our research indicate that novel transition metal (Au, Pd, Pt) complexes with berenil and nitroimidazole may constitute a new interesting class of compounds with potential antitumor activity.

Keywords: anticancer compounds; transition metal complexes; nitroimidazoles; breast cancer

Abstract No. PPP.009

Safety of useing soy isoflavonoid-based products in women during and after breast cancer treatment

Magda Pawlicka[1], Agata Filip[1], Patrycja Głaz[2], Magdalena Kołodziejczyk[3]

[1] Department of Cancer Genetics with Cytogenetic Laboratory, Medical University of Lublin, Poland
[2] Department of Synthesis and Chemical Technology of Pharmaceutical Substances, Medical University of Lublin, Poland
[3] Department of Biology and Genetics, Medical University of Lublin, Poland

E-mail address of presenting author: magda.pawlicka1@gmail.com

Objectives: In recent years there has been an increased interest in soybean isoflavonoids. Due to their strong antioxidant properties, these plant substances are now widely used in cosmetic products and dietary supplements. Breast cancer is the second most common malignancy in the world. Achievements of modern medicine have increased the survival rate of people with breast cancer, but patients during and after treatment are usually accompanied by a wide range of adverse effects, including skin disorders. Ongoing therapy often leaves scars and affects the function of the skin. Many patients decide to fight these unpleasant consequences of the therapy on their own, reaching for various cosmetics and supplements, very often containing phytoestrogens. There are many reports on anticancer properties of soy isoflavonoids, however, due to their wide spectrum of biological activity, the safety of these active ingredients should be studied, especially in oncological patients. Therfore, we conducted a study to examine the effects of genistein on estrogen-positive breast cancer cells line MCF-7.

Materials and Methods: After culture derivation, MCF-7 tumor cells and a control group, which was BJ dermal fibroblast cells, were treated with different concentrations of genistein (10-150 µM) for 24h and 48h. The cells were then subjected to trypan blue viability assays and cytotoxicity was checked using MTT assay. Apoptosis was detected using annexin and carboxyfluerescein assay. Additionally the expression of genes related to proliferation and apoptosis (*EGFR, MKI67, ACT1, BCL2* and *BIRC5*) was analyzed.

Results: The performed tests clearly indicated that genistein at concentrations above 20 µM had a negative effect on cancer cells at both exposure times. Additionally, it was observed that at 48h incubation, genistein concentrations of 50 µM and above also negatively affected the BJ fibroblast line. A genistein concentration of 10 µM had a stimulatory effect on both cell populations at both exposure times. Gene expression analysis showed that all genistein concentrations resulted in change of genes expression.

Conclusions: The performed experiments clearly indicate that soy isoflavonoid-based products are not fully safe for use in patients with breast cancer. However, genistein in strictly defined concentrations may be one of the methods of oncological or supportive treatment in cancer in the future.

Keywords: genistein, breast cancer, soy isoflavones

Abstract No. PPP.010

New benzamides as candidates for AChE and BACE1 inhibitors

Danuta Drozdowska[1], Dawid Maliszewski[1], Agnieszka Wróbel[1], Martyna Bogdańska[1], Justyna Wiśniewska[1]

[1] Organic Chemistry, Medical University, Poland

E-mail address of presenting author: danuta.drozdowska@umb.edu.pl

Objectives: Alzheimer's disease (AD) is a neurodegenerative disease, characterized by progressive loss of memory which is associated with other cognitive deficits. The growing number of cases, coupled with the lack of effective therapy, make AD a serious social, medical and economic challenge. There are various hypotheses about the causes of AD, but cholinesterases still remain key biological targets in the search for AD therapy. It has been postulated that β-amyloid aggregation is one of the major causes of Alzheimer's disease, and thus its inhibition may modify the course of the disease. β-secretase (BACE) initiates the production of Aβ, so its inhibition provides a valid target for the AD, therefore, the second most commonly chosen mechanism is to inhibit β-amyloid aggregation by inhibiting BACE1. The present study reports the synthesis of multifunctional ligands with both cholinergic and secretase inhibitory activities. The main goal of research was design, synthesis and biological evaluation of the novel benzamide derivatives. The designed compounds contained additional methoxy groups.

Methods: Aromatic amines were used as starting material for the synthesis. Benzamides were obtained by stirring of amines solved in dichloromethane (DCM) with N,N-diisopropylethylamine (DIPEA) in 0°C. Then solutions of aromatic acyl chlorides in dry DCM were added dropwise under atmosphere of inert gas. White precipitations were filtered under vacuum and washed three times with 10% HCl and 3 times with 10% $NaHCO_3$. The solid states were crystalized from boiling mixture of ethyl acetate and hexane. Products were filtrated and dried under vacuum. The structures of obtained compounds were confirmed by 1H and 13C NMR

The AChE inhibitory activity studies were carried out using Ellman's colorimetric method, and the BACE1 inhibitory activity studies were carried out using fluorescence resonance energy transfer (FRET).

Result and Conclusions: All compounds displayed considerable AChE and BACE1 inhibition.

Keywords: : Alzheimer Disease, AChE inhibitors, BACE1 inhibitors, benzamides

Abstract No. PPP.011

Novel derivatives of dipyridothiazines - synthesis and ADME analysis

Emilia Martula[1], Beata Morak-Młodawska[2], Małgorzata Jeleń[2]

[1] Department of Organic Chemistry, Doctoral School of The Medical University of Silesia, Poland
[2] Department of Organic Chemistry, The Medical University of Silesia in Katowice, Poland

E-mail address of presenting author: d201074@365.sum.edu.pl

Objectives: Phenothiazines (dibenzo-1,4-thiazines) are class of heterocyclic compounds with wide spectrum of biological properties. Recent reports showed promising anticancer, antiplasmid, antibacterial, anti-inflammatory and immunosuppressive activities of classical and new phenothiazines [1]. Previously synthesized dipyridothiazine derivatives (1,6-, 1,8-, 2,7- and 3,6-diazaphenothiazines) were shown to possess interesting antiproliferative, anticancer, antioxidant and immunosuppressive activity [2-7]. The aim of our project is obtaining new derivatives of dipyridothiazines – bis-dipyridothiazines possessing promising anticancer activity.

Methods: New compounds were obtain in the reactions of selected dipyridothiazines with selected linkers, in the presence of sodium hydride. Using 1H and 13C NMR, two-dimensional spectroscopy (1H-1H COSY, ROESY, HSQC, HMBC), mass spectrometry (HR MS) the right structure of the products were determined. For all new compounds, preliminary pharmacokinetic and lipophilicity studies were performed using available Internet servers: SwissADME, SwissTargetPrediction, showing promising properties [8].

Conclusions: Drug-likeness properties of novel compounds were evaluated using a predictive bioavailability radar model from the SwissADME web tool. The descriptors of physicochemical properties for selected compounds were projected next on the optimal range for each property to be considered drug-like. Further studies of biological activity have been planned.

Keywords: phenothiazines, biological properties, dipyridothiazines

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Abstract No. PPP.012

Novel Drug Design Framework As a Response to Neglected and Emerging Diseases

Stanisław Kulczyk[1], Mariola Koszytkowska-Stawińska[1]

[1] Faculty of Chemistry, Warsaw University of Technology , Poland

E-mail address of presenting author: stanislaw.kulczyk.stud@pw.edu.pl

Objectives: The emergence of new drug targets, as well as the abundance of uncharacterized drug targets is considered to leave a lot of space for the design of new pharmaceutics. The objective of our study was to create an *in silico* procedure to be used in the design of inhibitors of relatively unexplored targets. The feasibility of the developed procedure was to be shown on the example of SARS-CoV-2 main protease in response to global need for effective COVID-19 therapeutics.

Materials and Methods: The drug candidates were designed by linking small molecular fragments localized in their energy minima in the binding site. The proposed structures were modified following an in-house developed, unambiguous algorithm. Several verification steps were implemented to ensure high quality of the newly designed molecules. Lastly, a retrosynthetic analysis using an open access tool revealed two alternative retrosynthetic routes leading to each of the identified compounds.

Results: The *de novo* drug design algorithm yielded almost 200,000 potential inhibitors. Following a screening, rigorous verification and systematic modification, three compounds were chosen as potential drug candidates. These three compounds – novel chemical entities – were characterized with molecular descriptors that indicated high gastrointestinal bioavailability and predicted IC_{50} in nanomolar range. The conducted molecular dynamics analyses confirmed the suitability of the chosen compounds, and the conducted retrosynthetic analysis suggested that they were synthetically feasible.

Conclusions: The proposed protocol can be applied in the design of inhibitors of under researched targets, such as newly discovered proteins, neglected tropical diseases or emerging diseases. Owing to our novel approaches, the new potent inhibitors could be designed easily and efficiently. We hope that our procedure might be of interest to a wide range of researchers who conduct practical investigations, but are not primarily engaged on the field of computational chemistry. We find that their experimental investigations can be enriched by our simple, intuitive and openly available procedure.

Acknowledgements: The publication is a part of the "Szkoła Orłów" project, co-financed by the European Social Fund under the Knowledge-Education-Development Operational Programme, Axis III, Higher Education For The Economy And Development, Measure 3.1, Competences In Higher Education.

Abstract No. PPP.013

Validation a new LC-MS/MS method of tacrolimus concentration measurment in the blood samples collected by volumetric-absorptive microsampling

Arkadiusz Kocur[1], Dorota Marszałek[1], Tomasz Pawiński[1]

[1] Department of Drug Chemistry, Medical University of Warsaw, Poland

E-mail address of presenting author: arkadiusz.kocur95@gmail.com

Objectives: Volumetric-absorptive microsampling (VAMS) in a new approach to small volume of the whole blood collecting in the clinical practice. That procedure is beneficial in often blood sampling during immunosuppressive therapy, especially for the pediatric patients. Tacrolimus (TAC) is the primary drug in post-transplant pharmacotherapy and monitoring of its blood concentration levels is required due to the narrow therapeutic index. Home-based microsampling by Mitra® device seems to be a more advantageous for young transplant recipients, than classic venous sampling in the hospital. For this purpose, a new method of TAC determination in the samples collected by VAMS were developed and validated.

Materials and methods: The analyses were performed using a Shimadzu®-8050 mass spectrometer coupled with Nexera®-X2 HPLC system. The structural analogue of TAC- ascomycin (ASC) was used as internal standard (IS). The $[M + NH_4]^+$ adducts were monitored with mass transitions: 821.5 → 768.4 m/z and 809.5 → 756.4 m/z for TAC and ASC respectively. The samples after drying were diluted by deionized water, while the obtained extracts were treated with zinc sulphate: acetonitrile mixture (50:50; v/v). During validation of the method, the linearity, accuracy, carry-over, precision examination were evaluated. In addition, samples stability during stopped preparation to analyse and storage in autosampler and freezer has been checked.

Results: The method was successfully validated in 0.5–60 ng/mL range of TAC concentration. The imprecision and accuracy were satisfactory for the low (0.5 ng/mL), middle (7.5 ng/mL; 25 ng/mL) and high (60 ng/mL) concentration of the calibrators. The carry-over effect was not significant during analysis. The standard solutions of TAC and ASC were stable during freezing and in the autosampler storing. The all-tested parameters were fitted in the acceptance criteria range (according to the EMA guidelines).

Conclusions: The validated method based on VAMS-collected samples may be useful for TAC's concentration monitoring in the whole blood during immunosuppressive therapy. Young patients may perform sampling without leaving home, which in case of pandemic reality and limited pediatric transplant centers in Poland seems to be good solution. In the next step, the utility of developed method will be checked in the VAMS-samples obtained from the pediatric patients.

Keywords: tacrolimus, VAMS, TDM

Abstract No. PPP.014

Evaluation of the anticancer activity of a novel selenoester in MDA-MB-231 triple negative breast cancer cells

Dominika Radomska[1], Dominik Radomski[1], Robert Czarnomysy[1], Krzysztof Bielawski[1]

[1] Department of Synthesis and Technology of Drugs, Medical University of Bialystok, Poland

E-mail address of presenting author: dominika.radomska@umb.edu.pl

Objectives: Every year the number of new breast cancer cases is increasing, and its resistance to standard treatment is becoming a growing problem and at the same time a challenge for the modern scientific world. Lately, more attention has been concentrated on compounds containing selenium in their structure. It turns out that these compounds are highly cytotoxic, strongly induce apoptosis, and can even break multidrug resistance (MDR) in cancer cells (Radomska et al., International Journal of Molecular Sciences, 2021, 22(3), 1009). Therefore, our team undertook to evaluate the biological activity of a novel selenoester (EDA-71) in MDA-MB-231 triple-negative breast cancer cells.

Materials and Methods: At first, the cytotoxicity of compound EDA-71 and cisplatin against MDA-MB-231 breast cancer cells and MCF-10A normal breast epithelial cells was determined by MTT assay according to Carmichael's method. Afterward, flow cytometer analysis of apoptosis induction and mitochondrial membrane potential (MMP) was performed using annexin V/propidium iodide and JC-1 cationic dye, respectively.

Results: Results revealed that novel selenoester is more cytotoxic against cancer cells than cisplatin. Additionally, it is worth emphasizing that the cytotoxicity of the tested compound in normal cells was lower than in MDA-MB-231 cells. Moreover, further studies showed that EDA-71 induces apoptotic cell death and reduces MMP to a greater extent than cisplatin.

Conclusions: We observed that the high cytotoxicity of compound EDA-71 in MDA-MB-231 triple-negative breast cancer cells correlated with strong induction of apoptosis. We also found that apoptotic cell death occurs via the mitochondrial pathway that was associated with a decrease of MMP. The tested compound appears to be a promising candidate for an anticancer drug in the future, but knowledge of its detailed molecular mechanism of action still requires more extensive studies.

Abstract No. PPP.015

New chitosan composites with BODIPY compounds as potential photosensitizing drug form for PDT

Aleksander Smolarkiewicz-Wyczachowski[1], Szymon Bocian[2], Marta Ziegler-Borowska[1], Dorota Chełminiak-Dudkiewicz[1]

[1] Department of Biomedical and Polymer Chemistry, Nicolaus Copernicus University in Toruń, Poland
[2] Department of Environmental Chemistry and Bioanalysis, Nicolaus Copernicus University in Toruń, Poland

E-mail address of presenting author: asmolarkiewicz@gmail.com

Objectives: Synthesis of chitosan composites with the addition of BODIPY dyes, as an example of potential matrices for a photosensitizer-drug used in photodynamic therapy.

Materials and methods: Chitosan films containing BODIP-y compounds were obtained by pouring acetic acid solution according to the method described by H. Kaczmarek et. Al. [1], so that the concentration of the compound contained in the film would be 5%. The obtained materials were characterized using UV-Vis spectroscopy and spectrofluorimetry (quantum yields of fluorescence and singlet oxygen generation were determined). Surface morphology was characterized using Scanning Electron Microscopy (SEM) and Atomic Force Microscopy (AFM). The photostability and thermostability of the composites were also tested. The final stage of the research was to determine the kinetics of BODIPy type dye release from the chitosan membrane.

Results: The SEM results confirmed the homogeneity of the obtained materials. AFM showed differences in the roughness of the obtained chitosan materials with the addition of dyes compared to pure chitosan. Spectroscopic examination of the dye in the membrane gave results not significantly different from the results obtained based on the characteristics of the dye in the solution. It has also been proved that the compound in the chitosan membrane can generate singlet oxygen. Studies of the dye release kinetics from the film showed that the dye was released without any difficulty.

Conclusions: The results are the material that meets the conditions that allow it to be used as a drug delivery system. The fact that the compound in the chitosan membrane can generate singlet oxygen gives hope for potential use in photodynamic therapy. The material obtained could be used as a form of the drug-anchored patch in targeted therapies.

Acknowledgements: Gratefully acknowledge the Center of Excellence "Towards personalized medicine" for financial support of research.

Keywords: chitosan, BODIPY, photodynamic therapy

Reference:

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Abstract No. PPP.016

Physiologically based pharmacokinetic model for informing ropinirole new formulation development

Olha Shuklinova[1], Barbara Wiśniowska[1], Gabriela Wyszogrodzka-Gaweł[1], Bartosz Lisowski[1], Sebastian Polak[1]

[1] Social Pharmacy, Jagiellonian University Medical College, Poland

E-mail address of presenting author: shuklinova@doctoral.uj.edu.pl

Objectives: One of the desirable characteristics of an antiparkinsonian drug is smooth, non-pulsatile stimulation of dopamine receptors which allows to delay the onset of therapy-related side effects such as dyskinesia. The development of prolonged release formulations is one of the ways to provide such drug behavior. Physiologically based pharmacokinetics modeling (PBPK) is a tool which allows to conduct virtual PK trials and inform the formulation development. The purpose of this work was to develop a PBPK model for ropinirole prolonged release formulation to predict behavior of a new formulation in the human body based on in vitro dissolution data. Additional purpose of the study was to analyse ropinirole PK parameters and confirm the assumptions regarding its absorption, distribution, and metabolism.

Materials and Methods: The model was developed and verified using available physico-chemical, ADME, and clinical data for ropinirole and its formulations with immediate and prolonged release. The modeling and simulation activities were conducted using Simcyp® Simulator (V.20, Certara, Sheffield, UK) and such virtual population libraries as Sim-Healthy Volunteers and Sim-NEurCaucasian.

Results: The ropinirole PBPK model, which included mechanistic description of metabolic clearance, whole body distribution, and in vitro dissolution data (extracted from the drug product certificates of analysis), adequately predicted the concentration-time profiles of the ropinirole both in healthy volunteers and in Parkinson's disease patients. The verification of the model showed that the predicted versus observed Tmax, Cmax, and AUC ratios were within the 2-fold which is a commonly accepted criterion of the PBPK model performance estimation.

Conclusions: In this study the PBPK model for ropinirole was developed and successfully verified. The model was able to predict ropinirole pharmacokinetic behavior when administered as an immediate release and prolonged release formulation by healthy volunteers and Parkinson's disease patients. In the next step the developed model will be used to inform the development of new ropinirole formulation.

Acknowledgements: The current project has been financed from the Polish National Science Center OPUS project number OPUS 2018/31/B/NZ7/03238

Keywords: : ropinirole, physiologically based pharmacokinetic modeling, prolonged release formulation

Abstract No. PPP.017

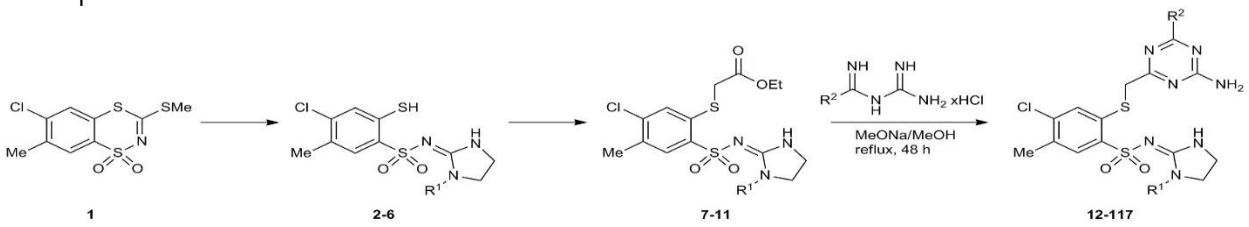
Design, synthesis and anticancer activity of novel 2-[4-amino-6-R2-1,3,5-triazin-2-ylmethylthio]-N-(1-substituted-imidazolidin-2-ylidene)-4-chloro-5-methylbenzenesulfonamide derivatives

Lukasz Tomorowicz[1], Jarosław Sławiński[1], Beata Żołnowska[1], Krzysztof Szafrański[1], Anna Kawiak[2]

[1] Organic chemistry, Medical University of Gdansk, Poland
[2] Biotechnology, University of Gdańsk, Poland

E-mail address of presenting author: lukasz.tomorowicz@gumed.edu.pl

Objectives: The aim of the project was to search for anti-cancer compounds among 2-(4-amino-6-R²-1,3,5-triazin-2-ylmethylthio)-*N*-(1-R¹-imidazolidin-2-yl)benzenesulfonamide derivatives (Scheme 1), which are a combination of two pharmacophore moieties: 2,4,6-trisubstituted 1,3,5-triazine and 2-mercaptobenzenesulfonamide.



Scheme 1. Multistep synthesis derivatives (**12**–**117**).

Materials and methods: The final compounds (**12**–**117**) were obtained in a multistep reaction starting from 3-methylthio-1,1-dioxo-1,4,2-benzoditiazine (**1**), which was transformed into *N*-substituted 2(ethoxycarbonylmethylthio)benzenesulfonamides (**7**–**11**) and in final step subjected to a cyclocondensation with the appropriate biguanide hydrochlorides in MeONa/MeOH solution (Scheme 1). Anticancer activity was evaluated *in vitro* for all obtained compounds using the MTT method on cancer cell lines of colon cancer (HCT-116), breast cancer (MCF-7) and cervical cancer (HeLa) and non-cancer line of human keratinocytes (HaCaT). After synthesized each series of derivatives, were generated QSAR models for HCT-116 cell line, which correlated IC_{50} value with chemical parameters molecule.

Results: The QSAR equations were generated for three series of compounds (**12**–**32**, **33**–**59**, **60**–**79**), that allowed to correlate the parameters of the molecule with the activity against the colon cancer cell line HCT-116. From QSAR model for compounds **12**–**32** (R^1 = H) shows that the activity for the HCT-116 cell line correlates with number of rings in the molecule and partial charge of molecule. QSAR model for compounds **33**–**59** (R^1 = Bn) indicates that the IC_{50} value depends on number of rotatable single bonds, the distance between the atoms, the charge of molecule and the number of oxygen and nitrogen atoms. QSAR model for compounds **60**–**79** (R^1 = 4-F-Bn) showed a relationship between the hydrophobic van der Waals surface and the IC_{50} value among HCT-116 cell lines.

Conclusions: The most active series of derivatives **103**–**117** bearing R^1 = 3,5-bis(trifluoromethyl)benzyl showed outstanding activity HCT-116: 3.6–13.0 μM, MCF-7: 4.5–13.0 μM and HeLa: 5.5–18.0 μM. Compound **110** R^2 = 4-phenylpiperazin ($IC_{50\ HCT\text{-}116}$ = 3.6 μM), exhibited high cytotoxic effect against the cell line HCT-116 comparable to effect of Cisplatin ($IC_{50\ HCT\text{-}116}$ = 3.8 μM).

Keywords: benzenesulfonamide; synthesis; 1,3,5-triazines; imidazole; QSAR; anticancer activity

Abstract No. PPP.018

Synthesis and antimicrobial activity of new derivatives of 4-tert-butylbenzoic acid hydrazide

Kinga Paruch[1], Anna Biernasiuk[2], Łukasz Popiołek[1]

[1] Chair and Department of Organic Chemistry, Medical University of Lublin, Poland
[2] Chair and Department of Pharmaceutical Microbiology, Medical University of Lublin, Poland

E-mail address of presenting author: kinga.paruch@umlub.pl

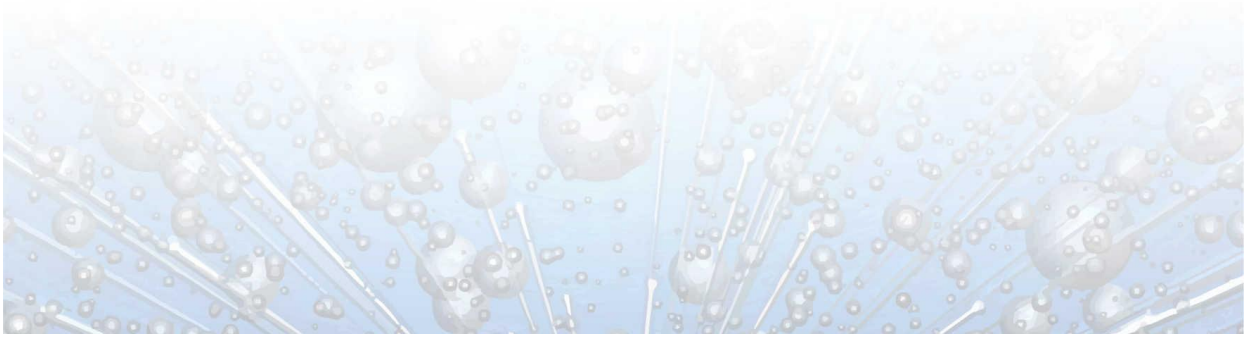
Objectives: In the era of broadly understood antibiotic resistance, it is necessary to search for new molecules with potential antimicrobial activity, capable to counteract the problem of growing bacterial resistance to standard antibiotics. This problem particularly affects the medicine resistant strains, which causes severe infections during inpatient and palliative treatment. Due to this fact, a series of new derivatives of 4-*tert*-butylbenzoic acid hydrazide has been designed and obtained.

Materials and Methods: The 4-*tert*-butylbenzoic acid hydrazide was used as a starting compound for the synthesis, which was subjected to condensation reactions with appropriate aldehydes. These reactions resulted in the synthesis of a series of 17 new acylhydrazones. The obtained compounds were subjected to a cyclization reactions with acetic anhydride, which allowed to obtain 17 new 2,5-disubstituted-*N*-acetyl-1,3,4-oxadiazoles. The chemical structure of all obtained substances was confirmed by spectroscopic methods (IR, ^{1}H NMR, ^{13}C NMR) and subjected to antimicrobial activity assays in order to confirm their bioactivity.

Results: The antimicrobial activity tests showed that the one compound showed the good antibacterial activity against *Micrococcus luteus* ATCC 10240 strain. It was observed that the activity of the studied group of derivatives was significantly higher against Gram-positive strains than for Gram-negative strains and fungi.

Conclusions: In summary in this research, we present the synthesis of a series of new 4-*tert*-butylbenzoic acid hydrazide derivatives, which show significant microbiological activity, especially against Gram-positive bacterial strains (staphylococci and micrococci) with 125 - 1000 ug/mL.

Keywords: acylhydrazones, *N*-acetyl-1,3,4-oxadiazoles, antimicrobial activity



Abstract No. PPP.019

Cornus mas fruit – searching of its role in inflammation-related metabolic disorders and the fate of its metabolites after gastrointestinal transformation in vitro

Agata Olędzka[1], Katarzyna Cichocka[1], Konrad Woliński[2], Matthias F. Melzig[3], Monika E. Czerwińska[4]

[1] Department of Biochemistry and Pharmacogenomics, Student Scientific Association "Farmakon", Medical University of Warsaw, Poland
[2] Centre for Biological Diversity Conservation in Powsin, Polish Academy of Sciences Botanical Garden, Poland
[3] Institute of Pharmacy, Freie Universitaet Berlin, Germany
[4] Department of Biochemistry and Pharmacogenomics, Medical University of Warsaw, Poland

E-mail address of presenting author: monika.czerwinska@wum.edu.pl

Objectives: Targeting inflammation as well as pancreatic lipase and α-amylase by ethanolic-aqueous (60%, *v*/*v*) extract from *Cornus mas* (cornelian cherry) fruit (CME) or digestion-derived fractions in relation to prevention and control of metabolic disorders, including diabetes, was the first purpose of the present study. In addition, we attempted to identify metabolites of CME after gastrointestinal digestion *in vitro* (GID) as well as their kinetic changes upon gut microbiota treatment to identify the potentially bio-accessible metabolites of CME.

Materials and methods: The effect of standardized CME on cytokines secretion in neutrophils/PMN (tumor necrosis factor α, TNF- α; interleukin 8, IL-8; interleukin 1β, IL-1β), peripheral blood mononuclear cells/PBMC (TNF- α, IL-6, IL-10), and in human colorectal adenocarcinoma cell line Caco-2 (IL-8) was established with enzyme-linked immunosorbent assays. The digestion of CME was simulated with digestive enzymes *in vitro* and human gut microbiota *ex vivo* (1h, 3h, 6h, 24h), followed by chromatographic analysis using the UHPLC-DAD-MS^n method. Next, the effect of GID fractions on the activity of pancreatic lipase and α-amylase was studied with fluorescence-based assays.

Results: CME showed the propensity to increase TNF-α and IL-1β secretion in PMN, whereas it inhibited the release of IL-8 in PMN and pro-inflammatory cytokines secretion in PBMC. The inhibition of IL-8 in Caco-2 cells was also noted. The gastric and intestinal fractions obtained after GID of CME inhibited pancreatic lipase and α-amylase activity. Loganic acid as the main constituent of CME (16.97 mg/g d.w.) was digested in the experimental conditions contrary to cornuside. Phenolic acids, flavonoids (e.g. aromadendrin), and tannins were detected in the late phases of digestion.

Conclusions: The immunomodulatory effect of CME and inhibition of digestive enzymes observed *in vitro* may partially confirm the traditional use of *Cornus mas* fruits in protection against the development of diabetes-derived inflammatory complications and decrease of glucose and lipids absorption. Cornuside, aromadendrin, and phenolic acids may be potentially bio-accessible compounds of preparations from cornelian cherries.

Acknowledgments: This project was financially supported by the National Science Centre research grant Sonata 12 No. 2016/23/D/NZ7/00958 (Poland).

Keywords: diabetes, metabolism, inflammation

Abstract No. PPP.020

Solubilizer tag effect on PD-L1/inhibitor binding for m-terphenyl derivatives

Ewa Surmiak[1], Julia Zaber[1], Grzegorz Wojtanowicz[1], Justyna Kocik[1], Oskar Kruc[1], Damian Muszak[1], Bogdan Musielak[1], Ismael Rodriguez[1], Lukasz Skalniak[2], Katarzyna Magiera-Mularz[1], Tad Holak[1], Justyna Kalinowska-Tłuścik[1]

[1] Faculty of Chemistry, Uniwersytet Jagielloński, Poland
[2] Uniwersytet Jagielloński, Poland

E-mail address of presenting author: ewa.surmiak@uj.edu.pl

Objectives: The PD1-PD-L1 pathway serves as a negative regulator of T cells and helps to maintain control of inflammation and self-aggression. Moreover, inhibition of PD-L1 leads to the reactivation of T cells against cancer cells. Several small molecules, PD-L1 inhibitors, have been reported, including the most prominent compound A, which induces PD-L1 dimerization and internalization. Although the main pressure is placed on the evaluation of the central hydrophobic core of inhibitors, there are limited examples of the structure-activity relationship based on the solubilizer tag.

Materials and Methods: The characteristic and purity of the compounds were assess with LC-MS, NMR, and IR. PD-L1 activity was validated by HTRF biochemical assay using a Cis-Bio reagents kit and an immune checkpoint blockade (ICB) assay. For this, the culture of Jurkat-Effector Cells (Jurkat-ECs) and CHO/TCR-Act/PD-1 was carried out according to the known protocols. The X-ray diffraction data were collected at the BL14.1 beamline operated by the Helmholtz Zentrum Berlin at the BESSY II. Molecular docking based on 7NLD PD-L1 crystal structure was performed in GOLD with GA used for conformer generation and PLP scoring function applied.

Results: In our study, we synthesized and tested several m-terphenyl derivatives with the cyclic amino acid solubilizer tag. The most prominent compounds contain beta-proline and isonipecotic acid tags and resulted in up to 90% dissociation of the PD-1/PD-L1 complex at 5 nM inhibitor concentration in the HTRF assay. However, all the studied compounds did not show suspected activity in the cellular system, which led us to investigate their binding to PD-L1 by protein crystallography and molecular modeling.

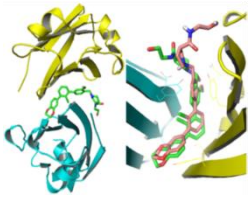


*Figure 1. PD-L1 dimer stabilized by **2f** compound (upper panel); superposition of **2f** observed in the crystal (green) and docked pose (lower panel)*

Conclusions: Analysis of ligand-protein interactions in the experimental and modeled complexes showed that the tertiary amine of the solubilizer tag in protonated form, may interact with Asp122 of PD-L1, creating salt bridge and by this provides additional binding force stabilizing the homodimeric PD-L1 complex. This finding revealed another feature which should be considered in the PD-L1 inhibitors design.

Acknowledgements: Research was supported by Sonata Grant No. UMO-2020/39/D/ST4/01344 from the National Science Centre, Poland.

Keywords: m-terphenyl, PD-1/PD-L1, cancer

Abstract No. PPP.021

In Vitro/In Vivo translation of synergistic combination of MDM2 and MEK inhibitors in melanoma using PBPK/PD modeling

Jakub Witkowski[1], Sebastian Polak[2], Zbigniew Rogulski[3], Dariusz Pawelec[4]

[1] Faculty of Chemistry/Preclinical Development Department, University of Warsaw/Adamed Pharma, Poland
[2] Faculty of Pharmacy, Jagiellonian University, Poland
[3] Faculty of Chemistry, University of Warsaw, Poland
[4] Preclinical Development Department, Adamed Pharma, Poland

E-mail address of presenting author: j.witkowski6@uw.edu.pl

Objectives: Development of method allowing to translate in vitro observed synergy between HDM201 (MDM2 inhibitor) and Trametinib (MEK inhibitor) into in vivo synergistic efficacy between these molecules in order to determine the most effective doses and dosing schedules which could be used in the clinical settings.

Materials and Methods: HDM201 and Trametinib combination cytotoxicity was evaluated in vitro against melanoma A375 cells in time with the use of RealTime-Glo assay. Analysis of drug combination matrix was performed with the use of Synergy and Synergyfinder packages. Female CD-1 Nude mice were utilized for the pharmacokinetic (PK)/pharmacodynamics (PD) studies. Analysis of plasma and A375 tumour profiles (PK) alongside with tumour volume change (PD) were examined in mice xenografted with A375 cells. ADME parameters were taken from literature or estimated in the Simcyp Simulator (V21). PBPK/PD modelling was performed for HDM201, Trametinib and their combination at various dose levels.

Results: The developed PBPK/PD models took into account PK interactions between HDM201 and Trametinib (Area Under Curve ratios: 1.14 and 0.80 respectively for the highest tested doses) and verified which synergistic PD interaction parameter from in vitro studies will be translational and could serve as PD interaction parameter (concentration independent parameter ß from Synergy package analysis – 23.1% of increase of combined drug efficacy or concentration dependent mean õ parameter from Synergyfinder analysis - 7.5% of increased of combined drug efficacy). Final models indicate that relationship between HDM201 and Trametinib concentrations and efficacy in combination could be explained with the dose dependent PK interaction and PD interaction with ß parameter from MuSyC model. This approach allowed for reasonable estimation of the most synergistic and efficacious dosing schedules and dose levels for those drugs in the mice in vivo setting.

Conclusions: PBPK/PD modelling is a powerful tool allowing for proper estimation of in vivo efficacy of the anticancer drug combination based of in vitro studies results. Such approach may indicate the most efficacious schedule and dose levels which allows for better planning of the clinical trials and estimation of drug combination efficacy in such trial on virtual representation of cancer patients.

Acknowledgements: The authors would like to thank Adamed Pharma for support.

Keywords: Drug combination, PBPK/PD modelling, MDM2

Abstract No. PPP.022

Dual GSK-3β and IKK2 kinase inhibitors in the group of 2(cyclopropanecarboxamido)pyridin-4-yl substituted benzenesulfonamides – a new direction in the search for Alzheimer's disease therapy

Tomasz Wichur[1], Justyna Godyń[1], Izabella Góral[1], Anna Więckowska[1]

[1] Department of Physicochemical Drug Analysis, Jagiellonian University Medical College, Poland

E-mail address of presenting author: tomasz.wichur@uj.edu.pl

Objectives: Complex and incurable Alzheimer's disease (AD) requires urgent approval of new effective drugs. However, despite the huge efforts on preclinical development and clinical trials, there are only a few approved drugs on the market. Furthermore, their effectiveness is limited to temporary slowing down the disease progression. The multifactorial and ununderstood nature of the disease makes the drug development pipeline challenging, with many potential targets involved. Among the promising goals, two enzymes are highlighted, glycogen synthase kinase 3β (GSK-3β) and IKK2 kinase, as their dysregulations play an undoubtedly significant role in the pathogenesis of AD. Under AD conditions, GSK-3β is associated with pathological hyperphosphorylation of tau protein, resulting in the formation of toxic neurofibrillary tangles (NFTs), a possible cause of neuronal death [1]. IKK2 activation may stimulate inflammatory gene expression in glial cells, thus being closely related to neuroinflammatory processes in the brain, and actively contributing to neuronal loss [2]. Therefore, modulating the activity of both enzymes may be an important step in the search for a possible disease-modifying therapy of the disease.

Materials and Methods: In our studies we designed, synthesized, and evaluated *in vitro* a series of novel GSK-3β and IKK2 dual inhibitors. The compounds, as presented in Figure 1, are derivatives of 4-substituted benzenesulfonamides with the *N*-(pyridin-2-yl)cyclopropanecarboxamide scaffold.

Results: The research results revealed promising biological activity of target compounds toward both kinases, with the obtained nanomolar range of IC_{50} values for most potent derivatives.

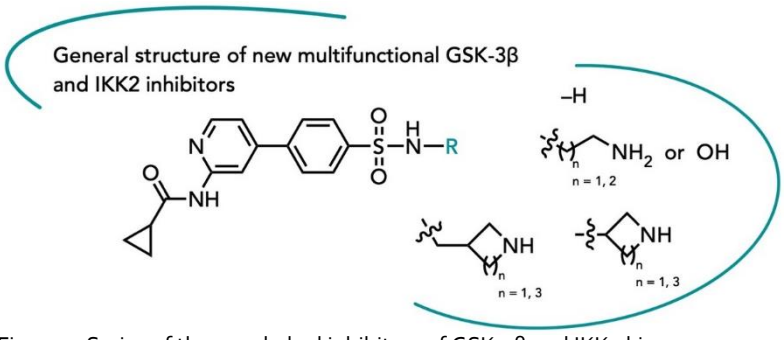


Figure 1. Series of the novel, dual inhibitors of GSK-3β and IKK2 kinases.

Conclusions: Within the presented group of compounds we identified potent GSK-3β and IKK2 dual inhibitors for further optimization and development in the search for AD causal treatment.

Acknowledgements: This research was funded by National Science Center, Poland grant No. 2019/34/E/NZ7/00090.

References:

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Abstract No. PPP.023

Synthesis of hydrazide starch for the selective release of anti-cancer drugs

Paweł Nowak[1], Kinga Mylkie[1], Marta Ziegler-Borowska[1]

[1] Department of Biomedical and Polymer Chemistry, Nicolaus Copernicus University in Toruń, Poland

E-mail address of presenting author: nowak19981411@wp.pl

Objective: Synthesis of hydrazide starch capable of forming a hydrazone bond with an anti-cancer drug.

Materials and Methods: Sodium carboxymethyl starch (CMS[Na]) synthesis was carried out according to the procedure developed by Stojanowić et al. with minor modifications [1]. The sodium form of carboxymethyl starch CMS[Na] was converted to acid form CMS[H] in the second step. At this modification stage, the degree of substitution in CMS [H] was determined by titration according to the procedure described by Stojanowić et al. [2]. In the last step of starch modification, CMS[H] was coupled with hydrazine leading to the formation of hydrazide starch (HMS). Then, the modified starch was used to attach doxorubicin (DOX) with the formation of a hydrazone bond. The percentage of drug bound to the carrier was examined by spectrofluorimetry.

Results: The multi-stage modification of starch led to the formation of hydrazide polysaccharide. At each synthesis stage, the structure of obtained material has been characterized by ATR FTIR spectroscopy, thermal and XRD analysis. Electron microscopy photos were done for the surface morphology characterization. Finally, an HMS-DOX conjugate with a pH-sensitive hydrazone bond was obtained, and the sensitivity of this combination for pH changes was detected.

Conclusions: The obtained materials were able to attach an antineoplastic drug using a pH-sensitive hydrazone bond. The resulting system may constitute a potential carrier of an anti-cancer drug, which will selectively release the drug in tumor cells.

Acknowledgements: Authors gratefully acknowledge the UMK Center of Excellence "Towards personalized medicine" for financial support of this research.

Keywords: starch, hydrazone bond, anticancer drug carrier

References:

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Abstract No. PPP.024

Immobilization of alpha-1-acid glycoprotein on magnetic nanoparticles functionalized with starch and boronic acids

Kinga Mylkie[1], Paweł Nowak[1], Marta Ziegler-Borowska[1]

[1] Faculty of Chemistry, Nicolaus Copernicus University in Toruń, Poland

E-mail address of presenting author: kinga.mylkie@doktorant.com.pl

Objective: Synthesis of magnetic nanoparticles coated with starch and boronic acids for effective immobilization of glycoproteins.

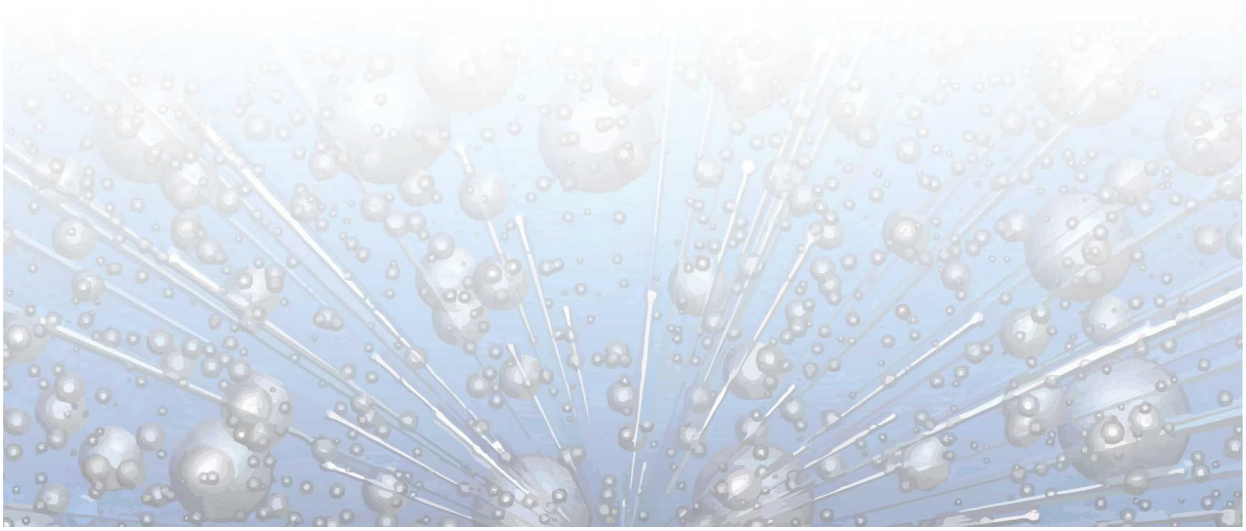
Materials and methods: The project's first stage involved the synthesis of starch-coated magnetite nanoparticles by in situ co-precipitation, which was then oxidized to a dialdehyde starch coating. Next, the reaction of obtained magnetite nanoparticles with 3-aminophenylboronic acid was carried out. The final stage was the immobilization of alpha-1-acid glycoprotein on the obtained material. The amount of immobilized protein was determined using the UV-Vis method.

Results: Prepared materials were composed of a magnetite core coated by a modified polysaccharide, with boronic acids on the surface. Due to the presence of dihydroxyboryl groups, synthesized material was able to binding glycoprotein. All obtained materials were fully characterized - Scanning Electron Microscopy photos, Transmission Electron Microscopy photos, thermal analysis, X-ray analysis and ATR-FTIR were taken. The amount of immobilized alpha-1-acid glycoprotein on magnetic nanoparticles coated with starch enriched in dihydroxyboryl groups is 33,5 mg glycoprotein/g nanoparticles.

Conclusion: Magnetic nanoparticles coated with starch enriched in free dihydroxyboryl groups are materials useful for rapidly binding the sugar part of glycoproteins.

Acknowledgements: Authors gratefully acknowledge the NCU Center of Excellence "Towards personalized medicine" for financial support of this research.

Keywords: glycoproteins, magnetic nanoparticles, modified starch



Abstract No. PPP.025

Impact of lignans on the secretion of heme oxygenase and pro-inflammatory cytokines

Małgorzata Kołtun-Jasion[1], Agnieszka Wrzesień[2], Andrzej Patyra[2], Anna Kiss[2]

[1] Warszawski Uniwersytet Medyczny, Poland
[2] Department of Pharmacognosy and Molecular Basis of Phytotherapy, Warszawski Uniwersytet Medyczny, Poland

E-mail address of presenting author: malgorzata.koltun@wum.edu.pl

Introduction: Lignans can be naturally found in measurable quantities in plants and plant products, including fruits, vegetables, seeds, and herbs. On the basis on the latest research, lignans may play an important role in maintaining health and even reversing the progression of chronic diseases, with anti-inflammatory and antioxidant effects as the important underlying mechanism. Heme oxygenase (HO-1) plays pivotal role in cellular stress response and homeostasis. It is a microsomal enzyme catalyzing heme degradation, in which biliverdin/bilirubin, carbon monoxide (CO), and free iron are released. The deregulation of its expression is implicated in pathophysiology of a wide spectrum of human disorders, including cardiovascular disease, obesity, diabetes, pulmonary disease, or kidney dysfunction, thus heme oxygenase has been considered as one of the most prominent targets in the management of many human diseases Both HO-1 as an antiinflammatory factor and also proinflammatory cytokines (IL-6, TNF-α, IL-1β and MCP-1) play an important role in anti-oxidant defenses and in the anti-inflammatory response.

Objectives: The aim of our project was to elucidate the precise mechanism of action for tested lignans in terms of secretion pro and anti-inflammatory cytokines in the model of LPS-stimulated, human monocytes/macrophages.

Materials and Methods: Monocytes/macrophages were isolated from human peripherial blood using density gradient centrifugation on Pancoll. After 24-h incubation with tested lignans (20 μM) cell lysates or supernatants were studied respectively on HO-1 and IL-6, TNF-α, IL-1β, MCP-1 by ELISA test. Results were presented with respect to LPS- control and positive control with dexometazone.

Results: The best results indicating inhibition of pro-inflammatory cytokine secretion were demonstrated, for pinoresinol glucoside, arctigenin, diarctigenin, cyclolariciresinol, and secoisolariciresinol diglucoside, while SECO and hydroxymatairesinol stimulated HO-1 secretion.

Conclusion: Our study shows that lignans have anti-inflammatory properties that are exerted through the induction of HO-1 and pro-inflammatory cytokines supression, which may be an introduction to further research.

Keywords: : lignans, heme oxygenase, cytokines

Abstract No. PPP.026

Structure-activity relationship in a series of glycogen synthase kinase 3β inhibitors based on N-(pyridin-2-yl)carboxamide scaffold

Izabella Góral[1], Natalia Szałaj[1], Dawid Panek[1], Tomasz Wichur[1], Justyna Godyń[1], Anna Więckowska[1]

[1] Department of Physicochemical Drug Analysis, UJCM, Poland

E-mail address of presenting author: natalia.guzior@uj.edu.pl

Objectives: Alzheimer's disease (AD), the most frequent cause of dementia, is a fatal and incurable neurodegenerative disorder, which remains an unmet medical need. Tau pathology is one of the most common AD-associated proteinopathies. Physiologically, tau is a microtubule-associated protein that stabilizes axonal microtubule structure and neuronal connectivity. In AD, it loses this function, because it is hyperphosphorylated by the tau-phosphorylating enzyme – glycogen synthase kinase 3β (GSK-3β). Therefore, the inhibition of GSK-3β is one of the pursued approaches in the search for an effective anti-AD treatment that aims at combating tauopathy as one of the causes underlying the disease.

Materials and Methods: In our research, we focused on the development of a new series of GSK-3β inhibitors. Based on the structure of the known GSK-3β inhibitor, we designed two series of compounds with *N*-(pyridin-2-yl)carboxamide scaffold linked to 2,2-dimethyl-2,3-dihydrothieno[3,2-d]pyrimidin-4(1*H*)-one or 3-ureidothiophene-2-carboxamide (Figure 1). Compounds' modifications included different substituents in cyclopropane carboxamide fragment. The designed series were synthesized and tested *in vitro* using Kinase-Glo luminescence assay.

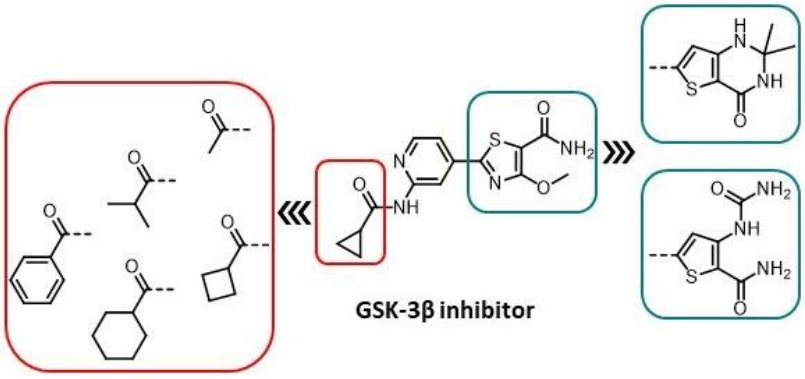


Figure 1. Design of GSK-3β inhibitors with N-(pyridin-2-yl)carboxamide scaffold.

Results: The analysis of potency of the synthesized compounds against GSK-3β indicated the key structural elements responsible for high inhibitory activity. The most potent inhibitor with IC_{50} value of 10 nM consist of 2,2-dimethyl-2,3-dihydrothieno[3,2-d]pyrimidin-4(1*H*)-one ring and cyclopropyl moiety.

Conclusions: Due to the promising results of the preliminary biological evaluation, the obtained compounds serve as an excellent starting point for further development and optimization.

Acknowledgements: This research was funded by National Science Center, Poland grant No. 2019/34/E/NZ7/00090.

