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Abstracts of Sponsored Lectures

Abstract SL No. 01

PKL-021 as a new treatment for snakebite envenoming

Stanisław Pikul[1], Anna Krause[1], Hanna Kieronska[1], Joanna Lipner[1], Anna Palubicka[1], Katarzyna Sidoryk[1], Magdalena Tyszkiewicz[1]

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Snakebite envenoming remains a major global health challenge and constitutes a serious medical emergency associated with high mortality and long-term morbidity. The burden is particularly pronounced in rural and tropical regions, where timely access to effective and affordable treatment is often limited. Current standard-of-care therapies, such as antivenoms, face significant logistical and clinical limitations, highlighting the need for alternative or complementary therapeutic approaches.

PKL-021, a broad-spectrum inhibitor of metalloproteinases, has emerged as a promising candidate for the treatment of snakebite envenoming due to its ability to inhibit snake venom metalloproteinases (SVMPs), which play a key role in venom-induced tissue damage and systemic toxicity. In preclinical in vivo studies conducted in mouse models, PKL-021 has demonstrated strong efficacy against venoms from several medically important viper species, supporting its potential as a therapeutic intervention.

In this presentation, we will summarize the most recent advancements in the development of PKL-021 and outline our strategy for bringing this new therapeutic approach to the market.

Keywords: Snake venom metalloproteinases (SVMPs), PKL-021, snake envenoming

Abstract SL No. 02

State-of-the-Art Preparative Purification of Organic Compounds: Selecting the Optimal Technology for Complex Separation Challenges

Piotr Drelich[1], Rafael Campos[2]

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Preparative purification remains one of the most critical and resource-intensive stages in modern chemical and pharmaceutical workflows. Selecting the appropriate purification strategy directly impacts product purity, process scalability, solvent consumption, and overall project efficiency. This presentation will provide a practical overview of how to select the optimal purification technology — including Flash Chromatography, Preparative HPLC, and Preparative SFC — for a wide range of separation challenges, with selected examples based on modern purification platforms developed by Teledyne LABS.

The presentation will cover purification strategies for diverse compound classes, ranging from small organic molecules to complex biopolymers such as peptides and nucleotides, while highlighting the strengths and limitations of each technique in relation to sample complexity, polarity, loading capacity, throughput, and required purity. In addition, practical approaches for reducing solvent consumption, shortening purification and analysis times, and maximizing product recovery and purity will be presented through real-world examples and modern workflow strategies.

Abstract SL No. 03

The Evolution of Modern UHPLC Systems: Where Are We Going Next?

Marcin Gawryś

[1]Shim-pol, ul. Lubomirskiego 5, 05 080 Izabelin

Ultra-high-performance liquid chromatography has evolved from a pressure-driven extension of HPLC into a performance-driven platform focused on minimizing dispersion, stabilizing gradient delivery, and optimizing system volume. Control of extra-column effects is now critical to preserve peak efficiency, resolution, and sensitivity, particularly when using small-particle and narrow-bore columns. Reduced column internal diameters enable significant solvent savings and lower environmental impact while maintaining analytical performance if system dispersion is tightly controlled. Modern UHPLC systems therefore prioritize low-volume flow paths and reproducible fast gradients to support high-throughput workflows, including LC–MS applications. This shift redefines UHPLC development toward integrated control of dispersion, flow stability, and gradient precision, allowing faster analyses without compromising data quality and positioning UHPLC as a key technology for efficient and sustainable analytical laboratories.

Abstract SL No. 04

Modern pharmaceutical analysis with Metrohm – precision, safety, compliance

Wiktor Lorenc

Metrohm

The pharmaceutical industry increasingly requires analytical technologies that combine high accuracy, operational efficiency, regulatory compliance, and flexibility across both R&D and routine quality control environments. This presentation by Dr. Wiktor Lorenc from Metrohm Poland will provide an overview of selected analytical solutions dedicated to pharmaceutical applications, with particular focus on Ion Chromatography, Raman spectroscopy, NIR spectroscopy, titration techniques, and Karl Fischer titration.

The lecture will discuss the major strengths of Metrohm solutions, including robust instrument design, advanced automation capabilities, user-friendly operation, and full support for compliant data management in regulated laboratory environments. Attention will also be given to workflow optimization and the implementation of integrated analytical platforms that help improve laboratory productivity and analytical reliability.

Practical pharmaceutical application examples will demonstrate the versatility of the presented techniques. Ion Chromatography will be presented as a powerful tool for impurity determination such as nitrosamines or PFAS and general ion analysis. Raman and NIR spectroscopy applications will include rapid raw material identification and fast, non-destructive quantitative analysis. Titration and Karl Fischer techniques will be presented as reliable methods for quantitative analysis and precise moisture determination in pharmaceutical substances and formulations.

The presentation will highlight how modern analytical instrumentation can support pharmaceutical laboratories in meeting increasing quality and regulatory expectations while maintaining efficiency, reproducibility, and confidence in analytical results.

Abstract SL No. 05

AD-O51: From preclinical rationale to clinical development of an anticancer apoptosis-targeting therapy

Maria Mazur, PhD; Principal Investigator AD-O51[1]

[1]Adamed Discovery, Adamed Pharma S.A.

AD-O51 is a fusion protein composed of a recombinant human TNF-related apoptosis-inducing ligand (TRAIL/Apo2L) and a peptide derived from vascular endothelial growth factor (VEGF). It is currently being evaluated in a first-in-human clinical trial in Poland for the treatment of multiple solid tumors.

AD-O51 was rationally designed to address key limitations of earlier death receptor (DR) agonists, including suboptimal pharmacokinetics, insufficient receptor clustering, and limited tumor penetration. Its compact format, improved stability, and enhanced DR4/DR5 clustering result in more efficient and physiologically relevant receptor activation, leading to increased apoptotic potency.

Non-clinical studies demonstrated potent anticancer activity across a broad panel of cancer cell lines and patient-derived samples, with 206 models (65%) classified as responders. Notably, AD-O51 demonstrated activity in Dulanermin-resistant models, with 55% of responsive cell lines lacking sensitivity to this first-generation DR agonist. Moreover, higher in vitro efficacy was observed compared to both first- and second-generation DR agonists.

In vivo, AD-O51 showed broad antitumor activity, with 75% of xenograft models achieving >50% tumor growth inhibition (TGI) and over half reaching TGI ≥80%, including patient-derived xenografts. Activity was also observed across multiple