Keywords: Alzheimer's disease, GSK-3β, tau protein

Abstract No. PPP.027

The Influence of Supplementation with Zinc in Micro and Nano Forms on the Metabolism of Fatty Acids in Livers of Rats with Breast Cancer

Agnieszka Stawarska[1], Małgorzata Czerwonka[1], Małgorzata Jelińska[1], Barbara Bobrowska-Korczak[1]

[1] Department of Bromatology, Medical University of Warsaw, Poland

E-mail address of presenting author: agnieszka.stawarska@wum.edu.pl

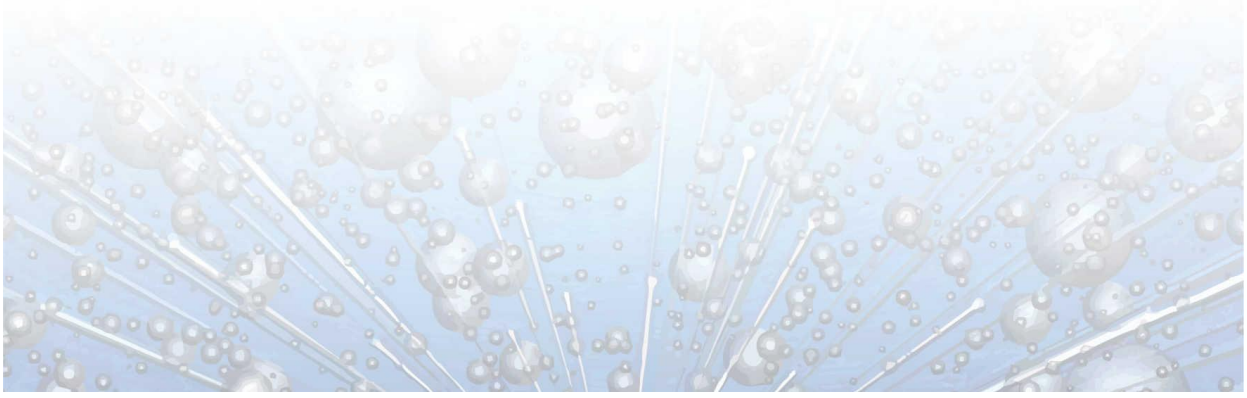
Objectives: The aim of the study was to investigate the effect of zinc supplementation (in form of nano or micro particles) on the profile and metabolism of fatty acids in the liver microsomes of rats with induced breast cancer. The activity of desaturases (Δ5, Δ6, Δ9) and the level of cholesterol and its oxidized derivatives were measured. The aim of this study was also to determine the effect of various forms of zinc supplemented to rats on 5-, 12- and 15-hydroxyeicosatetraenoic (5-, 12- and 15-HETE) and hydroxyoctadecadienoic (HODE) acids.

Materials and Methods: Female Spraque-Dawley rats (n = 24) were divided into 2 groups supplemented with zinc in the form of micro (342 nm) or nano (99 nm) particles, respectively, and a group with a standard diet (control group). All animals received 7,12-dimethylbenz[a]anthracene for induction of breast cancer. The analysis of the profile of fatty acids, cholesterol and cholesterol oxidation products (COPs) in rat liver microsomes was carried out using gas chromatography with a mass spectrometer. Desaturases activity and fatty acid lipoxygenase metabolites were analyzed with high performance liquid chromatography with UV detection.

Results: Zinc in form of nanoparticles inhibited the development of breast tumors that were chemically induced with DMBA in rats. Dietary Zn nano supplementation increased vaccenic acid content (p=0.032) and decreased Δ6-desaturase activity (p = 0.006), whereas Zn micro increased cholesterol (p=0.006), and total cholesterol oxidation products (p = 0.019) content. Dietary enrichment with Zn microparticles resulted in lower concentrations of metabolites: 15-, 12- and 5-HETE and HODE.

Conclusions: Our study indicates that the effect of zinc supplementation on fatty acids' metabolism in the liver microsomes under neoplastic conditions depends on the form in which it is administered.

Keywords: bromatology; food supplements



Abstract No. PPP.028

Zinc affects cholesterol oxidation products and fatty acids composition in rats' serum

Agnieszka Stawarska[1], Małgorzata Czerwonka[1], Barbara Bobrowska-Korczak[1]

[1] Department of Bromatology, Medical University of Warsaw, Poland

E-mail address of presenting author: agnieszka.stawarska@wum.edu.pl

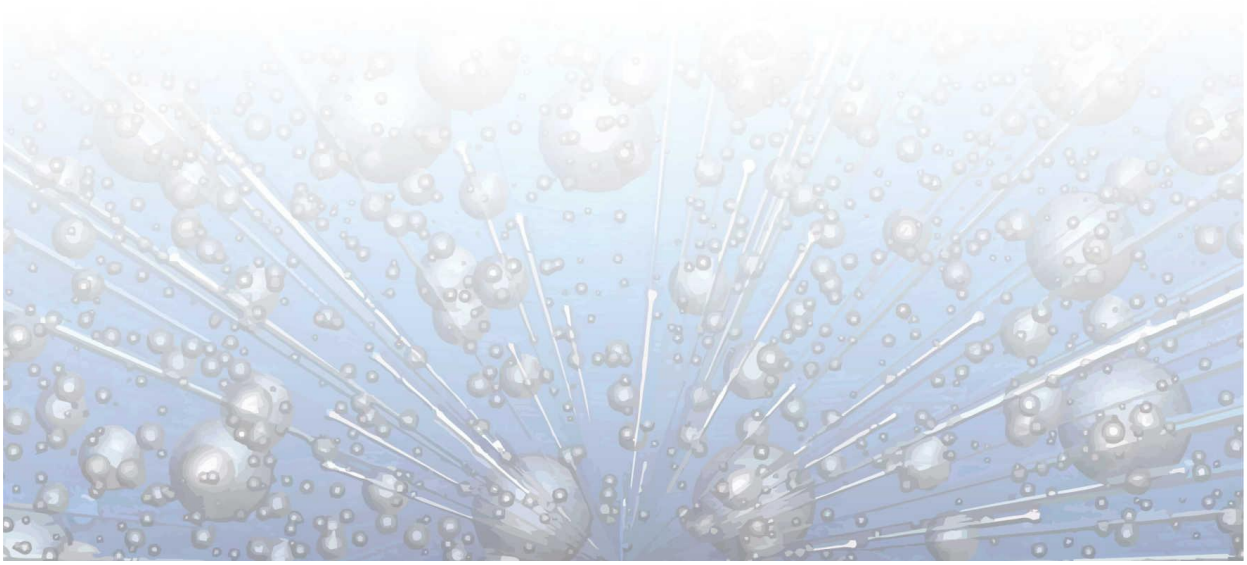
Objectives: The purpose of the work was to evaluate how the nanosized or microsized zinc particles affect fatty acids profile, enzymes activity and the level of cholesterol, squalene and oxysterols in rats with breast cancer.

Materials and Methods: Rats (female, Spraque-Dawley, n = 24) were divided into three groups (control, and test groups, such as animals supplemented with either zinc microparticles (342 nm) or zinc nanoparticles (99 nm). For induction of breast cancer, all animals received 7,12-dimethylbenz[a]anthracene. The analysis of the profile of fatty acids, cholesterol and cholesterol oxidation products was carried out using GC/TOFMS.

Results: Diet supplementation with zinc significantly reduced the cholesterol content ($P = 0.027$) and total cholesterol oxidation products level ($P = 0.011$). Enriching the diet with zinc microparticles also decreased the Δ6-desaturase activity ($P < 0.001$).

Conclusions: Zinc influences fatty acids' profile in rats' serum as well as inhibits desaturating enzymes. The beneficial effect of zinc supplementation in the form of nanoparticles has been confirmed, which may be promising when developing and using these compounds as dietary supplements.

Keywords: bromatology; food supplements



Abstract No. PPP.029

Ugi five-center four component reaction (U-5C-4CR) reaction of aminoaldehydes as a potential source of bioactive peptidomimetics

Maciej Dawidowski[1], Marta Splandesci[2]

[1] Department of Drug Technology and Pharmaceutical Biotechnology, Medical University of Warsaw, Poland
[2] Students' Scientific Association 'Synthesis', Medical University of Warsaw, Poland

E-mail address of presenting author: maciej.dawidowski@wum.edu.pl

Objectives: Over the past several decades, multicomponent reactions (MRCs) have emerged as attractive tools in modern medicinal chemistry and chemical biology. Among their many advantages, they allow the creation of large chemical libraries of diverse, complex molecular structures, starting from simple materials within a short time frame. One of them is an Ugi five-center four-component reaction (U-5C-4CR), which is based on the condensation of a carbonyl compound, an isocyanide, a nucleophile and an amino acid as a bifunctional component. Here, we focus on possible application of this reaction to synthesis of 2-piperazinone-based peptidomimetics.

Materials and methods: A two-step synthetic sequence to target peptidomimetics comprises of U5CR4CR and Boc-deprotection/intramolecular cyclocondensation, as shown below.

The final compounds and intermdiates are characterized by NMR and MS methods. The relative stereochemistries are assigned on basis of NOESY/ROESY experiments.

Results: U-5C-4CR step was optimized to give the condensation products in 40-50% yields. The one-step acid-triggered Boc removal protocol gives the target peptidomimetics in 70-90% yield ranges.

Conclusions: A two-step synthetic sequence comprised of U-5CR-4CR and Boc-deprotection/intramolecular cyclocondensation was developed. Although condensation yields are moderate, this route offers a short and easy assembly of complex molecular architectures with at least 5 derivatizationpoints.

Acknowledgements: This work was funded by Polish National Science Centre (OPUS grant UMO-2016/23/B/NZ7/03339). MS was funded from Erasmus student exchange program.

Abstract No. PPP.030

Structure elucidation of new compound Thiogenistein oxidation products - antioxidant chemistry and antitumor activity study

Elżbieta U. Stolarczyk[1], Weronika Strzempek[2], Marta Łaszcz[3], Andrzej Leś[4], Elżbieta Menaszek[2], Krzysztof Stolarczyk[4]

[1] Analytical Department, Lukasiewicz Research Network Industrial Chemistry Institute, Poland
[2] Faculty of Pharmacy, Jagiellonian University, Poland
[3] Department of Falsified Medicines and Medical Devices, National Medicines Institute, Poland
[4] Faculty of Chemistry, University of Warsaw, Poland

E-mail address of presenting author: elzbieta.stolarczyk@ichp.pl

Objectives: The cell contains various enzymes and antioxidants to provide protection and avoid damage. Flavonoids and their congeners isoflavonoids such as genistein (GE) are well-known antioxidants. Predictive biological activity of structurally new compounds such as thiogenistein (TGE) – a new analogue of GE becomes an interesting way to design new drug candidates with promising properties [1].

Materials and Methods: Two oxidation strategies were used to characterize TGE oxidation products, the first in solution and the second on the 2D surface of the Au electrode as a self-assembling TGE monolayer. The electrospray ionization mass spectrometry was used for identify the product of electrochemical and hydrogen peroxide oxidation in the solution. The FT-IR spectroscopy with ATR mode was used to identify a product after hydrogen peroxide treatment of TGE on the 2D surface. The density functional theory was used to support the experimental results for the estimation of antioxidant activity of TGE as well as for molecular modeling of oxidation products. The biological studies were performed simultaneously to assess the suitability of TGE for antioxidant and antitumor properties.

Results: Structure elucidation of products generated by different oxidation strategies were performed. It was found that TGE was characterized by a high cytotoxic activity toward human prostate and breast cancer cells. The research was also carried out on mice macrophages disclosing that TGE neutralized the production of the LPS-induced reactive oxygen species (ROS).

Conclusions: In the presented study, we identified the main oxidation products of TGE generated under different environmental conditions. The electroactive centers of TGE were identified and its oxidation mechanisms were determined. TGE redox properties can be related to its various pharmacological activity. Our new thiolated analogue of genistein neutralizes the LPS-induced ROS production better than GE. Additionally, TGE shows high cytotoxic activity against human prostate and breast cancer, and on the other hand it does not adversely affect the viability of healthy prostate cells [1].

Acknowledgments: The study has been supported by the Polish Ministry of Science and Higher Education grant no. 841343B. The calculations were performed at the ICM UW grant G18-6 and g85-962.

Keywords: antioxidant, antitumor, structure elucidation

Reference:

[1] E.U.Stolarczyk, et al.; Int.J.Mol.Sci, 22(2021)8783

Abstract No. PPP.031

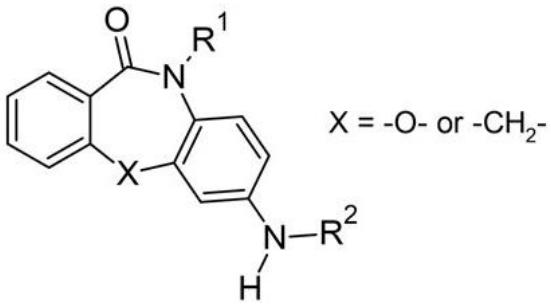
Dibenzo[b,e]azepines and dibenzo[b,f][1,4]oxazepines as novel PEX14-PEX5 protein-protein interaction (PPI) inhibitors

Michał Nowacki[1], Valeria Napolitano[2], Piotr Mróz[1], Emilia Pykacz[1], Mateusz Popiołek[1], Vishal Kalel[3], Ralf Erdmann[3], Oliver Plettenburg[4], Grzegorz Dubin[5], Grzegorz Popowicz[2], Maciej Dawidowski[1]

[1] Department of Drug Technology and Pharmaceutical Biotechnology, Medical University of Warsaw, Poland
[2] Institute of Structural Biology, Helmholtz Zentrum München;, Biomolecular NMR, Bayerisches NMR Zentrum and Center for Integrated Protein Science Munich at Chemistry Department, Helmholtz Zentrum München, Germany
[3] Department of Systems Biochemistry, Faculty of Medicine, Institute of Biochemistry and Pathobiochemistry, Germany
[4] Institute of Structural Biology, Helmholtz Zentrum München;, Institute of Medicinal Chemistry, Helmholtz Zentrum München, Germany
[5] Malopolska Center of Biotechnology, Jagiellonian University in Krakow, Poland

E-mail address of presenting author: mnowacki@wum.edu.pl

Objectives: Glycosomes – organelles characteristic for *Trypanosoma* protists, related to human peroxisomes – play the most distinct roles in parasite cells. Glycolysis – being an example – is the only source of energy for *T. brucei*, and not as important in a case of *T. cruzi*, which are the causatives of Human African Trypanosomiasis and Chagas disease, respectively.The parasite cell uses glycosomes also for compartmentalization e.g. importing enzymes from the cytosol due to peroxins (especially PEX14 and PEX5). Hence, blockade of PEX14-PEX5 complex formation resulting in inhibition of the individual glycolytic enzymes with 'drug-like' molecules results in parasite death. Impairing function of all of these enzymes at once by blocking their routing to target organelle should have comparable consequences for the cell. To design the new therapeutic strategy against *T.* diseases we develop agents based on dibenzo[*b,f*][1,4]oxazepin and dibenzo[*b*,*e*]azepine scaffolds. The overall goal is to obtain a new class of PEX14-PEX5 protein-protein interaction (PPI) inhibitors with high affinity to the molecular target and favorable ADME/PK properties with regard to the previously known chemotypes.



Materials and Methods: Ligand design is based on a high-res co-crystal structure of PEX14 with the previously obtained dibenzo[*b,f*][1,4]thiazepin-11(10H)-one derivatives. To synthesize target compounds we employed classical synthetic methods and multicomponent reactions, such as Petasis. The compounds are tested for their ability of interacting with PEX14 using AlphaScreen technology and 2D protein NMR. Selected compounds are tested for their cellular activity against *T. brucei* and *T. cruzi*.

Results: Numerous new derivatives were obtained with mid- to low-micromolar activities in disrupting PEX14-PEX5 PPI.

Conclusions: We have developed synthetic sequences leading to target compounds, derivatives of dibenzo[*b*,*e*]azepines and dibenzo[*b,f*][1,4]oxazepines. Basing on the obtained SAR information, next rounds of optimization are currently ongoing.

Acknowledgements: This work is funded by Polish National Centre for Science *(UMO-2018/31/B/NZ7/02089)*.
Keywords: TBDD, SAR, *Trypanosoma* diseases

Abstract No. PPP.032

Using the Design of experiments (DoE) approach to improve the efficiency of active compounds extraction from Polygoni Cuspidati Rhizoma et Radix

Magdalena Paczkowska-Walendowska[1], Judyta Cielecka-Piontek[1]

[1] Department of Pharmacognosy, Poznan University of Medical Sciences, Poland

E-mail address of presenting author: mpaczkowska@ump.edu.pl

Objectives: Japanese knotweed (Polygonum cuspidatum) is a rich source of polyphenols, especially stilbene (resveratrol, polydatin) and anthraquinone (emodin) derivatives, which conditions the health-promoting properties. Therefore, it is extremely important to determine the best extraction parameters in order to obtain active compounds with suitable efficiency.

Materials and Methods: By applying 3^2 full factorial design approach (Statistica 13.3, TIBCO Software Inc.), experimental factors of ultrasound-assisted extraction (UAE) such as percentage of methanol (from 0% up to 70%), temperature (in the range of 30–70°C) and the cycles number (form 3 up to 5 cycles) were determined. As dependent variables, phytochemical parameters as the total polyphenols content (TPC) and the sum of active compounds (resveratrol, polydatin, emodin and parietin), as well as biological activity as antioxidant (DPPH) and anti-inflammatory (inhibition of hyaluronidase activity) activities were analyzed. TPC, DPPH and inhibition of hyaluronidase activity were carried out in accordance with the methodology described by Paczkowska-Walendowska et al. [https://doi.org/10.3390/antiox10121945], while the evaluation of the contents of active compounds were carried out using the high-performance liquid chromatography method [https://doi.org/10.3390/pharmaceutics13111916].

Results: On the basis of the conducted experiments, it was shown that the concentration of methanol used in the extraction mixture and the extraction temperature were statistically significant factors affecting the properties of the obtained extracts in regards to TPC and active compounds contents (resveratrol, polydatin, emodin and parietin).

Conclusions: Using the Design of experiments (DoE) approach, it was determined that the best properties of the antioxidant potential and anti-inflammatory effect (expressed in hyaluronidase inhibition) are found in the extracts obtained with the use of an extractant containing 70% methanol (*v/v*) at 70°C for 20 minutes (4 cycles). The analysis of the composition of the extracts obtained in these conditions indicated the content of stilbene and anthraquinone analogues (over 5 mg per 1 g of plant material), which should be associated with the biological effect of the extracts.

Acknowledgements: This research was funded by National Science Center (Poland), under Sonata grant (number 2020/39/D/NZ7/01824).

Abstract No. PPP.033

NLX-101, a biased 5-HT1A receptor agonist, shows antidepressant-like effect in corticosterone-induced model of depression in mice

Angelika Jagielska[1], Anna Mardosz[1], Joanna Fąfara[1], Kinga Sałaciak[1], Joanna Śniecikowska[2], Marcin Kołaczkowski[2], Adrian Newman-Tancredi[3], Karolina Pytka[1]

[1] Department of Pharmacodynamics, Jagiellonian University Medical College, Faculty of Pharmacy, Poland
[2] Department of Medicinal Chemistry, Jagiellonian University Medical College, Faculty of Pharmacy, Poland
[3] Neurolixis SAS,Castres, France

E-mail address of presenting author: angelika.karnas@student.uj.edu.pl

Objectives: Depressive disorders often coexist with cognitive impairments. Previous studies have shown that an innovative group of compounds that are functionally selective 5-HT1A receptor agonists, possess antidepressant-like activity after just a single administration in a mouse model of depression. Interestingly, NLX-101 (F15599), a biased 5-HT1A receptor agonist reversed learned helplessness in the forced swim test, while it did not reverse anhedonia assessed by the sucrose preference test. It appears that the potential anti-anhedonic activity of compound NLX-101 in mice may require a longer application. In addition, depression may be characterised by recurrence so evaluating the duration of the antidepressant-like effect of the drugs is important. Thus, we investigated the anti-anhedonic and procognitive activity of a functionally selective 5-HT1A receptor agonist, compound NLX-101, in a mouse model of corticosterone-induced depression.

Material and methods: The experiments were performed on male Swiss Alb mice. Krf. CD-1. For the first two weeks, a model of depression was induced by subcutaneous administration of corticosterone (20 mg/kg). For the following two weeks, in addition to corticosterone injection, the compounds were administered orally with NLX-101 8 mg/kg and 4mg/kg, vortioxetine 10 mg/kg (reference compound) or saline (control group). Five days after the first administration of the compounds, behavioural tests (sucrose preference test and novel object recognition test) were performed and repeated weekly until the observed pharmacological effects of the test compounds disappeared.

Results: NLX-101 at 8 mg/kg (but not 4 mg/kg) showed a significant effect in sucrose preference test starting from the first week of administration. Despite promising assumptions, statistical significance for procognitive effect was not reached in the novel object recognition test.

Conclusions: Our results suggest that NLX-101 shows antidepressant-like properties in the mouse depression model, which persist after treatment cessation. However, contrary to the results reported in rats, NLX-101 did not improve cognitive function in mice in the corticosterone-induced model of depression. Explaining the differences between the two species will require further research.

Acknowledgement: This study was supported by the Jagiellonian University Students Scientific Association grant number:300995361.

Abstract No. PPP.034

NLX-101 does not negatively affect intermediate-term memory consolidation in mice

Sameh Abouzahra[1], Angelika Jagielska[1], Klaudia Lustyk[1], Marcin Kołaczkowski[2], Adrian Newman-Tancredi[3], Karolina Pytka[1]

[1] Department of Pharmacodynamics, Jagiellonian University Medical College Faculty of Pharmacy, Poland
[2] Department of Medicinal Chemistry, Jagiellonian University Medical College Faculty of Pharmacy, Poland
[3] Neurolixis SAS, Castres, France

E-mail address of presenting author: sameh.abouzahra@student.uj.edu.pl

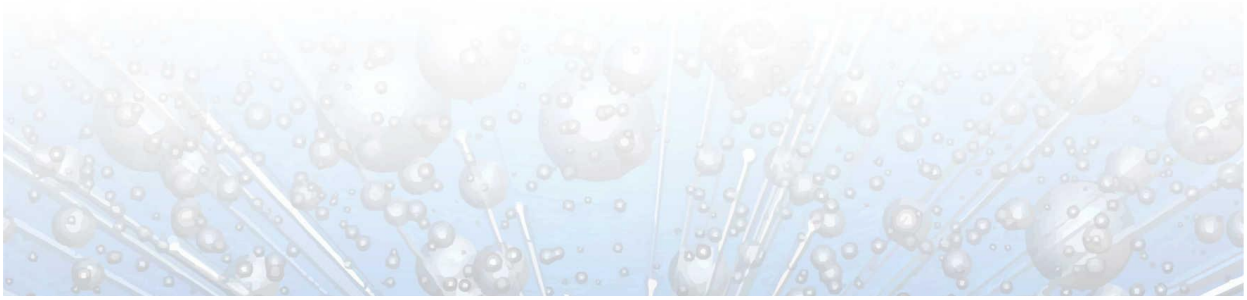
Objectives: NLX-101, also known as F15599, is characterized as a functional 5HT1A receptor agonist capable of selectively stimulating the phosphorylation of the intracellular signal-regulated kinase ERK1/2 relative to adenylate cyclase inhibition in the frontal cortex and inhibiting a similar response in the hippocampus. *In vivo* studies demonstrated that the compound rapidly reversed depression-like behaviors and improved rodent memory. In addition, NLX-101 reversed PCP-induced deficits in the long-term memory in rats. However, there are no reports of its effect on emotional memory in mice. Therefore, in this study, we aimed to investigate the influence of NLX-101 on intermediate-term emotional memory consolidation in mice.

Material and methods: The experiments were performed on male CD-1 mice. We used a step-through passive avoidance task in mice to determine the influence on intermediate-term emotional memory consolidation.

Results: NLX-101 at the dose range 1-16 mg/kg did not decrease latency to enter the dark compartment in the familiarization or retrieval phase of the step-through passive avoidance task in mice.

Conclusions: NLX-101, a biased 5-HT1A receptor agonist, did not impair intermediate-term emotional memory consolidation in mice. The study suggests that preferential activation of ERK1/2 phosphorylation in the prefrontal cortex does not negatively affect emotional memory consolidation. However, the effect of the compound on acquisition and retrieval phases of the learning process requires further studies.

Acknowledgement: This study was supported by Jagiellonian University grant N42/DBS/000140.



Abstract No. PPP.035

Possibility of using hetero-derivatives of fullerene C60 as carriers of drugs containing benzene, naphthalene or anthracene ring - endohedral complexes @C54Y6 and @C55Y5

Monika K. Franczak-Rogowska[1], Krzysztof Stępień[2], Tomasz Pieńko[2], Magdalena E. Grudzień[3], Miłosz D. Grudzień[4], Agnieszka A. Mazurek[5], Aleksander P. Mazurek[6]

[1] Department of Drug Chemistry, Faculty of Pharmacy, Medical University of Warsaw, Poland
[2] Faculty of Pharmacy, Medical University of Warsaw, Poland
[3] Warsaw University of Life Sciences, Poland
[4] University of Warsaw, Poland
[5] Jagiellonian University, Poland
[6] Department of Basic and Applied Pharmacy, National Medicines Institute, Poland

E-mail address of presenting author: monika@wum.edu.pl

Objectives: The effects of the aromatic compounds on the boron, silicon and sulphur fullerene C_{60} heteroderivatives stability and possibility of using created endohedral heterofullerenes as the carriers of compounds with pharmacological activity were analized in this preliminary study.

Materials and Methods: The C_{60} heteroderivatives were created by substitution of 5 or 6 carbon atoms of the fullerene cage by boron, silicon or sulphur atoms and all the heteroatoms were placed in the same pentagonal or hexagonal ring respectively (*Fig.1*). The following endohedral complexes were studied: $C_6H_6@C_{55}B_5$, $C_6H_6@C_{55}Si_5$, $C_6H_6@C_{55}S_5$ $C_{10}H_8@C_{55}B_5$, $C_{10}H_8@C_{55}Si_5$, $C_{10}H_8@C_{55}S_5$, $C_{14}H_{10}@C_{55}B_5$, $C_{14}H_{10}@C_{55}Si_5$ and $C_{14}H_{10}@C_{55}S_5$ and $C_6H_6@C_{54}B_6$, $C_6H_6@C_{54}Si_6$, $C_6H_6@C_{54}S_6$ $C_{10}H_8@C_{54}B_6$, $C_{10}H_8@C_{54}Si_6$, $C_{10}H_8@C_{54}S_6$, $C_{14}H_{10}@C_{54}B_6$, $C_{14}H_{10}@C_{54}Si_6$, $C_{14}H_{10}@C_{54}S_6$. The energies of stabilization ($\Delta E_{stab.}$), deformation (ΔE_{def}) as well as total energy (ΔE) of each examined heterofullerenes C_{60} and their complexes (ΔE_{cx}) with benzene, naphthalene and anthracene were calculated with the use of the molecular modeling technique (HF with STO-3G basis set, Spartan '14 software).

Results: There were slight changes in the shape of the structures $C_6H_6@C_{55}B_5$, $C_{10}H_8@C_{55}B_5$, $C_{14}H_{10}@C_{55}B_5$, $C_6H_6@C_{54}B_6$, $C_{10}H_8@C_{54}B_6$, $C_{14}H_{10}@C_{54}B_6$ and the flat structures of naphthalene and anthracene were strongly distorted. The shape of the $C_6H_6@C_{55}S_5$, $C_6H_6@C_{55}Si_5$, $C_6H_6@C_{54}S_6$, $C_6H_6@C_{54}Si_6$ and $C_{14}H_{10}@C_{54}Si_6$ structureis similar to a "barrel" but in the $C_{14}H_{10}@C_{55}Si_5$, $C_{10}H_8@C_{55}Si_5$, $C_{10}H_8@C_{55}S_5$, $C_{14}H_{10}@C_{55}S_5$, $C_{10}H_8@C_{54}Si_6$ and $C_{14}H_{10}@C_{54}S_6$ complexes, five-membered or six-membered heteroatom rings were very stretched and deformed, and the C-Si and C-S bonds were significantly elongated. In case of $C_{10}H_8@C_{54}S_6$ significantly elongation of six-membered heteroatom ring results in naphthalene release.

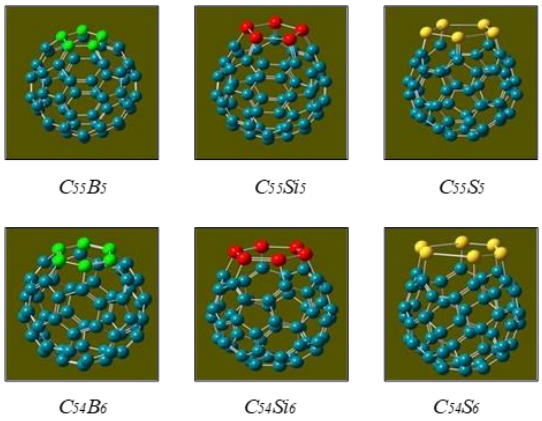


Figure 1. The hetero-derivatives of fullerene C_{60} after the optimization

Conclusions: The results reveal that examined systems are thermodynamically unstable However, calculation suggests that those systems could exist despite positive energy values and therefore the possibility of using them as drug carriers may be assumed.

Abstract No. PPP.036

Kinetics of the conformational changes of the valeric acid in the nitrogen matrix: experiment meets theory at finite temperature

Joanna Stocka[1], Rasa Platakyte[1], Jogile Macyte[1], Justinas Ceponkus[1], Valdas Sablinskas[1], Paweł Rodziewicz[2]

[1] General Physics and Spectroscopy, Vilnius University , Lithuania
[2] Institute of Chemistry, Jan Kochanowski University of Kielce, Poland

E-mail address of presenting author: joanna.stocka@ff.vu.lt

Objectives: Pentanoic acid or valeric acid is a minor constituent of Valeriana officinalis, a well-known flowering plant. Derivatives of valeric acid-like volatile esters are used in perfumes and cosmetics. Valeric acid (VA) plays also a role as a drug against stress and problems with insomnia. The main goal of our study was to investigate the structural diversity of the isolated VA molecule and possible conformational changes, in particular upon the solute-solvent interactions.

Materials and methods: Valeric acid sample was analyzed utilizing Bruker Vertex 70 spectrometer equipped with MCT detector and globar source. The spectra were collected for Argon and Nitrogen matrix gasses at different temperatures before and after annealing of the sample. All static calculations have been performed utilizing density functional theory (DFT/B3LYP). The time evolution of the system was studied by Car-Parrinello molecular dynamics (CP-MD) simulations.

Results: FTIR spectra recorded in the matrix isolation experiment showed the valeric acid dimers formation upon annealing proces. Initially we probed 250 possible conformers and finally considered 15 stable local energy minima structures. The relative energy difference between the global energy minimum structure and the least stable structure was 15,8 kJ/mol. The dynamical structural changes and lifetime of different conformers at finite temperature was studied. Utilizing the nitrogen matrix model the formation and lifetime of weak hydrogen bonds was traced in the CP-MD simulations but no conformational changes were detected.

Conclusions: The valeric acid may occur in several local energy minima and the most stable is tttt conformation. In the matrix experiment VA easily forms dimers upon annealing to 30 K (Ar) and 35 K (N_2). The CP-MD simulations of the the valeric acid molecule embedded in the nitrogen matrix proved the existence of the weak C-H···N and O-H···N hydrogen bonds stabilizing the chosen conformation in the matrix.

Acknowledgments: Calculations were performed utilizing the "VU HPC" supercomputer of Vilnius University at the Faculty of Physics. The research was financially supported by the Jan Kochanowski University of Kielce (grant SUPB.RN.22.060)

Keywords: Car-Parrinello molecular dynamics, Static calcuolations, conformational analysis, valeric acid

Abstract No. PPP.037

TLC fingerprinting of 70 grass species growing in Poland

Joanna Wróbel-Szkolak[1], Anna Cwener[2], Łukasz Komsta[1]

[1] Medicinal Chemistry, Medical University of Lublin, Poland
[2] Botanical Garden of Maria Curie-Skłodowska University in Lublin, Poland

E-mail address of presenting author: joasiaa932@wp.pl

Objectives: Due to the fact that in the literature it is very difficult to find thin layer chromatography of grasses, an attempt was made to carry out such an analysis. All articles encountered present one plant only, several of them describe TLC of two or three plants.

The aim of our research was to conduct phytochemical screening of grasses, with particular emphasis on wild grasses from Poland, which may be of particular interest due to their phytochemical composition.

Materials and methods: 70 species of grasses family (*Poaceae*) obtained in the 2020 season in Poland were used for the analysis. Almost all species were collected from natural stands, coming from genera:*Agrostis*, *Alopecurus*, *Anthoxanthum*, *Apera*, *Arrhenatherum*, *Avena*, *Brachypodium*, *Briza*, *Bromus*, *Calamagrostis*, *Corynephorus*, *Cynosurus*, *Dactylis*, *Danthonia*, *Deschampsia*, *Digitaria*, *Echinochloa*, *Elymus*, *Eragrostis*, *Festuca*, *Glyceria*, *Helictotrichon*, *Hierochloe*, *Holcus*, *Hordeum*, *Koeleria*, *Leymus*, *Lolium*, *Milium*, *Molinia*, *Nardus*, *Panicum*, *Phalaris*, *Phleum*, *Phragmites*, *Poa*, *Saccharum* and *Setaria* were fingerprinted on TLC with densitometric scanning. 5 µL of plant extract were applied to the plates by means of a microsyringe. Chromatography was performed on Silica Gel F254 plates using ethyl acetate – methanol – water (8:2:2) in sandwich mode. Densitometric scanning was performed at 210, 254, 312 and 366 nm in extinction mode, as well as in fluorescence mode with 312/370, 366/420 and 366/550 nm of excitation and emission filter, respectively. Data fusion of the above 7 modes of scanning was investigated chemometrically to check the redundance and amount of information in each mode.

Results: By using Principal Component Analysis (PCA) to identify and interpret 6 orthogonal trends in phytochemical composition. Since the data was difficult to compress with PCA, and the first 6 PCs explained only about half of the variance, hierarchical cluster analysis (HCA) was used to divide the studied species into 5 phytochemical classes. Points of interest, which were missed by the PCA and HCA, were found by grouping k-means and visual inspection.

Conclusions: The performed chromatographic analysis and the applied chemometric procedures made it possible to carry out a phytochemical screening of 70 species of grasses.

Keywords: Chemometrics, data fusion, fingerprinting

Abstract No. PPP.038

Optimization of Se and Zn -enriched mycelium of Lentinula edodes (Berk.) Pegler as a immunostimulatory preparation

Małgorzata Kałucka[1], Marzenna Klimaszewska[1], Marek Król[1], Sandra Górska-Jakubowska[1], Aleksander Roszczyk[2], Beata Kaleta[2], Aleksandra Prus[2], Radosław Zagożdżon[2], Jadwiga Turło[1]

[1] Department of Drug Technology and Pharmaceutical Biotechnology, Medical University of Warsaw, Poland
[2] Department of Clinical Immunology, Medical University of Warsaw, Poland

E-mail address of presenting author: jadwiga.turlo@wum.edu.pl

Objectives: Submerged cultures of *Lentinula edodes*, an edible and medicinal mushroom, have been used in our previous research to obtain immunomodulatory preparations enriched in selenium. Our current attempts to obtain a new medicinal preparation containing both selenium and zinc, two micronutrients necessary for the functioning of the immune system, extended our interest in the interactions between selenite and zinc(II) ions in the substrate and their effects on the transport of these ions into the cell. The analysis of the simultaneous accumulation of selenium and zinc by mycelia growing in media enriched with sodium selenite and zinc bromide allowed us to show the biosorption characteristics of these ions by mushroom cells and to optimize the conditions of the submerged mycelial culture in order to obtain a preparations enriched in zinc and selenium. Subsequently, we studied the effects of *L. edodes* mycelium water extracts with different proportions of selenium and zinc concentrations on human T cells activation. Flow cytometry analysis was used to measure CD69, CD25, PD-1, and Tim-3 expression on human $CD4^+$ and $CD8^+$ T cells.

Results: It was demonstrated that all extracts with different proportions of selenium and zinc upregulated the expression of all activation antigens on both T cells populations, however, statistically significant changes were observed for PD-1 and CD25 antigens on $CD8^+$ T cells. The expression of PD-1 on $CD8^+$ T cells was upregulated by *L. edodes* extracts except extract containing only Se: 19.50% ± 6.84% (control), 27.02% ± 10.37% (extract without Se or Zn), 23.57% ± 5.35% (extract Se/Zn with a significant predominance of selenium content), 25.73% ± 4.88% (extract Zn/Se with a significant predominance of zinc content), 24.11% ± 6.16% (extract with Se), 23.82% ± 6.76% (extract with Zn). As shown, extract Zn/Se has the strongest activating effect ($P < 0.01$). Moreover only this extract upregulated CD25 expression on $CD8^+$ T cells (control: 5.28% ± 1.89% vs Zn/Se: 20.06% ± 5.44%, $P<0.001$). Extracts Se/Zn and Zn showed tendency to upregulate expression of CD25 ($P = 0.051$, and $P=0.080$, respectively).

Conclusions: The obtained results confirm that the content of selenium and zinc in the examined preparations modifies immunomodulatory activity of polysaccharides contained in the aqueous extracts of *L. edodes* mycelium. It is interesting, however, that the mechanisms of action of various active ingredients of the mycelial extracts seem to be different.

Abstract No. PPP.039

Application of Hot Melt Extrusion technology in the development of innovative formulation containing amorphous form of Active Pharmaceutical Ingredient

Hanna Kierońska[1], Michał Kretkiewicz[1], Katarzyna Rogut[1], Joanna Lipner[1], Stanisław Pikul[1], Anna Krause[1]

[1] Pikralida sp. z o.o., Poland

E-mail address of presenting author: a.krause@pikralida.eu

The purpose of the project was to improve the solubility of the antidiabetic drug substance by using Hot Melt Extrusion Technique and to investigate and optimize the effects of different formulations on dissolution profile.

Oral drug delivery is the preferred route for drug administration, however any drug substance to be absorbed must be present in the form of solution to permeate the intestinal epithelial wall at the site of absorption. It allows to achieve the required drug concentration in systemic circulation and thus an appropriate pharmacological response. Drug absorption from the gastrointestinal tract can be limited by various factors but it is controlled by two fundamental parameters: drug solubility and permeability. The solubility of the drug substance is one of the most critical aspects to be considered during the drug development process especially for solid dosage forms. The solubility of adrug can be increased through many techniques. One of them is amorphization by Hot Melt Extrusion. Along with other applications such as taste masking, the HME technique is mainly used to improve the dissolution rate of poorly water-soluble drugs by forming solid dispersions and solid solutions and consequently to enhance their oral bioavailability. In addition to physicochemical properties of the extrudate itself, deep understanding of fundamental biopharmaceutics properties of different types of formulations are critical in the product development. The integrated in vitro methodology was implemented to study the impact of different excipients and different processing parameters. Application of this approach improved our ability to observe the relationship between different formulations and their *in vitro* parameters and assisted us in selection of the final, stable formulation.

Project "Establishment of a R&D Laboratory of Super Generic Drugs" no. RPWP.01.02.00-30-0047/19-00 is implemented under the Regional Operational Programme for Wielkopolskie Voivodeship 2014-2020; Priority Axis I: Innovative and Competitive Economy, Measure 1.2 Strengthening innovation potential of enterprises in of the Wielkopolskie Voivodeship.

Abstract No. PPP.040

Stability of aripiprazole in human plasma from healthy volunteers

Elżbieta Gniazdowska[1], Edyta Gilant[2]

[1] Department of Bioanalysis and Drugs Analysis, Doctoral School, Medical University of Warsaw, Sieć Badawcza Łukasiewicz Instytut Chemii Przemysłowej imienia Profesora Ignacego Mościckiego, Poland
[2] Sieć Badawcza Łukasiewicz Instytut Chemii Przemysłowej imienia Profesora Ignacego Mościckiego, Poland

E-mail address of presenting author: elzbieta.gniazdowska@ichp.pl

Objectives: Aripiprazole is used in the treatment of schizophrenia and bipolar disorder. The determination of the concentration of a drug is an important part of toxicology study and pharmacokinetic studies in clinical trials. It is particularly important to determine the stability of the analyte in biological samples at expected storage temperatures. According to The Good Laboratory Practice guideline, samples should be retained for 10 years [1]. The storage period time could be shortened if the quality of the sample does not permit evaluation of it again [1]. So far, the data on the stability of aripiprazole was reported only for the quality control samples stored after 2 years at a temperature of -20 ºC [2]. No data on clinical samples exist. The study aimed to examine the stability of aripiprazole in human plasma collected from volunteers. It is critical to assess whether the quality of the sample allows for a re-analysis that would confirm the validity of the ten-year storage of research samples after the clinical trial is completed. [1].

Materials and Methods: The aripiprazole concentration in human plasma was determined by the liquid chromatography coupled to mass spectrometry method after liquid-liquid extraction with hexane: isopropanol after seven years of storage at temperature ≤-65 ºC.

Results: The aripiprazole concentrations in clinical samples stored for 7 years were compared with the results of fresh samples obtained during the clinical trial. The stability of aripiprazole was evaluated by using incurred sample reanalysis test described in the European Medicine Agency guideline [3]. Samples are regarded as stable if the % difference has not exceeded 20% for at least 67% of the samples.

Conclusions: The results confirm the stability of aripiprazole in human plasma for the seven years stored at a temperature ≤-65 ºC. Thus, study samples can be reliably analyzed again during this period.

Keyword: stability, aripiprazole, clinical trails

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Abstract No. PPP.041

Microbial biotransformation of lipid-lowering drugs fenofibrate and gemfibrozil by Cunninghamella species

Karolina Słoczyńska[1], Kamil Piska[1], Dorota Żelaszczyk[2], Agnieszka Gunia-Krzyżak[2], Justyna Popiół[1], Agnieszka Ładyka[1], Paweł Żmudzki[3], Elżbieta Pękala[1]

[1] Department of Pharmaceutical Biochemistry, Jagiellonian University Medical College, Poland
[2] Department of Bioorganic Chemistry, Chair of Organic Chemistry, Jagiellonian University Medical College, Poland
[3] Department of Medicinal Chemistry, Jagiellonian University Medical College, Poland

E-mail address of presenting author: karolina.sloczynska@uj.edu.pl

Objectives. Fenofibrate and gemfibrozil, two lipid-lowering drugs, reduce the level of triglycerides in the blood circulation and decrease the risk of hyperlipidemia. Due to widespread occurrence of both drugs in the aquatic environments, there is a growing concern in their role in water quality and aquatic biota. Biological treatments use living organisms such as bacteria and fungi to degrade environmental pollutants into less toxic or non-toxic substances. *Cunninghamella* spp. are fungi that are widely studied in relation to their ability to transform xenobiotics including pesticides and drugs in the same manner as mammals. The main purpose of this study was to employ *Cunninghamella* strains to biotransform fenofibrate and gemfibrozil. Moreover, Derek Nexus software was used to estimate toxicological endpoints of the obtained fungal transformation products.

Materials and Methods. Three strains of *Cunninghamella*: *C. elegans, C. echinulata* and *C. blakesleeana* were used in the study. The biotransformation was carried out for 7 days, and its progress was monitored with liquid chromatography coupled with tandem mass spectrometry.

Results. The results indicated that main transformation products produced by *Cunninghamella* spp. were known metabolites in mammals. Fenofibrate was transformed mainly to fenofibric acid, whereas gemfibrozil to hydroxymethyl gemfibrozil. *In silico* the biotransformation products did not generate any structural alerts with respect to end points including mutagenicity, carcinogenicity, and neurotoxicity.

Conclusions. The presented biotransformation profile of lipid-lowering drugs by *Cunninghamella* spp. suggests that these compounds may follow a similar biodegradation fate when released into the environment.

Acknowledgements: The project was supported by the National Science Center Grant No 2020/37/B/NZ7/02546.

Keywords: Cunninghamella, fenofibrate, gemfibrozil

Abstract No. PPP.042

Synthesis of novel 4-aryl-pyrido[1,2-c]pyrimidine chiral derivatives as potential $5HT_{1A}$/5-HTT ligands

Grzegorz Ślifirski[1], Marek Król[1], Franciszek Herold[1], Jadwiga Turło[1]

[1] Department of Drug Technology and Pharmaceutical Biotechnology, Medical University of Warsaw, Poland

E-mail address of presenting author: jadwiga.turlo@wum.edu.pl

Introduction: Depression is a mental illness that affects over 250 million people worldwide. Emotional (depressed mood, irritability, anhedonia), somatic (sleep, appetite, libido), and functional disorders (suicidal thoughts, slowed speech and movement, learning, memory and attention deficits) make this disease the main cause of disabilities in the general population.

There is a need for further exploration of the neurochemical causes of depression. The search for new generations of antidepressants using the combination of serotonin reuptake inhibition with affinities for various 5-hydroxytryptamine (5-HT) receptor subtypes broadens the knowledge in this field. Clinical trials show that the combination of SSRIs with both partial agonism and antagonism of the $5\text{-}HT_{1A}$ receptor may result in an improvement in the speed and efficacy of the antidepressant effect. This can be confirmed by the drugs recently introduced into the pharmacotherapy of depression – vilazodone and vortioxetine.

Objectives: Research on synthesis and biological evaluation of pyrido[1,2-c]pyrimidine derivatives has been carried out for the last decade in the Department of Drug Technology and Pharmaceutical Biotechnology, Medical University of Warsaw. Earlier work in the mentioned research described a series of derivatives of pyrido[1,2-c]pyrimidine with 3-(piperidin-3-yl)-1H-indole residue in the pharmacophore element. A series of compounds with a high affinity to both molecular targets ($5\text{-}HT_{1A}$ and 5-HTT) were obtained. The aim of current research was to synthesize pure enantiomers of the most active derivatives to determine the role of asymmetry in their biological activity.

Materials and methods: Novel chiral 4-aryl-pyrido[1,2-c]pyrimidine derivatives were obtained in a way of a multi-step chemical synthesis with use of chiral auxilaries and subjected to analytical studies, using the methods of ^{1}H NMR and ^{13}C NMR spectroscopy as well as HRMS.

Results: The results of planned in vitro and in vivo pharmacological studies will allow to draw conclusions regarding structure-activity relationship in the tested group of compounds and to select compounds for further pre-clinical evaluation.

Acknowledgements: We acknowledge the financial support of the Polish National Science Center grant, OPUS 6, No. UMO-2013/11/B/NZ7/01638.

Abstract No. PPP.043

Structure-based design of new pyrazolo[4,3-c]pyridine as PEX14-PEX5 Protein-Protein Interaction (PPI) inhibitors

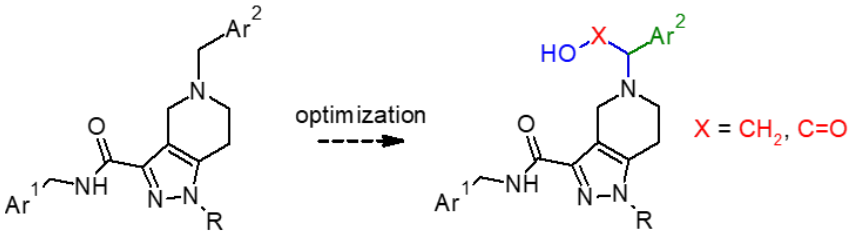
Michał Nowacki[1], Magdalena Pochoda[2], Dominika Kacprzak[2], Natalia Ogonowska[2], Valeria Napolitano[3], Sara Rioton[3], Martyna Wróbel[1], Vishal Kalel[4], Ralf Erdmann[4], Grzegorz Dubin[5], Grzegorz Popowicz[3], Michael Sattler[3], Oliver Plettenburg[6], Maciej Dawidowski[1]

[1] Department of Drug Technology and Pharmaceutical Biotechnology, Medical University of Warsaw, Poland, Poland
[2] Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Poland, Poland
[3] Biomolecular NMR, Bayerisches NMR Zentrum and Center for Integrated Protein Science Munich at Chemistry, Institute of Structural Biology, Helmholtz Zentrum München, Germany, Germany
[4] Institute of Biochemistry and Pathobiochemistry, Department of Systems Biochemistry, Faculty of Medicine, Ruhr-University Bochum, Germany, Germany
[5] Małopolska Center of Biotechnology, Jagiellonian University, Poland, Poland
[6] Institute of Organic Chemistry, Leibniz Universitat Hannover, Germany, Institute of Medicinal Chemistry, Helmholtz Zentrum München, Germany, Germany

E-mail address of presenting author: martyna.wrobel@wum.edu.pl

Objectives: Our research group seeks for new methods to disturb metabolic processes in *Trypanosoma*. The parasites of this genus infect humans and domestic mammals causing severe mortality and huge economical losses in poorly developed countries. In this context, new chemical compounds acting through innovative mechanism of actions can contribute greatly to development of new therapeutic strategies for combating these deadly diseases. More specifically, we target PEX14-PEX5 PPI to disrupt the glycosomal import in parasites. Glycosomes are organelles characteristic for *Trypanosoma* parasites, needed for execution of essential metabolic pathways in parasites' lifecycle. On the other hand, peroxisomes, the human *vis-à-vis* of glycosomes, are not critical for the survival of healthy cells. Glycosomes cannot express enzymes taking part in metabolic processes. Therefore, all enzymes that are active in this compartment need to be translocated form the cytosol post-translationally; disruption of this transport potentially leads to very severe metabolic consequences for parasite cell. Currently, we aim to improve the previously developed pyrazolo[4,3-c]pyridine series of small-molecule inhibitors of PEX14-PEX5 PPI, by applying a new type of structural modifications in the linker between the pyridine nitrogen and aromatic moiety targeting the phenylalanine pocket of PEX14.

Materials and Methods: The inhibitors were designed basing on the available crystallographic data. The compounds are obtained using the robust Petasis multicomponent-type reaction in a key step to introduce polar moieties for high binding to the target and improved drug-likeness profile.



Results: Numerous new derivatives were obtained with mid- to low-micromolar activities in disrupting PEX14-PEX5 PPI. In addition, two high-res ligand-protein co-crystal structures were obtained.

Conclusions: The presented research gives a solid basis for new round of optimization of pyrazolo[4,3-c]pyridine scaffold, which may result in finding new pre-clinical candidates against Trypanosoma-related diseases.

Acknowledgements: This work is funded by Polish National Centre for Science *(UMO-2018/31/B/NZ7/02089)*.

Keywords: TBDD, SAR, *Trypanosoma* diseases

Abstract No. PPP.044

Helical mimetics as novel inhibitors of PEX14-PEX5 protein-protein interaction

Piotr Mróz[1], Monika Marciniak[1], Roberto Fino[2], Valeria Napolitano[2], Grzegorz Popowicz[2], Maciej Dawidowski[1]

[1] Department of Drug Technology and Pharmaceutical Biotechnology, Faculty of Pharmacy, Medical University of Warsaw, Poland
[2] Institute of Structural Biology, Helmholtz Zentrum Munich, Germany

E-mail address of presenting author: pmroz@wum.edu.pl

Objectives: *Trypanosomatids* infect humans and domestic mammals, causing considerable mortality and huge economic losses. *T. brucei* causes *Sleeping Sickness*, called also *African Trypanosomiasis*, while *T.cruzi* is the pathogen for the *Chagas Disease*. Existing medications have serious side effects, require long treatment schedules and often fail to eliminate parasitemia. For that reasons new therapeutic strategies targeting trypanosomiases are needed [1,3].

The aim of current research is to synthesize small-molecule inhibitors of PEX14-PEX5 protein-protein interaction (PPI). PEX14 and PEX 5 are the two crucial peroxins involved in the post-translational import of matrix enzymes into glycosomes in *Trypanosoma*. It has previously been shown that disruption of complex formation between these two proteins results in mislocalisation of glycosomal enzymes, ATP depletion and parasite death [1,2].

Materials and methods: The development of inhibitors is carried out collaboratively with Institute of Structural Biology (STB) and Institute of Medicinal Chemistry (IMC), Helmholtz Zentrum Munich. The compounds are designed by docking to the high-resolution X-ray structures of PEX14-PEX5 PPI interface. They are synthesized and optimized by use of classical synthesis as well as multicomponent reactions. The efectiveness of the compounds in disrupting the PEX14 and PEX5 PPI is measured by AlphaScreen technology.

Results: Several groups of piperazinone derivatives were designed and synthesized, having the inhibitory activity in mid-micromolar ranges.

Conclusions: Helical mimetics display potential for disrupting PEX14-PEX5 PPI, thus they may serve as a template for design of novel agents trypanocidal agents.

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Abstract No. PPP.045

Photostability and phototoxicity of diflunisal and betamethasone

Karolina Lejwoda[1], Anna Gumieniczek[1], Agata Filip[2], Anna Berecka-Rycerz[1], Natalia Data[1]

[1] Department of Medicinal Chemistry, Medical University of Lublin, Poland
[2] Department of Cancer Genetics with Cytogenetics Laboratory, Medical University of Lublin, Poland

E-mail address of presenting author: k.lejwoda94@gmail.com

Objectives: Diflunisal (DIF) and betamethasone (BET) absorb radiation in the UV-Vis range over 270 nm, which may induce their photodegradation and phototoxicity.

Materials and Methods: The drugs were exposed to radiation in the range 300-800 nm (2.699, 18.902, 37.804, 56.7006, 75.608 and 94.510 kJ/m^2) under different pH values (1-13). The stressed samples were quantified with validated HPLC methods to obtain respective kinetic parameters. Generation of ROS, i.e. singlet oxygen (SO) and superoxide anion (SA) was also examined to estimate their phototoxic risks according to ICH guidelines. DIF, BET and chlorpromazine as a positive control (20-200 µM) were irradiated (4.9, 23.4, 68.7, 136.0 and 268.4 J/cm^2) in the presence of respective chemicals. Next, changes in absorbance at 440 nm for N,N-dimethyl-4-nitrosoaniline reacting with SO, and changes in absorbance at 560 nm for nitrotetrazolium blue reacting with SA, both formed during photodecomposition of the tested substances were monitored.

Results: Photodegradation of DIF and BET followed the first-order kinetics (Figures 1-2). Stability of DIF was the lowest at pH 1-4 and 13 (> 90% degradation), and the highest at pH 7-10 (32.15-45.61% of degradation).

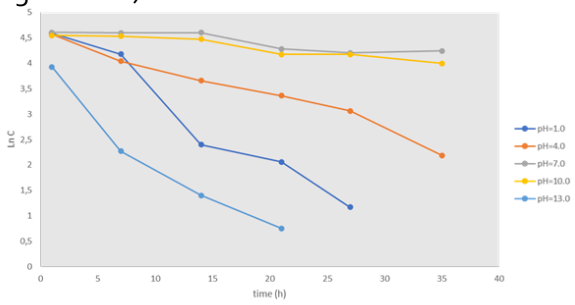

Figure 1. First-order degradation of DIF.

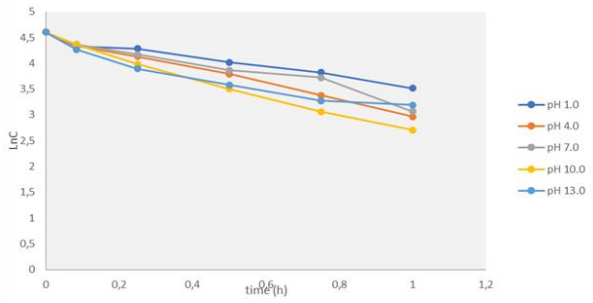

Figure 2. First-order degradation of BET.

BET had to be exposed to lower irradiation (234, 687, 1360, 2047 kJ/m^2). Despite this, it was shown as photolabile over the entire pH range

The molar extinction coefficient (MEC) for both DIF and BET exceeded the limit value (>1000). In addition, the results of ROS assays showed that DIF and BET may be potentially phototoxic (SO is ≥ 25 and the amount of SA is ≥ 70 according to ICH guidelines).

Conclusions: Because DIF and BET were photolabile, the presented data could be helpful for the rational design of their new formulations including topical drugs. In addition, they were shown as potentially phototoxic substances. Therefore, in vitro phototoxicity tests (MTT and 3T3-NRU tests) will be used in the next stage of this project.

Keywords: diflunisal and betamethasone; photodegradation; ROS assays

Abstract No. PPP.046

Application of GCMS and LCMS/MS techniques for direct analysis of amines in pharmaceutical substances

Anna Witkowska[1], Elżbieta U. Stolarczyk[1], Aleksandra Groman[2]

[1] Analytical Department, Łukasiewicz – Industrial Chemistry Institute, Poland
[2] Military Institute of Armament Technology, Poland

E-mail address of presenting author: anna.witkowska@ichp.pl

Objectives: Amines are widely spread organic chemicals commonly used as intermediates and solvents in industry. Various application of amines include: pharmaceuticals, rubber and agricultural chemicals, water disinfection products and solvents. For the safety of the patients ICH (International Conference on Harmonization) guidelines recommend acceptable amounts for amines in pharmaceuticals which have not been completely removed in manufacturing process.

The quantitative analysis of amines can be very challenging due to their properties. Basic nature, high polarity, tendency to form hydrogen bridges and interaction with actives sites in analytical columns can cause peak tailing which make separation difficult. To overcome problems in analysis of amines a wide range of derivatization/extraction procedures have been developed.

Analysis of amines can be performed by several techniques such as: gas chromatography with mass-spectrometry (GCMS) or flame ionization detection (FID), liquid chromatography coupled to a UV-VIS, fluorescence or mass spectrometry detector and capillary electrophoresis.

Materials and Methods: In our study sensitive GCMS and LCMS/MS methods (liquid chromatography with tandem mass spectrometric detection) for determination of amines are presented. In active pharmaceutical ingredients (APIs) manufactured in our production department various amines have been investigated including: tert-butylamine (TBA), pyrrolidine, 4-fluoroaniline, diisopropyl-ethylamine and tetrabutylammonium hydrogensulfate.

Results: Described methods have been validated according to ICH requirements and the validation acceptance criteria have been fulfilled. Fast and direct methods using GCMS and LCMS/MS has been successfully applied to analysis of harmful amines in production process of APIs.

Conclusion: Validation results demonstrate that new developed GCMS and LCMS/MS methods are sensitive and simple, and are suitable for quality control of active pharmaceutical ingredients. These analytical procedures are particularly important because amines are known to be precursors of cancerogenic N-Nitrosamines.

Acknowledgements: The study was supported by the European Union under the European Regional Development Fund No. UDA-POiG.01.03.01-14-069/08-00 and UDA-POIG.01.03.01-14-068/08-00.

Keywords: amines, GCMS, LCMS/MS, API

Abstract No. PPP.047

Chemical stability of linagliptin using HPLC-UV, FT-IR, NIR and DSC methods with chemometric assessment

Karolina Lejwoda[1], Anna Berecka-Rycerz[1], Anna Gumieniczek[1], Hanna Trębacz[2], Angelika Barzycka[2], Edyta Leyk[3], Marek Wesołowski[3]

[1] Department of Medicinal Chemistry, Medical University of Lublin, Poland
[2] Department of Biophysics, Medical University of Lublin, Poland
[3] Department of Analytical Chemistry, Medical University of Gdańsk, Poland

E-mail address of presenting author: k.lejwoda94@gmail.com

Objectives: Determination of chemical stability of linagliptin (LINA), an oral antidiabetic drug from gliptins was performed that is crucial in the rational design of new formulations.

Materials and Methods: The stability tests were performed in 1M HCl, 1M NaOH, 6% H_2O_2, buffers (pH 4-10) at 70°C. Next, a validated HPLC-UV method was used to quantify the no degraded LINA in the presence of its degradation products. Stability of LINA was also assessed in a solid state in the presence of excipients (lactose=LAC, mannitol=MAN, magnesium stearate=MgS and polyvinylpyrrolidone=PVP), using FT-IR, NIR and DSC with chemometric evaluation. High temperature and high humidity were applied as stressors in order to accelerate the potential interactions (60°C and 70% RH for 60 days).

Results: In the 1M HCl and 1M NaOH environments, decomposition of LINA was 65.34% and 92.00% (Figure 1) while using 6% H_2O_2, it was 14.28%. However, in the buffers (pH 4.15-10.35), degradation of LINA was only in the range 2.49-5.46%.

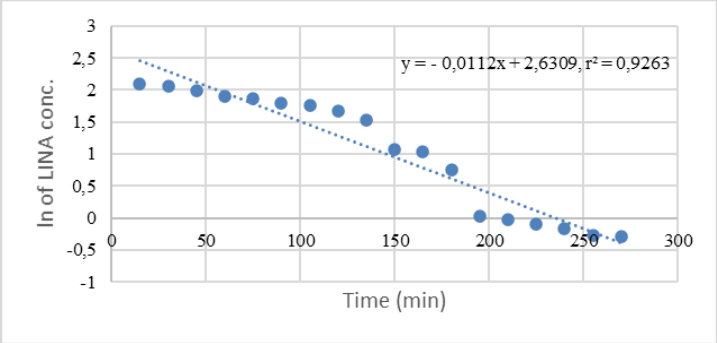

Figure 1. Degradation of LINA in 1M NaOH.

Visible effects of LAC, MAN, MgS and PVP on the substance stability were observed in the FT-IR spectra of the stressed binary mixtures (Figure 2).Using the NIR method, LINA was shown more sensitive to degradation in the presence of LAC and MAN while DSC experiments showed that all binary mixtures of LINA were affected. The observed changes were confirmed using chemometric assessment by combined *Principal Component Analysis*(*PCA*)and Hierarchical Cluster *Analysis* (*HCA*).

Conclusions: High degradation of LINA following the pseudo first-order kinetics was observed at 70°C for acidic, alkaline and oxidative conditions. The experiments in a solid state showed visible interactions of LINA with typical excipients, probably due to the presence of the amine group of LINA.

Keywords: Linagliptin degradation; pH, temperature and humidity; excipients

Abstract No. PPP.048

Lysine as effective amorphous stabilizer for sinapic acid

Ewa Garbiec[1], Natalia Rosiak[1], Judyta Cielecka-Piontek[1], Ewa Tykarska[2], Przemysław Zalewski[2]

[1] Department of Pharmacognosy, Poznan University of Medical Sciences, Poland
[2] Department of Chemical Technology of Drugs, Poznan University of Medical Sciences, Poland

E-mail address of presenting author: 62309@student.ump.edu.pl

Objectives: Sinapic acid is a promising natural compound with a wide range of biological properties resulting from antioxidant and anti-inflammatory activity. Those properties are limited by poor solubility and in the result poor oral bioavailability. A promising approach to overcome this limitation is to transform a substance from a crystalline form to an amorphous dispersion. However, amorphous dispersions are thermodynamically unstable and show tendency to recrystallize. While amino acids, such as lysine, can be used as low molecular weight co-formers to stabilize the amorphous dispersion.

The aim of this study was to obtain amorphous dispersion of sinapic acid with lysine and to evaluate its physical stability and antioxidant potential.

Materials and Methods: Amorphous dispersions of sinapic acid and lysine at molar ratios 1:1 and 1:2 were obtained by ball milling, solvent evaporation and freeze drying methods.

Amorphous dispersions were stored at 30°C, 40°C and 50°C under uncontrolled humidity. Using XRPD, the recrystallization tendency of amorphous sinapic acid and lysine dispersions was assessed after 2, 4 and 6 weeks. The antioxidant activity was studied by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging test and cupric ion reducing antioxidant capacity (CUPRAC) assay.

Results and conclusions: Significant physical stability was confirmed for the investigated amorphous dispersions of sinapic acid and lysine. The least tendency to recrystallization was shown by the sinapic acid systems with lysine dispersions which were obtained by grinding technique and freeze-drying process. They were stable for the period of 6 weeks in 30°C. Moreover, the obtained amorphous dispersions of sinapic acid and lysine were characterized by enhanced potential of antioxidant activity.

Acknowledgements: This study was supported by the grant OPUS from the National Science Centre Poland UMO-2020/37/B/NZ7/03975

Keywords: sinapic acid, co-amorphous dispersion, amino acids

Abstract No. PPP.049

Amorphous dispersion of diosmin and hesperidin

Kamil Wdowiak[1], Natalia Rosiak[1], Ewa Tykarska[2], Judyta Cielecka-Piontek[1]

[1] Department of Pharmacognosy, Poznan University of Medical Sciences, Poland
[2] Department of Chemical Technology of Drugs, Poznan University of Medical Sciences, Poland

E-mail address of presenting author: 76739@student.ump.edu.pl

Objectives: Diosmin (Dio) and hesperidin (Hes) are flavonoid glycosides that possess biological activities such as antioxidant, anti-inflammatory, anti-diabetic, anti-cancer and neuroprotective. They are also active components aiming at strengthening venous vessels and symptomatic treatment of venous circulation insufficiency. However, their biological activity is limited by poor solubility. The purpose of the study was to obtain an amorphous dispersion of Dio and Hes and to evaluate changes in the solubility rates of both polyphenols in the case of their compacted forms.

Materials and methods: Preparation of amorphous dispersions of Dio and Hes was carried out with PVP K30 in the following mass ratios: 1:1:4, 1:1:8, 1:1:12, 1:1:16 by the means of ball milling. The identification of the amorphous systems was performed using X-ray Powder Diffraction and Fourier-transform infrared spectroscopy. The compacted forms containing amorphous dispersions of Dio and Hes were prepared using a Natoli NP-RD10A tablet press (compacting force – 100 N). Additionally, the compacted forms consisted of lactose monohydrate as a filler and crospovidone as a disintegrating agent (5%). The solubility rate studies were conducted in paddle apparatus in phosphate buffer (pH 6.8) at 37 ± 0.5 °C. The concentration changes of polyphenols' were measured using high-performance liquid chromatography. The determination was accomplished using a stationary phase column: Dr. Maisch ReproSil-Pur Basic-C18 (250mm x 4.6 mm, 5 um) and isocratic elution of mobile phase methanol - 0.1% acetic acid (55:45, v/v). The UV absorbance detections were measured at 347 nm for Dio and 284 nm for Hes.

Results and conclusions: The amorphous dispersions of Dio and Hes were successfully obtained using ball milling in all mass ratios as confirmed by XRPD and FT-IR analysis. Obtaining amorphous dispersions of Diosmin and Hesperidin considerably improved solubility rates of compact forms (22-fold for Dio and 6,5-fold for Hes). Amorphous dispersions of Diosmin and Hesperidin can be considered a promising method to boost their solubility.

Acknowledgements: This study was supported by the grant OPUS from the National Science Centre Poland UMO-2020/37/B/NZ7/03975.

Keywords: Diosmin, Hesperidin, Amorphous Dispersions

Abstract No. PPP.050

Searching for molecular target(s) of AC27 compound

Agata Barzowska[1], Barbara Pucelik[1], Maciej Dawidowski[2], Anna Czarna[1]

[1] Jagiellonian University, Poland
[2] Medical University of Warsaw, Poland

E-mail address of presenting author: agata.barzowska@doctoral.uj.edu.pl

Objectives: Diabetes is a public health problem that has reached epidemic proportions on a global scale. β-cell dysfunction and insulin resistance are interrelated defects in the pathophysiology of diabetes. Regeneration of β-cell mass is an ultimate goal yet to be achieved. Our previous study of small-molecule inhibitors of DYRK1A kinase provides and validates a proof-of-concept that β-cell proliferation via kinase inhibition is achievable, especially after treatment with our best inhibitor, AC27. Therefore, a comprehensive characterization of its other targets (i.e., are there any other mechanisms of action beyond DYRK1A targeting?) that influence the β-cell function is important. Herein, we want to use proteome profiling to deconvolution possible targets of AC27 to fully understand its mechanisms of action. We try to synthesize an innovative, photoaffinity AC27 - based probe consisting of a click chemistry probe skeleton for target binding and enrichment and a photoaffinity group to fix the binding between the probe and the targets

Materials and Methods: The project emploied two innovative approaches. AC27 was decorated with an alkyne-containing moiety to give a probe that non-covalently binds to the target protein(s). In another approach, the AC27 derivative was synthesized having a 'minimal' photo-affinity linker (PAL) with a diazirine moiety and a terminal alkyne residue, to covalently bind to the target protein(s) upon irradiation with UV light. Both probes will be used for the in-cell conjugation with biotin-PEG-azide, followed by cell lysis, streptavidin enrichment, gel electrophoresis, and MS analysis of the identified target(s).

Results: AC27 restored β-cell function, where the compound may be useful in the treatment of diabetes. Specifically,, we aim to obtain molecular probes that will be used to analyze the possible mechanisms of cell-restoring action of the compound other than DYRK1A kinase.

Conclusion: Uncovered targets can be used as a proteomic signature resource for further analyses of the effects of AC27 in diabetes and furnish invaluable mechanistic information on AC27. Thus, the proposed approach can facilitate drug discovery efforts in diabetes treatment.

Acknowledgements: The authors thank for the financial support of the PRAs of the Jagiellonian University no. PSP U1U/P03/NO/03.03 and NCN no. 2019/34/E/NZ1/00467

Keywords: diabetic kinome; proteomics; diabetic-related proteins

Abstract No. PPP.051

Compatibility between formulation and container in pharmaceutical foam formulation

Justyna Dąbrowska[1], Piotr Bilski[2], Ewelina Kowalska[3], Bartłomiej Kubiak[1]

[1] Preformulation Department, Adamed Pharma S. A., Poland
[2] Department of Pharmaceutical Technology, Collegium Medicum Nicolaus Copernicus University, Poland
[3] Analytical Department, Adamed Pharma S. A., Poland

E-mail address of presenting author: justyna.dabrowska2@adamed.com

Objectives: The aim of this study was to check quality of aluminum cans coated with different internal coatings and their compatibility with foam formulation. Proper choice of excipients and containers is crucial for stability and safety of pharmaceutical products. In formulations developed with propellent, reactions between components could be accelerated by internal pressure.

Materials and Methods: Four cans from different suppliers were checked. Two of them had epoxy internal coating (one gold and one beige), other two were coated with Polyacrylamide (PAM) and Polyamide (PAI). Foam formulation was poured into cans and closed by valves and actuator with 5% propellent inside. The cans were placed in conditions 25°C, 60% RH, and 40°C, 75% RH. Samples from each condition were tested at the start, and after 1, and 3 months. Purity and API content was tested using pharmacopeial methods, additionally, the foam was visually assessed and its pH was measured.

Results: Most of the cans tested proved to be compatible with the formulation. Only the Epoxy beige coating gave bad results in the stability tests. After 1 month in 40°C, 75% RH conditions the content of API dropped below 95% and a further decrease was noticed. After 3 months of storage in this condition, total impurity was 14,63%, the foam changed color from white to yellow and its pH was raised from 5,55 to 6,47. Changes in the appearance of the coating were visible after opening the can. Yellowing and peeling of the coating from the can were observed.

Conclusions: Assessment of compatibility between formulation and container is important to develop safe pharmaceutical foam products. In this particular case the material from which the coating was made and its quality were crucial for the stability of the medicinal product.

Keywords: Cans, foam, compatibility

Abstract No. PPP.052

Search for first-in-class multifunctional kinase inhibitors targeting neurodegenerative pathogenesis of Alzheimer's disease

Emilia Sługocka[1], Adam Bucki[1], Justyna Godyń[1], Izabella Góral[1], Marcin Kołaczkowski[1], Anna Więckowska[1]

[1] Pharmaceutical Chemistry, Faculty of Pharmacy, Jagiellonian University Medical College, Poland

E-mail address of presenting author: emilia.slugocka@doctoral.uj.edu.pl

Objectives: Neurodegenerative disorders, with Alzheimer's disease (AD) being the most prevalent, are characterized by complex etiology that combines disruptions in diverse signaling pathways. The current paradigm based on the pivotal role of amyloid-B plaques and neurofibrillary tangles is insufficient, as there is no effective disease-modifying treatment. To address the unmet medical need, a combination of multiple targets was deployed. Inflammation of nervous tissue and amyloid-B plaques contribute substantially to neuropathological changes that underlie AD. Glycogen synthase kinase-3B (GSK-3B) activity, upregulated in AD, leads to hyperphosphorylation of tau, and decay in synaptic plasticity. IKKB, as an integrated part of the IκB kinases complex (IKK), plays a crucial role in modulating a pro-inflammatory signaling pathway, controlling the release of the NF-κB. The aim of the study is to identify new chemotypes of dual ligands, targeting both kinases.

Materials and Methods: Prepared and validated via virtual screening benchmark structural models of both proteins have been employed. Interaction models were developed by structure-based drug design methods. The optimization of GSK-3B and IKKB based on the PDB structures 4PTG, 4KIK, respectively, was followed by a docking-based retrospective virtual screening. Conformational characterization of the prepared models, followed by energy minimization based on molecular dynamics simulation, led to the selection of the final structures for prospective screening with a CNS-oriented database. The fingerprint-guided clustering enabled the selection of molecules with potential dual-inhibitor activity and drug-like properties.

Results: To this end, the catalytic domains analysis of both models revealed that a mutual core for a ligand with dual inhibitory activity is the hinge-binding moiety responsible for anchoring the molecule via multiple hydrogen bonds. The residues exploring the ribose and phosphate region of the binding site are responsible for the selectivity. Further *in vitro* studies are aimed at confirming the cellular activity of selected compounds.

Conclusions: Structure-based drug discovery methods and virtual screening provide strong support to the drug discovery process. Selected chemotypes of dual-active GSK-3B and IKKB ligands mark a starting point for further optimization with the final goal of discovering biologically active compounds.

Keywords: GSK-3B, IKKB, virtual screening

Abstract No. PPP.053

Implementation of QbD approach to the development of chromatographic method for the determination of complete impurity profile of the CPL409116 innovative substance, dual JAK/ROCK inhibitor

Lidia Gurba-Bryśkiewicz[1], Urszula Dawid[1], Damian A. Smuga[1], Wioleta Maruszak[1], Monika Delis[1], Bartosz Stypik[1], Krzysztof Szymczak[1], Aleksandra Moroz[1], Aleksandra Błocka[1], Michał Mroczkiewicz[1], Krzysztof Dubiel[1], Maciej Wieczorek[1]

[1] Medicinal Chemistry Department, Celon Pharma SA, Poland

E-mail address of presenting author: lidia.gurba@celonpharma.com

Objectives: The purpose of this work was to demonstrate use of the AQbD approach the methodical step-by-step development of a UHPLC method for quantitative determination of impurity profile of new CPL409116 substance on the preclinical and clinical step of drug discovery studies.

Materials and Methods: The critical chromatographic method parameters (CMPs) were kind of stationary phase (8 different columns), pH of aqueous mobile phase (2.6, 3.2, 4.0, 6.8), start (20% - 25%) and stop (85% - 90%) percentage of organic mobile phase (ACN). The critical method attributes (CMAs) are the resolution between the peaks (≥ 2.0), and peak symmetry of analytes (≥ 0.8 and ≤ 1.8). In the screening step, the effects of different levels of CMPs on the CMAs were evaluated based on full fractional design 2^2 (two factors: start and end gradient composition, two-level). The robustness tests were established from the knowledge space of the screening step. Fractional factorial design (2^(4-1)) with a centre point and repetition was applicated to the robustness test of the method. Method operable design region (MODR) was generated. The probability of meeting the specifications for the CMAs was calculated by Monte-Carlo simulations.

Results: Optimal separations conditions were achieved using a Zorbax Eclipse Plus C18 column with 10 mM formic acid pH 2.6 as an aqueous mobile phase. The final working conditions were as follows: 20% ± 1% of ACN at the start of gradient, 85% ± 1% of ACN at the end of the gradient, the concentration of formic acid 10 mM ± 1 mM, and the column temperature 30°C ± 2°C. Validation of the method was carried out in compliance with ICH guideline Q2(R1).

Conclusions: Using AQbD with DOE approach allowed for rapid and efficient selection of chromatographic conditions to the development of a robust, meeting regulatory requirements, fit for purpose UHPLC analytical method to the quantitative determination of impurity profile of new CPL409116 substance.

Acknowledgements: This work was supported by National Centre for Research and Development (Poland) Grant POIR.01.01.01-00-0382/16

Keywords: Analytical Quality by Design (AQbD), Design of Experiment (DOE), pharmaceutical impurity profiling

Abstract No. PPP.054

Development and validation of a new chromatographic method for the analysis of metformin-related substances in innovative drug

Urszula Dawid[1], Lidia Gurba-Bryśkiewicz[1], Damian Smuga[1], Dagmara Hołowińska[1], Krzysztof Mulewski[1], Małgorzata Wąsińska-Kałwa[1], Kinga Gałązka[1], Mateusz Mach[1], Krzysztof Wiśniewski[1]

[1] Medical Chemistry, Celon Pharma, Poland

E-mail address of presenting author: urszula.dawid@celonpharma.com

Objectives: A simple, sensitive, and reproducible ultra-high-performance liquid chromatographic (UHPLC) method has been developed and validated for quantitative estimation of metformin and related substances from tablets of new GPR40/FFA1 (G-protein coupled receptor 40/ Free Fatty Acid Receptor 1) agonist [1].

Materials and Methods: : The separation was performed on Waters Acquity BEH Amide (2.1 x 150 mm; particle size 1.7 µm) column. The mobile phase consisted of 20 mM potassium dihydrogen phosphate (pH = 2.3) with 10 mm NH_4F: acetonitrile (20:80 v/v), pumped at a flow rate of 0.5 mL/min and oven temperature at 50°C. Metformin and related substances (Imp: A, B, C, D, E) were detected by ultraviolet absorbance at 218 nm. Samples were subjected to ultrasonic extraction. For extraction from the tablets, acetonitrile: water (80:20; v/v) was used for the Imp A and ethanol for the other test compounds.

Results: : The resolution was > 2.8 between the neighbouring peaks. The method was linear (R^2 > 0.99 for all compounds) over the concentration range 0.006 (LOQ) - 0.009 µg/mL for Imp A and 0.015 (LOQ) - 0.022 µg/mL for Imp: B, C, D, E. The best recoveries for Imp A from tablets were in a diluent of acetonitrile: water (80:20; v/v) and for the other compounds in ethanol. The recoveries range was from 77 - 114% and met the acceptance criteria.

Conclusions: This method can be successfully used in the determination of metformin-related substances to new GPR40/FFA1 agonist (CPL207280) because no chromatographic interferences from the tablet excipients are found. The method is found to be selective, linear, and precise with an apposite detection limit. The suitability of the method for the quantitative determination of metformin-related substances is proven by validation in accordance with the requirements of the International Conference on Harmonization (ICH) guidelines. The statistical treatment of the data, together with validation results, demonstrated the reliability of this method.

Acknowledgements: This work was supported by The National Centre for Research and Development (POIR.01.01.01-00-0334/17).

Keywords: metformin, new UHPLC method, tablets.

Reference:

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Abstract No. PPP.055

Sustainable synthesis and in vitro biological evaluation of novel inverse agonists at the 5-HT6 receptor-operated Cdk5 signaling pathway

Vittorio Canale[1], Sévérine Chaumont-Dubel[2], Wojciech Trybała[1], Grzegorz Satała[3], Andrzej J. Bojarski[3], Paulina Koczurkiewicz-Adamczyk[1], Elżbieta Pękala[1], Philippe Marin[2], Paweł Zajdel[1]

[1] Jagiellonian University Medical College, Poland
[2] Institut de Génomique Fonctionnelle, Université de Montpellier, CNRS, INSERM, Montpellier, France
[3] Maj Institute of Pharmacology, Polish Academy of Sciences, Poland

E-mail address of presenting author: pawel.zajdel@uj.edu.pl

Objectives: The 5-HT_6R belongs to the family of GPCRs, which are positively coupled with adenylyl cyclase. Its interactome has been extensively characterized revealing that 5-HT_6R is linked to several cellular signaling cascades involved in cognitive processes and neurogenesis, such as mammalian target of rapamycin (mTOR) and cyclin-dependent kinase 5 (Cdk5) pathways. An important feature of the 5-HT_6R is its high level of ligand-independent constitutive activity. Preclinical studies have revealed that abnormal activation of the kinase Cdk5 might contribute to the pathogenesis of Alzheimer's disease through hyperphosphorylation of amyloid precursor protein. Based on these findings, herein we present the design, synthesis and *in vitro* biological evaluation of a focused library of inverse agonists at 5-HT_6R-operated Cdk5 signaling pathway.

Materials and Methods: The mechanochemical synthesis of designed compounds was performed in ball mill Retsch MM400. The affinity for 5-HT_6R and selectivity over 5-HT_{1A},

5-HT_{2A}, 5-HT_7Rs and D_2R were determined in radioligand binding studies. Next, the antagonism of selected compounds at the 5-HT_6R was assessed in cAMP-based cellular assay using 1321N1 cell line, whereas the intrinsic activity profile at the Gs and Cdk5 signaling pathways was evaluated on NG108 cells using the cAMP-BRET method and the measurement of neurite lengths, respectively. Finally, the metabolic stability of the most promising compound was investigated in RLM assay followed by evaluation of its potential hepatotoxicity on HepG2 cells in MTT assay.

Results: An application of mechanochemical procedure enabled the synthesis of 15 isoindoline-based derivatives in a sustainable manner. Radioligand binding assays showed that tested compounds behaved as potent and selective inverse agonists at the 5-HT_6R-dependent Cdk5 pathway and simultaneously displaying neutral antagonism at the Gs signaling The compound, displayed high metabolic stability and did not induce hepatotoxicity in a wide range of concentrations (0.1–10 μM).

Conclusions: The study identified the molecular probe to investigate the impact of selective inhibition of the 5-HT_6R-operated Cdk5 signaling pathway in the pathomechanism of neurodegenerative diseases.

Acknowledgements: The project was supported by the National Science Center, nr grant: 2021/05/X/NZ7/01847.

Keywords: Mechanochemistry, 5-HT_6R inverse agonists, Neurodegenerative disorders

Abstract No. PPP.056

Chitosan-based materials with natural active substance for wound healing

Dorota Chełminiak-Dudkiewicz[1], Aleksander Smolarkiewicz-Wyczachowski[1], Kinga Mylkie[1], Marta Ziegler-Borowska[1]

[1] Department of Biomedical Chemistry and Polymer Science, Faculty of Chemistry, Nicolaus Copernicus University in Toruń, Poland

E-mail address of presenting author: dorotachd@umk.pl

Objectives: This study focuses on obtaining and characterizing a novel chitosan-based biomaterials containing cannabis oil for potentially promoting wound healing. The primary active substance in cannabis oil is the non-psychoactive cannabidiol, which has many beneficial properties. Three chitosan-based films containing different concentrations of cannabis oil were prepared.

Materials and Methods: The obtained materials were characterized with several different technique and methods, including: Fourier transform infrared spectroscopy (FTIR), Scanning electron microscopy and atomic force microscopy (SEM, AFM), Thermogravimetric analysis (TGA), Contact angle and surface free Energy. Their mechanical properties, antioxidant effects, degradation and swelling properties were also examined. An important consideration in the design of wound dressings is the adsorption and accumulation of proteins at the solid-liquid interface of the biological fluid and wound dressing fiber. Hence, in this study were investigated the interactions of obtained biomaterials with human serum albumin using spectofluorimeter. The prepared films were subjected to acute toxicity test using the *Microtox*.

Results: All samples exhibited good mechanical and hydrophilic properties and a high swelling ratio. Degradability tests showed that the films lost their maximum weight within eight days. Moreover, increasing the concentration of cannabis oil in the samples promoted the adsorption of human albumin. The obtained biomaterials showed good antimicrobial activity against *A.fisheri* and a good drug release profile.

Conclusion: The development of novel biomaterials using natural polymers and active substances with antimicrobial properties is helpful in wound dressing. The present study focuses on preparing novel natural chitosan-based films incorporated with cannabis oil for wound dressing. Cannabis oil is rich in many active compounds, and its main component cannabidiol-provides antimicrobial, antioxidant, regenerative, and antithrombotic properties. The results showed that chitosan based films with cannabis oil might be an excellent candidate for wound treatment and dressing.

Acknowledgements: Authors are members of Center of Excellence ,,Towards Personalized Medicine" operating under Excellence Initiative – Research University.

Keywords: chitosan, natural active substance, wound dressing

Abstract No. PPP.057

In vitro ADME-Tox profiling and anti-inflammatory activity of 7,8-disubstituted 1,3-dimethyl-7H-purine 2,6-dione-based pan-PDE inhibitors in murine model of allergic asthma

Katarzyna Wójcik-Pszczoła[1], Krzysztof Pociecha[2], Elżbieta Wyska[2], Małgorzata Szafarz[2], Grażyna Chłoń-Rzepa[3], Hanna Plutecka[4], Paulina Koczurkiewicz-Adamczyk[1], Karolina Słoczyńska[1], Kamil Piska[1], Natalia Kocot[1], Elżbieta Pękala[1]

[1] Department of Pharmaceutical Biochemistry, Faculty of Pharmacy, Jagiellonian Univerity Medical College, Poland
[2] Department of Pharmacokinetics and Physical Pharmacy, Faculty of Pharmacy, Jagiellonian Univerity Medical College, Poland
[3] Department of Medicinal Chemistry, Faculty of Pharmacy, Jagiellonian Univerity Medical College, Poland
[4] Department of Internal Medicine, Faculty of Medicine, Jagiellonian Univerity Medical College, Poland

E-mail address of presenting author: katarzynaanna.wojcik@uj.edu.pl

Objectives: Phosphodiesterase (PDE) inhibitors represent promising anti-inflammatory and anti-remodeling agents. Based on theophylline, a non-selective PDE inhibitor structure we synthesized a group of its 7,8-disubstituted derivatives and characterized them as pan-PDE inhibitors potent against PDE isoforms that may be important for asthma treatment. Here, we examined the ADME-Tox properties using several in vitro methods and anti-inflammatory effects of a representative compound 38 and 145 (both PDE3, 4, 5, 7, and 8 inhibitors) in murine model of allergic asthma.

Materials and Methods: To achieve this goals we analyzed 38 and 145 hepatotoxicity, neurotoxicity, cardiotoxicity, mutagenicity, genotoxicity, and metabolic stability in vitro, as well as examined 38 and 145 effects on several inflammatory responses in Balb/c mice, sensitized and challenged with ovalbumin (OVA).

Results: Both pan-PDE inhibitors demonstrated to have no cytotoxic, mutagenic, and genotoxic activity and revealed moderate metabolic stability. Simultaneously, 38 and 145 significantly reduced the number of inflammatory cells, eosinophilia, and the level of pro-inflammatory cytokines in the bronchoalveolar lavage fluid from asthmatic mice. Both, total IgE and OVA-specific IgE levels in plasma were also diminished after treatment with these pan-PDE inhibitors. Additionally 38 and 145 significantly decreased goblet cell hyperplasia and α-smooth muscle actin expression in murine lungs.

Conclusions: In vitro ADME-Tox analysis confirmed favorable 7,8-disubstituted 1,3-dimethyl-7*H*-purine 2,6-dione-based pan-PDE inhibitor safety profiles. The obtained data strongly indicate anti-inflammatory activity of both tested compounds. Taken together we conclude that 7,8-disubstituted theophylline derivatives represent a promising group of compounds for the use in asthma therapy.

Acknowledgements: This study was supported by the National Science Centre, Poland, grant: UMO-2017/27/B/NZ7/01633

Keywords: phosphodiesterase inhibitors, theophylline derivatives, asthma, safety profile, anti-inflammatory activity

Abstract No. PPP.058

Permeation of albumin through human skin depending on its concentration and the substrate used

Wioletta Siemiradzka[1], Barbara Dolińska[1], Lucyna Bułaś[1]

[1] Department of Pharmaceutical Technology, Faculty of Pharmaceutical Sciences in Sosnowiec, Medical University of Silesia, Kasztanowa 3, 41-200 Sosnowiec, Poland, Medical University of Silesia in Katowice, Poland

E-mail address of presenting author: wsiemiradzka@sum.edu.pl

Objectives: Albumin, a natural polymer, is non-immunogenic, biodegradable and biocompatible. Albumin-based nanoparticles target specific cells as effective carriers in various therapies. Various routes of administration are also being tested, including on the skin [1]. It has been found that albumin applied to the skin as a hydrogel can penetrate this barrier and induce a systemic effect [2]. When introduced into a hydrogel containing corticotropin, it may contribute to increased porcine skin penetration of corticotropin [3]. This study aimed to investigate the permeation of albumin through human skin in simulated *in vivo* conditions depending on its concentration and the base used.

Materials and Methods: 4 formulations were prepared for skin application based on different hydrogels: methylcellulose (MC), sodium alginate, hypromellose and chitosan with methylcellulose, obtaining a final concentration of albumin in the hydrogel of 20 mg/g. In addition, a study of the effect of albumin concentration on skin permeation was carried out for the hydrogel, which released the greatest amount of albumin. The albumin permeation study through the skin was carried out in simulated *in vivo* conditions using Franz diffusion chambers. The Statistica module Pharmaceutical Kit: "Release Profiles" Weibull method was used to analyse the results. Statistically significant was calculated at $p<0.05$.

Results: Albumin applied as a hydrogel, penetrates through the skin. The greatest amount of albumin penetrated from the MC-based hydrogel. The effect of albumin concentration on its permeation was confirmed.

Conclusions: It was confirmed that albumin, despite its high molecular weight, permeated skin. It can therefore be applied on the skin as a carrier for therapeutic substances. The highest amount of albumin penetrated from a hydrogel with MC. The concentration of albumin affects the amount and rate of permeation of this protein through skin.

Keywords: Albumin, human skin permeation

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Abstract No. PPP.059

Targeting agonist-activated state of 5-HT$_6$ receptor by 2-arylpyrrole derivatives: potential application for the treatment of neurodegenerative disorders

Marcin Drop[1], Grzegorz Satała[2], Paulina Koczurkiewicz-Adamczyk[1], Sévérine Chaumont-Dubel[3], Xavier Bantreil[4], Wojciech Pietruś[2], Ophélie Bento[3], Vittorio Canale[1], Elżbieta Pękala[1], Andrzej Bojarski[2], Philippe Marin[3], Frédéric Lamaty[4], Paweł Zajdel[1]

[1] Faculty of Pharmacy, Jagiellonian University Medical College, Poland
[2] Maj Institute of Pharmacology, Polish Academy of Sciences, Poland
[3] Institut de Génomique Fonctionelle, Centre National de la Recherche Scientifique, France
[4] Institut des Biomolécules Max Mousseron, Centre National de la Recherche Scientifique, France

E-mail address of presenting author: marcindrop.son@gmail.com

Objectives: The serotonin type 6 receptor (5-HT$_6$R) belongs to the family of G protein-coupled receptors (GPCRs) and stands out a substantial level of constitutive activity [1]. The various conformational states of 5-HT$_6$R, which depend on the nature of the bound ligand, affect specific signal transduction pathways [2]. Considering these facts, the goal of our investigations was developing 5-HT$_6$R ligands with neutral antagonist and inverse agonist properties to explore distinct pharmacological responses associated with a particular functional profile.

Materials and methods: The new compounds were synthesized employing both classical and modern methods of organic chemistry (microwave-assisted and flow chemistry). The affinity for 5-HT$_6$R was determined using radioligand binding assays, impact on 5-HT$_6$R constitutive activity at Gs signaling was assessed using bioluminescence resonance energy transfer (BRET) method, while influence on 5-HT$_6$-operated Cdk5 kinases was determined by measurement of Cdk5-dependent neurite growth.

Results: The study identified a series of 2-phenyl-1*H*-pyrrole-3-carboxamide derivatives [3], which target constitutively active 5-HT$_6$R and displayed inverse agonist properties at receptor-operated Gs and Cdk5 signaling pathways. Structural rearrangement around the central core provided compounds with different geometries that target agonist-activated state of 5-HT$_6$R. The elaborated 5-HT$_6$R neutral antagonists at Gs signaling were investigated in cells-damage models characterized for neurodegenerative disorders.

Conclusions: Herein, we present 2-phenyl-1*H*-pyrrole-3-carboxamide as an interesting structural frame for developing 5-HT$_6$R inverse agonists. Further degradation of the 3-carboxamide moiety and shifting of the basic centre in the terminal aromatic fragments provide new scaffold for 5-HT$_6$R neutral antagonists.

Acknowledgements: The authors thank the financial support from the National Science Centre, Poland (grant no 2019/33/N/NZ7/01875, 2016/21/B/NZ7/01742), Centre National de la Recherche Scientifique and French Government Scholarship.

Keywords: 5-HT$_6$ receptor, constitutive activity, 2-arylpyrrole

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Abstract No. PPP.060

Influence of selected ointment bases on benzocaine release and penetration through model membranes

Lucyna Bułaś[1], Wioletta Siemiradzka[1], Olga Filuś[1], Barbara Dolińska[1]

[1] Department of Pharmaceutical Technology, School of Pharmacy with the Division of Laboratory Medicine in Sosnowiec, Medical University of Silesia in Katowice, 41-200 Sosnowiec, Poland, Medical University of Silesia in Katowice,, Poland

E-mail address of presenting author: lbulas@sum.edu.pl

Introduction: Benzocaine is included in aerosols, liquid preparations, suspensions, liquid powders, pastes, lozenges, gels, ointments (including rectal), suppositories. A wide range of media is used when preparing a semi-solid form of a drug containing benzocaine for the individual needs of the patient.

Objectives: Improvement of drug forms prepared according to the individual needs of the patient by analyzing the influence of the applied vehicle, formulation method and the membrane used on the release rate of the active substance from semi-solid drug forms.

Materials and methods: Six different formulations with a concentration of 2% of benzocaine were prepared with the use of two vehicles (a commonly used absorptive medium and a multi-component medium containing liposomes). The method of preparation different in the technique of introducing the active substance: without prior levigation; using the levigation process and after dissolving the raw material in ethanol and emulsifying it into the substrate. The USP apparatus 2 with enhancer cells was used to study release of benzocaine from obtained ointments. As a model membranes were used synthetic Spectra/Por 2 cellulose membrane and natural membrane isolated from porcine ear skin.

Results: The analysis of the release profiles through the synthetic membrane and natural skin revealed the existence of statistical differences in the release of benzocaine between different types of ointments. The release of benzocaine through the natural skin was consistent with the zero-order kinetics ($R2 > 0.96$). Statistical analysis showed that the particle size did not affect the degree of benzocaine release at a significance level of $p < 0.05$.

Conclusions: Benzocaine can penetrate the cellulose membrane and natural skin. The degree of benzocaine release depends on the type of ointment and the vehicle used. The highest amount of benzocaine released for the emulsion-type prepared on a commonly used absorption vehicle. With the suspension type ointment, more benzocaine was released from the liposome-containing multicomponent medium;

Keywords: release from semi-solid forms, benzocaine, suspension ointment; emulsion ointment

Abstract No. PPP.061

The effect of fluctuations in the vaginal pH on tenofovir disproxyl fumarate permeation and release from chitosan/poly(ethylene oxide) nanofiber film

Emilia Szymańska[1], Michał Wojasiński[2], Tomasz Ciach[2], Katarzyna Winnicka[1]

[1] Department of Pharmaceutical Technology, Medical University of Bialystok, Poland
[2] Department of Biotechnology and Bioprocess Engineering, Warsaw University of Technology, Poland

E-mail address of presenting author: emilia.szymanska@umb.edu.pl

Objective: This study aimed at evaluating the effect of vaginal pH (over physiological range) on the *ex vivo* tenofovir disproxyl fumarate (TDF) permeability and drug release behavior from chitosan (CS)/poly(ethylene oxide) (PEO) nanofiber film as a novel delivery platform for sexually transmitted infections prophylaxis.

Method: Nanofiber mat was produced by solution blow spinning (flow rate 1 ml/h; pressurized air 0.08 MPa; collector rotation speed 3000 rpm) from 12 % (w/w) solution of TDF/CS/PEO in the mass ratio 1:1:4. Dissolution study was conducted in simulated vaginal fluid (SVF) using paddle dissolution tester Agilent 708-DS. TDV permeation behavior across excised human vaginal epithelium (Local Bioethics Committee approval R-I-002/462/2018) was assessed by the in-line diffusion system SES Gmbh Analysesysteme, and drug content was examined by the HPLC method.

Results: TDF dissolution from CS/PEO formulation was gradual at SVF with pH 5.0 with less than 15 % of the drug dose released within the first 30 min. The release was prolonged up to 180 min with 80 % of TDF released after 150 min. In contrast, rapid dissolution was observed at pH 3.8 with about 60 % and 80 % of TDF present in the acceptor medium after 30 and 60 min, respectively. Differences in TDF permeation behavior were noticed concerning the applied pH environment. The permeated drug fraction evaluated at pH 5.0 reached about sixfold higher concentrations after 6 h incubation. About 40 % increase in drug accumulation in vaginal tissue compared to TDF in SVF with pH 3.8 was also noticed.

Conclusion: Fluctuations in the vaginal pH over the physiological range substantially affect TDF permeation and release from CS/PEO nanofiber film.

Acknowledgment: Special thanks to Dr. Magdalena Novicka (Noviline Clinic, Białystok) for providing tissue specimens.

Key word: vaginal nanofiber film, drug release, *ex vivo* permeation

Abstract No. PPP.062

Interactions of antiviral drugs with model lipid envelope of influenza virus

Dorota Matyszewska[1]

[1] Faculty of Chemistry, University of Warsaw, Poland

E-mail address of presenting author: dorota.matyszewska@chem.uw.edu.pl

Objectives: The aim of the research is to study the interactions of antiviral drugs with model membranes with composition typical for the lipid envelope of the H1N1 influenza virus. The changes in surface properties of model systems would point to a possible additional mechanism of action of antivirals, apart from the inhibition of neuraminidase.

Materials and Methods: The composition of the lipid envelope model included phosphatidylethanolamine (DOPE), phosphatidylserine (DMPS) and sphingomyelin (SM) found in the H1N1 virus envelope. The effect of oseltamivir, an antiviral drug used in the treatment of influenza was investigated by means of Langmuir technique, which allows for the formation of phospholipid monolayers at the air-water interface. Oseltamivir was dissolved in the different concentrations in the subphase, on which the lipid envelope monolayer was formed. The changes in surface physicochemical properties of the model layer were followed by Brewster angle microscopy (BAM) and grazing incidence X-ray diffraction (GIXD).

Results: Oseltamivir incorporates both into the monolayers of individual components of the lipid envelope and into a mixed ternary monolayer. The surface properties of the monolayers such as organization of lipids change, which influences fluidity of such layers. The strongest interactions were observed for negatively charged DMPS monolayers due to the presence of electrostatic attractions between the polar head and the positively charged drug. However, their extent is limited by higher concentrations of monovalent cations in the subphase, which screen the negative charge of DMPS. The electrostatic attractions lead to the changes in the domain formation, proving the increased fluidity of the model layers as shown by BAM images. The penetration of the model membranes induced by electrostatic interactions leads to the changes in the 2D crystal structure of the components of the lipid envelope.

Conclusions: The observed changes in the organization, structure and fluidity of model layers due to the interactions with oseltamivir may lead to the changes in the stability of the lipid envelope. It may be treated as an additional mechanism of action of antivirals on the influenza virus.

Acknowledgements: This work was partially financially supported by the "Excellence Initiative – Research University (2020-2026)" (New Ideas in Priority Research Area I).

Keywords: antivirals, lipid envelope, Langmuir technique

Abstract No. PPP.063

Polypharmacology towards diabetes

Przemyslaw Grygier[1], Katarzyna Pustelny[1], Grzegorz Dubin[1], Anna Czarna[1]

[1] Malopolska Centre of Biotechnology, Jagiellonian University, Poland

E-mail address of presenting author: p.grygier@doctoral.uj.edu.pl

Objectives: Diabetes is a long-term disease, that has become one of the leading causes of death globally. Two main types, type 1 diabetes (T1D) and type 2 diabetes (T2D), share a common mechanism: β-cells disfunction. Insulin supplementation treats the symptoms but does not cure the disease. Restoration of β-cells function has been proposed as a way towards the cure. Dual-specificity tyrosine phosphorylation-regulated kinase 1A (DYRK1A) and glycogen synthase kinase 3β (GSK3β) were identified as potential regulators of β-cells proliferation. DYRK1A suppresses the proliferation of β-cells through controlling the phosphorylation NFAT-family transcription factors, which act as a cell cycle activators. GSK3β is involved in a number of cellular pathways and requires its substrate to be primed, with DYRK1A being one of its priming kinases. Dual inhibition of DYRK1A and GSK3β leads to improvement in glucose homeostasis and induces β-cells proliferation.

Materials and methods: ATP competitive kinase activity assay (Cook assay), Microscale Thermophoresis (MST), NFAT activity luciferase assay, NFAT translocation assay, MTT cytotoxicity assay, X-ray crystallography.

Results: We show compounds with different molecular scaffolds that possess dual inhibitory activity against DYRK1A and GSK3β. *In vitro* validation, including Cook assay and MST, was used to determine the kinetic parameters of inhibitors. NFAT activity luciferase assay, NFAT translocation assay and MTT cytotoxicity assay were used to further assess the potency of the inhibitor. Selected compounds were cocrystallized with both DYRK1A and GSK3β to determine their binding modes.

Conclusions: We characterized a number of chemical compounds for their ability to inhibit both DYRK1A and GSK3β. Obtained cocrystal structures of either DYRK1A or GSK3β with the most potent inhibitors might lead, in the future, to the modifications of their chemical scaffolds for the sake of improving their efficacy and selectivity. Our results may be of further benefit in diabetes treatment.

Acknowledgements: This work was financially supported by the National Science Centre (grant no. 2019/34/E/NZ1/00467) and NAWA Polish Returns 2018 (grant no. PPN/PPO/2018/1/00046/U/00001)

Keywords: DYRK1A, GSK3β, diabetes, X-ray crystallography, kinase inhibitors

Abstract No. PPP.064

Studies on the influence of ionizing radiation on new, biodegradable drug delivery systems containing paclitaxel

Izabela Domańska[1], Aldona Zalewska[2], Andrzej Plichta[2], Monika Łyczko[3], Krystyna Cieśla[3], Marcin Sobczak[1]

[1] Department of Biomaterials Chemistry, Chair of Analytical Chemistry and Biomaterials, Faculty of Pharmacy, Medical University of Warsaw, Poland
[2] Faculty of Chemistry, Warsaw University of Technology , Poland
[3] Institute of Nuclear Chemistry and Technology, Poland

E-mail address of presenting author: idomanska@wum.edu.pl

Objectives: To investigate the influence of ionizing radiation in relation to sterilization process of new anticancer Drug Delivery Systems (DDSs).

Materials and methods: The polymeric matrices were synthesized *via* ring opening polymerization (ROP) of ε-caprolactone (CL), glycolide (Gly) and L-lactide (LA) in various monomer compositions, in the presence of bismuth 2-ethylhexanoate ($BiOct_3$) as a catalyst. Paclitaxel (PTX)-loaded microparticles were obtained by a single-solvent evaporation technique. The samples were irradiated in a linear electron accelerator (Elektronika 10/10) with an electron beam of 10 MeV energy to dose 25 kGy (sterilization dose). Gamma-irradiation was performed in a Gamma Chamber 5000 (^{60}Co source) with 25 kGy at a dose rate of 1.8 kGy h^{-1}. The influence of ionizing radiation on the microstructure of the polymers was evaluated by ^{1}H- or ^{13}C-NMR. GPC, DSC and TGA were used to determine physicochemical and thermal properties of the polymers. The quantitative and qualitative studies of drug release were performed using HPLC method.

Results: Various biodegradable polymeric matrices were synthesized *via* ROP of CL, Gly and LA. The copolymers obtained: poly(LA-*co*-CL) and poly(CL-*co*-Gly), had a random distribution and short sequences of comonomer units along the polymer chain. The study revealed a negligible effect of ionizing radiation on the microstructure of the copolymers. A slight influence of radiation on thermal properties was observed. A 6 % to 9 % change in average molecular weights (M_n) of poly(L-lactide) and poly(ε-caprolactone) was observed. A greater change in M_n was observed for the copolymers. Additionally, some influence of ionizing radiation on obtained PTX-loaded microparticles was observed.

Conclusions: The results confirm a great potential of $BiOct_3$ to create random copolymers. Most importantly, the polymers formed were non-toxic in terms of cyto- and genotoxicity, and thereby suitable for pharmaceutical and medical applications. The study revealed a negligible effect of the possible sterilization process on the structure of synthesized polyesters, while some alteration of the physicochemical properties as well as kinetics of drug release were noticed.

Acknowledgements: The contribution of Izabela M. Domańska was realized within the Project No POWR.03.02.00-00-I009/17-00.

Keywords: anticancer drug delivery systems, biodegradable polymers, polyester, radiation sterilization, gamma irradiation, e-beam

Abstract No. PPP.065

Synthesis and physicochemical characteristics of three new febuxostat cocrystals

Anita Sarna[1], Izabela Domańska[2], Izabela Madura[3], Edyta Pindelska[2]

[1] Scientific Circle "Spektrum" at Department of Analytical Chemistry and Biomaterials, Faculty of Pharmacy, Medical University of Warsaw, Poland
[2] Analytical Chemistry and Biomaterials, Faculty of Pharmacy, Medical University of Warsaw, Poland
[3] Inorganic Chemistry, Faculty of Chemistry, Warsaw University of Technology , Poland

E-mail address of presenting author: s075509@student.wum.edu.pl

Objectives: Cocrystals may affect manufacturability (flow, compaction, processability) as well as solubility/dissolution, hygroscopicity and stability properties of drugs. That kind of drug modification could be used to improve pharmaceutical properties of the II class BCS (*Biopharmaceutical Classification System*) drugs, such as febuxostat (FEB), poorly water-soluble drug. In this work we present a method of a synthesis of three novel FEB cocrystals and their structural and pharmaceutical studies.

Materials and Methods: FEB is used in the treatment of gout and hyperuricemia. The selection of cocrystal former molecules such as malonamide, lactamide, and diacetamide was based on the presence of complementary functional groups capable of forming hydrogen bond and the ΔpKa difference between them and FEB. The liquid assisted milling method has been used successfully to obtain FEB cocrystals. Fourier transformed infrared spectroscopy (FT-IR) and powder X-ray diffraction (PXRD) were used to provide information about the formation of cocrystals. Structural studies were supported by solubility tests.

Results: PXRD patterns and FT-IR spectra of the FEB cocrystals are evidently different from that of starting materials. Changes of the positions, intensity of the peaks in the PXRD patterns indicate that these were not just ordinary physical mixtures. The cocrystals successfully formed by the hydrogen bonding interaction between API and coformers. Different hydrogen bond interactions in the cocrystals result in changes in the FT-IR spectra. The water solubility and the effect of pH on the solubility and dissolution rate of FEB cocrystals were measured using a ultraviolet-visible spectroscopy and high-performance liquid chromatography.

Conclusions: Solubility and dissolution research show that three newly obtained cocrystals exhibited higher solubility than the FEB. Therefore, it can be concluded that cocrystallization can improve the properties of the pharmaceutical solids, which showed a poor solubility or low dissolution rate.

Acknowledgments This work was financially supported by Medical University of Warsaw, grant number FW231/2/F/GW/N/21.

Keywords: febuxostat, solubility, cocrystals

Abstract No. PPP.066

Effect of multiple-emulsion therapy on genotoxic and oxidative stress induced by doxorubicin and UV radiation in human fibroblasts

Konrad Kosicki[1], Agnieszka Markowska-Radomska[2], Elżbieta Speina[3], Ewa Dłuska[2]

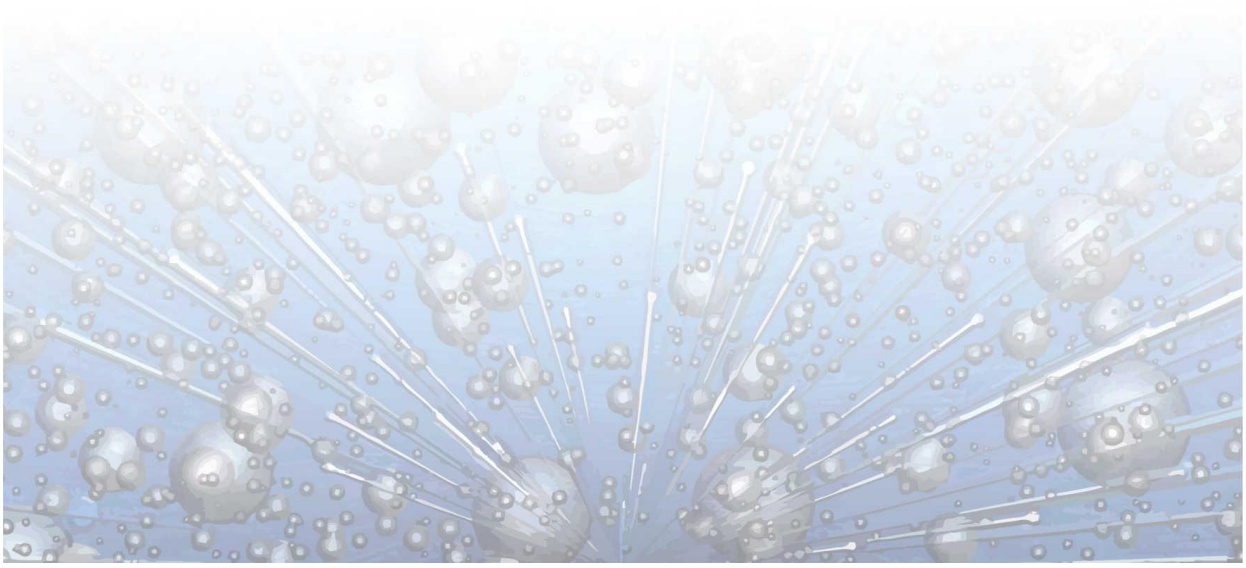
[1] Institute of Genetics and Biotechnology, Faculty of Biology, University of Warsaw, Poland
[2] Faculty of Chemical & Process Engineering, Warsaw University of Technology, Poland
[3] Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Poland

E-mail address of presenting author: konrado@biol.uw.edu.pl

Doxorubicin chloride (DOX) is a popular anti-cancer drug. DOX intercalates between DNA nitrogen base pairs, inhibits topoisomerase II, and ultimately induces oxidative DNA damage such as DNA double breaks (DSBs). The accumulation of DSB in cancer cells leads to genome instability and triggers the process of apoptosis. In turn, UV radiation (UVR) is the main etiological factor in the development of skin cancer. UVR induces the formation of DNA damage such as pyrimidine dimers (CPDs), 6-4 photoproducts (6-4PPs). These lesions are both toxic and mutagenic. Both DOX and UVR induce oxidative stress in cells by producing reactive oxygen species (ROS).

In our experiments, we use the K21 human fibroblast cell line as a laboratory model to study the effect of Multiple Emulsion Based Therapy (MEBT) on the effects of genotoxic and oxidative stress induced by DOX and UVR. To this end, the viability of K21 cells treated with DOX and UVR before and after MEBT treatment was tested. The kinetics of the recovery of the proliferative potential by fibroblasts is also shown. Additionally, the status of oxidative stress in K21 cells was examined before and after MEBT.

Keywords: Doxorubicin chloride (DOX), UV radiation (UVR), Multiple Emulsion Based Therapy (MEBT)



Abstract No. PPP.067

Effect of copper on the function of isolated pig kidneys stored in simple hypothermia

Aneta Ostróżka-Cieślik[1], Barbara Dolińska[1], Florian Ryszka[2]

[1] Department of Pharmaceutical Technology, School of Pharmacy and the Division of Laboratory Medicine in Sosnowiec, Medical University of Silesia, Katowice, Poland
[2] "Biochefa" Pharmaceutical Research and Production Plant, Sosnowiec, Poland

E-mail address of presenting author: bdolinska@sum.edu.pl

† In memory of Professor Florian Ryszka

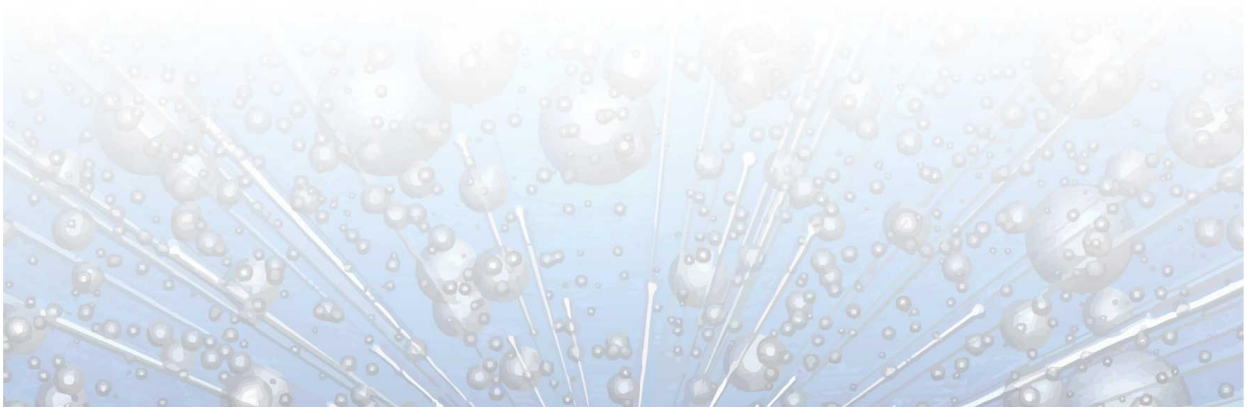
Objectives: Preservation solutions are used to rinse and store organs in the peri-transplant period. Our team is conducting advanced research on the development of an optimal solution formulation that will improve the efficiency of organ preservation in hypothermia and show a protective effect on grafts. The aim of this study was to analyse the protective effect of copper, as a potential component of Biolasol solution, in the prevention of nephron damage occurring during ischemia.

Materials and Methods: Pilot studies were performed in the isolated porcine kidney model Polish "Large White", with the approval of the II Local Ethics Committee Krakow; number 1046/2013. Biolasol solution (FZNP "Biochefa", Poland) was modified with the addition of Cu^{2+} at a dose of 1 µg/l. The kidneys were rinsed and stored using the static method for 48h under hypothermia (4°C). Biochemical markers of renal function were determined in the collected perfusates.

Results: Modified Biolasol + Cu^{2+} fluid (vs Biolsol) did not significantly affect the reduction in biochemical activity of ALT and AST enzymes ($p<0.05$). The presence of copper affected the occurrence of hyperkalaemia ([K^+]: 48h30'/18±2 mEq/l; $p<0.05$). The concentration of [Na^+] ions (48h30'/142±10 mEq/L; $p<0.05$) remained normal.

Conclusions: The addition of copper ions to the composition of Biolasol solution did not significantly improve its effectiveness.

Keywords: copper, kidney, preservation solution



Abstract No. PPP.068

Anti-inflammatory activity of Cannabis sativa L. extract in experimental atopic dermatitis

Renata Wolińska[1], Karolina Frączek[1], Piotr Poznański[2], Agata Nawrocka[2], Maria Zalewska[1], Mariusz Sacharczuk[2], Magdalena Bujalska-Zadrożny[1]

[1] Department of Pharmacodynamics, Centre for Preclinical Research and Technology, Medical University of Warsaw, Poland
[2] Department of Experimental Genomics, Institute of Genetics and Animal Biotechnology, Polish Academy of Sciences in Jastrzebiec, Poland

E-mail address of presenting author: rwolinska@wum.edu.pl

Objectives: *Cannabis sativa L.* (Cannabaceae) is one of the oldest plants known and used by humanity for multiple therapeutical purposes. The main active substances in this plant are phytocannabinoids, among others cannabidiol (CBD) and Δ-9-tetrahydrocannabinol (THC).

Non-psychoactive CBD is one of the most valuable cannabinoids because of its anti-inflammatory, analgesic, anxiolytic and antiepileptic effects. Moreover, topical formulations with CBD are used in the treatment of various dermatological diseases, despite the fact that current data regarding their efficacy and safety is limited.

Atopic dermatitis (AD) is a chronic skin disease, manifested by inflammation and intense pruritus. The gold standard of AD treatment are topical corticosteroids which possess a number of side effects, e.g. telangiectasia, rosacea, skin or impaired wound healing.

Therefore, our aim was to evaluate the anti-inflammatory effect of CBD-enriched *Cannabis sativa L.* extract in the model of AD.

Materials and methods: The model of AD was established by repeated application of 2,4-dinitrochlorobenzene (DNCB) to the skin of the rat's ear. The therapeutic effect of the studied extract was evaluated in behavioral and histopathological studies following topical application as an ointment containing 2% CBD.

Results: Application of 2% CBD ointment resulted in attenuation of DNCB-induced inflammation. Interestingly, treatment with the CBD extract caused a reduction in ear thickening, to a higher extent than the 1% hydrocortisone ointment. However, the CBD extract did not reduce the frequency of DNCB-induced scratching, in contrast to a visible antipruritic effect following 1% hydrocortisone application. Histopathological analysis revealed that both 2% CBD and 1% hydrocortisone ointments significantly decreased mast cell count, compared with the vaseline control group.

Conclusions: Taken together, our results demonstrate the anti-inflammatory properties of topically administered *Cannabis sativa L*. extract, suggesting its therapeutic potential for the treatment of AD.

Acknowledgements: The study was supported by statutory funds received from the Medical University of Warsaw, Poland. This study was carried out with the use of CePT infrastructure financed by the European Union – the European Regional Development Fund within the Operational Programme 'Innovative economy' for 2007–2013.

Keywords: Dermatitis, *Cannabis sativa L.*, cannabidiol (CBD)

Abstract No. PPP.069

Novel potential depigmenting agents from the group of cinnamic acid derivatives

Magda Borczuch-Kostańska[1], Popiół Justyna[2], Elżbieta Pękala[2], Henryk Marona[1], Dorota Żelaszczyk[1], Agnieszka Gunia-Krzyżak[1]

[1] Department of Bioorganic Chemistry, Chair of Organic Chemistry, Jagiellonian University Medical College, Poland
[2] Department of Pharmaceutical Biochemistry, Jagiellonian University Medical College, Poland

E-mail address of presenting author: justyna.popiol@uj.edu.pl

Objectives: Skin hyperpigmentation disorders constitute one of the most common dermatological problems. The currently available methods for their treatment are not effective enough. Moreover the organic compounds such as kojic acid or arbutin used in topical formulations often cause adverse effects. Kojic acid similarly to benzophenone derivatives, parabens and triclosan was identified as a very high-risk cosmetic ingredient with potential endocrine disrupting properties [1]. Thus we focused on searching for new potential depigmenting agents, which may be an alternative to currently available compounds. The aim of the study was the examination of ability of obtained compounds to inhibit tyrosinase - the key enzyme in the process of synthesis of the skin pigment (melanin).

Materials and Methods: A series cinnamic acid derivatives was synthetized and next they were tested *in vitro* as tyrosinase inhibitors (inhibition of tyrosinase monophenolase activity). The most frequent mushroom tyrosinase was used in this study, kojic acid was used as reference compound. The concentrations of the compounds that caused 50% tyrosinase inhibition (IC_{50}) were determined. For most promising compounds the kinetic analysis was carried out to evaluate the type of inhibition.

Results: Tested compounds showed various inhibitory activity, the IC_{50} values ranged from 36,98 to 1032,93 µM. Compound A-111 showed the highest activity that was comparable to kojic acid. Kinetic analysis of selected compounds showed that they could act as competitive (H-427) or mixed-type (A-111, H-331, H-300) enzymatic inhibitors.

Conclusions: The most active compounds will be further tested in more advanced models like cell lines and pigmented human epidermis. They could be potentially used in cosmetic products for hyperpigmentation disorders.

Acknowledgements

The research was financed by National Centre for Research and Development within LIDER XI program (contract number LIDER/26/0094/L-11/19/NCBR/2020).

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Abstract No. PPP.070

New colchicine derivatives with improved anticancer potency and selectivity

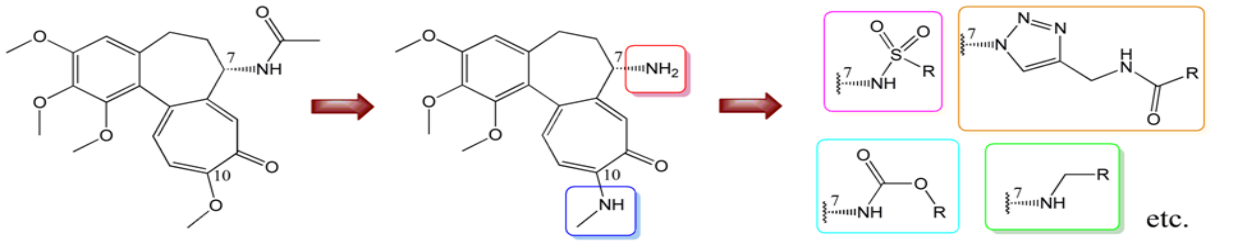
Julia Krzywik[1], Witold Mozga[2], Przemysław Pilaszek[2], Ewa Maj[3], Anna Nasulewicz-Goldeman[3], Joanna Wietrzyk[3], Adam Huczyński[4]

[1] TriMen Chemicals, / Department of Medical Chemistry, Adam Mickiewicz University, Poland
[2] TriMen Chemicals, Poland
[3] Hirszfeld Institute of Immunology and Experimental Therapy , Polish Academy of Sciences, Poland
[4] Department of Medical Chemistry, Adam Mickiewicz University, Poland

E-mail address of presenting author: julia@trimen.pl

Objectives: Colchicine is an inhibitor of mitosis that disrupts microtubules and inhibits tubulin polymerization. Many research centers are working on the development of an analogue based on colchicine skeleton, which could become the anticancer agent in the future. We joined this research and developed several series of new, patent-free double-modified colchicine derivatives.

Materials and Methods: Chemical modification of the colchicine was performed using several methods of organic synthesis. LC-MS and NMR spectroscopy were used to confirm the structure and purity of the obtained compounds. Cytotoxicity studies have been performed *in vitro* using various tumor cell lines and one normal cells.



Results: We have successfully obtained and characterized over 110 chemically diverse colchicine derivatives. The most of the new compounds showed high cytotoxicity with low nanomolar IC_{50} values. We also calculated the selectivity of the action of analogues and compared it with cisplatin and doxorubicin, most common anticancer drugs, as well as unmodified colchicine [1-6]. Some of the compounds showed favorable selectivity coefficients with low IC_{50} values at the same time, which makes them promising candidates for further research.

Conclusions: An appropriate modification of the colchicine molecule can lead to compounds with improved antiproliferative activity and higher selectivity of action. The results of the conducted research allowed us to observe the relationship between the structure and antiproliferative activity of colchicine derivatives, select compounds for further preclinical studies and indicate rational routes for further modifications of colchicine.

Keywords: anticancer agents, antiproliferative activity, colchicine derivatives.

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Abstract No. PPP.071

Adenosine Bisphosphonate Analogs as P2Y1 Receptor Antagonists with Antiproliferative Activity

Marta Fordymacka[1], Przemysław Pilaszek[1], Marta Switalska[2], Joanna Wietrzyk[2], Roman Blaszczyk[1]

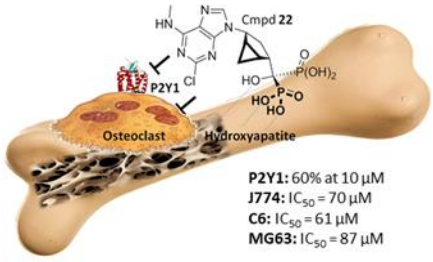
[1] Arendi, Poland
[2] Ludwik Hirszfeld Institute of Immunology and Experimental Therapy, Poland

E-mail address of presenting author: marta@trimen.pl

Objectives: Herein we present our results on the synthesis and activity of nucleotide derivatives bisphosphonates (BPs) that act as P2Y1 receptor antagonists and thus can be potential antiresorptive agents. The structures of the compounds were designed to be bone-targeted through the presence of bisphosphonic moiety and simultaneously blocked P2Y1 locally in the bone microenvironment. Additionally, because BPs are known to be also anticancer agents the final compounds were tested for cytotoxicity toward selected cancer cell lines.

Materials and methods: The antagonist effect toward P2Y1 receptor were evaluated using human recombinant 1321 N1 cells fluorimetric assay, measuring the intracellular mobilization of Ca^{2+}. The antiproliferative activity (IC_{50}) were evaluated toward selected cell lines using SRB assay. Mouse macrophage cell line J774 was used to determine antiresorptive activity *in vitro*. Rat C6 glioma and human MG63 osteosarcoma cell lines were used to determine anticancer activity *in vitro*.

Results: The synthesized compounds can be divided into three structural classes with a different linkage between the bisphosphonate group and adenine: acyclic, simple cyclic and ring constrained analogs of carbanucleotides. The only compound that combines high potency against P2Y1 with high antiproliferative activity was the enantiomerically pure bicycle **22**. In the cellular models of action it showed increased potency compared to both references compounds MRS2179 (known P2Y1 receptor antagonist) and zoledronic acids.



Conclusions: We have discovered a series of potent bisphosphonic acids P2Y1 receptor antagonists with a potentially dual mechanism of antiresorptive activity. In tested cellular models, the designed compounds showed superior activity compared to MRS2179 and clinically used zoledronate. Thus some of these compounds can be regarded as novel antiosteoporotic agents as well as anticancer agents especially related to bone cancers.

Acknowledgements: Studies were supported by the National Centre for Research and Development (NCBiR; Poland) in the framework of INNOTECH II; nr HI2/181925.

Keywords: Bisphosphonates, P2Y1 receptor antagonists, anticancer drugs

Abstract No. PPP.072

Preparation and assessment of new drug carriers based on silver nanoparticles containing hydrogels

Oleg M. Demchuk[1], Maciej Masłyk Masłyk[1], Konrad Kubiński[1], Kamila Górka[1], Monika Janeczko[1], Aleksandra Martyna[1], Mateusz Kwaśnik[1], Mariusz Borkowski[2], Anna Boguszewska-Czubara[3], Joanna Kowalczuk[4]

[1] Faculty of Science and Health, The John Paul II Catholic University of Lublin, Poland
[2] Jerzy Haber Institute of Catalysis and Surface Chemistry, Polish Academy of Sciences, Poland
[3] Department of Medical Chemistry, Medical University of Lublin, Poland
[4] Institute of Molecular Physics, Polish Academy of Sciences, Poland

E-mail address of presenting author: oleg_demchuk@hotmail.com

Objectives: The attempts to find medical applications for silver nanoparticles (AgNP) are still popular mostly due to the assumed antimicrobial activity of AgNP and well-documented antimicrobial properties of silver ions. On the other hand AgNPs could be used as an efficient drug carrier.For the presented studies, the AgNPs were synthesized through a method that consisted of reducing the ionic silver with sodium citrate solution, and characterized by XPS, UV-VIS, DLS, Z-potential, SEM, and AFM. It was proved that nanoparticles with a narrow deviation from 26 nm size were obtained after the standard purification procedure.

Materials and methods: AgNPs were stabilised in biologically neutral C_{12}ALA hydrogel. As the analyses shown in prepared C_{12}ALA-AgNP the AgNPs are located in the liquid phase trapped between the fibers of C_{12}ALA gel. AgNPs could be separated by long time centrifuging while the C_{12}ALA-hydrogel remains generally stable after such treatment.

Results: The thermal properties and kinetics of C_{12}ALA-AgNP and C_{12}ALA-hydrogels were examined by thermogravimetry technique using Perkin Elmer TGA 8000 analyzer. The gel sample undergoes thermal conversion at a specific rate and at a given temperature. This process can be analyzed in a light of OWF theory. As a results the total temperature of decomposition or time dependence of sample conversion as a function of temperature were obtained. On the basis of these information we can conclude about strength and thermal stability of the gel. The AgNPs and C_{12}ALA-AgNP were tested against nine bacteria and one fungus, and they showed inactivity up to the concentration of 27.5 µg/ml. The low activity of the nanoparticles was also observed in tests against human normal (CCD-11Lu) and cancer (HeLa, SW480) cell lines, the IC_{50} values were >200 µM. Finally AgNPs show a trace hemolytic activity against human erythrocytes, and under tested concentrations show no ecotoxicity on *Danio rerio.*

Conclusions: In comparison with the biological activity AgNPs published elsewhere, our nanoparticles show high level of biological neutrality and may be taken into consideration as promising drug carrier developing no side effects.

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

Keywords: nanoparticle, hydrogel, drug carrier, AgNP, biological activity

Abstract No. PPP.073

Derivatives of 4-AN as effective antifungal agents

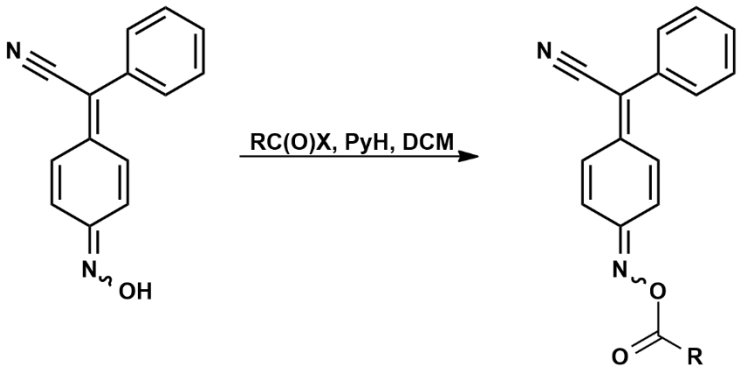
Monika Janeczko[1], Aleksandra Martyna[1], Antonina Kurowska[1], Maciej Masłyk[1], Konrad Kubiński[1], Oleg M. Demchuk[1]

[1] Faculty of Science and Health, The John Paul II Catholic University of Lublin, Poland

E-mail address of presenting author: oleg_demchuk@hotmail.com

Objectives: From last few decades an increasing occurrence of fungal antibiotic resistance has been noticed. That is why there is an urgent need to search and develop new effective therapeutic strategies to overcome this phenomena. Recently we have presented a new properties of a molecule 4-AN (arylcyanomethylenequinone oxime) such as high anti-Candida activity showing reduction of the relative expression of genes engaged in fungal virulence. Moreover, it presents no toxicity towards human cells [doi: 10.3390/molecules25122928].

Materials and methods: Here we present a new series of 4-AN derivatives. Using efficient synthetic approach 7 new compounds were prepared.



R = CH_3, n-$C_{11}H_{23}$, $(CH_2)_3C(O)OMe$, CH_2Cl, Ph, $(CH_2)_2CO_2H$, OCH_2Ph

Results: New compounds were tested against Candida species as well as *C. albicans* clinical strains. The substance with highest activity was analyzed in order to verify the mechanism of activity, influence on the level of expression of genes engaged in virulence. The toxicity influence on human cells was also checked in order to verify the toxicity level.

Conclusions: The research revealed the antifungal properties of 4-AN derivatives. It effectively kills clinical isolates of Candida, affects mature biofilm, and moderately disrupts membrane permeability. The results of those studies and some additional issues will be discussed.

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

Keywords: anti-Candida activity, 4-AN, therapeutic strategies

Abstract No. PPP.074

The influence of sirtuin 1 (SIRT1) on the anticancer activity of vitamin D in lung cancer cells

Dominika Lewoń-Mrozek[1], Ewa Maj[1], Joanna Rossowska[2], Joanna Wietrzyk[1]

[1] Laboratory of Experimental Anticancer Therapy, Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Poland
[2] Inter-departmental Laboratory of Cytometry and Confocal Microscopy, Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Poland

E-mail address of presenting author: dominika.lewon@hirszfeld.pl

Objectives: The aim of the study was to investigate the impact of sirtuin 1 (SIRT1) expression on the anticancer activity of vitamin D (vD) in lung cancer cells, that differ in EGFR and KRAS mutation status. SIRT1 is a NAD^+-dependent class III histone deacetylase, that deacetylates histones and non-histone proteins, including vD receptor (VDR), which is an effector protein for biologically active form of vD - calcitriol. Deacetylation of VDR potentiates its activity as a transcription factor, thus SIRT1 may influence cell response to calcitriol. Understanding the proteomic mechanisms influencing vD anticancer activity would provide a rationale for targeted therapy and vD supplementation in management and prevention of lung cancer.

Materials and Methods: Four lung cancer cell lines were included in the study: HCC4006 and NCI-H1975 representing EGFR lung cancer subtype, NCI-H1792 and NCI-H23 representing KRAS lung cancer subtype. Cancer cells were genetically modified via the lentiviral transduction to obtain stable cell lines with overexpression (OE) or knockdown (KD) of SIRT-1. Three different sequences of shRNA were tested to choose the most effective sequence in SIRT-1 silencing. Anticancer activity of calcitriol in the concentration of 1000, 100, 10, 1 nM was measured in the antiproliferative test with sulforhodamine B. Levels of SIRT1 expression in generated cell sublines were evaluated by western blot.

Results: We obtained lung cancer cell lines with stable OE and KD of SIRT1, sh3 was the most effective in silencing SIRT1 out of all shRNA. SIRT1 KD with sh3 caused a decrease in cell sensitivity to calcitriol in HCC4006 NCI-H1792 and NCI-H23. SIRT1 OE also caused a decrease in cell sensitivity to calcitriol in HCC4006 and in NCI-H1792.

Conclusions: SIRT1 may play an important role in the response of lung cancer cells to anticancer activity of vD. Silencing of SIRT1 might result in the increase of VDR acetylation status, thus lower its activity as a transcription factor. However, further research is necessary to understand mechanisms standing behind proteomic interactions between VDR and SIRT1 in lung cancer cells which lead to decrease of anticancer properties of vD after KD as well as OE of SIRT1.

Acknowledgements: National Science Center, grant No SONATA 2018/31/D/NZ3/01739.

Keywords: sirtuin 1, vitamin D, lung cancer.

Abstract No. PPP.075

Synthesis and characterization of multifunctional collagen-nanoapatite composite with a potential haemostatic effect

Barbara Kołodziejska[1], Łukasz Pajchel[1], Joanna Kolmas[1]

[1] Department of Analytical Chemistry, Medical University of Warsaw / Faculty Of Pharmacy, Poland

E-mail address of presenting author: bkolodziejska@wum.edu.pl

Objectives: The main goal was to develop a new, multifunctional collagen-apatite composite with a high degree of biocompatibility and bioactivity with bone tissue. The designed material was to be additionally used as an anti-hemorrhagic drug carrier - tranexamic acid (TXA). Thanks to this, the synthesized biomaterial gains a local hemostatic effect while minimizing side effects occurring in the systemic administration of this drug and it speeds up the wound healing process.

Materials and Methods: The first step was to obtain biomimetic calcium phosphate apatite containing Zn^{2+} and Mg^{2+} ions, the osteogenic agents. The apatitic fraction was used to obtain two types of composites differing in the type of collagen: collagen type I and atelocollagen. TXA was introduced into the composite by two methods. Then all materials were lyophilized.

Results: The obtained composites were characterized using the following methods: FTIR spectroscopy, Scanning Electron Microscopy, Atomic Absorption Spectrometry and Powder Xray Diffraction. The drug substance release profile was examined by HPLC. The obtained composites differed in the mutual arrangement of collagen fibers and apatite crystals (Figure 1), as well as in the mechanical strength of the samples depending on the method of synthesis. There were also differences in the amount of foreign ions in individual samples. Significant differences in the release profiles of TXA, zinc and magnesium ions were detected.

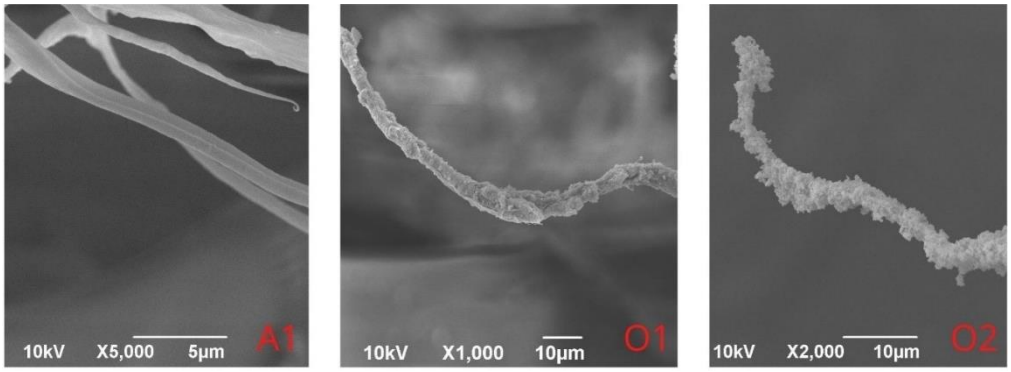


Figure 1. SEM images of the atelocollagan/apatite composite synthesized by first method (A1), collagen/apatite synthesized by first method (O1) and collagen/apatite synthesized by second method (O2).

Conclusions: In the present study, various biomimetic apatite/collagen composites were synthesized. Their composition and crystallinity are similar to bone tissue structure. Some differences in physicochemical properties depending on the type of collagen and synthesis method were observed. Drug release profiles vary with the type of composite. In vitro studies on its biological properties are in progress and will contribute to the full evaluation of the obtained materials. It seems that the use of collagen-based materials with the addition of zinc ions as well as a TXA may be a promising combination in the wound healing process.

Acknowledgements: This study was financed from a research grant (Project FW23/F/MB1/N/20) from Medical University of Warsaw.

Keywords: biomaterials, collagen, multifunctional composite

Abstract No. PPP.076

Radiosynthesis study of the novel 131I-labelled 1,2,3-dithiazole-based radiopharmaceutical for L-amino acid transporters molecular imaging and therapy

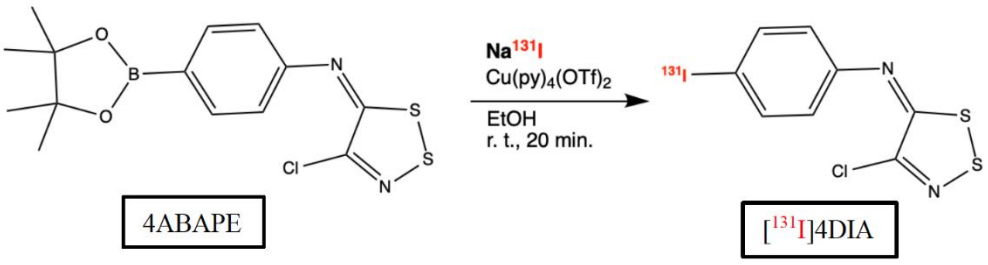
Mateusz Pocięgiel[1], Justyna Pijarowska-Kruszyna[2], Antoni Jaroń[2], Piotr Garnuszek[2], Renata Mikołajczak[2]

[1] National Centre for Nuclear Research, Poland
[2] Radioisotope Centre POLATOM, National Centre for Nuclear Research, Poland

E-mail address of presenting author: acke@op.pl

Objectives: The *L*-type amino acid transporter (LAT1) can serve as the pharmacological target of tumors. This system is highly expressed in many types of cancers and contributes to tumor growth and survival. The new inhibitors of human LAT1 transporter based on 1,2,3-dithiazole scaffold were reported. We hypothesize that their ^{131}I-labelled derivatives could be synthesized at high yield as a result of aromatic nucleophilic substitution (S_NAr) reaction using sodium iodine-131. Therefore, we selected the iodine containing compound 4DIA (4-chloro-*N*-(4-iodophenyl)-5H-1,2,3-dithiazol-5-imine), with high inhibition of LAT1 (>90%) as potential radioligand. The specific aim of this work was to develop an efficient and convenient method for its radiosynthesis with ^{131}I.

Materials and Methods: Radiosynthesis of [^{131}I]4DIA was based on the copper (II) mediated nucleophilic radioiodination of previously obtained aryl boronic acid pinacol ester 4ABAPE (4-chloro-*N*-(4-pinacolboronatephenyl)-5H-1,2,3-dithiazol-5-imine). This procedure was performed under mild conditions (EtOH, r.t., 20 min.) using 5 MBq Na^{131}I, 100 mg of 4ABAPE precursor and 1.4 mg $Cu(py)_4(OTf)_2$. The crude radioligand [^{131}I]4DIA was purified using SiO_2 cartridge. Radiochemical purity (RCP) was determined by radio-TLC and -HPLC.



Scheme 1. Radiosynthesis reactions of [^{131}I]4DIA.

Results: New radiotracer [^{131}I]4DIA was synthesized with 90% yield (n=15). Both radio-TLC and HPLC analyses demonstrated RCP of more than 98% (n=4). The identity of [^{131}I]4DIA (R_t=7.48 min) was confirmed by co-injection with non-radioactive standard 4DIA (R_t=7.35 min).

Conclusions: The method of synthesis based on a new drug precursor with the boronic acid pinacol ester mediated by copper (II) resulted in high yields, obtained in a short time (20 min.) and under mild conditions. First *in vitro* tests in glioblastoma and prostate cancer metastases to brain (U87 and DU145 cell lines) are in progress.

Acknowledgements: This work was realized within Project No POWR.03.02.00-00-I009/17-00 (Operational Project Knowledge Education Development 2014–2020 co-financed by European Social Fund).

Keywords: : Radiopharmaceuticals, LAT1, molecular imaging

Abstract No. PPP.077

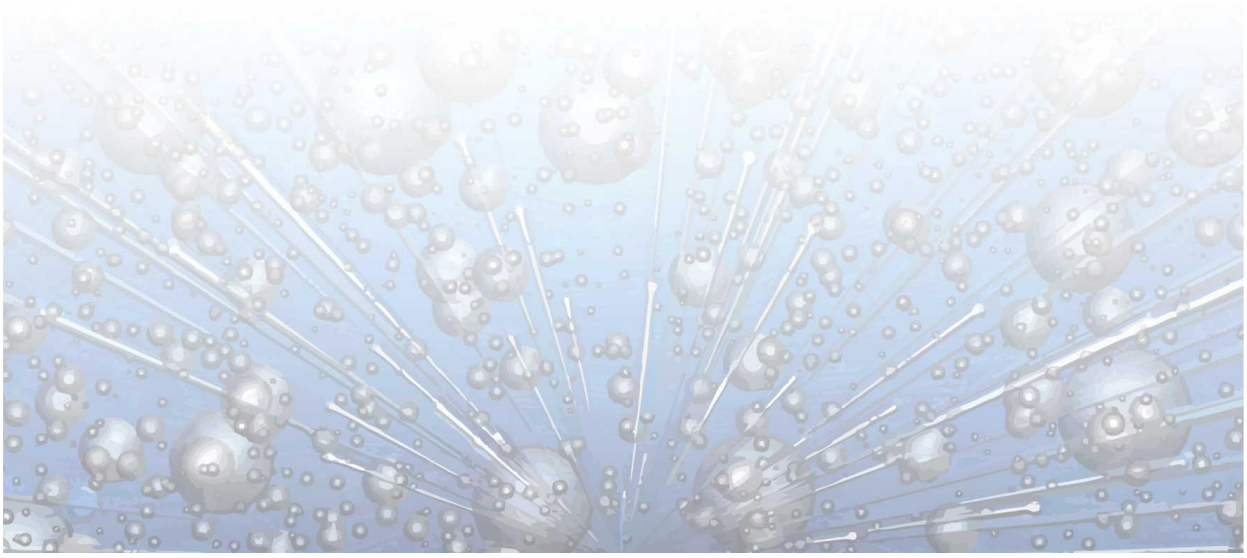
Protein expression changes assay as a tool in monitoring the fluorinated drug treatment

Andrzej Gawor[1], Bożena Czarkowska-Pączek[2], Zdzisław Gajewski[3], Ewa Bulska[1], Leszek Pączek[4]

[1] Biological and Chemical Research Centre, Faculty of Chemistry, University of Warsaw, Zwirki i Wigury 101, 02-089 Warsaw, Poland, Poland
[2] Department of Clinical Nursing, Medical University of Warsaw, Ciolka Street 27, 01-445 Warsaw, Poland, Poland
[3] Center for Translational Medicine, Warsaw University of Life Science, Nowoursynowska 100, 02-797 Warsaw, Poland, Poland
[4] Department of Immunology, Transplantology and Internal Diseases, Medical University of Warsaw, Nowogrodzka 59, 02-006 Warsaw, Poland, Poland

E-mail address of presenting author: agawor@chem.uw.edu.pl

Cinacalcet belongs to type II calcimimetics and is metabolised mainly in the liver. Unfortunately, Cinacalcet has some adverse effects for which the mechanism is not fully explained. With this study, we aimed to examine the effects of Cinacalcet on protein expression in rat brain and liver tissues. A mass-spectrometry-based label-free differential proteomics approach was used to evaluate the changes in protein expression in examined tissues. Interestingly, among the treatment subgroup, ten up-regulated and three down-regulated proteins were detected in the liver and one upregulated protein in the brain compared to the control group. These proteins were involved in the enzyme regulator activity (36%), binding (31%) and catalytic activity (19%). Accordingly, the results obtained in the pilot study indicate that chronic cinacalcet therapy could impair phase II of enzymatic detoxication and may cause disturbances in blood hemostasis, lipid metabolism, and inflammatory mediators or contribute to the acceleration of cognitive dysfunction; therefore, appropriate patient monitoring should be considered.



Abstract No. PPP.078

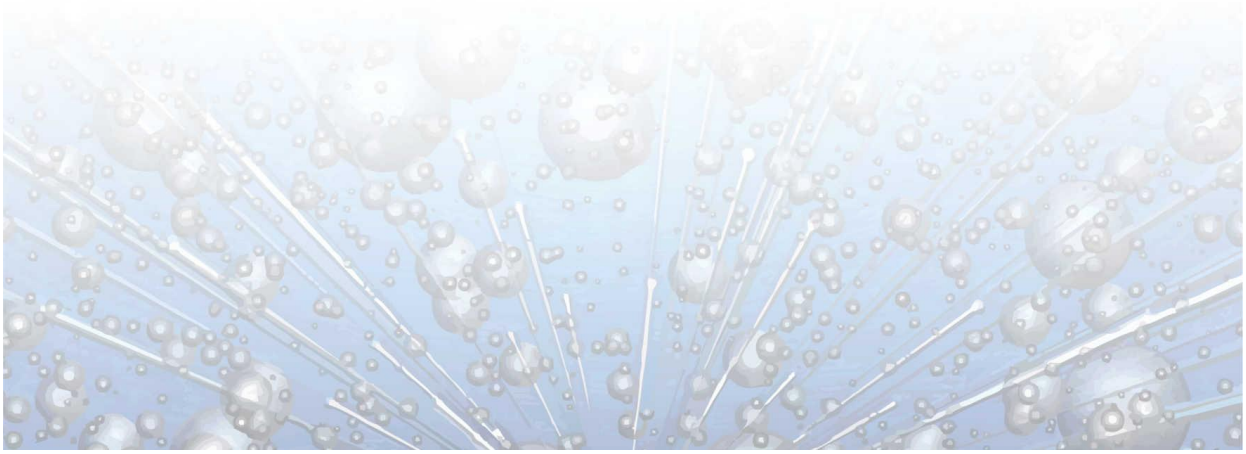
Fluorination of selected proteins in rats as a result of fluorine-containing drug administration

Ewa Bulska[1], Andrzej Gawor[1], Zdzisław Gajewski[2], Bożena Czarkowska-Pączek[3], Leszek Pączek[4]

[1] Biological and Chemical Research Centre, Faculty of Chemistry, University of Warsaw, Zwirki i Wigury 101, 02-089 Warsaw, Poland, Poland
[2] Center for Translational Medicine, Warsaw University of Life Science, Nowoursynowska 100, 02-797 Warsaw, Poland, Poland
[3] Department of Clinical Nursing, Medical University of Warsaw, Ciolka Street 27, 01-445 Warsaw, Poland, Poland
[4] Department of Immunology, Transplantology and Internal Diseases, Medical University of Warsaw, Nowogrodzka 59, 02-006 Warsaw, Poland, Poland

E-mail address of presenting author: ebulska@chem.uw.edu.pl

In a number of pharmaceuticals, a hydrogen atom or hydroxyl group is replaced by fluorine to increase bioavailability and biostability. Although fluorine can easily penetrate the cell membranes of hard and soft tissues, there is not much care on the potential interaction of fluorine released from fluorine-containing drugs with proteins. With this project, we aimed to evaluate the possible fluorination of proteins after administration of the fluorinated drug cinacalcet. As model objects, we selected a rat's liver and brain. In the total of 18 Wistar rats were randomised into a control group and a group treated with cinacalcet. In order to investigate the presence of fluorinated proteins the MS-based proteomics approach was used to identify proteins in examined tissues (rat liver and brain). The applied label-free proteomic approach consisted of: chromatographic separation and analysis by high-resolution mass spectrometry; peptide/protein identification using the Mascot search algorithm; manual verification of an experimentally generated MS/MS spectrum with the theoretical MS/MS spectrum of identified fluorinated peptides. Three fluorinated proteins (spectrin beta chain; carbamoyl-phosphate synthase [ammonia], mitochondrial; 6-phosphofructo-2-kinase/fructose-2, 6-bisphosphatase 1) were identified in the liver and four (spectrin beta chain, dihydropyrimidinase-related protein 4, prominin-2, dihydropyrimidinase-related protein 4) in the brain tissue after 21 days of cinacalcet treatment, but not in the control group. Thus we could conclude that administration of fluorinated drugs results in tissue-specific fluorination of proteins.



Abstract No. PPP.079

Determination of rivaroxaban concentration in human plasma using LC-MS/MS technique

Krzysztof Abramski[1], Beata Mantur[1], Michał Kaza[1], Piotr J. Rudzki[1]

[1] R&D Finished Dosage Form, Celon Pharma S.A., Poland

E-mail address of presenting author: krzysztof.abramski@celonpharma.com

Objectives: Development of a bioanalytical method suitable to determine concentration of rivaroxaban in human plasma samples. GLP-compliant validation of the method according to international regulatory guidelines [1, 2].

Materials and methods: Ultra high performance liquid chromatograph (Agilent Technologies 1290 Infinity II quaternary pump with multisampler and multi wash option) hyphenated with electro spray ionization tandem mass spectrometer (Sciex QTRAP 6500+). Phenomenex Synergi Fusion-RP as chromatographic column. Rivaroxaban and rivaroxaban-d4 (internal standard).

Results: The analytical method with isocratic elution proved to be linear in the range from 2 to 600 ng/mL of rivaroxaban in human plasma using linear equation with 1/x weighing. Precision expressed as relative standard deviation was better than 6.56%. The recovery/accuracy was in the range from 103.1% to 111.6% and the matrix effect was negligible (1.008 to 1.023). Stability of rivaroxaban in standards solutions and in samples stored in different conditions was confirmed (stock solution up to 15 days at 2-8 °C, sample solution at room temperature up to 48 h and 162 h in an autosampler). The method reliability was confirmed by 239 of 240 incurred samples reanalysis meeting acceptance criteria.

Conclusions: The validation parameters met acceptance criteria. The method was validated according to the respective EMA [1] and FDA [2] guidelines. The method was successfully applied to the analysis of clinical samples.

Acknowledgements: The authors acknowledge Marta Rucińska and Justyna Czajkowska (Celon Pharma S.A., Research and Development Centre) for excellence in quality assurance monitoring.

References:

[1] European Medicines Agency. *Guideline on bioanalytical method validation* (EMEA/CHMP/EWP/192217/2009 Rev.1 Corr. 2**), London, 21 July 2011
[2] Food and Drug Administration. *Bioanalytical Method Validation. Guidance for Industry*, U.S. FDA/CDER/CVM, May 2018

Abstract No. PPP.080

De novo foldameric mini-protein as inhibitors of PD-1/PD-L1 interaction

Juan Lizandra Pérez[1], Łukasz Berlicki[1]

[1] Department of Bioorganic Chemistry, Politechnika Wroclawska, Poland

E-mail address of presenting author: juan.lizandra-perez@pwr.edu.pl

Objectives: Interaction of programmed cell death protein 1 (PD-1) and its ligand (PD-L1) is an immune checkpoint and one of the mechanisms employed by cancer to evade the immune system. The blockade of this interaction has proven to be complex to target. Such complexity of the interaction is because it is based on large hydrophobic flat surface. In the present work, we intend to develop a *de novo* foldameric mini-protein with an βαββ topology. Such topology will generate a triple stranded antiparallel beta-sheet stabilized with a helix, via a hydrophobic core, and the residues of the outer surface will be mutated to attain affinity towards PD-L1.

Materials and methods: The mini-protein designs are synthesized by Solid Phase Peptide Synthesis, purified by High Performance Liquid Chromatography (HPLC), and characterized with mass spectrometry, analytical HPLC, Circular Dichroism, Differential Scan Calorimetry, and 2D NMR. The affinity studies towards PD-L1 are done with BioLayer Interferometry (BLI), and the inhibition studies by two methods, Homogenous Time Resolved Fluorescence (HTRF) and Promega PD-1/PD-L1 blockade bioassay.

Results: We have designed EHEE (E-extended, H-helix), a foldameric mini-protein which folds cooperatively into the topology of interest. The developed inhibitors of PD-1/PD-L1 immune checkpoint, based on this scaffold, have shown levels of nanomolar affinity towards PD-L1 by BLI and nanomolar inhibition of PD-1/PD-L1 interaction by HTRF studies. Our most recent work with Promega PD-1/PD-L1 blockade bioassay, showed T-Cell activation at micromolar concentrations of one of the inhibitors, a thermally hyper-stable inhibitor with T_m of 105 °C.

Conclusions: Here, we successfully designed a novel mini-protein with βαββ topology. By modification of solvent exposed residues, from the beta sheet, we have generated active inhibitors, in protein and cell-based assays, towards PD-1/PD-L1 interaction These results are a lead to develop potential candidates for anticancer immunotherapy.

Acknowledgments: Project financed by National Science Center "De novo designed, structurally extended peptide foldamers and their use for construction of PD-1/PD-L1 interaction inhibitors"; NCN, OPUS 2018/31/B/ST5/02631

Keywords: Mini-proteins, Cancer, Immune therapy

Abstract No. PPP.081

Miniprotein-based PD-1/PD-L1 interaction inhibitors

Agnieszka Ciesiolkiewicz[1], Łukasz Berlicki[1]

[1] Department of Bioorganic Chemistry, Wroclaw University of Science and Technology, Poland

E-mail address of presenting author: agnieszka.ciesiolkiewicz@pwr.edu.pl

Objectives: Immune checkpoints inhibition has been identified as a promising target for the development of anticancer therapeutics. One of the immune checkpoints, consisting of the programmed cell death protein 1 (PD-1), and its ligand (PD-L1), has been shown to be a beneficial but difficult immunotherapy target due to its large, flat, and hydrophobic interacting surfaces. Noteworthy, the effectiveness of the PD-1/PD-L1 interaction inhibition in anticancer immunotherapy has been already evidenced for two groups of inhibitors: monoclonal antibodies and small molecules. In this work, we provide an alternative approach using miniproteins to block the PD-1/PD-L1 interaction.

Materials and Methods: Here, miniproteins were designed based on the engrailed homeodomain scaffold consisting of three helices. The surface made up of two antiparallel helices was used to create interaction interface with PD-L1 at the PD-1 binding site. Miniproteins were synthesized using microwave-assisted automated solid phase synthesis. Their secondary structure and thermal stability were determined by circular dichroism (CD). Binding kinetic analysis and inhibition measurements were performed using biolayer interferometry (BLI) and homogeneous time-resolved fluorescence (HTRF).

Results: In this study, we showed that the obtained inhibitors exhibit a micromolar binding affinity towards PD-L1 in BLI assay and micromolar inhibitory activity against PD-1/PD-L1 interaction in HTRF studies. CD studies did not reveal any significant changes in the CD spectrum and denaturation plots compared to the results of the original miniprotein. These results suggest that sequence mutations that introduced to achive anti-PD-L1 affinity did not lead to significant changes in the tertiary structure.

Conclusions: Our work has led to the discovery of novel group of rationally designed PD-1/PD-L1 interaction inhibitors based on a miniprotein scaffold stabilized by a hydrophobic core. Therefore, the presented inhibitors will be considered as a lead compound for further optimization toward more potent anticancer drugs.

Acknowledgements: Project financed by the National Science Center ("De novo designed, structurally extended peptide foldamers and their use for the construction of PD-1/PD-L1 interaction inhibitors"; NCN, OPUS 2018/31/B/ST5/02631).

Abstract No. PPP.082

First-in-human pharmacokinetic study of a JAK/ROCK dual inhibitor CPL409116

Michał Kaza[1], Dorota Włodarczyk[2], Krzysztof Abramski[1], Katarzyna Jarus-Dziedzic[3], Beata Mantur[1], Daniel Rabczenko[4], Piotr J. Rudzki[1], Agnieszka Segiet[4], Maciej Wieczorek[5]

[1] R&D Finished Dosage Form, Celon Pharma S.A., Poland
[2] Clinical Department, Celon Pharma S.A., Poland
[3] Clinical Site, BioResearch Group, Poland
[4] Instytut Edukacji, Poland
[5] Celon Pharma S.A., Poland

E-mail address of presenting author: michal.kaza@celonpharma.com

Objectives: A novel, selective JAK/ROCK dual inhibitor CPL409116 successfully completed the preclinical program. The compound has been classified as a good clinical candidate for treatment of various inflammatory diseases [1], including rheumatoid arthritis or psoriasis. The aim of the study was to assess CPL409116 human pharmacokinetics after a single and multiple doses in healthy subjects.

Materials and methods: CPL409116 and its M3 metabolite concentrations in human plasma were measured by validated LC-MS/MS method at GLP-certified laboratory of Celon Pharma S.A. Non-compartmental model was selected to assess pharmacokinetics. In phase I clinical study ascending CPL409116 doses were studied in 21 healthy subjects after single dose and in 24 subjects after multiple doses.

Results: Blood sampling points defined in the clinical trial protocol enabled reliable assessment of PK profiles. Linear pharmacokinetics were observed in dosing range from 10 mg to 300 mg. Metabolite-to-parent ratio was constant across studied doses. Median T_{max} was dose independent. Due to short terminal elimination half-life, low potential for CPL409116 accumulation was observed.

Conclusions: Pharmacokinetic parameters were consistent across all parts of the first-in-human study in healthy subjects. Results of phase I clinical trial support further development of orally administered CPL409116.

Acknowledgements: This project was supported by European Funds under National Centre for Research and Development "Program Operacyjny Inteligentny Rozwój 2014–2020" (grant POIR.01.01.01-00-0382/16). The authors acknowledge Marta Rucińska and Justyna Czajkowska (Celon Pharma S.A., Research and Development Centre) for excellence in quality assurance monitoring.

Keywords: Inflammatory disease; clinical drug development; human pharmacokinetics

References:

1. Dulak-Lis M. et al. A novel JAK/ROCK inhibitor, CPL409116, demonstrates potent efficacy in the mouse model of systemic lupus erythematosus. Journal of Pharmacological Sciences 2021, 145(4), 340-348.

Abstract No. PPP.083

Utilizing isothermal spectral shift detection to quantify challenging biomolecular interactions with Monolith X

Andreas Langer[1], Tanja Bartoschik[1], Jakub Nowak[1], Natalia Kubisa[1], Claire Hatty[1]

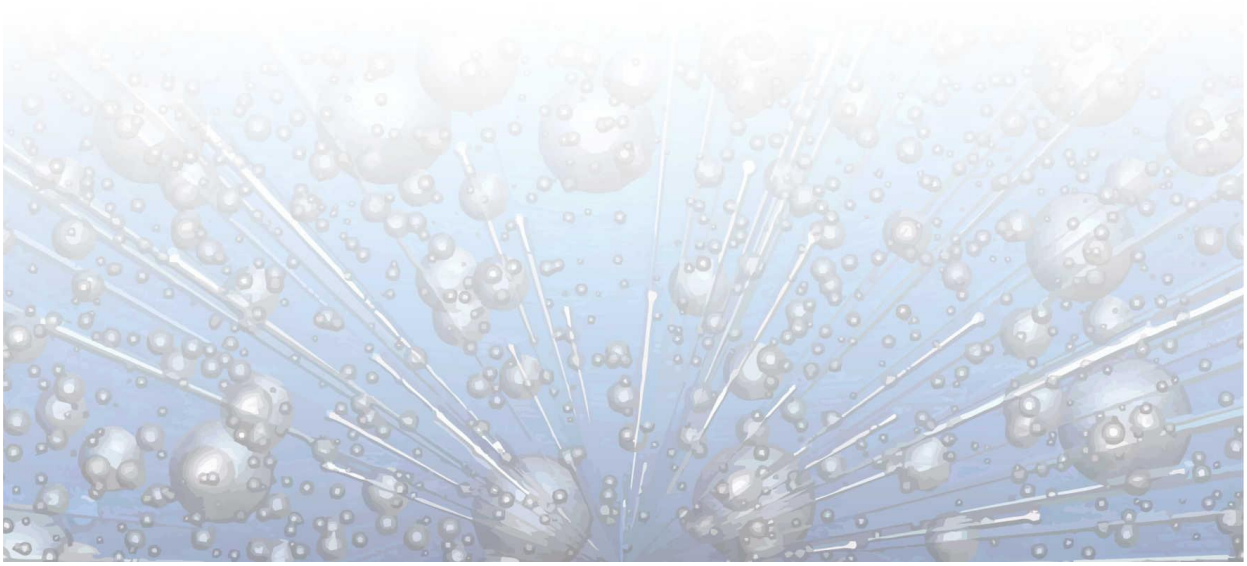
[1] NanoTemper Technologies, Germany

E-mail address of presenting author: natalia.kubisa@nanotempertech.com

Objectives: Monolith X is the latest addition to the Monolith product line, combining isothermal spectral shift detection with MST (MicroScale Thermophoresis) technology to characterize biomolecular interactions in solution. When a target is labelled with a fluorophore it generates a particular emission spectrum, and if a ligand binds to this labelled target, the fluorophore's chemical environment is changed, causing a shift in fluorescence spectra. Monolith X exploits this phenomenon by performing ratiometric measurements at two emission wavelengths of a labelled target in the presence of various concentrations of an unlabeled ligand to derive the affinity constant (Kd) for the interaction.

Materials and Methods: MicroScale Thermophoresis and Isothermal Spectral Shift were used to analyze interactions with membrane proteins, small molecules and antibodies.

Results and Conclusions: Isothermal spectral shift detection enables characterization of in solution interactions for a wide range of biomolecules, even for challenging samples such as membrane proteins, intrinsically disordered proteins, and cell lysates. Since the binding partners are in solution, there is no lost activity due to immobilization, and evaluation is size independent. Measurements can be performed in any buffer, including detergents, using low sample volumes and concentrations. The spectral shift analysis also facilitates the evaluation of competition assays and ternary binding events. Monolith X provides a valuable orthogonal method to validate your results from other biophysical methods and to characterize your most challenging interactions.



Abstract No. PPP.084

Tryptophan dietary supplements - release test, targeted and untargeted screening

Krzysztof Stępień[1], Joanna Giebułtowicz[1]

[1] Department of Bioanalysis and Drugs Analysis, Faculty of Pharmacy, Medical University of Warsaw, Poland

E-mail address of presenting author: krzysztof.stepien@wum.edu.pl

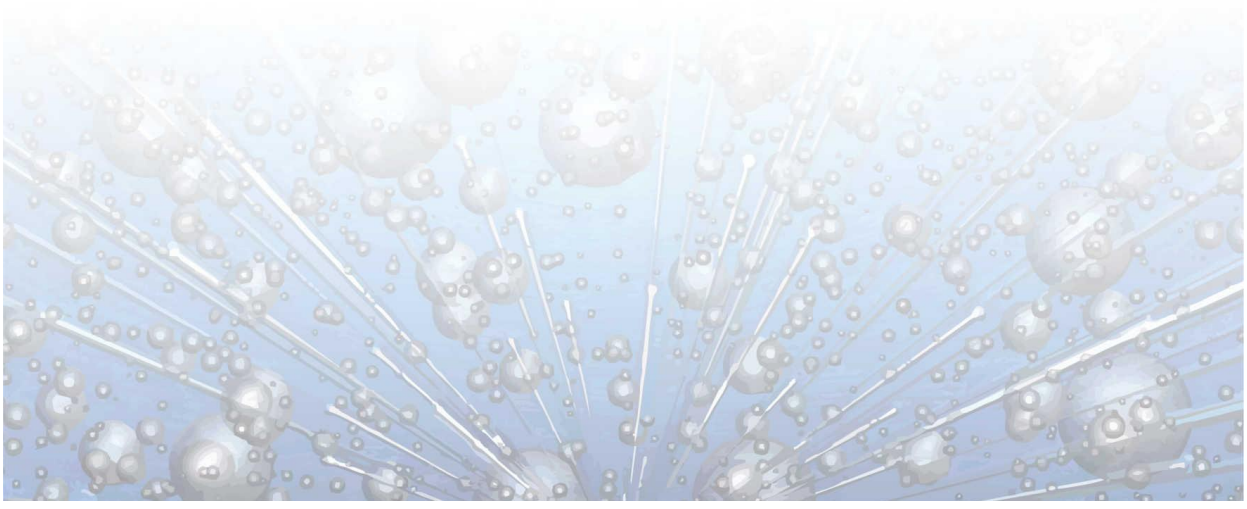
Introduction: Food analysis is essential to ensure food safety and quality. One of the least studied food categories is dietary supplements, widely consumed in the EU and USA. To fill this gap, the quality of dietary supplements containing tryptophan (Trp) was analyzed.

Materials and Methods: We examined 22 dietary supplements in the form of tablets or capsules, produced in the USA, Great Britain, Germany, France, Czech Republic, and Poland. Trp release, crucial for bioavailability and efficiency, was assessed. Additionally, we performed a qualitative analysis of the main ingredient and screening for contaminants, with the use of high-performance liquid chromatography coupled with mass spectrometry.

Results: Among the contaminants, we detected Trp's metabolites, condensation products of Trp and carbonyl compounds, Trp degradation products, degradation products of kynurenine, and other contaminants like glucosamine and melatonin. The main ingredient content was in the range of 55%-100% in capsules and 69%-87% in tablets. Very surprisingly, almost no Trp release was noted from some supplements.

Conclusions: The quality of supplements is lower than that of pharmaceuticals with lower than claimed amounts of the main ingredient and lack of uniform distribution between units. Sometimes, the release of the main ingredient is low, resulting in a lower probability of absorption and physiological effect. The study confirms issues with the quality of dietary supplements and provides an important contribution to the discussion on the regulation of dietary supplements.

Keywords: Tryptophan, dietary supplement, food supplement analysis



Abstract No. PPP.085

Theoretical insight into Loganic Acid Methyltransferase activity: a combined MD and QM study

Mateusz Jędrzejewski[1], Dariusz Maciej Pisklak[2], Łukasz Szeleszczuk[2]

[1] Department of Physical Chemistry, Faculty of Pharmacy, Medical University of Warsaw, Centre of New Technologies, University of Warsaw, Poland
[2] Department of Physical Chemistry, Medical University of Warsaw, Poland

E-mail address of presenting author: s078210@student.wum.edu.pl

Jędrzejewski Mateusz[1,2], Pisklak Dariusz Maciej[1], Szeleszczuk Łukasz[1]

[1] Faculty of Pharmacy, Medical University of Warsaw, Poland
[2] Centre of New Technologies, University of Warsaw, Poland

Objectives: Methyltransferases (MTs) are enzymes responsible for various biological processes, including biosynthesis, gene expression, and post-translational modifications. One type of MTs is carboxyl methyltransferases. This group of enzymes uses S-adenosylmethionine as a methyl donor and methylates the oxygen atom in the carboxylic acid. There is growing interest in using these MTs as biocatalysts in drug synthesis because they can replace toxic alkylating agents. To provide an insight into this matter, we studied the molecular details of the catalytic activity of Loganic Acid Methyltransferase (LAMT) - an enzyme involved in the biosynthesis of anticancer drugs, by catalyzing the methylation of loganic acid (LA).

Materials and Methods: Based on the crystal structure of LAMT, molecular dynamic simulations (MDs) were performed to analyze the conformational landscape of the enzyme. The selected snapshots were used to build cluster models of the active site of the enzyme. QM calculations with the DFT method were applied to analyze the energetics of methyl transfer.

Results: Molecular dynamics simulations were used to identify the substrate binding residues. In the crystal structure of LAMT, the side chain of GLN38 is not in hydrogen bonding distance from the carboxylate group, but in simulations, LA forms a hydrogen bond with this residue. This observation suggests a role of GLN38 in substrate binding, recognition and explains why the mutation of glutamine to alanine has a large effect on LAMT activity. Calculations also showed that TRP163 in the active site can form a hydrogen bond with LA directly or water-mediated. Obtained results from active site models were validated by experimental data from the kinetic study and mutational analysis.

Conclusions: Using a combined approach of MD simulation and QM calculations, LA-binding residue was identified and its role in the catalytic mechanism was revealed. Results show that the combined QM/MD approach allows getting deeper insight into the molecular mechanism of MT catalytic activity and could be applied to engineering novel MT enzymes.

Acknowledgments: This research was supported in part by PL-Grid Infrastructure.

Keywords: Methyltransferases, QM/MD, reaction pathway

Abstract No. PPP.086

Dissolution assessment of oral locally acting peptide drug product

Bartłomiej Milanowski[1], Małgorzata Sosnowska[1]

[1] GENERICA R&D Lab, Regionalne Centrum Zdrowia Sp. z o.o., Poland

E-mail address of presenting author: b.milanowski@rcz-zbaszyn.pl

Objectives: Linaclotide (LIN) is a 14-amino acid, guanylate cyclase C receptor agonist structurally related to the endogenous guanylin peptide family and was approved in 2012 to treat chronic idiopathic constipation and constipation-predominate irritable bowel syndrome in adults. *In vitro* digestion studies showed that, as a peptide, linaclotide was susceptible to degradation by intestinal fluids. Moreover, neither the parent drug nor its metabolite is detectable in blood plasma. One of the options for demonstrating bioequivalence (BE) of linaclotide generic drug products is based solely on comparative *in vitro* dissolution testing. Thus, the purpose of the study was to develop a validated RP-HPLC-UV method and conduct multi-point, multi-media dissolution testing using USP 1 and USP 4 apparatuses for reference listed drug product (Constella 290µg).

Materials and Methods: LIN (98.2% pure) and Constella 290µg hard gelatine capsules were purchased from Ontores Biotechnologies (Zhejiang, China) and a local pharmacy. Shimadzu LC-2030C Plus (Kyoto, Japan), equipped with a UV detector, was used for HPLC analysis. The chromatographic separation was performed on a Luna Omega Polar C18 1.6 µm 100Å, 50 × 2.1 mm column (Phenomenex Ins., CA, USA) with an injection volume of 100 µL. Dissolution testing of Constella 290 µg capsules was performed applying USP 1 and USP 4 apparatuses (both from SOTAX, Aesch, Switzerland) in a set of compendial (pH: 1.2, 2.0, 4.5 and 6.8) and biorelevant (SGFsp pH 1.2, FaSSGF pH 1.6, FaSSIF pH 6.5) media.

Results: Two RP-HPLC-UV methods were developed and validated: the isocratic method for linaclotide quantification in compendial dissolution media and the gradient method for assays in biorelevant media. The fastest dissolution kinetics of LIN was demonstrated in HCl solutions with a pH range of 1.2-2.0, slightly slower in 0.05M phosphate buffer pH 6.8, and the slowest in 0.05M acetate buffer pH 4.5 (f_2 < 50). We observed the similarity of LIN dissolution profiles (f_2 > 50) in biorelevant media (FaSSGF vs. FaSSIF).

Conclusions: Constella 290µg product exhibits very rapid (> 85% within 15 min using USP 1 apparatus) or similarly rapid (> 85% within 30 min using USP 4 apparatus) *in vitro* dissolution characteristics.

Acknowledgments: The authors are grateful for the financial support received from the Polish National Centre for Research and Development grant number POIR.01.02.00-00-0075/18.

Keywords: Linaclotide, HPLC, biorelevant dissolution

Abstract No. PPP.087

3D printing by selective laser sintering and characterization of composite highly drug-loaded floating tablets with insoluble matrix

Piotr Kulinowski[1], Piotr Malczewski[1], Marta Łaszcz[2], Ewelina Baran[1], Bartłomiej Milanowski[3], Mateusz Kuprianowicz[3], Przemysław Dorożyński[4]

[1] Institute of Technology, Pedagogical University of Cracow, Poland
[2] Department of Falsified Medicines and Medical Devices, National Medicines Institute, Poland
[3] GENERICA R&D Lab, Regionalne Centrum Zdrowia Sp. z o.o., Poland
[4] Department of Drug Technology and Pharmaceutical Biotechnology, Medical University of Warsaw, Poland

E-mail address of presenting author: b.milanowski@rcz-zbaszyn.pl

Objectives: 3D printing by selective laser sintering (SLS) of high-dose drug delivery systems using pure brittle crystalline active pharmaceutical ingredients (API) is possible but impractical. A composite material consisting of sintered polyamide (PA12) and metronidazole was applied to obtain printlets to overcome the issue.

Materials and Methods: The printlets were characterized using DSC and IR spectroscopy together with an assessment of their mechanical properties. Functional properties of the printlets were evaluated, i.e., drug dissolution in USP 3 and USP 4 apparatus, together with floatation assessment.

Results: The resulting printlets were good quality with an internal porous structure, which assured flotation. The thermal stability of the composite material was confirmed using DSC and IR. Elastic PA12 mesh maintained the shape and form of the printlets during drug release and floatation. Laser speed and the addition of an osmotic agent in low content influenced drug release changing release profiles virtually not changing the composition of the printlet.

Conclusions: Printlets were manufactured as a composite material consisting of elastic insoluble PA12 mesh filled with high content of crystalline metronidazole using additive manufacturing by SLS. The range of possible dissolution modifications was demonstrated. Obtained composite has potential for further development from a scientific and technical point of view.

Acknowledgments: The authors are grateful for the financial support received within the local project from the Pedagogical University of Cracow BN.610-432/PBU/2020 (P.K. & P.M.), National Science Centre Poland grant number UMO-2018/31/B/NZ7/03238 (P.D. & E.B.) and the Polish National Centre for Research and Development grant number POIR.04.01.04-00-0142/17 (M.K. & B.M).

Keywords: pharmaceutical additive manufacturing, powder bed fusion, 3D printing, personalized medicine, composite materials, selective laser sintering, nylon, polyamide 12, metronidazole, drug delivery, drug release, flotation

Abstract No. PPP.088

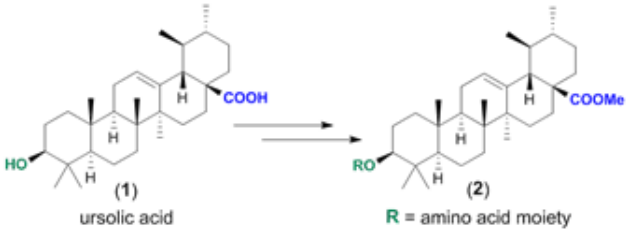
Synthesis and cytotoxicity of novel ursolic acid derivatives containing an amino acid moiety

Olga Michalak[1], Piotr Krzeczyński[1], Wojciech Szymanowski[2], Marek Kubiszewski[3], Marcin Cybulski[1], Krzysztof Bielawski[2], Anna Bielawska[2]

[1] Department of Pharmacy, Cosmetic Chemistry and Biotechnology, Łukasiewicz Research Network–Industrial Chemistry Institute, Poland
[2] Department of Biotechnology, Medical University of Bialystok, Poland
[3] Analytical Department, Łukasiewicz Research Network–Industrial Chemistry Institute, Poland

E-mail address of presenting author: olga.michalak@ichp.pl

Objectives: Ursolic acid is a pentacyclic triterpenoid present in plants, vegetables and fruits. This compound has been found to have some antitumor and chemopreventive activities in breast cancer models [1]. In order to improve the preexisting antitumor activity of ursolic acid (**1**), a series of novel ursolic acid derivatives with amino acid moieties (**2**) were obtained and their antitumor activity was evaluated *in vitro* against selected breast cancer cell lines.



Methods: Synthesis of the target compounds involved the esterification (DCC, DMAP, DCM, rt) of (**1**) with corresponding *N–t*–butoxycarbonyl amino acid. Then, Boc group was removed (HCl_{gas}/AcOEt. Dipeptide derivatives were synthesized using the "step by step" method. The cytotoxicity of all compounds was determined by the MTT assay. To prove that all tested compounds have antiproliferative activity, we measured DNA synthesis by the inhibition of [^3H]–thymidine incorporation in tested breast cancer cells.

Results and Discussion: Highest cytotoxic activity *in vitro* against MDA–MB–231 cells (IC_{50} 15 μM, 14 μM, 17 μM), was found for **Ser–URS–OMe**, **Pro–URS–OMe** and **Ala–Ile–URS–OMe**. The activity against MCF–7 cells was lower (32, 42 and 50 μM respectively). **Ser–URS–OMe**, **Pro–URS–OMe** and **Ala–Ile–URS–OMe** were also found to have a higher than reference ursolic acid ability to inhibit DNA biosynthesis in MDA–MB–231 breast cancer cells. IC_{50} for MDA–MB–231 cells were 17.34, 15.5 and 14.9 μM, respectively (**URS**: 32.5 μM). In the case of MCF–7 cells, compounds **Ser-URS-OMe**, **His-URS-OMe** and **Pro-URS-OMe** were found to have a higher antiproliferative potential compared to ursolic acid. IC_{50} found to be: 32.46, 41.89, and 40.2 μM, respectively (**URS**: 55.0 μM).

Conclusion: All analogues have been evaluated *in vitro* for their antiproliferative profile against cancer cell lines (MCF–7, MDA–MB–231). The IC_{50} values indicate that the MDA–MB–231 breast cancer cells are slightly more sensitive to the tested compounds than the MCF–7 cells. Analogue **Ser–URS–OMe** and **Pro–URS–OME** show the highest cytotoxic activity towards MCF–7 and MDA-MB–231 cells.

Acknowledgements: This work was supported under the framework of a statutory project of the Łukasiewicz–Industrial Chemistry Institute (№ 841333) funded by the Polish Ministry of Science and Higher Education.

Keywords: ursolic acid, cancer, anti–tumor activity.

References:

[1] A. Bishayee et al, *Front Biosci*, **2011**, 16, 980–996.

Abstract No. PPP.089

Multicomponent new crystals of erlotinib - structural and pharmaceutical studies

Cristina Martin Suarez[1], Izabela Domańska[2], Izabela Madura[3], Edyta Pindelska[2]

[1] Pharmacy, Universidad de Alcala (UAH), Spain
[2] Analytical Chemistry and Biomaterials, Medical University of Warsaw, Poland
[3] Faculty of Chemistry, Warsaw University of Technology , Poland

E-mail address of presenting author: crimarsu98@gmail.com

Objectives: Erlotinib (ETB) is the drug used to treat non-small cell lung cancer, and several other manifestations of the disease including pancreatic cancer. It is classified as class II drugs in the Biopharmaceutical Classification System (BCS), which characterized by low solubility and high permeability. Even the marketed hydrochloride salt, the most stable form B exhibits solubility only up to 0.4 mg L −1 in pH ≃ 2 medium. Therefore, it is justified to search for and research new salts or cocrystals of ETB. In this study we present a method of a synthesis of new multicomponent crystals of ETB and their structural and pharmaceutical studies. Due to the pending patent procedures, we are not able to disclose any more information on the ingredients used.

Materials and Methods: The liquid assisted milling method has been used successfully to obtain new crystals of ETB. Fourier transformed infrared spectroscopy (FT-IR) and powder X-ray diffraction (PXRD) were used to provide information about the formation of salts. Single crystals were obtained from recrystallization from MeOH solvent. The crystal structure of the new obtained crystals was determined by single crystal X-ray studies. Structural studies were supported by solubility tests.

Results: PXRD patterns and FT-IR spectra of the FEB crystals are evidently different from that of starting materials. Changes of the positions, intensity of the peaks in the PXRD patterns indicate that these were not just ordinary physical mixtures. The new multicomponent crystals successfully formed. Different hydrogen bond interactions in the new obtained crystals result in changes in the FT-IR spectra. The water solubility and dissolution rate of ETB compound were measured using a ultraviolet-visible spectroscopy and high-performance liquid chromatography.

Conclusions: Solubility and dissolution research show that newly obtained multicomponent crystal of ETB exhibited almost three times higher solubility and dissolution rate than the free base of ETB.

Acknowledgments: These studies were financially supported by Medical University of Warsaw, grant number FW231/2/F/GW/N/21.

Keywords: : erlotynib, solubility, dissolution rate, crystal structure

Abstract No. PPP.090

DNA Encoded Libraries at Ryvu – preliminary design

Marcin Król[1], Krzysztof Baczyński[1], Iwona Mames[1], Adrian Zarębski[2], Aleksandra Sabiniarz[1], Maja Potocki[1]

[1] Chemistry, Ryvu Therapeutics, Poland
[2] Biology, Ryvu Therapeutics, Poland
E-mail address of presenting author: maja.potocki@ryvu.com

DNA Encoded Libraries at Ryvu – design and setup
Maja Potocki*, Krzysztof Baczyński, Marcin Król, Iwona Mames, Adrian Zarębski, Aleksandra Sabiniarz
Ryvu Therapeutics, R&D Center for Innovative Drugs
Leona Henryka Sternbacha 2, 30-394 Kraków, Poland

Objectives: Hit identification remains one of the major challenges in drug discovery. Broadening the scope of targetable proteins beyond enzymes and GPCRs, traditional drug discovery targets, requires novel techniques for hit finding. The well-established source of bioactive chemical matter, high-throughput screening (HTS) is limited in size and contains only a minute fraction of the available chemical space.

In the current work we present design and implementation of a novel hit finding technique - DNA Encoded Libraries (DELs). DELs are characterized by potentially higher efficiency of hit finding where all library members are simultaneously screened against a target of interest. The aim of the current work is to perform a feasibility study of DEL application for hit finding campaigns in Ryvu

Materials and Methods: Virtual DEL libraries were prepared and analysed using rdkit and scikit-learn libraries. Dask was used for code parallelization. Database and screen-related data were designed in PostgreSQL and implemented on a dedicated Linux Container LXC. Overlap of chemical space was visualized using in-house python scripts and dimensionality reduction was performed using t-distributed stochastic embedding algorithm.

Results: In the current presentation we will discuss early assessment of DEL design and implementation at Ryvu, with the comparison of chemical space covered by Ryvu HTS libraries and proposed DELs. We will show optimization of DELs in the physicochemical space and briefly touch on the design of databases and computational analytical tools to mine DEL generated data.

Conclusions: DNA Encoded Libraries may become an integral tool for hit identification in Ryvu drug discovery projects that may complement standard HTS small molecule campaigns. DELs are amenable for sophisticated improvement and analysis strategies using cheminformatics approaches.

Keywords: DNA-encoded libraries, hit identification, cheminformatics

Abstract No. PPP.091

Synthesis and physicochemical characterization of an Mesoporous Silica material/Hydroxyapatite/Natural Polymer composite for potential use like drug delivery system

Anna Żychowska[1], Łukasz Pajchel[1]

[1] Faculty of Pharmacy, Medical University of Warsaw, Poland

E-mail address of presenting author: lukasz.pajchel@wum.edu.pl

Synthesis and physicochemical characterization of an Mesoporous Silica material/Hydroxyapatite/Natural Polymer composite for potential use like drug delivery system
A. Żychowska[1], Ł. Pajchel[1],
1. *Medical University of Warsaw, Faculty of Pharmacy, Chair of Analytical Chemistry and Biomaterials, Warsaw, Poland*

Objectives: Hydroxyapatite are similar to natural bone, mesoporous silica material have 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 ensures the controlled release of a variety of drug molecules, so they are very useful for bone tissue regeneration. Natural polymers show high biocompatibility. Composites containing this three biomaterials most likely will have better properties, so in this study they were synthesized.

Materials and Methods: Composites were synthesized with three silica mesoporous materials (MCM-41, MCM-48, SBA-15) previously synthesized, hydroxyapatite and two natural polymers: alginate and gelatin (figure 1). Drug was added using two different ways: soaking the composites in a solution of the ciprofloxacin antibiotic and adding the antibiotic directly to composites. The obtained products were examined using various analytical methods: powder X-ray diffractometry (PXRD), infrared spectroscopy (IR) and transmission electron microscopy (TEM). The previously prepared composites were subjected to the drug release studies in phosphate buffer (pH = 7.4). After fixed periods of time part of the solution was collected for ciprofloxacin hydrochloride analysis on high-performance liquid chromatography (HPLC).

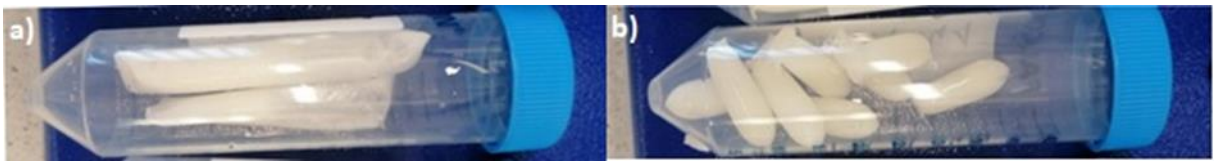


Figure 1. Composites with alginate (a) and gelatin (b).

Results: Successfully synthesized composite containing three biomaterials and they showed very good properties. They are analyzed on high performance liquid chromatography (HPLC) and the results have been interpreted.

Conclusions: This study shown that composites especially with silica mesoporous materials increase drug release and they are very useful as implants for bone tissue regeneration.

Keywords: biomaterials, drug release, silica mesoporous materials

Abstract No. PPP.092

Analytical characterization of oligonucleotides by Liquid Chromatography - (LC) High Resolution Mass Spectrometry (HRMS) and State-of-the-Art analytical software

Brill Laurence M.[1], Nelson Lorne[1], Veale Lawrie[2], Maria Basanta-Sanchez[2], Brian Rivera[1], Aleksandra Pietracho[1]

[1] Phenomenex, Inc., United States
[2] Protein Metrics Inc, United States

E-mail address of presenting author: jakubm@phenomenex.com

Objectives: The primary method for quantitation and characterization of synthetic oligonucleotides is liquid chromatography-mass spectrometry (LC-MS). However, because of their inherent complexity, high charge state envelopes, and cation adduct formation, MS1 spectra are typically difficult to interpret. Further complicating spectral elucidation are the variety and complexity of product and process related impurities, which may lead to spectral overlap.

Here we demonstrate the use of HRMS and Protein Metrics Inc. Byos® Software to identify process and product related impurities with three different therapeutic synthetic oligonucleotide platforms: Nusinersen, an anti-sense oligonucleotide (ASO), Patisiran, a duplex RNA, and Givosiran, a GalNAc conjugated RNA duplex with hairpin loop.

Materials and methods: Oligonucleotides were purchased from Integrated DNA Technologies (Coralville, IA). LC-HRMS was performed using BioZen 2.6 µm Oligo column (Phenomenex, US), a Thermo Vanquish™ Flex with detection using an Orbitrap Q-Exactive Plus (Waltham, MA). Raw m/z data was deconvolved to neutral masses and matched to Oligo components using Protein Metrics, Inc. Byos® software (Cupertino, CA).

Results: Sets of total ion chromatograms.

Conclusions: Ion pair reversed phase LC-MS analysis of oligonucleotides presents unique challenges. By leveraging HRMS, along with software using a parsimonious deconvolution algorithm, high quality results may be obtained to better characterize impurities that are related to synthetic oligonucleotides. This is particularly important for therapeutic oligonucleotide platforms, which may be especially difficult because of the inherent complexity due to various chemical modifications.

Keywords: Biopharmaceuticals, Novel bioanalytical methods

Abstract No. PPP.093

Development of a Workflow for Deep Proteome Profiling in Human Plasma by Micro-LC-MS/MS

Roxana Eggleston-Rangel[1], Jason Anspach[1], Jennifer E. Van Eyk[2], Simion Kreimer[2], Angela Mc Ardle[2], Aleksandra Binek[2], Alejandro Rivas[2], Danica Manalo[2], Connor Phebus[2], Blandine Chazarin[2], Annie Moradian[2], Cory Bystrom[2], Aleksandra Pietracho[1]

[1] Phenomenex, Inc., United States
[2] Cedars Sinai, United States

E-mail address of presenting author: jakubm@phenomenex.com

Objectives: Blood and plasma are complex high-dynamic range mixture of proteins which presents an especially difficult challenge to proteomics. The mixtures are mostly composed of high abundance proteins such as albumin and immunoglobulins which obscure detection and quantitation of biologically significant but low abundance proteins such as those leaked from tissue. The ability to robustly and reproducibly quantify hundreds of proteins during a single LC-MS/MS analysis in complex biological samples provides an opportunity to discover novel biological markers and therapeutic targets and provides invaluable insight into human biology. Thus reproducibility, accuracy and precision are key aspects to be considered when developing proteomic assays in order to deliver reliable information regarding the human proteome and to increase our understanding of it. An important aspect of the LC-MS/MS assay is the HPLC column, which should be carefully chosen to maximize assay performance and reliability. This study presents a workflow for deep proteome profiling in human plasma which implements a Phenomenex Luna® Omega Polar C18 as the column of choice while addressing its lifetime, performance and reproducibility.

Materials and methods: Human blood samples were obtained and processed at Cedars-Sinai Medical Center, Smidt Heart Institute, CSMC. High select TOP14 depletion slurry was purchased from Thermo Scientific®, and all other chemicals were obtained from Sigma-Aldrich Company® (St. Louis, MO, USA).

Results: Total ion chromatograms for each sample type. Total number of proteins and peptides identified per sample type. The total number of proteins quantified were 504, 473, 578 in plasma, depleted plasma and blood, respectively. Total number of peptides quantified were 2378, 2029, 1622 in plasma, depleted plasma and blood, respectively.

Conclusions: The Luna Omega Polar Micro LC column of choice delivered appropriate peak widths, selectivity, and a good column lifetime to support the quantification of proteins with median intra-day coefficients of variations (CVs) ranging from 10- 13 %, 4-8 % and 10-13 % while inter-day CVs were 24%, 15 % and 18% in plasma, depleted plasma and blood, respectively.

Keywords: Biopharmaceuticals, Novel bioanalytical methods, Omic methods

Abstract No. PPP.094

Supramolecular ribbon-like ligands as drug delivery systems for BMS-354825 to bladder cancer cells of T24 line

Anna Jagusiak[1], Małgorzata Lasota[1], Daniel Jankowski[1]

[1] Chair of Medical Biochemistry, Jagiellonian University Medical College, Poland

E-mail address of presenting author: anna.jagusiak@uj.edu.pl

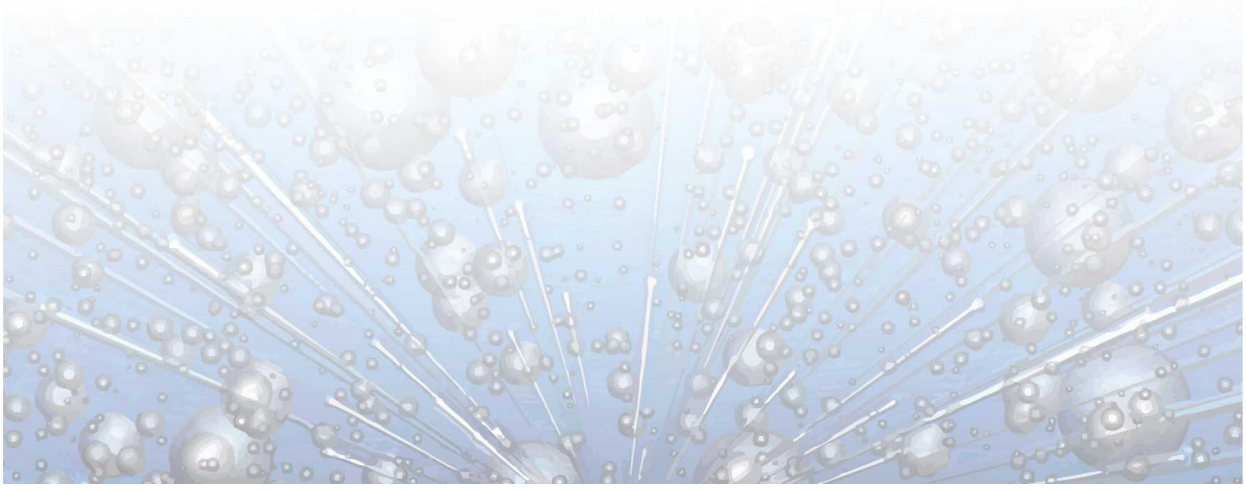
Introduction: Supramolecular chemistry is a new field of knowledge and supramolecular compounds with ribbon-like shapes are an interesting phenomenon. The ribbon-like structure of supramolecular ligands (on example of Congo red, CR) allows for intercalation of foreign particles, including drugs. This phenomenon is particularly interesting due to the selective binding of these ligands to antibodies, but only those engaged in the antigen-antibody complexes (with no corresponding binding to free antibodies). Therefore, it seems likely that supramolecular ligands may be used in immunotargeting.

Objectives: Description of the properties of complexes formed by CR with BMS and its effect on cells of the bladder tumor line T24.

Methods: electrophoresis, UV/VIS spectrophotometry, cell cultures: assessment of proliferation (MTS method), determination of cytostatic doses, assessment of the induction of apoptosis (FACS).

Results: The formation of complexes between CR and BMS was confirmed. The system inhibits the growth of T24 cells dose-dependently. Cytostatic doses were determined. CR-BMS complex induces apoptosis. The presented system offers a wide range of biomedical applications including drugs delivery to cancer cells. As carriers of drugs, these complexes present an interesting alternative to the currently used systems.

Acknowledgments: We acknowledge the financial support from the National Science Centre, Poland (grant no. 2016/21/D/NZ1/02763), the international project (no. U1C/P03/NO/03.23) in the strategic program Initiative of Excellence at the Jagiellonian University and the Polish Ministry of Science and Higher Education (grant no. N41/DBS/000715).



Abstract No. PPP.095

Deciphering the underlying causes of ineffective therapy with increased rivaroxaban doses using untargeted metabolomics

Sylwia Michorowska[1], Natalia Korytowska[1], Roman Piotrowski[2], Joanna Giebułtowicz[1]

[1] Department of Bioanalysis and Drugs Analysis , Medical University of Warsaw, Poland
[2] Department of Cardiology, Postgraduate Medical School, Grochowski Hospital, Poland

E-mail address of presenting author: ssolobodowska@wum.edu.pl

Objectives: Anticoagulants are invaluable treatment strategy used in controlling thromboembolic complications associated with the most common arrhythmia, atrial fibrillation (AF). Nowadays, vitamin K and low molecular weight heparins are being successively replaced with novel oral anticoagulants such as rivaroxaban, which improve both, life expectancy and life quality. Based on the results collected in ROCKET-AF study once-daily rivaroxaban 20 mg was approved for the reduction of the risk of stroke and systematic embolism in patients suffering from nonvalvular AF by European Medicines Agency and Food and Drug Administration. However, in some patients standard rivaroxaban treatment was shown to be ineffective, despite excellent compliance. Subsequent RIVA-TWICE study showed complete resolution of thrombus in the left atrial appendage (LLA) in some patients treated with 15 mg twice daily. Intriguingly 53.5% of patients did not respond to this modified dosage regimen, despite having therapeutic rivaroxaban plasma concentrations. Statistically significant differences in anti-Xa activity were excluded as a potential explanation. In pursuance of identifying the underlying cause of this observation metabolic profiles of responders and non-responders to increased rivaroxaban dose were compared using untargeted metabolomics.

Materials and Methods: Plasma was collected from 15 AF-patients with thrombus in LLA not responding to once-daily rivaroxaban 20 mg. Extracted metabolites were analyzed using LC-MS analysis employing Orbitrap Focus. Raw data were processed in Compound Discoverer 2.1. Next, Metaboanalyst 4.0 was used to perform statistical analysis.

Results: 33 metabolites were found to be differentially abundant in patients who responded to modified dosage regimen as compared to non-responsive patients. Identified metabolites are involved in alpha-linoleic acid pathway, fatty acid metabolism (acylcarnitines), carnitine synthesis, as well as degradation of proteins.

Conclusions: Effectiveness of rivaroxaban treatment may be affected by the levels of acylcarnitines, carnitine and its precursors. These metabolites may be thus used in the future as prognostic markers of the therapeutic effect of rivaroxaban treatment in AF. Additional quantitative data are needed to support these preliminary findings.

Acknowledgments: This work was supported by Medical University of Warsaw (**FW27/1/F/GW/N/20).**

Keywords: atrial fibrillation, rivaroxaban, untargeted metabolomics

Abstract No. PPP.096

Development and validation of analytical methods for determination of bromhexine hydrochloride and related impurities in novel orodispersible tablets

Mirosław Strózik[1], Magdalena Strzebońska[2], Katarzyna Skiba[1], Wiktor Tatara[1]

[1] F1 Pharma S.A., Poland
[2] Department of Environmental Protection; Faculty of Geology, Geophysics and Environmental Protection, AGH University of Science and Technology, Poland

E-mail address of presenting author: miroslaw.strozik@f1pharma.pl

Objectives: Bromhexine hydrochloride (BRX) is applied as an active pharmaceutical ingredient (API) of oral solutions and tablets for the treatment of the infections of the respiratory tract. (3*RS*)-6,8-dibromo-3-cyclohexyl-3-methyl-1,2,3,4-tetrahydroquinazolin-3-ium specified as impurity E by European Pharmacopeia, represents the recent addition to the well established set of impurities of BRX (A-D) described in the monograph. The presence of impurity E in pharmaceutical formulations may be a challenge to fulfill present regulations. The novel, containing low level of impurity E, BRX orodispersible tablets (ODT) with improved chemical stability were developed.

Materials and Methods: BRX working standard, Impurity A, Impurity B, Impurity C, were purchased from Ven Petrochem & Pharma Ltd., Impurity E, Bromhexine N-Oxide from LGC and BRX CRS standard from EP. ODT tablets containing 4 mg and 8 mg of BRX were developed by F1 Pharma.

The high performance liquid chromatography (HPLC) analysis for the determination of BRX was performed on Hitachi Primaide MERCK and Hitachi Elite LaChrom MERCK liquid chromatographs with DAD z detector.

Results: Stability of the product was tested in accelerated, intermediate and long-term conditions for ICH zone II and III (ACC, INT, LT). The degradant E was the most common impurity detected. In the samples stored in INT and LT conditions only a slight increase in impurities and a slight drop in the assay of the BRX was observed, however the results did not exceed the pre-established acceptance criteria. In the samples stored at ACC conditions, an increase of known impurities (mainly E, followed by B and N-oxide) was noted. Noteworthily, even after three years shelf-life of the product the level of the degradant E is still well below 0.2 % which corresponds to the ICH identification threshold for the BRX related impurities.

Conclusions: The novel analytical methods for determination of BRX and related substances in ODT tablets containing 4 mg and 8 mg of BRX were developed and validated. The methods were found to be specific, precise, accurate, linear and robust. Therefore they are suitable for testing the assay and purity of the drug product. Moreover, the conditions favoring the impurity E increase have been examined and identified.

Acknowledgements: The study was financially supported from the Intelligent Development Operational Program from the EU, Grant No POIR 01.01.01-00-0469/17-00.

Abstract No. PPP.097

Use of liquid chromatography associated with mass spectrometry (LC-MS/MS) to determine the composition of illegal pharmaceutical products containing anabolic-androgenic steroids

Agnieszka Kalicka[1], Krzysztof Stępień[1], Joanna Giebułtowicz[1], Zbigniew Fijałek[2]

[1] Department of Drug Analysis, Medical University of Warsaw, Poland
[2] Department of Forensic Pharmacy, Medical University of Warsaw, Poland

E-mail address of presenting author: agnieszka.kalicka@wum.edu.pl

Introduction: Illegal drugs that contain anabolic-androgenic steroids are often falsified. Preparations from illegal sources are not manufactured by the principles of Good Manufacturing Practice (GMP). Very often their actual composition differs significantly from that declared on the packaging. The presence of undeclared active substances and their analogs, impurities, and incorrect dosing can lead to serious side effects that threaten the life or health of patients. Very rarely, such illegal products are analyzed for the presence of ingredients not declared on the package.

Objectives: The aim of the study is an attempt to identify undeclared components (including impurities) in falsified medicinal products containing anabolic-androgenic steroids.

Materials and methods: The study material consisted of medicinal products from various law enforcement cases, including those manufactured in three illegal factories dismantled in Poland (in Koronowo, Kościan, and Brodnica). The pharmaceuticals studied were anabolic androgenic steroids containing stanozolol, testosterone propionate, testosterone enanthate, and methandienone. The scope of the study included a qualitative analysis of medicinal products for the content of active substances and potential impurities using LC-MS/MS method.

Results: In most cases, LC-MS / MS analyzes showed the poor quality of the counterfeit products. The samples contained many ingredients in addition to what was declared on the package. Some of them were present as impurities (e.g. dehyrochloromethylotestosterone or PEG) others, such as maltose, accounted for a significant percentage of the composition of the samples, indicating that they were likely to have been intentionally added to the products.

Conclusion: The LC-MS/MS method seems to be a good tool for the analysis of the composition of illegal pharmaceuticals containing anabolic-androgenic steroids. PCA analysis shows a clear grouping of samples retained in the same place, which proves that chemometric techniques can be an effective tool for creating a method for determining the place of a manufactured product based on its composition.

Funding: This research was funded by Medical University of Warsaw, grant number FW27/1/F/MBS/N/21.

Keywords: illegal pharmaceutical products, LC-MS/MS, anabolic-androgenic steroids

Abstract No. PPP.098

In vitro studies on 4-borono-L-phenylalanine uptake in non-small cell lung cancer and normal lung cells

Emilia Balcer[1], Joanna Giebułtowicz[1], Małgorzata Sochacka[1], Anna Ruszczyńska[2], Magdalena Muszyńska[3], Ewa Bulska[2]

[1] Department of Bioanalysis and Drug Analysis, Medical University of Warsaw, Poland
[2] Faculty of Chemistry, University of Warsaw, Poland
[3] Pro-Environment Polska Sp. z o.o., Poland

E-mail address of presenting author: ebalcer@wum.edu.pl

Objectives: 4-borono-L-phenylalanine (BPA) enriched with 10B isotope is currently one of the three clinically used compounds for boron-neutron capture therapy (BNCT), a type of cancer treatment that utilizes a nuclear reaction occurring between 10B isotope and thermal neutrons from the external beam. There is a great potential of BNCT application in different types of cancers, including lung cancer. Aim of this study was to investigate if BPA uptake in cells can be increased upon pre-loading with amino acid like phenylalanine and to determine how the uptake is influenced by the exposure length. BPA uptake was assessed in tumour (A549) and normal (V79) cell lines pre-treated with L-phenylalanine. A new method for boron concentration measurement was also explored with the use of single-cell inductively coupled plasma mass spectrometry (SC-ICP-MS).

Materials and methods: Boron uptake was evaluated in tumour and normal cell lines: human non-small cell lung cancer (A549) and Chinese hamster lung fibroblast (V79). Boron was delivered in the form of a BPA complex with D-fructose, with a concentration of 2 mM. The influence of several times of exposure ranging from 1 to 12 hours and the effect of pre-loading with L-phenylalanine were investigated. Boron concentration was measured using the inductively coupled plasma mass spectrometry (ICP-MS) and the initial tests with SC-ICP-MS were performed in order to establish the method for boron analysis.

Conclusions: The experiments have shown that the short term exposure within the range of 2-3 hours increased boron concentration in cells, although the correlations are yet to be determined. In the measurements involving L-phenylalanine pre-loading, an indirect influence of pre-treatment was observed. More experiments have to be conducted in order to formulate conclusive results. A new method for the assessment of boron uptake in cells was successfully established with the use of SC-ICP-MS.

Acknowledgements: The contribution Emilia Balcer has been done in the frame of the National Centre for Research and Development Project No POWR.03.02.00- 00-I009/17 (Radiopharmaceuticals for molecularly targeted diagnosis and therapy, RadFarm, Operational Project Knowledge Education Development 2014–2020 co-financed by European Social Fund).

Abstract No. PPP.099

Synthesis and physicochemical characterization of an SBA-16/HA composite for potential drug delivery

Maria Atienza Moratalla[1], Łukasz Pajchel[2]

[1] Faculty of Pharmacy,, Universidad San Jorge, Zaragoza,, Spain
[2] Department of Analytical Chemistry and Biomaterials, Faculty of Pharmacy, Medical University of Warsaw, Poland

E-mail address of presenting author: lukasz.pajchel@wum.edu.pl

Objectives: The main objective is the synthesis and physicochemical characterization of an SBA-16/HA composite for potential drug delivery. Synthesis of SBA-16, HA and SBA-16/HA composite has been optimized by different methods. Test the dissolution profile of the Ibuprofen in the composite obtained.

Materials and Methods: Synthesis of the SBA-16/HA composite by 2 different methods:

1st) First, reparation of the Ca-doped silica matrix, followed by the HA crystallization within the Ca-doped matrix.

2nd) Involves the precipitation of HA on the previously synthesized SBA-16. The sieve was synthesized from the templates: F127, P123, CTAB, BuOH and TEOS. The obtained composites have been characterized using transmission electron microscopy (TEM) and infrared (FT-IR).

Results: Fig.1 shows representative TEM images of the crystal and pore sizes, that are in line with the overviewed literature . FT-IR shows that the reaction has been obtained successfully and that the required compound has been characterised. The dissolution profile of the Ibuprofen is tested in the composites obtained.

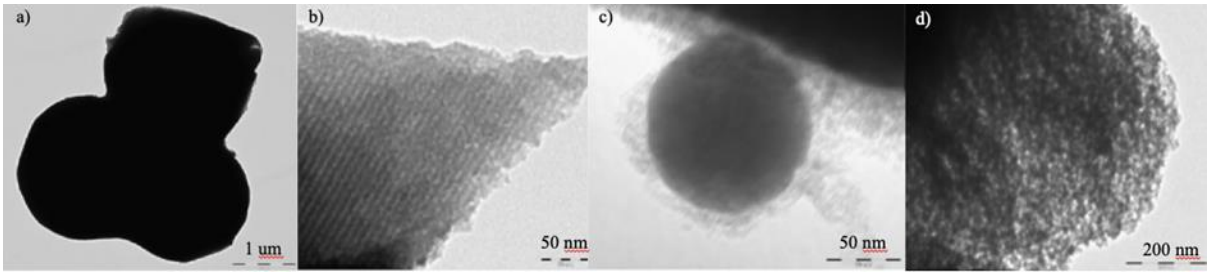


Fig 1. TEM pictures: a) Crystal size SBA-16, b) Pore size SBA-16, c) Shape of SBA-16/HA, d) Crystal size of SBA-16/HA

Conclusions: Testing and description through different methods to obtain SBA-16 has been performed. The synthesis of SBA-16/HA composite has been optimized using two step method. The compounds obtained by optimizing the reactions described in the literature have been correctly characterized for use in future research. The dissolution profile of Ibuprofen complies with the appropriate kinetics for the drug delivery system.

Abstract No. PPP.100

Structural studies of constitutional isomers of new arylpiperazine ligands for serotonin receptors

Izabela Madura[1], Edyta Pindelska[2], Mateusz Mogilnicki[2], Jolanta Jaśkowska[3]

[1] Faculty of Chemistry, Warsaw Univeristy of Technology, Poland
[2] Faculty of Pharmacy, Medical University of Warsaw, Poland
[3] Faculty of Chemical and Engineering and Technology, Cracow University of Technology, Poland

E-mail address of presenting author: izabela.madura@pw.edu.pl

Objectives: The purpose of this contribution was to confirm the molecular structure and the purity of novel serotonin ligands, namely three constitutional isomers (ortho, meta and para) of 3-[4-(2-methoxyphenyl)piperazin-1-yl]propoxy}benzamide, and to discuss the structural differences introduced by a varied placement of the functional group in the context of ligands' biological activity.

Materials and Methods: The compounds were investigated by combined techniques including ^{1}H and ^{13}C NMR spectroscopy in solution and the solid state, supported by *ab initio* and periodic calculations as well as X-ray diffraction methods. The crystal structures were determined from single-crystal X-ray measurements and analyzed with the aid of weak interactions energy calculations based on the molecular Hirshfeld surface analysis.

Results: The presence of various hydrogen bonds and other weak intermolecular interactions in the crystal lattice helped to the differences in chemical shifts in the ^{13}C and ^{15}N CP/MAS NMR spectra of the common fragments in all three isomers. Among the arylpiperazine derivatives tested, the highest affinity for the 5-HT1A/5-HT7 receptors is exhibited by the ortho isomer.

Conclusions: Based on the above studies, we concluded that constitutional isomerism has a big influence not only on the crystal structure but also on the electronic structure of the molecule, and in consequence on its bioactivity. Future studies concerning modifications of the crystal structure (e.g. by co-crystallization or salt formation) will focus mostly on ortho isomer.

Acknowledgements: IDM is grateful to the Scientific Council of Chemical Sciences at Warsaw University of Technology for the NChem3_2022 grant.

Keywords: 5-HT1A/5-HT7 receptor ligands, arylpiperazine derivatives, structural research

Abstract No. PPP.101

Synthesis of the modified biphasic calcium phosphate granules with antibacterial properties for potential use in bone repair surgery

Kamil Pajor[1], Łukasz Pajchel[1], Anna Zgadzaj[2], Joanna Kolmas[1]

[1] Department of Analytical Chemistry, Medical University of Warsaw, Poland
[2] Department of Environmental Health Sciences, Medical University of Warsaw, Poland

E-mail address of presenting author: kpajor@wum.edu.pl

Objectives: The risk of surgical site infection (SSI) during orthopedic surgery is one of the problems of modern medicine and direct delivery of antibiotic into targeted place seems to be its optimal solution. Calcium phosphates (CaPs), because of their similarity to inorganic bone tissue and easy absorption of many drug substances, can be used as antibiotic carriers in mentioned problem. In the following study biphasic granules composed of combination of different CaPs were synthesized. CaPs were doped with gallium or silver ions, which exhibit antibacterial properties.

Materials and Methods: 7 types of CaPs were synthesized using standard wet method with different molar ratios of reagents, different pH of reaction mixture and in some cases sintering precipitate in high temperature. 7 types of granules, composed of obtained CaPs, were prepared using alginate cross-linking method. Then, the granules were modified with ciprofloxacin and covered with polycaprolactone (PCL). The materials were subjected to physicochemical and biological studies.

Results: FT-IR and PXRD studies allowed to confirm identity of the synthesized powders. SEM images clearly present porous feature of the above-mentioned materials [Fig.1]. In the cross-section images numerous mesopores and macropores may be noticed, what can indicate a high porosity of the granules. The study of antibacterial properties of granules on two bacteria strains – E. coli and S. aureus – demonstrated significant inhibition of bacterial growth for granules without ciprofloxacin and total inhibition of bacterial growth in most cases for granules containing ciprofloxacin.

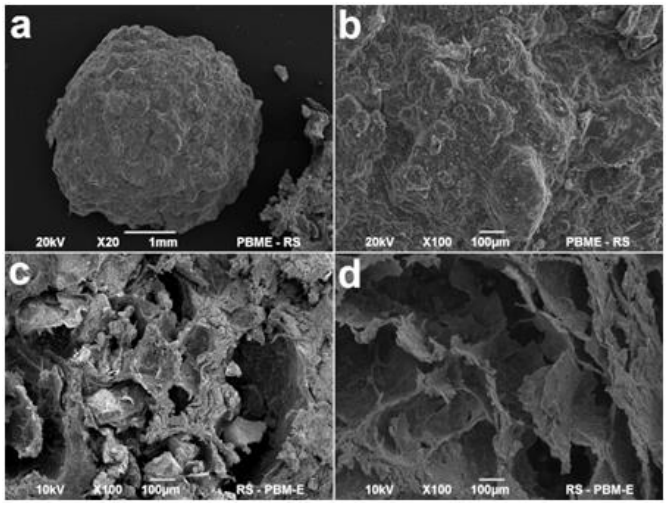


Fig. 1. SEM representative images of the samples: whole granule (a); surface of the granule (b); cross-section of the granule (c, d).

Conclusions: The studies show that obtained granules exhibit antibacterial properties and can be potentially used in preventing SSIs during orthopedic surgeries. Different types of CaPs used in granules ensure gradual release of antibacterial agents to local environment.

Acknowledgements: This work was supported by the research program UMO-2016/22/E/ST5/00564 of the National Science Centre, Poland.

Keywords: Drug delivery; Orthopedic surgery; Calcium phosphates

Abstract No. PPP.102

Method development and validation for targeted analysis of modified nucleosides and deoxynucleosides in urine samples obtained from patients diagnosed with bladder cancer

Małgorzata Artymowicz[1], Wiktoria Struck-Lewicka[1], Danuta Siluk[1], Marcin Markuszewski[2], Marcin Matuszewski[2], Michał J. Markuszewski[1]

[1] Department of Biopharmaceutics and Pharmacodynamics, Medical University of Gdańsk, Poland
[2] Department of Urology, Medical University of Gdańsk, Poland

E-mail address of presenting author: malgorzata.artymowicz@gumed.edu.pl

Objectives: Modified nucleosides and deoxynucleosides are products of RNA and DNA turnover. Extended DNA and RNA turnover is observed in various diseases, including cancer. Consequently, a correlation between elevated level of modified nucleosides and deoxynucleosides in urine and pathophysiological disorders development can be expected. The aim of the study was method development for targeted metabolomics analysis of 11 modified nucleosides and deoxynucleosides in urine samples collected from bladder cancer patients at 7 time points before and after tumor resection surgery.

Materials and methods: The first task of the research covered the development of method for simultaneous extraction of nucleosides and deoxynucleosides from urine matrix. This included comparison of two procedures: based on solid-phase extraction procedure (SPE) and methodology with no extraction step. Procedures were evaluated by their throughput, selectivity, recovery and ability to extract modified nucleosides and deoxynucleosides. Selected method was further validated.

Results: Procedure without extraction step was selected for further analysis. Developed method was validated in accordance with the guidance of FDA and EMA. Validated method was applied for the quantitative analysis of 11 nucleosides in urine collected from patients with bladder cancer (n=133) at different time points: before the surgery and 24 hours, 2 weeks, 3, 6, 9 and 12 months after the surgery. Samples were analyzed by HPLC-ESI-QqQ/MS technique.

Conclusions: Fast and reliable method for simultaneous analysis of modified nucleosides and deoxynucleosides was developed. Procedure was successfully utilized for analysis of urine obtained from patients diagnosed with bladder cancer. Next step includes advanced statistical analysis of obtained results as well as their biochemical interpretation. Proposed research may give some new information about the mechanism of bladder cancer development by the analysis of relation between changes of modified nucleosides in urine and stage of the disease. The observation of quantitative changes of nucleosides in bladder cancer patients before and after the surgery of tumor resection will verify their potential diagnostic and prognostic role in the cancer development.

Acknowledgements: The work has been supported by the National Centre of Science, project no 2018/29/N/NZ7/0229.

Keywords: Modified nucleosides, metabolomics, bladder cancer

Abstract No. PPP.103

Cinnamic acid derivatives as chmosensitizers that modulate activity of anthracyclines antibiotic- involvement of CBR1 and AKR1C3 inhibition mechanism

Paulina Koczurkiewicz-Adamczyk[1], Bartosz Gąsiorkiewicz[1], Anna Mróz[1], Agnieszka Gunia-Krzyżak[2], Kamil Piska[1], Benedykt Władyka[3], Marek Jamrozik[4], Katarzyna Wójcik-Pszczoła[1], Adam Bucki[4], Marcin Kołaczkowski[4], Elżbieta Pękala[1]

[1] Department of Pharmaceutical Biochemistry, Jagiellonian University Medical College, Poland
[2] Department of Bioorganic Chemistry, Chair of Organic Chemistry, Jagiellonian University Medical College, Poland
[3] Department of Analytical Biochemistry, Jagiellonian University, Poland
[4] Department of Medicinal Chemistry, Jagiellonian University Medical College, Poland

E-mail address of presenting author: paulina.koczurkiewicz@uj.edu.pl

Objectives: Doxorubicin (DOX) is an antracycline antybiotic widely used to treat haematological and solid cancers. Its clinical application is limited due to resistance development and cardiotoxicity. Recently, inhibition of carbonyl reduction of ANTs, catalyzed by enzyme from carbonyl reductase (CBR) and aldoketo reductase (AKR) families, emerged as a potential way to simultaneously bypass cancer resistance and alleviate cardiotoxicity of ANTs. In this context we evaluated cinnamic acid derivatives (CA).

Material and Methods: Inhibitory properties of the compounds toward CBR1and AKR1C3 were simulated by molecular modelling and confirmed in vitro using enzyme inhibition assay with recombinant proteins. Cytotoxicity, proliferation, migration analyses and proapoptotic activity were evaluated to checked chemosensitizing activity of compounds in human lung cancer cells model. The ability of compounds to DOX induced damage prevention was evaluated in rat cardiomyocytes.

Results: The tested compounds was found to chemosensitize A549 human lung cancer cell line towards DOX¬induced viability and proliferation reduction. Co-treatment with DOX + CA significantly inhibited the migration of A549 cells. The addition of CA alleviated DOX-induced viability reduction in H9c2 rat cardiomyoblast cell line. Accordingly, CA reduced DOX-induced reactive oxygen species (ROS) generation and increased glutathione levels.

Conclusion: Our results suggest that CA are promising candidates for DOX adjuvant therapy that may simultaneously chemosensitize cancer cells and alleviate cardiotoxicity.

Acknowledgements: This work was supported by funds granted by National Science Centre, Poland, SONATA No.2016/21/D/NZ7/01546.

Abstract No. PPP.104

Latex of *Chelidonium majus* – a rich source of potent bioactive compounds

Robert Nawrot[1], Joanna Gracz-Bernaciak[1], Oliwia Mazur[1], Natalia Kielich[1], Michalina Krakowiak[1], Sophia Bałdysz[1], Martyna Węglewska[1], Oskar Musidlak[1], Alicja Warowicka[1], Anna Goździcka-Józefiak[1]

[1] Faculty of Biology, Institute of Experimental Biology, Department of Molecular Virology, Adam Mickiewicz University, Poznań, Poland

E-mail address of presenting author: rnawrot@amu.edu.pl

Introduction: Latex is a milky emulsion produced by plants and after mechanical disruption of plant tissue it is immediately released as the first line of plant defense against pathogens. It is composed of two main groups of compunds: secondary metabolites (alkaloids among others) and proteins. *Chelidonium majus* L. is a model medicinal latex-bearing plant from the family *Papaveraceae*, with antiparasitic, insecticidal, anti-neoplastic, antiproliferative, antimycotic, immunomodulating, antibacterial and antiviral properties. Although the extracts from this plant have long been used in traditional folk medicine to treat visible symptoms of human papilloma virus (HPV) infection, the molecular mechanism of their action still remains unclear. In recent years two proteins - major latex protein (MLP) and glycine-rich protein (GRP) caught our attention as potential factors of latex antiviral and anticancer activity. Moreover, their interaction with alkaloids can modulate therapeutic effects.

Objectives: The main goal of current studies is to elucidate structure, functions and molecular mechanism of *Chelidonium majus* GRP and MLP proteins and their antiviral and anticancer activities.

Materials and Methods: Our research activity related to *C. majus* latex involves biotechnological production of both MLP and GRP proteins, molecular docking studies of several alkaloids to their pockets, and molecular testing of antiviral and antitumor activities of their combinations with alkaloids using tumor cell lines.

Results: Production and purification of recombinant CmGRP protein using a prokaryotic expression system was performed as well as CmMLP production conditions were established. The level of cytotoxicity of CmGRP itself and in combinations with selected alkaloids in different concentrations against HPV positive and HPV negative cell lines was determined also to assess the non-toxic concentrations for the use of combinations in further tests.

Conclusions: Herbal medicines can act synergistically with currently used therapeutics. Our results suggest that testing the combinations of recombinant *C. majus* latex proteins with low-molecular compounds can lead to the novel fixed-dose antiviral and antitumor plant-derived therapeutics.

Acknowledgements: This research was funded by the National Science Centre, Poland (grant number 2019/35/B/NZ9/03851).

Abstract No. PPP.105

The significance of microglial activation following mild traumatic brain injury (mTBI) in mouse lines selected for high (HA) and low (LA) intensity of depressive-like behaviors

Mateusz Rycerz[1], Piotr Poznański[2], Mariusz Sacharczuk[3], Magdalena Bujalska-Zadrożny[1], Anna Lesniak[1]

[1] Department of Pharmacodynamics, Medical University of Warsaw, Centre for Preclinical Research and Technology, Poland
[2] Department of Experimental Genomics, Institute of Genetics and Animal Biotechnology, Polish Academy of Sciences in Jastrzebiec, Poland
[3] Department of Pharmacodynamics and Department of Experimental Genomics, Medical University of Warsaw, Centre for Preclinical Research and Technology and Institute of Genetics and Animal Biotechnology, Polish Academy of Sciences in Jastrzebiec, Poland

E-mail address of presenting author: mateusz.rycerz1@gmail.com

Introduction: Microglial activation contributes substantially to the progression of neuropsychiatric disturbances with major depressive disorder (MDD), being the prime example. We hypothesize that disruption of blood-brain barrier (BBB) integrity by mild traumatic brain injury (mTBI) will preferentially trigger a neuroprotective microglia phenotype in mice with an innate susceptibility to depressive-like behavior.

Methods: Mild traumatic brain (mTBI) injury was induced by a weight-drop device in two mouse lines manifesting high (HA) and low (LA) intensity of depressive-like behaviors. Microglial secretory activity and polarization in the injured hemisphere were determined by measuring *Bdnf*, *Ngf*, *Iba1*, *CD86* and *CD206* mRNA levels with qPCR. Protein expression was determined by ELISA. Depressive-like behaviors were assessed in the tail suspension test (TST) and scored as immobility time.

Results: As expected, HA mice displayed longer immobility time in the TST test. mTBI exacerbated depressive-like behaviors in both mouse lines. However, a more prominent effect was seen in LA mice. Additionally, mTBI decreased *Bdnf* levels both at mRNA and protein levels in the LA line, while its expression was unaffected in HA mice. No changes in *Ngf* expression were detected in either line. The *Iba-1* microglial activation marker mRNA was significantly elevated in HA mice along with the M2-specific marker - *CD206*.

Conclusion: Loss of Bdnf in mTBI-subjected LA mice is positively correlated with depressive-like behavior severity and negatively with microglial activation. Thus, microglial activation could represent a protective response against mTBI-induced exacerbation of depressive-like behavior in HA mice with congenitally more pronounced depression-like symptoms.

Acknowledgements: This study was supported by statutory funds received from the Medical University of Warsaw and carried out with the use of the CePT infrastructure.

Keywords: Microglial activation, Depressive-like behavior, mTBI, HA/LA mice

Abstract No. PPP.106

Non-negative Principal Component Analysis in TLC Fingerprinting of 21 in-vitro grown Gentiana species

Sebastian Gadowski[1], Karolina Tomiczak[2], Łukasz Komsta[1]

[1] Department of Medicinal Chemistry, Faculty of Pharmacy, Medical University of Lublin, Poland
[2] Department of Conservation Biology of Plants, Polish Academy of Sciences Botanical Garden – Center for Biological Diversity Conservation in Powsin, Poland

E-mail address of presenting author: sebastiangadowski@gmail.com

Objectives: *Gentiana L.* (gentian) is a genus containing almost 400 species with worldwide distribution. Performing studies comparing phytochemical similarity of *Gentiana* species is difficult due to changes in phytochemical composition caused by the vegetation factors, such as light conditions, soil type, weather. However, it is possible to eliminate the influence of the environment by growing these plants *in vitro*. Therefore we performed TLC fingerprinting of gentians obtained by *in vitro* methods with chemometric interpretation of the differences in phytochemical composition

Materials and Methods: The extracts of different parts of twenty one *in vitro* grown Gentiana species (G. *affinis*, G. *andrewsii*, G. *bhutanica*, G. *burseri*, G. *cachemirica*, G. *capitata*, G. *crassicaulis*, G. *dahurica*, G. *decumbens*, G. *freyniana*, G. *frigida*, G. *gelida*, G. *grossheimii*, G. *kurroo*, *G. macrophylla, G. paradoxa,* G. *robusta*, G. *scabra*, G. *septemfida*, G. *siphonantha*, G. *tianschanica*) - roots, stems and three callus variants, were fingerprinted on silica gel plates with densitometric detection. Fresh material was extracted with methanol:acetone:water (3:1:1) mixture three times in 35C with ultrasonication and evaporated under vacuum to dryness, then dissolved in methanol. 5 uL aliquots of these extracts were chromatographed with mobile phase ethyl acetate - methanol - water (8:2:2) and scanned with densitometer in various extinction and fluorescence modes. Obtained fingerprints were smoothed using Savitzky-Golay filters, baseline was extracted with iteratively reweighted least squares (IRLS), and then they were warped using Parametric Time Warping algorithm. Various techniques was used for comparative analysis: hierarchical cluster analysis (HCA), principal component analysis (PCA), as well as non-negative variant of PCA.

Results and Conclusions: The samples both clustered against plant parts and several species was outlying. Four clusters of samples were the optimal number in clustering algotithms. The features responsible for differences were identified and discussed. Non-negative PCA gives less variance in first components, but these components contain only positively correlated peaks, so they are more easy to interpret and it gives additional insight to the data structure. Performed TLC fingerprinting study can be treated as a quick method of phytochemical screening, which encourages for further studies using other methods and compound identification.

Keywords: chemometrics, PCA, Gentiana

Abstract No. PPP.107

COX-2 binded fluorescence probe as cancer identification

Lukasz Milewski[1], Stefan Knippenberg[2], Silvio Osella[1]

[1] Laboratory of Chemical and Biological Simulations, Centre for New Technology, University of Warsaw, Poland
[2] Theory Lab, Universiteit Husselt, Belgium

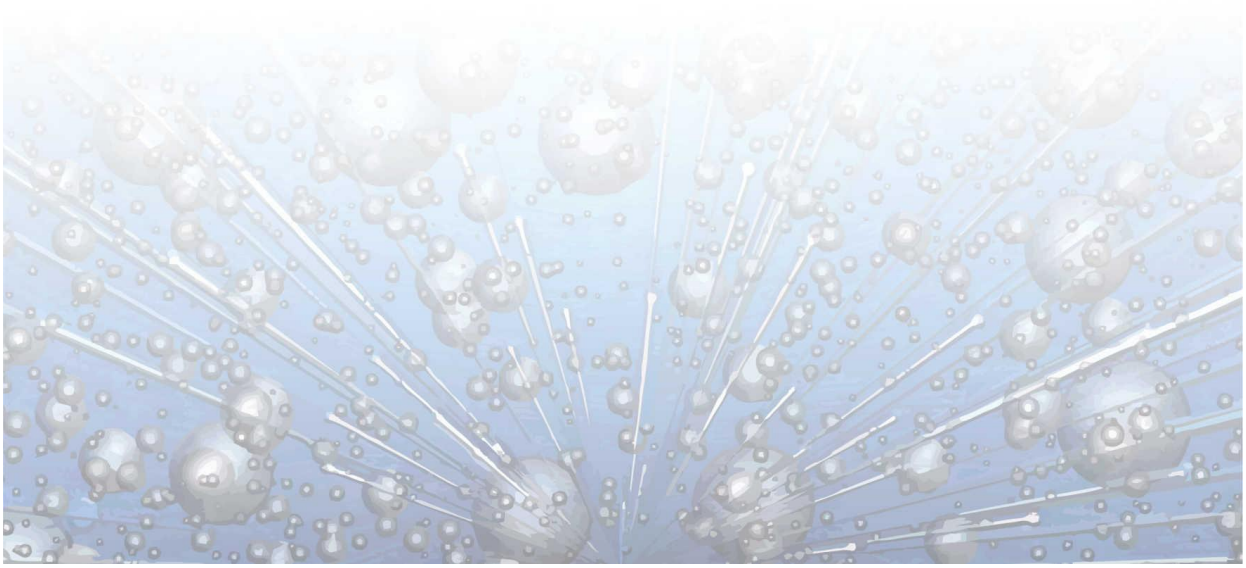
E-mail address of presenting author: l.milewski@cent.uw.edu.pl

Objectives: Cyclooxygenases are a family of enzymes consisting of the isoenzymes COX -1 and COX -2, which are important for the biosynthesis of prostaglandins in inflammatory reactions. In addition, COX -2 is overexpressed in all stages of cancer and therefore can be used to identify cancer cells through the use of molecular fluorescent probes. The aim of this study is to calculate the fluorescence properties of the probe ANQ-IMC -6, which consists of the fluorophore acenaphtho-1,2-b-quinoxaline (ANQ) and an indomethacin inhibitor (IMC).

Materials and methods: We use our CHARMM modified force field used for molecular dynamics calculations (MD) calculations. Molecular dynamics is approach to investigating molecular movements which gives us dynamics of the system using classical Newtonian mechanics. The next steps of the project will include a 300 ns MD simulation. Extracted snapshots from this simulation will be used as starting points for the excited state hybrid quantum molecular mechanics (QM-MM). In this method we calculate dynamics of whole system consisting molecular probe and its target protein using classical approach and probe using quantum mechanical approach. This can give us fluorescent properties of the probe.

Results: Preliminary 160 ns ground state MD after 90 ns was well equilibrated, which we which can be seen in constant energy plots and a low drift equal to -45 kJ/mol.

Acknowledgments: This work was supported by the National Centre of Science (NCN) SONATA-14 grant, number UMO-2018/31/D/ST4/01475 (Silvio Osella).



Abstract No. PPP.108

Innovative Anti-Cancer Therapeutics Based on Gold (III) Complexes in Colorectal Cancer – in vitro studies

Agata Gurba[1], Przemysław Szymański[2], Szymon Lipiec[2], Izabela Agnieszczak[2], Przemysław Taciak[1], Izabela Młynarczuk-Biały[3], Magdalena Bujalska-Zadrożny[1], Jakub Fichna[4]

[1] Departament of Pharmacodynamics, Medical University of Warsaw, Faculty of Pharmacy, Poland
[2] Histology and Embryology Students Association at the Department of Histology and Embryology, Medical University of Warsaw, Faculty of Medicine, Poland
[3] Departament of Histology nad Embryology, Medical University of Warsaw, Faculty of Medicine, Poland
[4] Medical University of Lodz, Faculty of Medicine, Deparrtment of Biochemistry, Poland

E-mail address of presenting author: agata.grabowska@wum.edu.pl

Objectives: Despite the constant development of knowledge on the prevention and treatment of neoplastic diseases, colorectal cancer (CRC) remains a significant challenge in medicine. The latest GLOBOCAN 2020 report estimated that only in 2020 approximately 1.2 million new cases of CRC would occur. Standard cisplatin and oxaliplatin-based treatment regimens that act through DNA intercalation are becoming less effective due to a lack of specificity for tumor cells, the occurrence of resistance, and tumor recurrence. Hence, the current goal of researchers in the field is to achieve higher efficacy, increased selectivity, and reduced toxicity of new metal-based complexes. Gold (III) complexes seem to be particularly promising due to their structural similarity to platinum (II). The investigated gold (III) cyanide complexes are relatively well soluble and stable in water (including serum and lymph), as well as possess good bioavailability. Our study aimed to investigate the antiproliferative properties of three innovative gold (III) derivatives named TGS 121, TGS 404, and TGS 512.

Materials and methods: Cytotoxicity of TGSs was evaluated with the PrestoBlue assay using the COLO-205 colon cancer cell line. Furthermore, we performed assays investigating cellular ATP levels, cell apoptosis and necrosis, colony formation assay, and cell cycle phase analysis.

Results: Investigated complexes showed a strong cytotoxic effect with a half-maximal inhibitory concentration (IC_{50}) at a range of 0,5 - 2 mg/L. The tested compounds induced cell death of the COLO-205 cell line in an apoptosis-independent mechanism inhibited clonal growth and induced a block in the G2/M phase of the cell cycle.

Conclusions: These results suggest that the evaluated innovative gold (III) complexes have strong anticancer properties in vitro against human colorectal cancer. Currently, the exact mechanism of this activity is under investigation.

Acknowledgements: This research was supported by the grant from the National Science Center (2017/25/B/NZ5/02848 to JF) and the Medical University of Lodz (#503/1-156-04/503-11-001-19 to JF). The research was funded by grant No. 1M15/3/M/MG/N/20 to P.T. The grant was supervised by I.M-B and was funded by a subsidy for science received by the Medical University of Warsaw

Keywords: gold (III) complex, colon cancer, cytotoxicity

Abstract No. PPP.109

Computer-aided design of carbonyl reductase 1 (CBR1) inhibitors as cancer chemosensitizing agents

Marek Jamrozik[1], Kamil Piska[2], Adam Bucki[1], Paulina Koczurkiewicz-Adamczyk[2], Michał Sapa[1], Marcin Kołaczkowski[1], Elżbieta Pękala[2]

[1] Department of Pharmaceutical Chemistry, Jagiellonian University Medical College, Poland
[2] Department of Pharmaceutical Biochemistry, Jagiellonian University Medical College, Poland

E-mail address of presenting author: kamil.piska@uj.edu.pl

The search for effective anticancer drugs is one of the biggest challenges of modern pharmacotherapy. Anthracycline antibiotics (ANT) are still among the most widely used group of anticancer drugs. Unfortunately, ANT metabolism, which consists in the two-electron reduction of a carbonyl moiety to a hydroxy group, performed mainly by carbonyl reductase 1 (CBR1), leads to the formation of metabolites with decreased activity. Additionally, it is postulated that those metabolites are responsible for the cardiotoxic effect that significantly limits the use of ANT. Inhibition of CBR1-related ANT metabolism can lead to improvement of the pharmacological action of this group of drugs.

This study presents the application of computational methods in the optimization of CBR1 crystal structures (PDB codes 1WMA and 3BHJ) to improve their ability to effectively distinguish CBR1 ligands from non-ligands. Optimized models were then used in prospective virtual screenings leading to a selection of new chemotypes of potential CBR1 inhibitors. The selected structures were than assayed with CBR1 recombinant enzyme to determine their inhibitory properties.

Optimization of CBR1 crystals was performed using Induced-fit docking. The obtained models were validated in retrospective virtual screening. Good-quality models were additionally checked in 20 ns molecular dynamics simulations to select the best ones, which then were used in prospective virtual screening with over 2 million compounds. After the virtual screening, ADMET properties of selected 52 best scored (based on scoring function) compounds were predicted and evaluated using ADMET Predictor software. Five the most promising compounds (considering both docking simulations and ADMET properties prediction) were purchased and submitted for *in vitro* research. Among them, one compound exhibited potent inhibitory properties against recombinant CBR1 activity.

Abstract No. PPP.110

The enhanced activity of doxorubicin in breast cancer therapy due to sulforaphane properties

Anna Pogorzelska[1], Pamela Krug[2], Maciej Mazur[2], Marta Świtalska[3], Joanna Wietrzyk[3], Katarzyna Wiktorska[1]

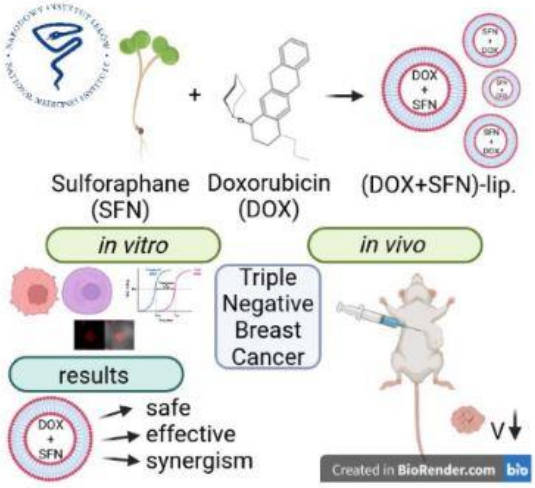
[1]Department of Biotechnology of Medicines and Bioinformatics, National Medicines Institute, Warsaw, Poland;
[2]Department of Chemistry. University of Warsaw, Warsaw, Poland
[3]Laboratory of Experimental Anticancer Therapy, Institute of Immunology and Experimental Therapy, Wrocław, Poland

E-mail address of presenting author: a.pogorzelska@nil.gov.pl

Objectives: This study aimed to define the effectiveness and safety of a novel formulation of isothiocyanate - sulforaphane (SFN) with a cytostatic - doxorubicin (DOX) together encapsulated in polydispersive liposomes in the aggressive and metastatic, triple-negative breast cancer treatment.

Materials and Methods: The liposomal formulations of doxorubicin hydrochloride alone and with the addition of sulforaphane (DOX–lip. and DOX+SFN-lip.) were prepared and characterized. The in vitro experiments were performed using triple-negative breast cancer (TNBC) cell line MDA-MB-231 and non-tumorigenic MCF-10A cell line as a normal breast tissue control. The anticancer effectiveness and synergism were determined using the MTT test and the intracellular distribution was performed using a confocal microscope. The safety and effectiveness were evaluated also in an in vivo model of TNBC Balb/c mice. Safety and effect on tumor growth were assessed, histopathology of tumors and blood biochemistry were also examined, and the hypothetical effect of combinational therapy was calculated.

Results: In vitro studies on TNBC cell growth inhibition showed that DOX and SFN act synergistically and dose reduction of DOX is possible when combined with SFN. The addition of SFN to DOX enhanced the internalization of DOX to cell nuclei - the target site for DOX, which can be a proposed mechanism of action of our formulation. In vivo results proved that compounds are safe and DOX showed stronger inhibition of tumor growth when encapsulated together with SFN in liposomes. These results indicate lower toxicity of (DOX+SFN)-lip. administration for normal cells, but stronger anticancer effectiveness than the administration of DOX-lip. separately encapsulated in polydispersive liposomes in the triple-negative breast cancer treatment.



Conclusions: The obtained results suggest that the proposed formulation of DOX encapsulated together with SFN in polydispersive liposomes is a promising drug candidate for the safe and effective treatment of TNBC.

Acknowledgments: This study was supported by 9/2020 National Medicines Institute Institutional funding, and National Science Centre, Poland, 2021/41/N/NZ7/02530.

Keywords: Breast cancer; liposomes; doxorubicin

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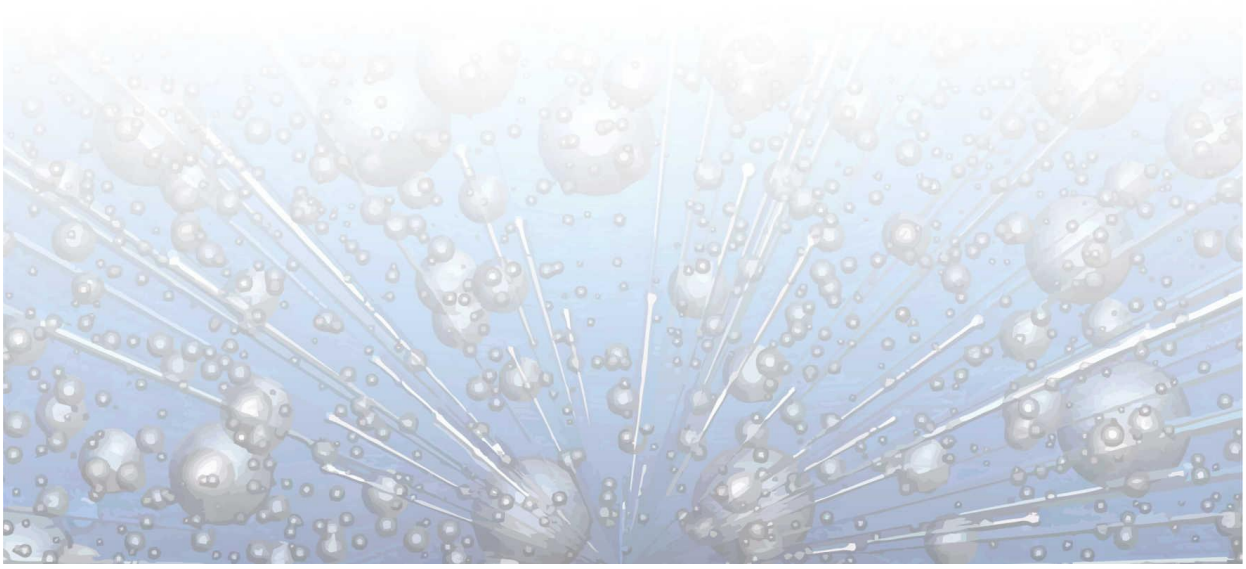


Abstract No. PSP.01

Phytochemical screening on biological study on the plant Linum usitatissimum Linn.

Ashu Guni[1]

[1] Department of Pharmacy, National Model College for Advanced Learning, Tribhuwan University, Nepal

E-mail address of presenting author: nishchalbaniya@gmail.com

Objectives: Dried seeds of flax were subjected to successive extraction with petroleum ether, chloroform, ethyl acetate, methanol and water using Soxhlet apparatus. Qualitative and quantitative phytochemical screening of the extract was carried out and biological activity was studied both in-vitro and in-vivo.

Results: Phytochemical screening revealed the presence of phenol, flavonoid, terpenoid, protein and amino acid in all five extracts. Saponin and fat and fixed oils were found absent only in petroleum ether and water extract respectively. Cardiac glycoside and carbohydrate were present in ethyl acetate, chloroform and methanol extract. Methanolic extract showed the highest TPC and TFC with the value of 205.32 ± 5.03 mg GAE/g and 5.49 ± 0.16mg QE/g respectively. *Staphylococcus aureus* was found to be susceptible towards ethyl acetate and water extract while fungal strain under test was found to be resistant. Methanolic extract possessed highest free radical scavenging activity with the IC_{50} value of 564.48 ± 1.37 µg/mL. Ethyl acetate extract was found toxic with LC_{50} value of 134.09 ± 1.44 µg/mL while petroleum ether and methanol extract were found to be moderately toxic.

Methanolic extract obtained by maceration was subjected for further tests which showed the potent inhibition of α amylase enzyme, with the IC_{50} value of 6.698 ± 0.40 mg/mL comparing to standard Acarbose with IC_{50} value 0.75 ± 5.64 mg/mL. Significant anti-inflammatory action was found at 400 mg/kg which showed inhibition of 59.36% with, with inhibition of 51.61% by Ibuprofen (10 mg/kg) used as standard. Also, potential analgesic activity was shown by methanolic extract with 41.84% inhibition at 400mg/kg and standard, Ibuprofen which showed 40% inhibition.

Conclusions: The study revealed the plant as potential candidate for drug development.

Key words: *Linum usitatissimum*, Phytochemical screening, Antioxidant, α amylase inhibition, Anti inflammatory activity, Analgesic activity

Abstract No. PSP.02

The role of oxytocin in mephedrone-induced social reward

Olga Wronikowska-Denysiuk[1], Agnieszka Michalak[1], Tymoteusz Słowik[2], Barbara Budzyńska[1]

[1] Independent Laboratory of Behavioral Studies, Chair of Biomedical Sciences, Medical University of Lublin, Poland
[2] Centre of Experimental Medicine, Medical University of Lublin, Poland

E-mail address of presenting author: olga.wronikowska-denysiuk@umlub.pl

Objectives: Mephedrone is one of the most frequently misused drug of abuse. Although many factors influencing its addictive potential have been identified, the impact of social factor on mephedrone-induced reward remains underexplored. The aim of this study was to assess the role of oxytocin (OT) in mephedrone-induced social conditioned place preference (social-CPP) assay.

Materials and Methods: The experiments were carried out on 8 weeks old Wistar rats. The social-CPP procedure was conducted in a two-compartment apparatus divided by guillotine doors and differing by tactile and visual stimulation (floor structures and wall patterns). During the pre-conditioning test an initial preference of rats was measured, which was followed by a 6-day social-conditioning with mephedrone (5 or 20 mg/kg, i.p.). During the post-conditioning test animals received acute injection of OT antagonist - L,368,899 (5 mg/kg, i.p.) and the post-conditioning preference was measured. An increase of time spent in the drug-associated compartment was considered as a rewarding effect of mephedrone.

Results: The results showed that single administration of OT antagonist - L,368,899 (5 mg/kg, i.p.) successfully blocked mephedrone (5 mg/kg)-induced social-CPP without affecting CPP scores of mephedrone in a higher dose (20 mg/kg).

Conclusions: The obtained findings suggest that mephedrone-induced social reward is, at least partially, mediated by OT, suggesting its potential role as novel therapeutic target for the treatment of mephedrone use disorder.

Acknowledgements: This study was supported by the Grants No NCN 2020/37/N/NZ7/01564 and No NCN 2017/25/B/NZ7/02410 from the National Science Centre (NSC, Poland).

Keywords: mephedrone, oxytocin, social reward

Abstract No. PSP.03

Identification of Novel Ligands of Cannabinoid Receptor 2: In Silico Screening Assay and In Vitro Evaluation

Adam Stasiulewicz[1], Joanna Sulkowska[2]

[1] Department of Drug Chemistry, Medical University of Warsaw, Poland
[2] Interdisciplinary Laboratory of Biological Systems Modelling, University of Warsaw, Poland

E-mail address of presenting author: a.stasiulewicz@cent.uw.edu.pl

Objectives: The endocannabinoid system (ECS) is a major regulatory system responsible for a vast array of physiological processes in the human organism. Cannabinoid receptor 2 (CB2) is one of the most important proteins of ECS, and as such—a very promising therapeutic target. CB2 agonists may be of use in the treatment of pain, neurodegenerative disorders, autoimmune and inflammatory diseases, and many other conditions. Moreover, targeting CB2 reduces the risk of encountering serious adverse effects related with the impact on CB1 transmission. CB2 tertiary structure was recently determined, thus enabling rational drug design with the effective application of structure-based methods. Therefore, the aim of this study is to find novel CB2 ligands and to establish an in silico protocol for identification of new ligands of similar receptors.

Materials and Methods: Herein, we present a multi-step, computational workflow to find new CB2 ligands, merging structure- and ligand-based methods. In the first part–pharmacophore screening, we utilized LigandScout. We conducted hybrid structure/ligand-based pharmacophore screening of over 7 mln drug-like compounds from ZINC database. Candidates with best values of scoring function were selected for the second part—molecular docking. In this phase, we used Schrödinger Glide and docked chosen molecules to three CB2 models, based on crystal structures and on a conformation generated with molecular dynamics simulation in GROMACS. Then, we conducted a double-step filtration with Glide SP function and molecular mechanics–generalized Born surface area (MM–GBSA) binding energy values. The third major part of the study consisted of machine learning binding affinity prediction with Schrödinger AutoQSAR. Finally, we selected 16 structurally diverse molecules and verified their affinity to CB2 with in vitro radioligand displacement assay.

Results: We identified two novel CB2 ligands with nanomolar affinity toward CB2. Both compounds are structurally diverse from to-date known CB2 ligands.

Conclusions: Two new CB2 ligands provide a promising starting point for optimization. The established virtual screening protocol will be useful for hit identification for CB2 and similar molecular targets.

Acknowledgements: This work was supported by National Science Centre, Poland grants no. 2019/35/N/NZ7/04258 to AS and no. 2020/01/0/NZ7/00244 to JS.

Keywords: CB2, drug design, virtual screening

Abstract No. PSP.04

Synthesis and anticancer evaluation of new 1,3,4-thiadiazole derivatives

Sara Janowska[1], Dmytro Khylyuk[1], Anna Bielawska[2], Anna Szymanowska[2], Krzysztof Bielawski[3], Monika Wujec[1]

[1] Department of Organic Chemistry, Medical University of Lublin, Poland
[2] Department of Biotechnology, Medical University of Bialystok, Poland
[3] Department of Synthesis and Technology of Drugs, Medical University of Bialystok, Poland

E-mail address of presenting author: sara.janowska@wp.pl

Objectives: Breast cancer is the most diagnosed cancer in women in the world. Most of the currently known pharmacological treatment methods are directed at estrogen receptors (ER) in their mechanisms of action. Therefore, these drugs are not effective in non-estrogen-dependent breast cancer. To look for substances showing activity independent of estrogen receptors we designed and synthesized new small-molecule substances with anticancer potential. We decided on 1,3,4-thiadiazole derivatives due to numerous reports on the antitumor activity of this group of heterocyclic compounds.

Materials and Methods: To develop new 1,3,4-thiadiazole derivatives, we have created a library of molecular standards and reports on their antitumor activity in tests. Based on the analysis of the collected data, we designed and synthesized a group of thiadiazole derivatives differing in the structure of substituents at C2 and C5 positions. The cytotoxic activity of the obtained compounds was then determined in biological studies with MCF-7 and MDA-MB-231 breast cancer cells. Moreover, in silico studies we determined the interaction of the compounds obtained with proteins involved in neoplastic processes.

Results: The strongest antiproliferative activity in both breast cancer cell lines was demonstrated by the compound ST10 with a trifluoromethylphenyl substituent. IC_{50} values of the compound against MCF-7 and MDA-MB-231 breast cancer cells were 49.6 µM and 53.4 µM, respectively. Docking simulations indicated a possible multi-directional mode of action. Nevertheless, the antitumor properties are probably mainly related to activity against Caspase 3, Caspase 8 and activation of BAX proteins.

Conclusions: The antitumor activity of the newly synthesized compounds, demonstrated in biological tests, indicates the validity of further research involving the modification of the structure to find more active compounds.

Keywords: 1,3,4-thiadiazole; anticancer activity; breast cancer

Abstract No. PSP.05

Proapoptotic activity of MM-129 in experimental colon cancer models

Iwona Kwiatkowska[1], Justyna Hermanowicz[1], Robert Czarnomysy[2], Anna Bielawska[2], Mariusz Mojzych[3], Krzysztof Bielawski[2], Dariusz Pawlak[1]

[1] Department of Pharmacodynamics, Medical University of Bialystok, Poland
[2] Department of Synthesis and Technology of Drugs, Medical University of Bialystok, Poland
[3] Department of Chemistry, Siedlce University of Natural Sciences and Humanities, Poland

E-mail address of presenting author: iwona.kwiatkowska@umb.edu.pl

Objectives: Colorectal cancer (CRC) is one of the most common tumor type, responsible for about one million deaths every year.

Materials and Methods: The goal of the study was to synthesize a novel 1,2,4-triazine sulfonamide derivative and evaluate its anticancer potential in DLD-1 and HT-29 colon cancer cells. The target compound MM-129 was obtained by a multi-step synthesis, starting from 1,2,4-triazine. The antiproliferative activity of MM-129 and the reference drugs roscovitine and 5-fluorouracil were examined by [3H]thymidine incorporation assay and in a zebrafish embryo model. To explore the cellular mechanism, by which the synthesized compound triggers induction of apoptosis, we examined the alterations of the mitochondrial transmembrane potential, phosphatidylserine externalization and caspase activity by using flow cytometry analysis.

Results: Screening results revealed that MM-129 exhibited strong inhibition activity toward DLD-1 and HT-29. It showed potent antiproliferative effects against colon cancer cell lines with IC50 values 3.1 μM compared to 5-fluorouracil and roscovitine with values above 10 μM. Flow cytometry analysis revealed that apoptosis was the main response of colorectal cancer cells to MM-129 treatment.

Conclusions: : These preclinical results suggest that this novel 1,2,4-triazine derivative, due to high proapoptotic activity, should be tested in the clinic for treatment of colon cancer.

Acknowledgements: This research was funded by National Science Center, Poland grant number 2018/31/B/NZ7/00875.

Keywords: zebrafish, apoptosis, colon cancer

Abstract No. PSP.06

Analysis on the use of personalized medicines produced via 2D and 3D printers

Ronald Terrazas Mallea[1]

[1] Institute of Physical Chemistry, Polish Academy of Sciences, Poland

E-mail address of presenting author: rtmallea@ichf.edu.pl

Objectives: Traditional medicines follow a one-dose-fits-all model as it is very complicated and expensive to study many different doses on different patient populations. As a result, between 75-85% of patients experience some degree of adverse effects. Personalized medicines aim to deliver the "right drug in the right dose" to the "right patient".

However, producing personalized medicines would also require flexible manufacturing means. Among the different possible options, 3D and 2D printers can produce pills and oromucosal films, respectively, with the right dose and right controlled pharmacokinetics. Additionally, they can be formulated in orodispersible form suitable for patients with swallowing problems.

In this work, I present an analysis on how both technologies could be implemented by a laboratory producing medicines. The analysis focuses mainly on the production costs and target market.

Methods: The reference 3D printer for this analysis is the M3DIMAKER produced by FABRX. Unfortunately, there is yet no 2D printer strictly focused on producing oromucosal films. Therefore, a large-scale digital printer Domino N610i was used as a reference. Their production costs are compared to a reference pill conversion cost of ~0.01 $.

In terms of the laboratory operations, a case is made on the most attractive market.

Results: The estimated pill conversion costs using 3D printers is 600% higher than the reference price at both small and large scale due to the linear production scaling. Using the 2D printers, the estimated costs at large scale can be similar, around 10% higher.

In terms of the market, patients that dangerously alter their drugs or refuse to take pills due to pain (~12%) could benefit from drugs in orodispersible form. Significantly adapting the dosage and PK have potential but cannot be done due to strict regulations.

Conclusions: 3D printed personalized pills would be prohibitively expensive. Several added benefits would be required to justify the cost. 2D printed films offer competitive production costs, so adding the personalization features would make them even more attractive.

Given the current regulations, the orodispersible characteristic would be the most profitable feature of the printed drugs.

Abstract No. PSP.07

Curcumin derivatives: Anti-inflammatory, analgesic, ulcerogenic, cyclooxygenase-2 inhibition and molecular docking studies

Mahmood Ahmed[1]

[1] University of Education, Pakistan

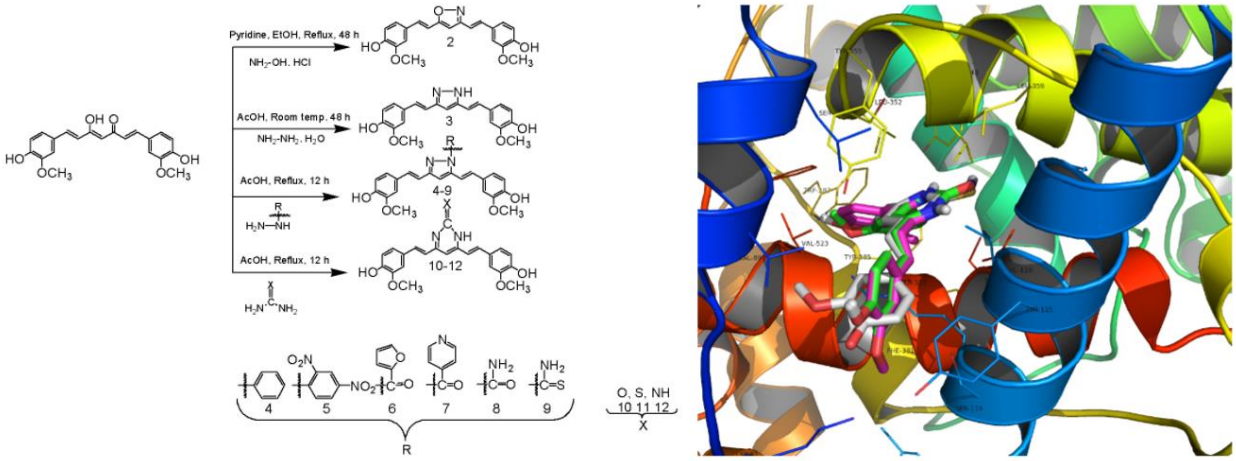
E-mail address of presenting author: mahmoodresearchscholar@gmail.com

Objective: Curcumin has shown pharmacological properties against different phenotypes of various disease models. Different synthetic routes have been employed to develop its numerous derivatives for diverse and improved therapeutic roles.

Materials and Methods: In present study, we have synthesized curcumin derivatives containing isoxazole, pyrazoles and pyrimidines then the synthesized molecules were evaluated for their anti-inflammatory and antinociceptive activities in experimental animal models. Acute toxicity of synthesized molecules was evaluated in albino mice by oral administration. Any behavioral and neurological changes were observed at dose of 10 mg/kg body weight. Additionally, cyclooxygenase-2 (COX-2) enzyme inhibition studies were performed through in-vitro assays.

Results: In vivo anti-inflammatory studies showed that curcumin with pyrimidines were most potent anti-inflammatory agents which inhibited induced edema from 74.7-75.9 %. Compound 7, 9 and 12 exhibited relatively higher prevention of writhing episodes than any other compound with antinociceptive activity of 73.2, 74.9 and 71.8 % respectively. This was better than diclofenac sodium (reference drug, 67.1 % inhibition). Similarly COX-2 in vitro inhibition assays results revealed that compound 12 (75.3 % inhibition) was the most potent compound.

Conclusion: Molecular docking studies of 10, 11 and 12 compounds in human COX-2 binding site revealed the similar binding mode as that of other COX-2 selective inhibitors.



Acknowledgements: I am indeed grateful to all the colleagues of School of Chemistry, University of the Punjab, Lahore-Pakistan especially my advisor Dr Muhammad Abdul Qadir for providing me good working environment and research facilities.

Keywords: Curcumin, Heterocyclics, Disease, Docking

Abstract No. PSP.08

The impact of vitrification method on the physico-chemical properties and dissolution rate of bosentan

Ewa Ozimina-Kamińska[1], Aldona Minecka[1], Barbara Hachuła[2], Karolina Jurkiewicz[3], Kamil Kamiński[3], Bartłomiej Milanowski[4]

[1] Faculty of Pharmaceutical Sciences in Sosnowiec, Medical University of Silesia in Katowice, Poland
[2] Institute of Chemistry, University of Silesia, 40-006 Katowice, Poland, Poland
[3] Institute of Physics, Faculty of Science and Technology University of Silesia in Katowice, Poland
[4] Faculty of Pharmacy, Poznan University of Medical Sciences, 60-780 Poznan, Poland, Poland

E-mail address of presenting author: ekaminska@sum.edu.pl

Objectives: Our purpose was thorough characteristics of thermal and structural properties, H-bonding pattern and molecular dynamics of amorphous bosentan, BOS (an endothelin-1 receptor antagonist dedicated to the treatment of pulmonary arterial hypertension in adults) prepared by two methods: vitrification and cryomilling. We also wanted to check whether the amorphization influences the dissolution rate of this active pharmaceutical substance (API), which is a class IIa drug within the Biopharmaceutical Classification System.

Materials and Methods: The vitrified and amorphous BOS were analyzed by X-ray diffraction (XRD), differential scanning calorimetry (DSC), thermogravimetry, Fourier transform infrared (FTIR) and broadband dielectric spectroscopy (BDS).

Results: Measurements with the use of XRD, FTIR and BDS indicated that both disordered samples are nearly identical in terms of the local structure, H-bonding scheme, and structural (α) dynamics in the supercooled liquid state. However, based on the analysis of α-relaxation times (BDS studies) predicted for temperatures below the glass-transition temperature (T_g), as well as results of DCS investigations, it was concluded that the cryoground sample is more aged and probably more physically stable compared to the vitrified one. Interestingly, such differences in physical properties of both amorphous systems were reflected in the lower intrinsic dissolution rate of BOS obtained by cryomilling (in the first 15 min of dissolution test) in comparison to the vitrified drug. Moreover, FTIR and thermogravimetric data clearly showed that cryomilling yields nearly anhydrous amorphous BOS (despite the fact that the initial crystalline sample is a monohydrate). This unique finding, not reported to date, was in contrast to the results obtained previously by Megarry et al. [1] for trehalose. We proposed and discussed two different hypotheses explaining water removal upon cryomilling of BOS.

Conclusions: Our studies revealed differences between amorphous BOS prepared via different methods, that influence the solubility of this API. Obtained results initiated an interesting discussion on the behavior of cryomilled pharmaceuticals and the mechanism of amorphization behind this experimental technique.

Acknowledgements: Sonata Bis project: Dec-2016/22/E/NZ7/00266

Keywords: bosentan, water removal, dissolution rate

References:

[1] Megarry, A.J. et al. *Carbohydr. Res.* **2011**, *346*, 1061-1064.

Abstract No. PSP.09

Solvent Front Position Extraction as a powerful sample preparation procedure for the quantitation of coccidiostats

Maciej Rybicki[1], Anna Klimek-Turek[1], Tadeusz Dzido[1]

[1] Medical University of Lublin, Department of Physical Chemistry, Poland

E-mail address of presenting author: maciek.j.rybicki@gmail.com

Introduction: Coccidiostats are veterinary drugs, which are commonly administered to animal feed. Unfortunately, it can lead to their presence in food products. European Union legislation established hygiene rules for the production of animal feed to prevent occurrence of drugs in food. Despite the significant reduction of the fraction of the contaminated food products to 0.32% (2019), the problem remains. Coccidiostats can be resistant to cooking in high temperatures, therefore the most important is to monitor and prevent unacceptable contamination of animal products intended for human consumption. In order to effectively detect a threat, many analytical methods are developed, among which method of choice is LC-MS/MS. However, it often requires sample preparation, which is considered as one of the most crucial stages of analysis.

Objectives: Solvent Front Position Extraction (SFPE) can be considered as an interesting alternative for sample preparation and it was expected that it could be potentially used as reference method for determination of coccidiostats, when coupled with LC-MS/MS. For this reason SFPE was validated according to the European Commission Regulation 2021/808 of 22 March 2021 parallelly with 3 other sample preparation techniques for coccidiostats, established by or with cooperation with European routine laboratories [1]. Obtained validation parameters were compared and used to evaluate usability of SFPE.

Materials and Methods: Determinations of coccidiostats using four methods of sample preparation were carried out with samples of the same feed, fortified at a constant level. In order to determine the validation parameters, replicates of extracts from feed were analyzed for three consecutive days, by one researcher with different batches of reagents and solvents.

Results: SFPE-LC-MS/MS quantitation results were very satisfactory (linearity $R^2 \geq 0.971$, recovery 90.1%-111.1%, RSDr: 8.7%-16.6%, RSDR: 9.0%-17.7%) and fulfilled requirements described in mentioned Commission Regulation.

Conclusions: SFPE has great potential in sample preparation of coccidiostats in animal feed.

Keywords: Chromatography, mass spectrometry, coccidiostats, sample preparation with TLC (SFPE)

Abstract No. PSP.10

Parametrization of the AMOEBA polarizable force field for 17-β-estradiol and cyclodextrin basic unit. Step into the Free Energy Perturbation calculations and Endocrine Disrupting Chemicals analysis

Anna Mazurek[1], Thomas Simonson[1]

[1] Biology Department, Ecole Polytechnique de Paris, France

E-mail address of presenting author: anna.mazurek@wum.edu.pl

Objectives: Cyclodextrins (CDs) are cyclic oligosaccharides which are used both as drug carriers and as removing agents for toxins like Endocrine Disrupting Chemicals (EDCs). Nowadays, experimental research in this topics is supported by the computational molecular modelling. One of the most developing methods for the binding free energy calculations is the Free Energy Perturbation method which requires the parametrization of the applied force field (FF). Wide databases for the classical FFs are available. However, it is believed that the polarizable FFs might deliver more accurate results. Unfortunately, in the latter case, the databases still lack a lot of information. Therefore, the goal of this project was to parametrize the polarizable AMOEBA FF for the drug and EDC example: 17-β-estradiol (EST) and for a basic CD unit: α-D-glucopyranoside (GYP).

Materials and Methods: For parametrization the Tinker software has been used. Geometry optimization and single point calculations have been performed in Gaussian 09 software.

Results: Despite the complexity of the EST structure, parametrization of both the geometry parameters (bond length, angle size, dihedrals) and the electronic polarization description have been successfully completed for EST and GYP. RMSD values show that the obtained data is in good agreement with the Density Functional Theory (DFT) results.

Conclusions: It is possible to parametrize the AMOEBA FF for EST and basic CD unit within the acceptable RMSD range, when compared to the DFT results. The obtained data significantly enriches the available AMOEBA FF parameters database. This allows to perform the Free Energy Perturbation calculations of the steroids/EDCs-CD complexes. This in turn will lead to better understanding of the molecular nature of such bindings and help to define possible guests for CDs used as drug carrier or toxin removing agent.

Acknowledgements: The presented work is part of the International Interdisciplinary Dual PhD studies performed at the Medical University of Warsaw and Ecole Polytechnique, Paris, France. This research has been conducted in France, under the supervision of Prof. Thomas Simonson and the research stay has been financed from the French Government Scholarship.

Keywords: force filed, cyclodextrin, binding free energy

Abstract No. PSP.11

Quantum Chemistry in a Pocket

Filipe Menezes[1], Grzegorz Popowicz[1]

[1] Institute for Structural Biology, Helmholtz Zentrum Muenchen, Germany

E-mail address of presenting author: filipe.menezes@helmholtz-muenchen.de

Objectives: The basis of structure based drug design is the formation of complexes between protein and ligands. The relative position of atoms in this complex are not direct observables. They result from fitting to experimental data: electronic densities, chemical shifts, *etc.* Inconsistencies in the underlying models and inaccuracies in the experimental data may lead to tensions on the structure. These may be disguised in, for instance, overly stretched/contracted bonds or incorrect angles. While current force fields can describe quite accurately the inner physics of proteins, these methods lack enough generality for the ligands. This is the domain of quantum chemical methods. Though the full optimization of protein-ligand complexes is affordable with some methods, the computational cost is nevertheless high: in optimistic cases one requires at least three weeks of calculation time. On the other hand, the optimization of the free-ligand may result in a conformer that is not experimentally observed.

We introduce in-pocket optimization, a new technique to optimize the structure of ligands in protein's pockets without requiring the latter. The computational cost is kept at a minimum, while allowing full quantum mechanical optimization of ligands in protein's pockets.

Materials and Methods: In-pocket optimization is a new computational technique implemented in our in-house quantum chemical package ULYSSES. The key to success is the introduction of a penalty that allows the molecules to minimize strain, while retaining the conformation stabilized by the pocket.

Results: In-pocket optimization allows strain minimization in experimentally determined structures. It enables the calculation of physico-chemical properties of ligands as they are in the protein pocket. Group-specific tension and the real molecular tension accumulated upon binding is easily accessible. We use it also for scaffold hopping and to understand the physical reasons for binding.

Conclusions: We introduce a new computational technique, which we believe will be extremely helpful for medicinal chemists in structure based drug development. This technique improves not only the quality of the underlying molecular structures chemists work with, but it also opens the door to further insights at the molecular level that relate to binding.

Acknowledgments: Financial Support: BMWi ZIM. KK 5197901TS0; BMBF SUPREME, 031L0268

Keywords: Quantum-Chemistry, Pocket, Structure based drug discovery

Abstract No. PSP.12

Fruits of Hippophaë rhamnoides – a question about their preventive role in gut barrier leakage, epithelial inflammation, and glucose transport

Anna Laskowska[1], Aleksandra Wilczak[2], Weronika Skowrońska[3], Piotr Michel[4], Matthias F. Melzig[5], Monika E. Czerwińska[6]

[1] Department of Pharmaceutical Microbiology, Medical Univeristy of Warsaw, Poland
[2] Department of Pharmacognosy and Molecular Basis of Phytotherapy, Student Scientific Association "Herbarium", Medical University of Warsaw, Poland
[3] Department of Pharmacognosy and Molecular Basis of Phytotherapy, Medical University of Warsaw, Poland
[4] Department of Pharmacognosy, Medical University of Lodz, Poland
[5] Institute of Pharmacy, Freie Universitaet Berlin, Germany
[6] Department of Biochemistry and Pharmacogenomics, Medical University of Warsaw, Poland

E-mail address of presenting author: monika.czerwinska@wum.edu.pl

Objectives: In traditional medicine, *Hippophaë rhamnoides* L. (HR; sea buckthorn) is considered in complaints such as gastric ulcers and wound healing [1]. It has been recently noted that HR reduces blood glucose and alleviates insulin resistance [2]. The aim of the study was an assessment of anti-inflammatory activity of an aqueous extract of HR fruit (HRE) expressed as inhibition of cytokines (IL-8, IL-1*β*, IL-10, IL-6, TNF-*α*) secretion by human neutrophils (PMN), peripheral blood mononuclear cells (PBMC), and human colorectal adenocarcinoma cell line (Caco-2) stimulated with LPS (lipopolysaccharide from *Escherichia coli*)/IL-1*β*. HRE influence on LPS-leakage through the Caco-2 monolayer and GLUT2 translocation was also evaluated.

Materials and methods: HRE composition was determined with HPLC-DAD-MS^n. Secretion of cytokines by cell cultures was established with ELISA. LPS concentration in the apical and basolateral compartments of the Caco-2 monolayer was evaluated with *Limulus amebocyte lysate* assay. The transepithelial electrical resistance of the Caco-2 monolayer was monitored with a voltmeter. GLUT2 translocation was evaluated using an immunostaining assay (IF).

Results: HRE (100 µg/mL) inhibited the secretion of TNF-*α* and IL-8 in PMN and PBMC. It increased the release of anti-inflammatory cytokine IL-10 in PBMC. The concentration of IL-8 was significantly decreased in the Caco-2 model after the treatment with HRE (50-500 µg/mL). The extract in the concentration of 500 µg/mL significantly inhibited LPS leakage through epithelial monolayer *in vitro* in comparison with non-treated control. The treatment of Caco-2 with HRE in the concentrations of 50 and 100 µg/mL showed GLUT2 expression similar to non-treated control and phloretin used as a positive control, whereas in the higher concentrations of HRE GLUT2 expression in Caco-2 cells was increased.

Conclusions: HRE might prevent low-grade chronic inflammation through the decrease of chemotactic factors released by immune and epithelial cells, which support its use in diabetes-linked complications.

Acknowledgments: This project was financially supported by the National Science Centre research grant Sonata 12 No. 2016/23/D/NZ7/00958 (Poland).

Keywords: inflammation, epithelium protection

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Abstract No. PSP.13

Could Oligourea Foldamers Become New Antibiotics?

Damian Dziubak[1], Kinga Burdach[1], Sławomir Sęk[1]

[1] Biological and Chemical Research Centre, Faculty of Chemistry, University of Warsaw, Poland

E-mail address of presenting author: ddziubak@chem.uw.edu.pl

Objectives: Since the 1940s medicine has changed a lot. The bacterial infections were no longer so deadly, and surgical procedures so risky. This was due to the increased availability of antibiotics. However, during the 80 years, everything has substantially changed. The bacteria create the ability to defeat the drugs designed to kill them. Therefore, there is a need to find new substances which might be helpful with treating bacterial infection. Our proposition of a new class of antibiotics involves the use of oligoureas foldamers. Oligoureas consist of interconnected urea units which can be substituted with groups analogous to those present in amino acids. Lipooligoureas are known to be resistant to the enzymes responsible for cleaving the peptide bonds and might possess enormous potential in the area of antibacterial applications.

Materials and Methods: The biomimetic model of the *Staphylococcus aureus* bacterial cell membrane was composed of DPPG, POPG, and cardiolipin (CL) in molar ratio 1:1:2. All lipids were purchased from Avanti Polar Lipids. To verify the potential activity of new antimicrobial substances, surfe-sensitive techniques such as have been used: electrochemistry, quartz crystal microbalance with dissipation monitoring (QCM-D), and attenuated total reflectance spectroscopy (ATR).

Results: ATR and QCM-D techniques confirmed the interaction between the membrane and the lipooligoureas. As a result of the interaction, there are differences in the conformation of lipids and thus in the permeability of the membrane itself. Moreover, the changes in hydration of the membrane and fluidity are observed. According to the electrochemical measurements, lipooligorures display the depolarization effect on the membrane, similar or even more efficient compared with known antibiotic drug - daptomycin.

Conclusions: Our results indicate the potential membranolytic nature of these compounds, which makes them good candidates for the new antimicrobial agents. The mechanism of interaction and the influence on the biomimetic membrane are very similar to the effect induced by daptomycin. Moreover, recent biological results confirmed the antimicrobial properties of these compounds.

Acknowledgments: This work was financially supported by Polish National Science Centre, Project No. 2019/35/B/ST4/01847

Abstract No. PSP.14

The new field of research in drug development - lipooligoureas based on peptidomimetic foldamers

Kinga Burdach[1], Joanna Juhaniewicz-Dębińska[1], Sławomir Sęk[1]

[1] Chemistry, Univeristy of Warsaw, Poland

E-mail address of presenting author: kburdach@chem.uw.edu.pl

Objectives: The discovery of antibiotics was a turning point for medical and societal advances. Current medical achievements would not be possible without the existence of effective antimicrobial agents. Also, antibiotics contributed to improvement in the quality and length of human life. This situation creates the need to develop the next generation of antimicrobials with new modes of action. Therefore, it is necessary to look for new, natural, or synthetic active substances with a broad spectrum of antimicrobial activity and at the same time with low cytotoxicity on the eukaryotic cells. Antimicrobial peptides and their alternatives like lipopeptides have certain drawbacks limiting their use as therapeutic agents. These include hemolytic activity and susceptibility to enzymatic degradation. As a possible pathway to overcome these limitations, we propose an entirely new class of fully artificial compounds based on oligourea foldamers coupled with a lipophilic moiety of fatty acid, further referenced as lipooligoureas.

Materials and Methods: *Staphylococcus aureus* model membrane was composed of lipids: DPPG, POPG, cardiolipin, and POPG-NBD purchased from Sigma Aldrich as well as ultrapure methanol and chloroform, which were used to dissolve lipids. Langmuir trough, quartz crystal microbalance with dissipation monitoring (QCM-D), and fluorescence microscopy were used to conduct the research.

Results: Langmuir monolayer technique shows the different effects of two lipooligoureas with a diverse length of the lipophilic moiety. Likewise, QCM-D experiments the influence of lipooligoureas on the more advanced model membrane. It is also observed that the formation of bilayer and giant unilamellar vesicles (GUV) composed of DPPG:POPG:CL depends on calcium ions.

Conclusions: Results show the comparison of two lipooligoureas. It is known that compounds with a longer lipophilic chain have more impact on the hemolytic properties, which is an undesirable effect. In my research, the opposite situation is observed, that lipooligourea with a shorter lipophilic chain has a greater influence on model bacterial membranes. These results are very satisfactory for us and show that we are going in the right direction against antibiotic resistance of bacteria.

Acknowledgements: This work was financially supported by Polish National Science Centre, Project No. 2019/35/B/ST4/01847.

Abstract No. PSP.15

Development of a sensitive screening method for stimultaneous determination of nine genotoxic nitrosamines in active pharmaceutical ingredients by GCMS

Anna Witkowska[1], Elżbieta U. Stolarczyk[2], Joanna Giebułtowicz[1]

[1] Department of Drug Analysis, Medical University of Warsaw Faculty of Pharmacy, Poland
[2] Analytical Department, Łukasiewicz – Industrial Chemistry Institute, Poland

E-mail address of presenting author: anna.witkowska@ichp.pl

Objective: Worldwide crisis with cancerogenic nitrosamines contamination in medical products began in 2018. Since then widespread investigations by European Medicines Agency (EMA) and United States Food and Drug Administration (US FDA) were undertaken for such drugs as angiotensin II receptor blockers (ARBs), ranitidine, metformin, rifampin and rifapentine. According to new established guidelines every marketing authorization holder have to identify risk of nitrosamines formation or cross-contamination in manufacturing/storage of chemical and biological medicines. The scale of the problem is revealed by the fact that till now more than one thousand batches of medical products have been recalled from the market due to detection of harmful nitrosamines. A wide number of studies also resulted in the introduction of new, more restrictive legislation that regulated concentration of nitrosamines in drug products. Therefore, a trace-level analysis of nitrosamines is becoming an emerging topic of interest in the field of quality control.

Materials and Methods: A new, fast, environment friendly method of extraction for nitrosamine in APIs with high-pressure direct injection GCMS has been developed in present study. Based on performed risk evaluation in manufacturing process in Łukasiewicz-IChP various APIs have been examined. Quantitative method for each active pharmaceutical ingredient (API) was validated according to the requirements of the ICH (International Conference on Harmonization) guideline.

Results: In our study sensitive screening GCMS method for simultaneous determination of nine nitrosamines has been described. Our novel analytical protocol for a trace level analysis of nitrosamines turned out to be specific, linear, accurate and precise. The LODs of nitrosamine were in the range 0.15 - 3 ppb.

Conclusions: The developed trace level GCMS method can be used for the routine quality control of APIs under GMP (Good Manufacturing Practice) rules ensuring the safety and effectiveness of pharmaceutical products.

Acknowledgements: The study has been supported by the Polish Ministry of Science and Higher Education grant No. 841343A.

Keywords: nitrosamines, gas chromatography-mass spectrometry (GCMS), API

Abstract No. PSP.16

Changes in surface properties of lipid raft models exposed to cholesterol-lowering drugs

Michalina Zaborowska[1], Dorota Matyszewska[2], Renata Bilewicz[1]

[1] Faculty of Chemistry, University of Warsaw, Poland
[2] Faculty of Chemistry, Biological and Chemical Research Centre, University of Warsaw, Poland

E-mail address of presenting author: mzaborowska@chem.uw.edu.pl

Objectives: The lipid rafts model of a biological membrane has been introduced by Simons and Gerrit van Meer. It is characterized by tighter lipid packing due to the enrichment of the membrane in sphingomyelin and cholesterol. These microdomains are able to anchor proteins in their structure and they are responsible for proper functioning of cells. 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) is one of the transmembrane proteins responsible for the production of cholesterol and its reductase (HMGR) is able to regulate cholesterol production cycle. Proper work of this enzyme reduces the risk of hypercholesterolemia and cardiovascular diseases, which are the main cause of death nowadays. In order to counteract the increased production of cholesterol, statins are used. They lower blood cholesterol level by inhibiting HMGR and their effectiveness depends on their physicochemical properties and interactions with cell membranes.

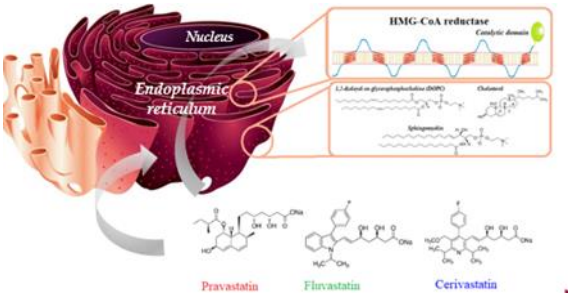


Fig.1. Mechanism of the action of statins in the cell

Materials and methods: The presented studies show a relationship between the degree of hydrophobicity of three selected statins (pravastatin<fluvastatin<cerivastatin) (Fig.1) and the extent of interactions with a lipid raft model consisting of phosphocholine, cholesterol, sphingomyelin. The mechanisms of molecular interactions between drugs and lipids were investigated by Langmuir technique, Brewster angle microscopy (BAM) and surface pressure measurements over time. The assessment of the influence of drugs on the model systems was also performed using fluorescence microscopy.

Results: The study proved significant differences in the surface characteristics of the DOPC:Chol:SM monolayers exposed to the three statins. The degree of their hydrophobicity has an impact on the ease of penetration of the lipid layers mimicking lipid raft.

Conclusions: Our studies suggest that the negative side effects often observed in the case of statin therapy may result from the changes of structure and functions of the biological membranes exposed to the drugs.

Acknowledgements: This work was financially supported by the Polish National Science Centre (Project No. 2018/31/B/ST4/00406).

Keywords: hypercholesterolemia, statins, model biological membranes

Abstract No. PSP.17

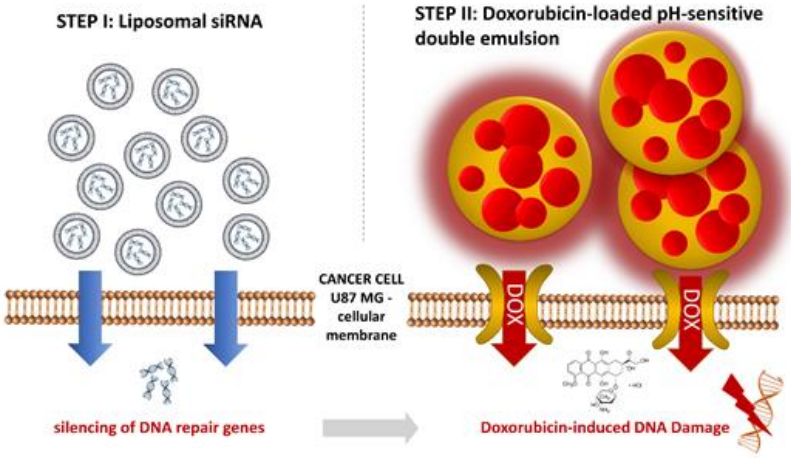
Cancer treatment based on a doxorubicin double emulsion delivery system aided by a mechanism of synthetic lethality

Agnieszka Markowska-Radomska[1], Ewa Dluska[1], Konrad Kosicki[2]

[1] Faculty of Chemical and Process Engineering, Warsaw University of Technology, Poland
[2] Faculty of Biology, Institute of Genetics and Biotechnology , University of Warsaw, Poland

E-mail address of presenting author: agnieszka.markowska@pw.edu.pl

Objectives: Glioblastoma multiforme (GBM) is the most common and aggressive malignant of the glial tumors. The development of an effective method for brain cancer therapy still remains a formidable challenge. The research aimed to develop an effective method for treating GBM tumors aided by a synthetic lethality mechanism. A two-step strategy was proposed: (I) silencing of DNA repair genes in cancer cells by siRNA-loaded liposomes; (II) cancer cells' DNA damage by doxorubicin (DOX) released from a double emulsion-"drops in drops" structure, Fig.



Materials and Methods: The experimental studies include: siRNA-loaded liposomes generation (Lipofectamine3000); double emulsions with DOX (in the internal droplets) preparation in a Couette-Taylor flow contactor; the characterization of emulsions (fluorescence microscope/image analysis software); the in vitro release of DOX (release profiles/spectrofluorometry method); cytotoxicity study (U87 MG glioblastoma cell line/PrestoBlue test).

Results: Double emulsions with DOX and pH-sensitive biopolymer (carboxymethylcellulose) were designed to be responsive to the acidic tumor microenvironment, and thereby trigger DOX release in the intra-tumoral space. DOX release studies were performed in the presence of transfected cells (+liposomal siRNA) and non-transfected. It was found that transfection did not affect the process of DOX release from emulsions. Verification of the effect of siRNA (synthetic lethality) on the viability of U87 MG cells was performed. A significant decrease in the cell viability (~25%) was observed for cells treated with siRNA and then emulsion with DOX, compared to cells treated with emulsion only.

Conclusions: A synthetic lethality mechanism was induced to enhance the efficacy of GBM therapy using an emulsion drug delivery system. This strategy provided promising results to improve the therapeutic effect of chemotherapeutics for brain tumor by decreasing tumoral cell viability.

Acknowledgements This work was supported by POB Biotechnology and Biomedical Engineering of Warsaw University of Technology within the Excellence Initiative; Research University (IDUB) programme (project BIOTECHMED-2)

Keywords: double emulsion, brain cancer, synthetic lethality

Abstract No. PSP.18

Flavone glycosides with a chlorine atom obtained by biotransformation as potential membrane integrity factors and antimicrobial agents

Agnieszka Krawczyk-Łebek[1], Monika Dymarska[1], Tomasz Janeczko[1], Edyta Kostrzewa-Susłow[1]

[1] Department of Food Chemistry and Biocatalysis, Wrocław University of Environmental and Life Sciences, Poland

E-mail address of presenting author: agnieszka.krawczyk-lebek@upwr.edu.pl

Objectives: Flavonoids are widespread in nature secondary metabolites of plants with numerous biological activities. Among a great variety of their derivatives, flavonoids with a chlorine atom exhibit great antimicrobial and immunomodulatory potential. In nature, flavonoids usually occur as glycosides and are more stable and bioavailable than their aglycone forms. The pharmacological application of flavonoids is limited by their low concentration in plants and low yield of flavonoid glycosides chemical synthesis. The main objective of the presented study was to obtain glycosylated flavonoids using combined chemical and biotechnological methods and to assess their biological activity and physicochemical properties using computer-aided simulations. The enzymatic glycosylation of the synthesized flavonoid aglycones with a chlorine atom was performed in the cultures of selected entomopathogenic filamentous fungi strains [1].

Materials and Methods: In the presented study, 6-chloroflavone and 2′-chloroflavone were obtained by synthesis and biotransformed in the cultures of *Isaria fumosorosea* KCH J2 and *Beauveria bassiana* KCH J1.5, respectively. The products were extracted from the post-reaction mixture and afterward, they were purified and separated using preparative thin-layer chromatography (PTLC). Their structures were established based on NMR spectroscopy methods.

Results: 6-Chloroflavone and 2′-chloroflavone were biotransformed into their glycosylated forms in an efficient manner. The predictions made with SwissADME online tool showed their improved water solubility and high gastrointestinal absorption. The Pass Online tool simulations showed that these compounds can act as membrane integrity agonists, antimicrobial agents, cardioprotectants, and anticancer agents.

Conclusions: The conducted studies indicate that *I. fumosorosea* KCH J2 and *B. bassiana* KCH J1.5 are able to glycosylate flavonoids. The location of a chlorine atom attached to the flavonoid core affects the biotransformation products. The glycosylated derivatives are new and show interesting potential biological activities that require further investigation.

Acknowledgements: The work was created as a result of the research project No 2021/41/N/NZ9/01195 financed by the National Science Center.

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Abstract No. PSP.19

Core Facility for Crystallographic and Biophysical Research to support the development of medicinal products

Jan Kutner[1], Maura Malińska[1], Marlena Kisiała[1], Szymon Sutuła[1], Krzysztof Woźniak[1]

[1] Biological and Chemical Research Centre, University of Warsaw, Poland

E-mail address of presenting author: j.kutner@uw.edu.pl

Objectives: As a result of the TEAM-TECH Core Facility Project from the Foundation for Polish Science, we have established the Core Facility for Crystallography and Biophysics (CFCB) at the Biological and Chemical Research Centre, University of Warsaw, under the supervision of Professor Krzysztof Woźniak (Head) and Jan Kutner, Ph.D. (Deputy Manager).

Results: The Core Facility services (Figure 1) are focused on the analysis of proteins and small molecule compounds leading to crystallization trials for academic and commercial users. The project enables studies of challenging biochemical and pharmaceutical problems, with an emphasis on drug development. Research at CFCB is carried out in an interdisciplinary way, including both wet biology ("BIO") and chemical crystallography ("CHEM") techniques as well as theoretical approaches including structure modelling, bioinformatics and computational methods. Biology and chemistry team members work in synergy complementing their knowledge, skills and experience. Apart from services and collaborations, postdoctoral and Ph.D. researchers carry out their research projects dedicated either to small-molecule or protein crystallography.

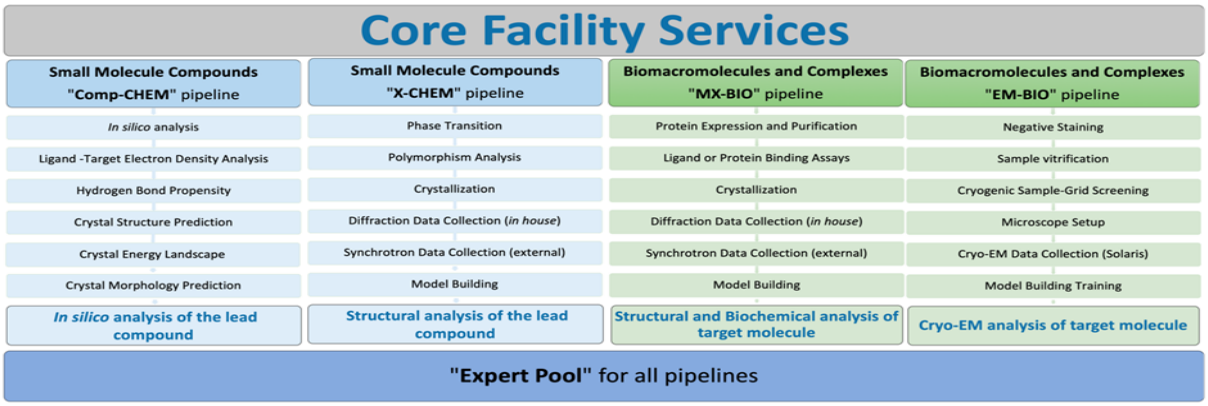

Figure 1. The main science services pipelines of the CFCB

Conclusions: Work in the Facility includes collaboration with other research groups and biotech/pharmaceutical companies, such as Celon Pharama, Moleculin Biotech, Inc., (USA), WPD Pharmaceuticals, OncoArendi Therapeutics , Pikralida, Innvigo, PGZ Nitrochem, Cellis, Biorad, Innox, Natoro, Leaderna Biostructures.

Moreover, we cooperate with Dr. Sebastian Glatt and Dr. Przemysław Grudnik (Structural Biology Core Facility, Jagiellonian University, Cracow) under the TT CF extension concerning on the commercial aspects (The Integrative Platform for Accelerated Drug Discovery – IPADD).

WE ARE OPEN TO DIFFERENT FORMS OF COLLABORATIONS WITH INDIVIDUAL RESEARCHERS, RESEARCH GROUPS OR BIOTECH/PHARMA COMPANIES.

Acknowledgments: The project is supported by Foundation for Polish Science/European Union under the European Regional Development Fund (TEAM TECH CORE FACILITY/2017-3/4, POIR.04.04.00-00-31DF/17-00)"

Keywords: crystallography, polymorph analysis, drug discovery, science services

Abstract No. PSP.20

Ebselen-based inhibitors of urease affect the viability of Cryptococcus neoformans and Helicobacter pylori

Wojciech Tabor[1], Paweł Krzyżek[2], Urszula Nawrot[3], Artur Mucha[1], Łukasz Berlicki[1], Agnieszka Grabowiecka[1]

[1] Department of Bioorganic Chemistry, Faculty of Chemistry, Wrocław University of Science and Technology, Poland
[2] Department of Microbiology, Wrocław Medical University, Poland
[3] Department of Pharmaceutical Microbiology and Parasitology, Wrocław Medical University, Poland

E-mail address of presenting author: wojciech.tabor@pwr.edu.pl

Objectives: In *Helicobacter pylori* and *Cryptococcus neoformans*, degradative enzyme urease has been recognized as an essential virulence factor. As urease is not present in mammalian cells, its inhibition is considered a promising approach in therapy. Organoselenium cysteine-reactive compounds affect urease activity by targeting the thiol group of Cys322. Ebselen has been shown to concomitantly inhibit urease and thioredoxin reductase, which led to viability loss in ureolytic microorganisms that lack glutathione/glutathione reductase system. Our aim was to verify the efficiency and identify the mode of inhibition of a set of ebselen derivatives towards urease from *C. neoformans* and *H. pylori*. We planned to determine the influence of selected highly active inhibitors upon ureolysis in living cells of both species, oxidative stress level in the yeast cells and biofilm formation ability in *H. pylori*.

Materials and Methods: Native yeast (IHEM 3969) urease and recombinant enzyme from *H. pylori* was obtained in 5-step chromatographic procedure. Oxidative stress was measured using dihydrorhodamine 123 fluorescent assay. *H. pylori* ATCC 51932 biofilm formation was evaluated in BioFlux 1000 system in the presence of compounds at the concentration equal to their MIC in planktonic cultures.

Results: Modification of the phenyl ring in ebselen led to identification of a set of urease inhibitors with IC_{50} below 1 μM towards whole cells of *C. neoformans* and *H. pylori*. The best inhibitors induced oxidative stress twice as efficiently than the lead compound ebselen and reduced *H. pylori* biofilm amount and viability more than ebselen and levofloxacin.

Conclusions: Selenoorganic compounds based on a lead structure of ebselen may serve as potential therapeutic agents for treatment of infections caused by ureolytic pathogens, especially the ones lacking glutathione/glutathione reductase system.

Acknowledgements: The work was financially supported by the National Science Centre, Poland, Grant No.2018/31/B/NZ6/02017 (to AM).

Keywords: urease, biofilm, inhibitor

Abstract No. PSP.21

Why the diet is so important during chemotherapy for lung cancer? Molecular modeling (and not only) of affinity of some Pt derivatives to B vitamins

Beata Szefler[1], Przemysław Czeleń[2]

[1] Department of Physical Chemistry, Collegium Medicum, Nicolaus Copernicus University, Poland
[2] Department of Physical Chemistry, Faculty of Pharmacy, Collegium Medicum, Nicolaus Copernicus University, Poland

E-mail address of presenting author: beatas@cm.umk.pl

Objective: The key aspect of the conducted research was related to the evaluation of Pt derivatives affinity to vitamins from the B group and the potential impact of such interactions on the reduction of therapeutic capabilities of chemotherapeutic agents in anticancer therapy. A theoretical study on the initial Pt-N_7(N_1) bond formation with vitamins from B group and their comparison with values characterizing native purines were performed.

Materials: Pt derivatives such as Cisplatin (CisPt), Carboplatin, Oxaliplatin and others have anticancer activity by interacting with DNA. Their mode of action has been linked to their ability to crosslink with the canonical purine bases, Guanine and Adenine found within double helical DNA, causing its damage and subsequently inducing apoptosis in cancer cells. However, theoretical and experimental studies performed on the molecular level suggest that such nonspecific interactions can also take place with many competitive compounds, such as vitamins containing aromatic rings with lone-pair orbitals.

Methods: Geometries of studied structures were optimized with an aid of Gaussian 09 using the B3LYP functional with the 6-31G** basis set in the PCM continuum model. Atomic orbitals of Platinum were represented by the lanl2dz basis. In the experimental part of study, the spectroscopic measurements were used.

Results: The affinities of Cisplatin and Carboplatin derivatives towards compounds belonging to the group of B vitamins were studied and compared to interactions with canonical purines. The estimated values of Gibbs free energy of reaction (ΔG_r) were found to be negative. The comparison of ΔG_r values obtained for compounds from vitamin B group and the ones characterizing complexes created by Guanine molecules indicates higher affinity of cisPt monomers towards purines. In spectroscopic studies it was revealed that after complexation of B6 vitamin and Carboplatin, the decrease of maximum absorbance for the vitamin relative to the baseline occurs.

Conclusion: Based on the observations, the regular intake of vitamin-rich beet root or carrot juices is strongly discouraged during anticancer therapy using Pt drugs.

Acknowledgements: This research was supported by PL-Grid Infrastructure (http://www.plgrid.pl/en).

Keywords: Cisplatin (CisPt), Carboplatin, platinum-based drugs, lung cancer, vitamin B, thiamine (B1), pyridoxal phosphate (B6), niacin (B3), riboflavin (B2), molecular modeling

Abstract No. PSP.22

Optimization of Dexibuprofen Loaded Nanostructured Lipid Carriers: Slow Release and Enhanced Anticancer Activity in In Vitro Studies

Vaikunthavasan Thiruchenthooran[1], Elena Sánchez-López[2], Anna Gliszczyńska[1]

[1] Department of Food Chemistry and Biocatalysis, Wrocław University of Environmental and Life Sciences, Poland
[2] Department of Pharmacy, Pharmaceutical Technology and Physical Chemistry, University of Barcelona , Spain

E-mail address of presenting author: thiruchenthooran.vaikunthavasan@upwr.edu.pl

Objectives: Dexibuprofen (DXI) is the active enantiomer of ibuprofen for which not only ant-inflammatory but also anticancer activity has been proven. Despite promising therapeutic potential, the usage of this drug as the anticancer agent is still limited by its low hydrophilicity and gastrointestinal toxicity. Strategy for the solution could be application of nanosystems such as nanostructured lipid carriers (NLC). This process requires optimization of crucial parameters what will be the subject of the presented work as well as the comparison of the antiproliferative activity of free drug and its fabricated nanoformulation towards selected cancer cell lines.

Materials and Methods: DXI-NLC were formulated by hot shear homogenization technique. Physiochemical parameters were characterized using Zetasizer Nano ZS. Encapsulation efficacy was measured using reverse phase higher performance liquid chromatography. Experimental design of the optimization was performed using central composite design in R Studio teams (2020). *In vitro* drug release was performed by direct dialysis technique. In addition, anticancer activity was evaluated by cytotoxic assay towards selected human cancer cell lines.

Results: Quadratic interaction model explains that concentration of compounds had significantly affected mean particle size (Z-Ave) (*p value* - 0.00, R^2 *value* - 0.99) and zeta potentials (ZP) (*p value* – 0.003, R^2 *value* – 0.89) of DXI-NLC formulations. However, polydispersity index (PI) and encapsulation efficacy (EE) were not represented by concentrations of used compounds and drug. Moreover, optimization provided the desired Z-Ave, PI, ZP and EE of DXI-NLC formulation, in values respectively 152.3 ± 1.6 nm, 0.149 ± 0.03, -19.8 ± 0.764 mV, 99.17%. In addition, in vitro drug release up to 24 h, comparatively DXI had been slowly released by formulation (B_{max} – 91.09, K_d – 2.458) than control (B_{max} – 100, K_d – 1.95). Moreover, cytotoxic assay confirms that DXI-NLC has low IC_{50} values than DXI in human cancer cell lines.

Conclusions: Optimization of the concentrations of the compounds and DXI provided < 200 nm size anionic DXI-NLC. It improves the stability and active release of DXI-NLC during delivery in cancer cells. In addition, cytotoxic assay confirms that DXI-NLC enhance the anticancer activity of DXI.

Keywords: Dexibuprofen, nanostructured lipid carriers, drug delivery system, anticancer activity

Abstract No. PSP.23

Pharmacokinetic/Pharmacodynamic Modeling of Selective PDE3, PDE4, and PDE7 Inhibitors in a Mouse Model of Autoimmune Hepatitis

Artur Świerczek[1], Bartosz Pomierny[2], Elżbieta Wyska[1]

[1] Department of Pharmacokinetics and Physical Pharmacy, Faculty of Pharmacy, Jagiellonian University Medical College, Poland
[2] Department of Toxicological Biochemistry, Faculty of Pharmacy, Jagiellonian University Medical College, Poland

E-mail address of presenting author: artur.swierczek@uj.edu.pl

Objectives: Autoimmune hepatitis (AIH) is a chronic life-threatening disorder for which corticosteroids in combination with immunosuppressives remain the standard therapy. Administration of these medications is often a lifelong therapy that has a limited efficacy and is associated with many severe dose- and time-dependent side effects. Mechanistic pharmacokinetic/pharmacodynamic (PK/PD) modeling is a methodology that aids in and accelerates the development of new drugs. In this study, a novel mechanistic PK/PD model was developed to assess the effects of PDE inhibitors as possible alternative therapeutics for AIH.

Materials and Methods: Female BALB/c mice were given an intraperitoneal dose of cilostazol, a PDE3-selective inhibitor, rolipram, a PDE4-selective inhibitor, or BRL-50481, a PDE7-selective inhibitor and, 30 min after compound administration, AIH was induced in mice by intravenous injection of concanavalin A (ConA). The concentrations of the studied compounds, IL-17, TNFα, and activities of aspartate and alanine transaminases were measured at several time points to obtain the time courses of the drugs and biomarkers in serum of mice. The PK/PD analysis was implemented using the ADAPT 5 computer program (BMSR, Los Angeles, USA).

Results: Based on the data obtained, a new PK/PD model of PDE inhibitor activities in mice with ConA-induced hepatitis was proposed. The presented mathematical model assumes inhibition of cAMP degradation in T-cells by PDE inhibitors, ConA-triggered production and release of IL-17 and TNFα, cAMP-mediated suppression of production of these cytokines, and stimulation of the release of transaminases from damaged liver cells by IL-17 and TNFα. Based on the model assumptions and predictions, selective blockage of PDE4 leads to the highest inhibition of cAMP degradation in T-cells and the most pronounced reduction of disease activity. Nonetheless, suppression of both PDE3 and PDE7 substantially contributes to this effect.

Conclusions: Balanced inhibition of PDE isoforms that are located in immune cells appears to be a promising treatment option for AIH; however, this hypothesis should be tested in humans. The presented PK/PD model may be used not only to assess and predict the activities of novel PDE inhibitors in ConA-induced hepatitis but also, upon modifications and translation into the clinical settings, to inform clinical trial designs.

Keywords: PK/PD modeling, autoimmune hepatitis, phosphodiesterase

Abstract No. PSP.24

Application of liquid chromatography coupled to mass spectrometry for the determination of dutasteride and its major metabolites in human plasma - pharmacokinetic approach and stability

Elżbieta Gniazdowska[1], Michał Kaza[2], Katarzyna Buś-Kwaśnik[2], Joanna Giebułtowicz[3]

[1] Łukasiewicz Research Network, Industrial Chemistry Institute, Doctoral School, Department of Bioanalysis and Drugs Analysis, Medical University of Warsaw, Poland
[2] Łukasiewicz Research Network, Industrial Chemistry Institute, Poland
[3] Department of Bioanalysis and Drugs Analysis, , Faculty of Pharmacy, Medical University of Warsaw, Poland

E-mail address of presenting author: elzbieta.gniazdowska@ichp.pl

Objectives: Dutasteride is a testosterone-5-alpha reductase inhibitor and a drug used in benign prostatic hyperplasia - ATC G04CB [1]. Dutasteride is extensively metabolized in humans by cytochrome P-450 3A4 and 3A5 to three major pharmacologically active metabolites, including monohydroxy metabolites (4′-hydroxydutasteride, 6β-hydroxydutasteride, and 1,2-dihydrodutasteride). The activity of 6β-hydroxydutasteride is comparable to that of dutasteride, whereas 4′-hydroxydutasteride and 1,2-dihydrodutasteride show less potency than dutasteride on both 5α-reductase isoforms [2]. Orally administered dutasteride is excreted unchanged in faeces (5.4%) and urine (< 1%). Although the drug is extensively metabolized in humans, there are no data on the concentrations of its major metabolites. Measurements of metabolite concentrations may be crucial to understanding variability in patient response to treatment [3]. Furthermore, data on the stability of dutasteride in frozen plasma samples from clinical trial participants are lacking. It has only been described for admixed samples not from clinical trials, with a storage time of only 59 days [4]. The study aimed to develop a new liquid chromatography coupled to tandem mass spectrometry method with liquid-liquid extraction to determine dutasteride and its active metabolites: 4′-hydroxydutasteride, 6β-hydroxydutasteride, and 1,2-dihydrodutasteride in plasma after a single administration of 0.5 mg of dutasteride. Another aim was to assess the long-term stability of dutasteride in clinical samples, after two and three years of storage in the freezer at ≤-65 °C.

Material and Methods: The range of linearity was 0.1-3.5 ng/mL for dutasteride and 0.08-1.2 ng/mL for 1,2-dihydrodutasteride, 4′-hydroxydutasteride, 6βhydroxydutasteride.

Results and Conclusions: The stability of dutasteride has been confirmed in clinical samples stored for up to three years in a freezer at ≤-65 °C. During this time, samples can be reanalyzed without the risk of unreliable results.

Keywords: 4′-hydroxydutasteride, dutasteride, 6β-hydroxydutasteride

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Abstract No. PSP.25

Novel lipid nanoparticles (LNP) as innovative mRNA carriers to various applications in medicine

Olga Długosz-Grochowska[1], Małgorzata Lipka[1], Dominik Lipka[1]

[1] SyVento sp. z o.o., Poland

E-mail address of presenting author: olga.dlugosz-grochowska@syvento.com

Objectives: Lipid nanoparticles (LNPs) are one of the most modern drug carriers currently used in the pharmaceutical industry. They enable to carry and effectively protect mRNA molecules, highly sensitive to external factors. LNPs are lipid vesicles made of ionizable lipid (encapsulates mRNA), pegylated lipid (supports particle stability), cholesterol and auxiliary lipid - responsible for the structure of final LNP. Syvento, as the first company in Poland, started to work on the use of microfluidization in the preparation of mRNA-delivering LNP. This technique results in great repeatibility and particle parameters (e.g. size, encapsulation efficacy).

The main objective of presented studies were to obtain a broad range of differentially composed LNPs, which may serve as effective mRNA delivery systems intended for either intramuscular/subcutaneous or systemic administration, targeting diversified therapeutic goals. Moreover, Syvento has developed the innovative LNP system based on plant origin lipid instead of animal derived cholesterol, which simultaneously reduces the size and polydispersity of LNP while increasing the affinity of LNP for the cell membrane.

Materials and Methods: Model LNPs were prepared using microfluidic method. Firefly luciferase (FLuc) coding mRNA was encapsulated into LNPs with varied lipid compositions. After preparation, basic nanoparticle parameters were characterized: diameter (d), polydispersity (PDI), mRNA encapsulation efficacy. Efficiency of *in vitro* FLuc transfection was investigated by treating cells by LNP formulations, with subsequent reading of cells' luminescence (in presence of D-luciferin).

Results: Developed mRNA-LNPs with different lipid compositions showed impresive parameters and release profile of mRNA inside the cells for both subcutaneous, intramuscular and systemic application. In addition, a successfull development of LNP formulation with a plant origin lipid instead of cholesterol has been proven by the increase in mRNA transfection efficiency.

Conclusions: Among many tested lipid compositions, Syvento has selected several configurations with particularly promising properties of LNP-mRNA, that may be used to deliver nucleic acid-based therapeutics tailored to the needs of specific therapies and mode of administration, although further investigations with therapeutic substances are needed to consolidate the obtained results.

Keywords: lipid nanoparticles, microfluidization, mRNA therapeutics

Abstract No. PSP.26

New salts of memantine with enhanced properties – synthesis, structure and physicochemical characteristics

Katarzyna Znajdek[1], Izabela Madura[2], Edyta Pindelska[3]

[1] Scientific Circle "Spektrum" at Department of Analytical Chemistry and Biomaterials, Faculty of Pharmacy, Medical University of Warsaw, Poland
[2] Department of Inorganic Chemistry, Faculty of Chemistry, Warsaw University of Technology, Poland
[3] Department of Analytical Chemistry and Biomaterials, Faculty of Pharmacy, Medical University of Warsaw, Poland

E-mail address of presenting author: znajdek.katarzyna@gmail.com

Objectives: We aimed to obtain new salts of memantine with reduced hygroscopicity and improved taste compared to currently used memantine hydrochloride, which is slightly hygroscopic and has a bitter taste. Another aim was to determine the structures of formed salts as well as their physicochemical properties.

Materials and Methods: The synthesis was performed by reacting memantine free base with sweet-tasting acids: acesulfame, saccharin, and anthranilic acid. Obtained substances were examined with: Fourier Transform Infrared Spectroscopy (FT-IR), Single Crystal X-ray Diffractometry (SCXRD), and Powder X-ray Diffraction (PXRD). Moreover, preliminary solubility, hygroscopicity, and taste tests were performed.

Results: Salts formation has been confirmed by FT-IR and SCXRD, while the homogeneity of the samples was verified by PXRD. Memantine acesulfamate crystallises in the space group Pbca, while memantine saccharinate hemihydrate crystallises in P1 (Fig. 1). Two polymorphs of memantine anthranilate: stable Form I and metastable Form II crystallise in space groups $P2_12_12_1$ and P1, respectively. The synthesis of the above salts resulted in a slight reduction in solubility compared to memantine hydrochloride. Each of the obtained substances has an improved taste. Memantine anthranilate and memantine saccharinate are also less hygroscopic.

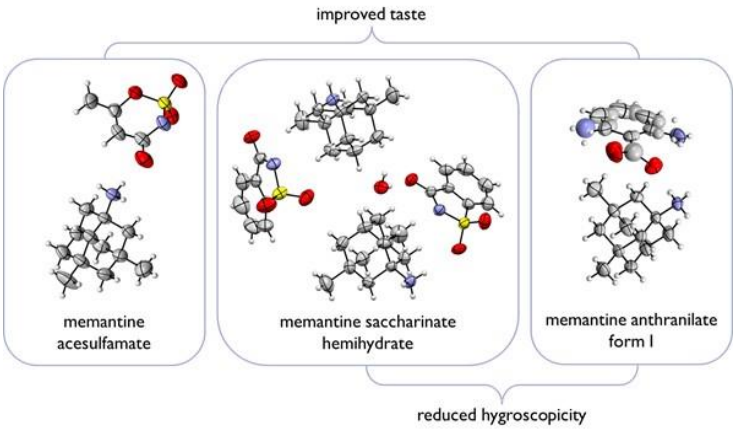

Figure 1. Crystallographic structures of memantine salts.

Conclusions: Memantine saccharinate and anthranilate salts with the most advantageous properties, can be used to develop a taste-masked drug formulation and, as a result, increase patients' compliance.

Acknowledgements: This work was financially supported by Medical University of Warsaw, grant number FW231/1/F/MG/N/21.

Keywords: crystal structure analysis; analysis of bulk drug materials; analysis of drug formulations

Abstract No. PSP.27

Glucocorticoids secretion in patients with cystic fibrosis

Rafał Podgórski[1], Marta Sumińska[2], Marta Rachel[3], Marta Fichna[4], Artur Mazur[3]

[1] Department of Biochemistry and General Chemistry, University of Rzeszow, Poland
[2] Department of Pediatric Diabetes and Obesity, Institute of Pediatrics, Poznan University of Medical Sciences, Poland
[3] Department of Pediatric, University of Rzeszow, Poland
[4] Department of Endocrinology, Metabolism and Internal Medicine, Poznan University of Medical Sciences, Poland

E-mail address of presenting author: rpodgorski@ur.edu.pl

Objectives: : The purpose of the work was to estimate the concentrations of urine cortisol metabolites in cystic fibrosis patients (CF) and the activity of specific enzymes involved in the steroidogenesis process.

Materials and Methods: Steroid profiles were determined in 24-h urine samples in 25 pediatric patients with CF and 73 healthy participants. Metabolites were analyzed by quantitative targeted GC-MS. Free and conjugated urinary steroids were extracted by SPE, conjugates were enzymatically hydrolyzed, and after the addition of known amounts of internal standards, methyloxime-trimethylsilyl ethers were formed.

Results: The secretion of the major cortisol metabolites and the enzymes involved in the formation and metabolism of glucocorticoids are shown in the Table.

	Boys			Girls		
Metabolites	**CF [median]**	**Control [median]**	**p**	**CF [median]**	**Control [median]**	**p**
Cortisol metabolites	3749,304	5142,761	**0,003**	2257,329	5165,592	**0,001**
C 21 metabolites	3383,846	4596,745	**0,003**	2089,224	4886,156	**0,001**
11β-HSD activity	**Boys**			**Girls**		
(THF+5αTHF)/THE ratio	1,006	0,719	**0,010**	0,913	0,737	0,240
F/E ratio	0,965	1,074	0,064	0,946	1,051	**0,039**
5α-reductase activity	**Boys**			**Girls**		
(11β-OH- Androsterone)/11ß-OH-Etiocholanolone	34,188	3,623	**<0,001**	22,506	4,484	**<0,001**
5α-dihydro-T/Testosterone (T)	0,182	0,572	**0,006**	0,655	0,685	0,663
20α-HSD activity	**Boys**			**Girls**		
[αC+αCl]/[THF+5αTHF+THE]	0,189	0,270	**0,029**	0,267	0,267	0,802

Cortisol metabolites- the sum of: Cortisol (F), Cortisone (E), TetrahydroC (THF), 5α-TetrahydroC (5αTHF), TetrahydroE (THE), α-Cortol (αC), ß-Cortol (ßC), α-Cortolone (αCl), ß-Cortolone (ßCl), 20ß-DihydroE, 20α –DihydroE, 20ß-DihydroF, 20α -DihydroF, 6ß-hydroxyF.

Conclusions: Characterization of glucocorticoid metabolism in CF patients compared to the control group has revealed significant differences in the excretion of cortisol metabolites in both gander. We have also found that the activity of the key enzymes involved in endogen glucocorticoid transformation is altered, especially in boys. Our findings may suggest that CF affects the function of adrenal glands or / and liver related to cortisol metabolism.

Abstract No. PSP.28

Safirinium - and Fmoc-based alkyne fluorescent probes for "click" labelling of zidovudine and structurally diverse azides

Patryk Kasza[1], Przemysław Szafrański[1], Jarosław Sączewski[2], Joanna Fedorowicz[2], Krzysztof Pociecha[1], Katarzyna Wójcik-Pszczoła[1], Vittorio Canale[1], Paweł Zajdel[1], Marek Cegła[1]

[1] Faculty of Pharmacy, Jagiellonian University Medical College, Poland
[2] Department of Chemical Technology of Drugs, Medical University of Gdańsk, Poland

E-mail address of presenting author: patryk.kasza@uj.edu.pl

Objectives: The aim of the study was to develop fluorescent probes for labelling of antiretroviral drug zidovudine (ZDV) and structurally diverse azides. For this purpose, we synthesized Safirinium- and Fmoc-based alkyne probes, optimized cycloaddition reaction conditions, and developed analytical procedures for ZDV, using UV and fluorometric detection.

Materials and Methods: A labelling was carried out using copper(I)-catalysed azide alkyne cycloaddition (CuAAC), a flagship "click chemistry" reaction, in the presence AMTC ligand.[1] The RP-HPLC analytical studies were carried out with UV and fluorescent detection. Cytotoxicity of the obtained conjugates against Hep-G2 cells was evaluated using the MTT test.

Results: We obtained a set of alkyne fluorescent probes based on the Safirinium core and used the propargyl-Fmoc probe.[2] For propargyl-Fmoc we demonstrated a sustainable mechanochemical approach. Within the analytical studies, we optimized precolumn derivatization of zidovudine with propargyl-Fmoc. We estimated limits of detection (LOD) and quantification (LOQ) for the optimized analytical methods using UV and fluorescence detection. Moreover, we found that the propargyl-Fmoc dye and its ZDV conjugate do not show cytotoxic effects.

Conclusions: We developed alkyne fluorescent probes based on Safirinium and Fmoc fluorophores for efficient labeling of zidovudine and structurally diverse azides. Subsequently optimized RP-HPLC methods allowed quantification of AZT using precolumn derivatization with the propargyl-Fmoc probe. The propargyl-Fmoc probe and its conjugates are not hazardous for analytical studies in terms of Hep-G2 cytotoxicity.

Acknowledgements: The project was financially supported by National Science Centre, Poland grant no 2018/29/N/NZ7/01918.

Keywords: Fluorescent labelling; Safirinium probes; Zidovudine.

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Abstract No. PSP.29

A breakthrough therapeutic solution protecting against the development of the post-traumatic and post-stroke epilepsy

Marta Magdycz[1], Magdalena Kania[1], Joanna Lipner[1], Marek Masnyk[1], Łukasz Mucha[1], Sylwia Piasecka[1], Katarzyna Sidoryk[1], Anna Krause[1], Stanisław Pikul[1]

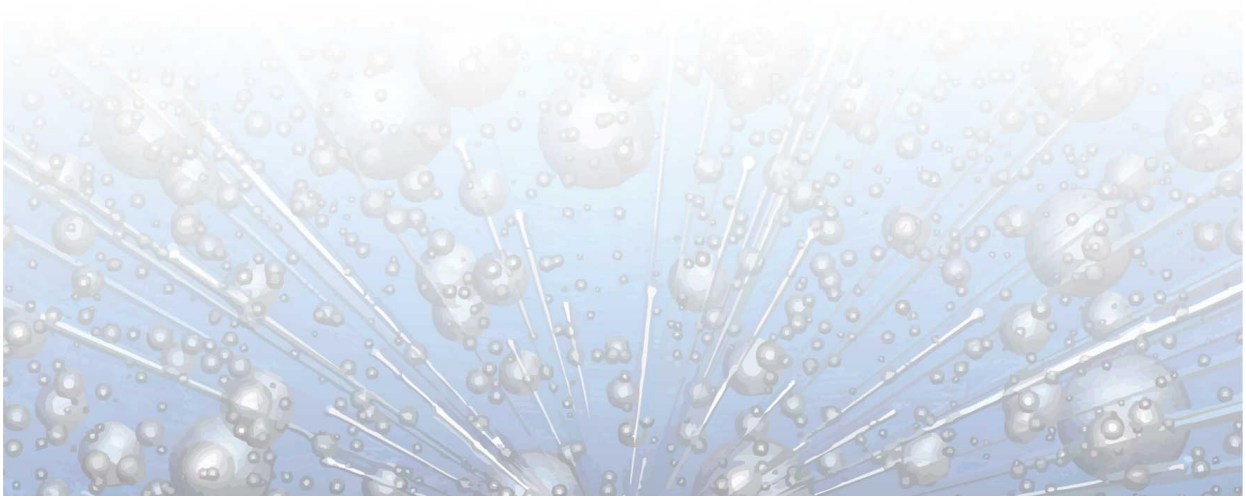
[1] Pikralida Sp. z.o.o., Poland

E-mail address of presenting author: m.magdycz@pikralida.eu

Epilepsy is a chronic neurological disease with recurrent epileptic seizures, which are an expression of transient brain dysfunction resulting from excessive, sudden, and spontaneous bioelectrical discharges in nerve cells. Currently, for about 30% of epilepsy cases there is no cure available and constant pharmacotherapy is required to relieve the symptoms. The manifestation of epileptic seizures and the constant use of medications cause significant limitations in the professional and social life of affected individuals. The development of epilepsy is a common complication after a traumatic brain injury or a stroke. Based on the current estimations, 2.3 million serious head injuries and up to 1.9 million strokes occur annually in Europe and the United States. Each of these events can initiate epileptogenesis leading, in the consequence, to the development of epilepsy.

No drugs inhibiting epilepsy development have been developed so far.

The aim of our Project is to develop a breakthrough therapeutic solution protecting against the development of post-traumatic and post-stroke epilepsy based on the inhibition of the epileptogenesis process through the modulation of the enzymatic protein, matrix metalloprotease 9 (MMP-9). MMP-9 inhibitor named PKL-021 selected within the project is a low molecular weight chemical compound with high activity and optimal drug-like properties. The project covers, among others, development of the active substance and investigational medicinal product manufacturing technology, analysis of the therapeutic potential of the MMP-9 inhibitor in the animal models, and carrying out the preclinical and phase I clinical studies.



Abstract No. PSP.30

Synthesis of Dibenzo[b,f][1,4]oxazepin-11(10H)-one derivative with potential Trypanocidal activity

Emilia Pykacz[1], Michał Nowacki[1], Maciej Dawidowski[2]

[1] Department of Drug Technology and Pharmaceutical Biotechnology, Medical University of Warsaw, Poland
[2] Department of Drug Technology and Pharmaceutical Biotechnology, Faculty of Pharmacy, Medical University of Warsaw, Poland

E-mail address of presenting author: s078273@student.wum.edu.pl

Objectives: *Trypanosoma brucei* and *Trypanosoma crusi* are parasitic species that infect humans and animals leading to severe mortality and economical loses throughout the world. Currently used drugs have serious side effects and are ineffective in the chronic phases of the diseases caused by the parasites.

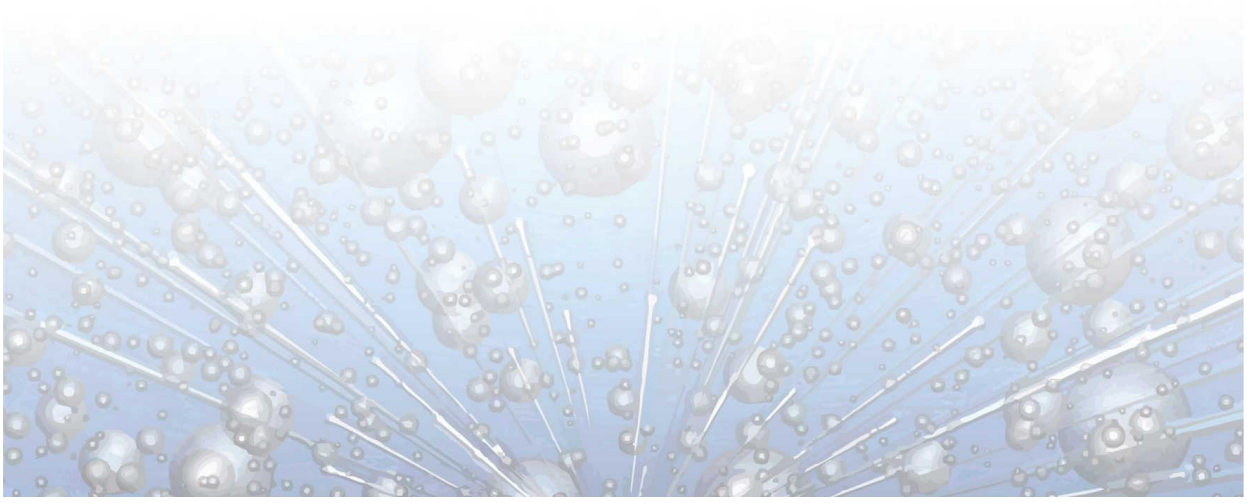
Materials and Methods: Recently, it was found that blocking PEX14-PEX5 protein-protein interaction in *Trypanosoma* parasites leads to ametabolic catastrophe causing death of parasite. Hence, inhibiting the PEX14-PEX5 complex formation emerged as a potential way to combat *Trypanosoma* infections.

Results: Dibenzo[*b*,*f*][1,4]oxazepin-11(10*H*)-one scaffold was identified through High-Throughput Screening as a promising PEX14 ligand. Ugi four-component reaction enables installation of complex residues derived from aldehydes and isocyanides onto the aforementioned scaffold.

Conclusions: Dibenzo[*b*,*f*][1,4]oxazepin-11(10*H*)-one derivative was successfully synthesized by Ulmann reaction followed by reduction and a phenylvinyl moiety was installed to increase the compound's affinity to binding pockets of PEX14 protein.

Acknowledgements: Acknowledgments: this study was supported by a NCN Opus grant (UMO-2018/31/B/NZ7/02089)

Keywords: Multicomponent reaction, Trypanosoma, Structure-based drug discovery



Abstract No. PSP.31

Pharmacokinetics of esketamine inhaled as dry powder in healthy subjects and patients

Piotr J. Rudzki[1], Sylwia Janowska[2], Piotr Gałecki[3], Katarzyna Jarus-Dziedzic[4], Daniel Rabczenko[5], Agnieszka Segiet[5], Maciej Wieczorek[6]

[1] R&D Finished Dosage Form, Celon Pharma, Poland
[2] Clinical Department, Celon Pharma, Poland
[3] Specjalistyczny Psychiatryczny Zespół Opieki Zdrowotnej w Łodzi, Poland
[4] Clinical Site, BioResearch Group, Poland
[5] Instytut Edukacji I.E. Sp. z o.o., Poland
[6] Celon Pharma, Poland

E-mail address of presenting author: piotr.rudzki@celonpharma.com

Objectives: Treatment resistance is a common problem in patients suffering from depression in the course of major depressive disorder and bipolar depression. Inhaled esketamine is a promising option in both conditions. The investigational medicinal product developed by Celon Pharma [1-5] uses inhaled route for convenient self-administration and to minimize the esketamine dose avoiding its extensive first-pass metabolism. Aim of the study was to evaluate pharmacokinetics of esketamine and its main metabolite noresketamine in healthy subjects and in patients.

Materials and methods: Esketamine and noresketamine concentrations in human plasma were measured by UPLC-MS/MS method aty Anapharm Europe. Non-compartmental model was selected to assess pharmacokinetics. In phase I clinical study esketamine doses ranging 12-48 mg were studied in 12 healthy subjects after single dose and in 25 subjects after repeated doses on days 1, 4, 8 and 11. In phase II studies esketamine doses ranging 24-48 mg were studied in pharmacokinetic population of 63 patients with treatment-resistant depression in the course of major depressive disorder and in 64 patients with treatment-resistant bipolar depression.

Results: Blood sampling points defined in clinical trial protocol enabled reliable assessment of pharmacokinetic profiles. Metabolite-to-parent ratio was constant across studied populations and doses, no accumulation was observed between days 1 and 11. Median T_{max} and mean $T_{1/2}$, CL/F and Vz/F for esketamine and esnorketamine are comparable between days 1 and 11 as well as across studied doses.

Conclusions: Pharmacokinetics of inhaled esketamine were comparable between healthy subjects and both populations of patients. Results of phase I and II clinical trials support further development of esketamine inhaled as dry powder.

Acknowledgements: This project was supported by European Funds under National Centre for Research and Development "Program Operacyjny Inteligentny Rozwój 2014–2020" (grant POIR.01.01.01-00-1021/15-00).

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Abstract No. PSP.32

The effect of environmental concentrations of selected pharmaceuticals on the metabolome of Ostracods

Dawid Kucharski[1], Sylwia Michorowska[1], Justyna Chojnacka[2], Grzegorz Nałęcz-Jawecki[2], Joanna Giebułtowicz[1]

[1] Department of Bioanalysis and Drugs Analysis , Medical University of Warsaw, Poland
[2] Department of Environmental Health Science, Medical University of Warsaw, Poland

E-mail address of presenting author: ssolobodowska@wum.edu.pl

Objectives: Pharmaceuticals are increasingly being detected in aquatic ecosystems. They enter the environment through the discharge of effluents from wastewater treatment plants, the disposal of unused medications and/or sludge and animal manure used in agriculture. Importantly, pharmaceuticals may undergo physical, chemical, and biological degradation as well as sorption in the environment. Therefore, their environmental water concentrations, in the range of ng-µg/L, are usually not high enough to induce acute toxicity. However, the continuous addition of pharmaceuticals to the environment in small but significant amounts, as well as the desorption of some of them from sediments make some of them environmentally persistent. This may result in chronic effects towards aquatic life, especially benthic organisms such as Ostracods, as the concentrations of pharmaceuticals in sediments and just above them are much higher than those in water. The identification of contaminant-induced metabolic biomarkers is thus of great importance, as they could be used for biomonitoring. This can be efficiently achieved using throughput and sensitive approaches such as metabolomics. Since data related to the effects of pharmaceuticals at environmentally relevant concentration are scarce the aim of this work was to assess the effect of amlodipine, carbamazepine, propranolol, tiapride, and tolperisone as a mixture on the metabolom of Ostracods.

Materials and Methods: Ostracods were exposed to environmental concentrations of amlodipine, carbamazepine, propranolol, tiapride, and tolperisone as a mixture for 6 days. Next, metabolites extracted from frozen Ostracods were subjected to LC-MS analysis using Q Exactive™ Focus. Raw data were processed in Compound Discoverer 2.1. Next, Metaboanalyst 4.0 was used to perform statistical analysis.

Results: In total 155 metabolites were assigned, among which 56 were found to be differentially abundant as determined by the ANOVA test (FDR<0.05). The three most significantly impacted pathways were alanine, aspartate and glutamate metabolism, starch and sucrose metabolism as well as arginine synthesis.

Conclusions: Environmental concentrations of pharmaceuticals were shown to significantly affect the metabolom of benthic organisms such ostracods.

Acknowledgements: This work was supported by the National Science Centre, Poland (MINIATURA 5, NCN47).

Keywords: pharmaceuticals, benthic environment, ostracods, untargeted metabolomics

Abstract No. PSP.33

Obtaining pure enantiomers of chiral drugs and chiral building blocks with biocatalytic tools

Natalia Kocot[1], Rafał Mastalerz[1], Jacek Dulęba[1], Tomasz Siódmiak[1], Michał Marszałł[1]

[1] Medicinal Chemistry Department, Collegium Medicum in Bydgoszcz, Poland

E-mail address of presenting author: natalia.kocot18@gmail.com

Objectives: The aim of the study was to separate enantiomers of a chiral drug: (*R*,*S*)-flurbiprofen and the enantiomers of chiral building block compound: (*R*,*S*)-1-phenyloethanol with the application of biocatalytic "green chemistry" tool - lipase B from *Candida antarctica* (CALB) immobilized on Octyl-Sepharose CL-4B carrier. Lipase CALB is enantioselective towards *R* enantiomer.

Materials and methods: Lipase CALB was immobilized on Octyl-Sepharose CL-4B carrier by a proper immobilization protocol. The obtained biocatalytic system was then applied as a biocatalyst of the kinetic resolution reactions. In the case of (*R*,*S*)-flurbiprofen, the compound was dissolved in organic solvent and then the solution was added to the catalyst, followed by adding methanol and the esterification reaction was performed. In the case of (*R*,*S*)-1-phenyloethanol, the second substrate of the reaction was isoprenyl acetate and the transesterification reaction was carried out. After the incubation, products and substrates were determined by HPLC.

Results: We obtained methyl ester of (*R*)-flurbiprofen with enantioselectivity expressed with enantiomeric excess of the products eep=80,2%. Reaction yield, expressed with conversion was C=35,1%. In turn, enantiomer *R* of 1-phenyloethanol was produced with eep= 92,6% and C=50,3%. It should be noted that in conventional kinetic resolution maximum value of conversion is 50% and enantiomeric excess can be at most 100%. Moreover, it was noticed that reactor's material has an influence on enantioselective reaction parameters.

Conclusions: The applied CALB-Octyl-Sepharose CL-4B biocatalytic system turned out to carry out presented reactions with excellent enantioselectivity and efficacy. The studies were performed in the laboratory scale, but the attempt to scale the process up can be made. It should be emphasized that enantiomers of the chiral drug often exhibit different pharmacological and toxicological profiles. Therefore, it is important to treat patients with pure enantiomers. Furthermore, obtaining them in environmentally friendly way should be considered. Additionally, during the study the potential industrial scale problem of choice of the reactor's material has been noted and solved.

Keywords: chiral drugs, biocatalysis, kinetic resolution

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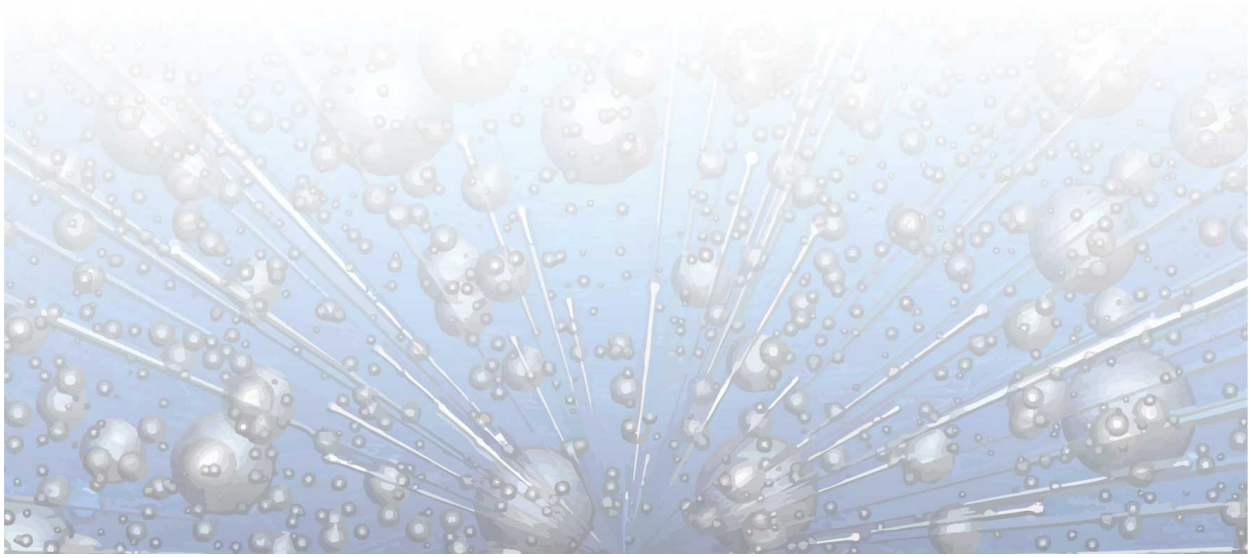
Abstracts of Short Communications



Abstract No. SC.1

The development of new isatin derivatives as promising competitive inhibitors of CDK-2 and GSK-3β

Przemysław Czeleń[1], Beata Szefler[1]

[1] Department of Physical Chemistry, Nicolaus Copernicus University Collegium Medicum in Bydgoszcz, Poland

E-mail address of presenting author: przemekcz@cm.umk.pl

Objectives: The glycogen synthase kinase 3β (GSK-3β) and cyclin dependent kinase 2 (CDK-2) are involved in regulating numerous physiological processes. The overexpression of these enzymes is related with many diseases such as schizophrenia, Alzheimer's disease, diabetes and cancer. One of the basic methods of treatment in these cases is the usage of ATP competitive inhibitors. A significant group of such compounds are indirubin and its analogues, e.g. isatin derivatives.

Materials and Methods: The new isatin derivatives were created by addition of active groups to the oxindole core presented in the Figure 1. For each of them were estimated values of toxicity and LogP. The preliminary analysis of binding capabilities of considered compounds towards both enzymes was conducted with the use of docking procedure. Based on obtained molecular properties and docking simulations, a selected group of complexes which were analysed in molecular dynamics stage was nominated.

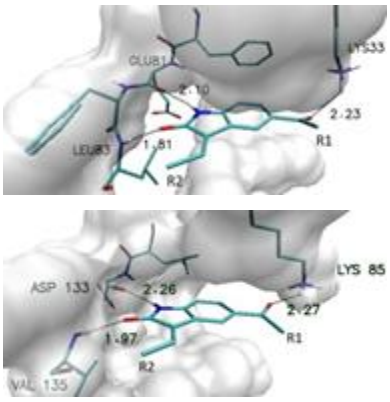


Figure1. The interactions of the oxindole core with amino acids from CDK2 (a) and GSK-3β (b) active site.

Results: Realised analysis allowed for identification of compounds which create stable complexes with CDK-2 and GSK-3β enzymes and are characterized by highest values of binding affinity. The key interactions responsible for stabilization of considered complexes were identified and their dynamic stability was also determined. In the case of both enzymes the highest binding affinity was stated for this same molecule namely indol_4_9. Analysis of structures of the rest of inhibitors exhibiting highest affinity towards both active sites show that they contain similar set of active groups. Comparative analysis including analysed compounds and reference isatin derivatives with a confirmed inhibitory potential, clearly indicates that the proposed compounds exhibit similar properties.

Conclusions: The group of isatin derivatives proposed in this paper exhibits significant affinity towards both enzymes. The proposed modifications of the native molecular core significantly influenced the binding capabilities of obtained molecules, however obtained compounds show low selectivity in the context of inhibition of both considered enzymes.

Acknowledgements: This research was supported by PL-Grid Infrastructure

Keywords: CDK-2; GSK-3β; isatin derivatives; docking; molecular dynamics; inhibition

Abstract No. SC.2

RVU330 best-in-class dual A2A/A2B adenosine receptor antagonist

Katarzyna Dziedzic[1], Michał Gałęzowski[2], Paulina Węgrzyn[1], Aniela Gołas[3], Magdalena Bońkowska[1], Karolina Grycuk[1], Aneta Bobowska[2], Stefan Chmielewski[1], Mateusz Ogórek[1], Joanna Szeremeta-Spisak[2], Marcin Nowogródzki[2], Grzegorz Satała[1], Iwona Łozińska-Raj[2], Przemysław Wyrębek[2], Marcelina Dudek[2], Anita Janiga[2], Jacek Reus[2], Marek Wronowski[2], Magdalena Zastawna[2], Mateusz Świrski[2], Luigi Stasi[2], Peter Littlewood[4], Krzysztof Brzózka[5], Mateusz Nowak[6]

[1] Biology, Ryvu Therapeutics, Poland
[2] Chemistry, Ryvu Therapeutics, Poland
[3] In Vivo Research, Ryvu Therapeutics, Poland
[4] DMPK, Ryvu Therapeutics, Poland
[5] Ryvu Therapeutics, Poland
[6] Early Discovery and Innovation, Ryvu Therapeutics, Poland

E-mail address of presenting author: katarzyna.dziedzic@ryvu.com

Objectives: Adenosine is the endogenous metabolite that is involved in signal transduction and maintaining homeostasis in diverse systems. In pathological states such as cancer, the immune homeostasis might be disturbed via adenosine upregulation which leads to local immunosuppression that prevents cancer from being eradicated by host defense systems. Immune cells respond to adenosine via two GPCR receptors expressed on the cell surface: A2A and A2B. The first one is expressed on both lymphoid and myeloid cells, the latter only on the second population of immune cells. Therefore, the full restoration of adenosine-mediated cancer immunosuppression is possible only by the employment of the dual A2A/A2B antagonist.

Materials and methods: Radioligand binding assays were performed with membrane fractions and [3H]-ZM-241385. cAMP was measured both in cell lines and human primary cells. Antagonists' activity was tested at low agonist concentrations (corresponding to EC80 values) and high adenosine concentrations (100 µM). Restoration of effector functions in primary human immune cells was also evaluated by cytokine detection. Finally, RVU330 efficacy was tested in *in vivo* study using MCA205 model.

Results: Here, we present the *in vitro* characterisation of the RVU330 best-in-class dual A2A/A2B antagonist. Our compound reverses the immunosuppression generated by adenosine on a large variety of immune primary cells like dendritic cells, macrophages, and lymphocytes. Moreover, it maintains the nanomolar activity in a high adenosine condition that mimics a tumor-like microenvironment. The extensive pharmacological characterization includes receptor binding, cAMP accumulation, and functional assays in primary human immune cells which demonstrate the reversibility of immunosuppression induced by adenosine.

Conclusions: RVU330 - the new best-in-class dual A2A/A2B adenosine receptor antagonist has the ability to inhibit both receptors at very high adenosine conditions which address distinct, cell type mediated mode of action of adenosine related immunosuppression in TME. The dual A2A /A2B antagonistic profile as well as the long residence time are the key features differentiating RVU330 from other adenosine receptor antagonists.

Acknowledgements: Project was supported by the European Regional Development Funds under the Measure 1.1.1. Operational Program - Smart Growth (POIR.01.01.01-00- 0987/16-00)

Keywords: adenosine immunosuppression, A2A/A2B dual antagonist

Abstract No. SC.3

Effect of food on human pharmacokinetics of CPL207280 – a new GPR40 agonist: *in vitro* and *in vivo* studies

Ewelina Juszczyk[1], Kamil Kisło[1], Ewa Tratkiewicz[1], Katarzyna Bazydło-Guzenda[1], Agnieszka Gierczak-Pachulska[2], Piotr J. Rudzki[1], Jadwiga Paszkowska[3], Dorota Danielak[4], Jaroslaw Sczodrok[5], Grzegorz Garbacz[3], Katarzyna Jarus-Dziedzic[6], Katarzyna Buś-Kwaśnik[7], Maciej Wieczorek[1]

[1] Celon Pharma S.A., Poland
[2] /, Celon Pharma S.A., Poland
[3] Physiolution Polska Sp. z o.o., Poland
[4] Department of Physical Pharmacy and Pharmacokinetics, Poznan University of Medical Sciences, Poland
[5] Physiolution GmbH, Germany
[6] Clinical Site, BioResearch Group, Poland
[7] Industrial Chemistry Institute, Łukasiewicz Research Network, Poland

E-mail address of presenting author: ewelina.juszczyk@celonpharma.com

Objectives: Food can change the rate and extent of drug absorption from gastrointestinal tract, influencing its clinical efficacy and safety of patients. During the development of a new medicinal product its administration in fasting and/or fed conditions should be evaluated. We studied the influence of food on human pharmacokinetics of a new G-protein coupled receptor 40 (GPR40) agonist CPL207280.

Materials and methods: CPL207280 sustained-release matrix tablets were manufactured by Celon Pharma S.A. The bio-relevant dissolution stress test device[1] (Physiolution GmbH) was used to simulate *in vitro* mechanical and physicochemical conditions of the gastrointestinal tract in fasting/fed conditions. Phase I clinical study included evaluation of food effect in 12 healthy human subjects.

Results: *In vitro* tests showed significant delay of the drug absorption in fed conditions[2]. It was well reflected by the median T_{max} shift from 1 to 8 hours in clinical study. Exposure was similar between fasted and fed conditions (geometric mean fed-to-fasting ratios of C_{max} and AUC_{0-24h} were 113% and 87%, respectively).

Conclusions: *In vitro* study well predicted absorption behavior of the drug candidate in fasting and fed conditions. Food seemed not to impact the extent of drug absorption of a novel potential antidiabetic medicine.

Acknowledgements: This project was subsidized from European Funds owing to The National Centre for Research and Development "Program Operacyjny Inteligentny Rozwój 2014–2020" (grant POIR.01.01.01-00-0334/17-00). DD and GG are supported by European Union's Horizon 2020 research and innovation program under the Marie Skłodowska-Curie grant 778051 "ORBIS-Open Research Biopharmaceutical Internships Support" and the Ministry of Science and Higher Education of Poland (grant 3899/H2020/2018/2).

Keywords: clinical drug development; human pharmacokinetics; biorelevant dissolution

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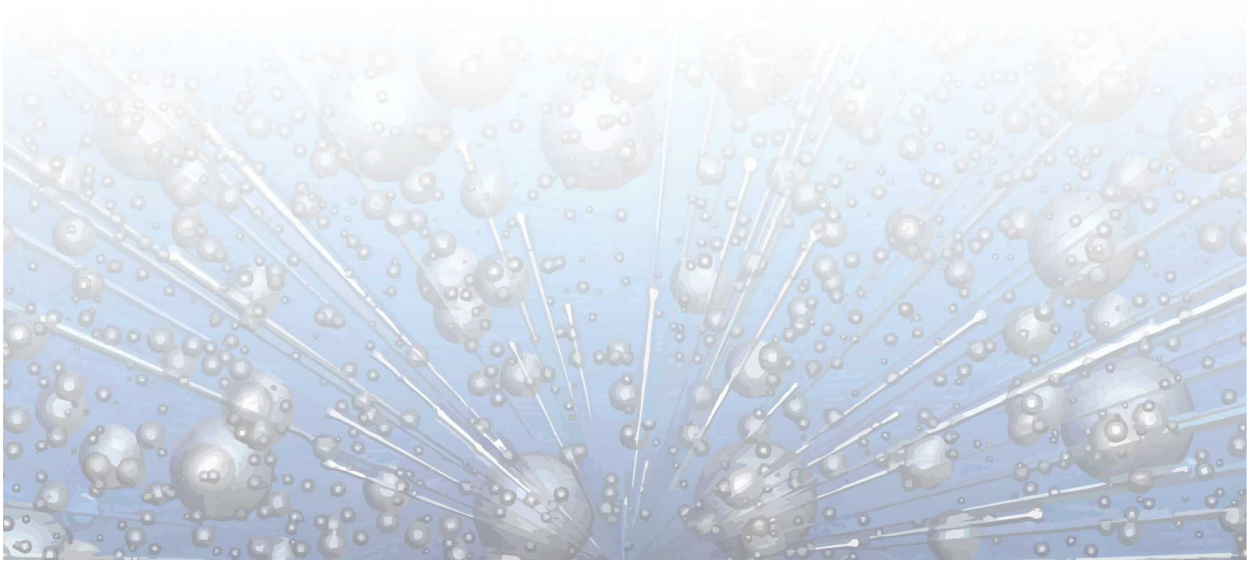
Abstracts of Sponsored Lectures



Abstract No. SL.2

HPLC method development according to Analytical Quality by Design using the Method Development Software

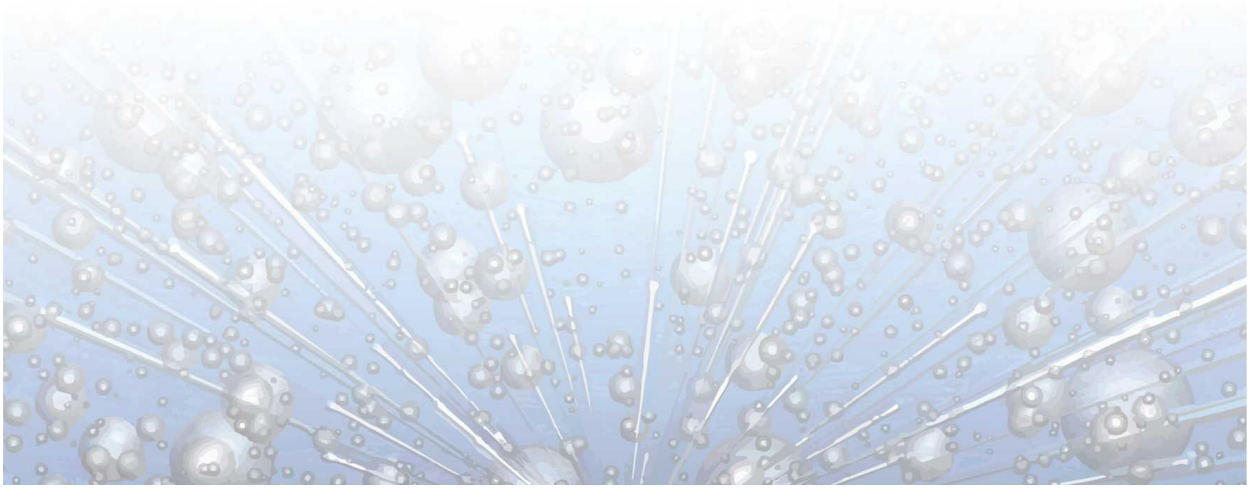
Marcin Gawryś[1]

[1] Dr Marcin Gawryś, Shim-Pol A.M. Borzymowski

E-mail address of presenting author: aleksandrab@shim-pol.pl

Liquid Chromatography is used in the quality testing of pharmaceuticals to ensure the safety and security of patients taking the drugs. Analytical methods for pharmaceuticals are developed in the Chemistry, Manufacturing and Control departments of pharmaceutical manufacturers, and after confirming their validity through analytical method validation, the technology is transferred to test sites such as the quality control departments of manufacturing plants for actual operation. In recent years, the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use has proposed Analytical Quality by Design (AQbD), which is a different perspective from analytical method validation, to evaluate and validate analytical methods by clarifying the background and rationale behind the analytical method. In the development of analytical methods based on AQbD, it is recommended to extract factors that affect analytical results from the results obtained by efficient experiments such as Design of Experiments using statistical methods, and to visualize the effective area of parameters as a design space. This approach, which is based on scientific evidence and risk, is expected to enable the development of robust and low-risk analytical methods, as well as the development of comprehensive analytical methods that do not rely on experience and intuition. Software, Method Development Solution, streamlines the entire analytical method development flow, from experiment planning to design space construction, and enables the development of optimal analytical methods without the need for skilled personnel.

Keywords: Liquid Chromatography, Analytical Quality by Design, Method Development Software



Abstract No. SL.4

Biophysical characterization of antibodies interacting with cells captured in microfluidic cell traps

Agnes Marshal[1], Stefanie Mak[1], Nena Matscheko[1], Kristina Popova[1]

[1] Dynamic Biosensors GmbH, Martinsried,, Germany

E-mail address of presenting author: popovak@dynamic-biosensors.com

The binding of antibodies to cell surface antigens is a pivotal step in the recruitment of the cellular immune response. The characterization of association and dissociation rates, affinity, and avidity is essential for the development of efficient multivalent immunotherapies. Binding kinetic measurements in physiological conditions are expected to have superior predictive value in the treatment of cancer, infection and autoimmunity. However, despite substantial efforts, straightforward methods to measure binding kinetics directly on target cells are still not available, which leaves a "biophysical gap" in the research and development of biological drugs.

Here, we present a novel method to capture cells and to measure the association and dissociation kinetics of fluorescently labeled antibodies to/from cell surface antigens in real-time. To this end, flow-permeable and bio-compatible cell cages were designed to accommodate and physically retain single cells in the microfluidic channel of a commercially available biochip. Suspension or adherent cells can be loaded into the cages by an automated workflow using only a few microliters of sample. Antibodies labelled with standard dyes are subsequently injected into the microfluidic chip under continuous flow.

We validated the method by investigating several different Bispecific T-cell Engagers (BiTE®) against antigens expressed on the surface of T-cells and target cells. Measurements of real-time on- and off-rates of BiTE® molecules against T-cells, tumor cells, and control cells are shown. All BiTE® molecules analyzed presented similar binding properties to CD_3 from T cells. In contrast, the molecules presented different binding behavior when interacting with tumor cells, not only in terms of engagement propensity (driven by the on rate) but also stability and residence time (indicated by the off rate). The presented method enables the investigation of antibody-cell interactions in a highly automated workflow that can characterize more the 30 new biological drugs per day.

Abstract No. SL.5

Biosimilars - from registration to use in everyday practice

Julia Feldman[1]

[1] Sandoz Poland, Poland

E-mail address of presenting author: julia.feldman@sandoz.com

A biological drug is a medicine whose active substance is produced by or derived from living organisms, tissues or cells.

Modern biological drugs were made possible by discoveries in the field of immunology and genetic engineering, which aimed to program cells such as bacteria, fungi or mammalian cells to produce not only their own proteins but also human proteins.

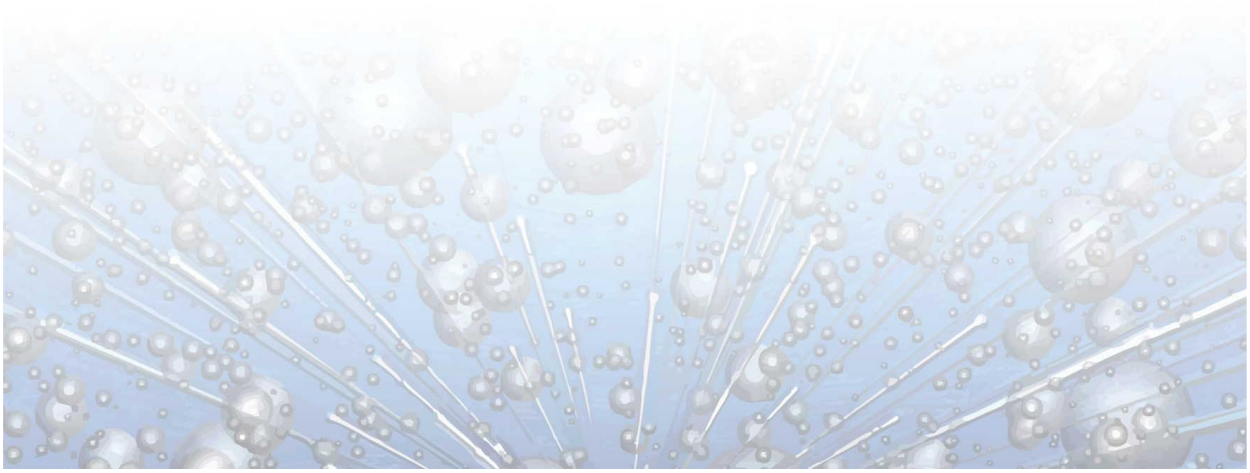
Biosimilar biological medicines are biologics that match the efficacy and safety profile of a reference (original) biological medicine already existing on the market. Small differences between a biosimilar biological medicine approved and registered in the European Union and the reference biological medicine cannot and do not affect the efficacy or safety of the therapy.

All living organisms are characterized by natural variability. This also applies to proteins produced by biotechnology and genetic engineering (biopharmaceuticals, biological medicines). Due to their protein structure, every biological drug shows a certain degree of variability ("microheterogeneity"), both within a batch and between batches of the same drug.

Microheterogeneity applies to all biologic drugs - both reference biologics and biosimilars.

The range of variability that is allowed for a biosimilar is the same as the variability between batches of the reference drug.

Since a biosimilar is equivalent to the reference medicine, doctors, pharmacists and patients can expect the same safety and efficacy.



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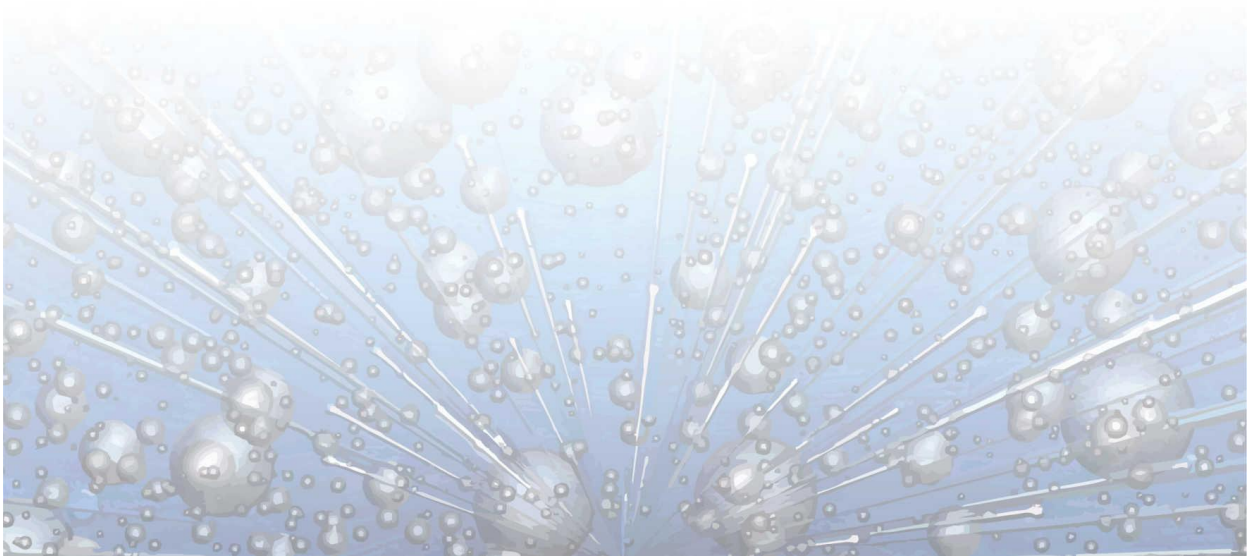
Abstract of Short Presentation



Abstract No. 'SP.1

Synergy of interdisciplinary innovations

Olaf Kelber[1]

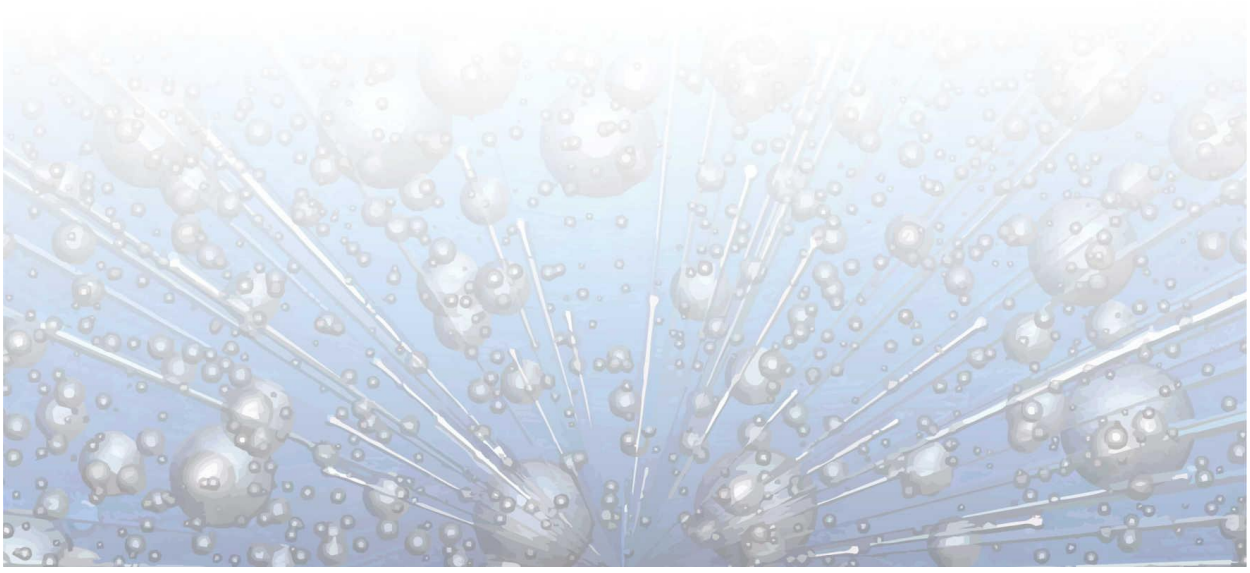
[1] R&D Phytomedicines, Steigerwald Arzneimittelwerk GmbH, Germany

E-mail address of presenting author: olaf.kelber@bayer.com

Synergy research teaches us, that the precondition of a synergistic action is a diversity of mechanisnm of actions. This has turned out to be the key of the efficacy of natural products, wich are characterized by the multitude of phytochemical constituents, which act in the sense of a multi drug-multi-target therapy.

In an analogous way, in modern drug development, a multitude of disciplines needs to work hand in hand to lead to innovations. This applies of course in a very general way, starting with lead discovery, the understanding of mechanism and action and of the safety profile, by first in vitro, and then in vivo research in pharmacological, pharmacokinetic and toxicological models, and in a similar way again along the different stages of clinical development, later followed by pharmacoepidemiological research during routine clinical use.

In natural products, which are in use based on tradition and clinical experiance, this applies in a special way, as there all these disciplines act simultaneously, in the sense of a reverse pharmacology approach, to which nowadays also microbiota research adds as a further key discipline. So this product group is an especially well suited example for ley role of an interdisciplinary approach for innovations.



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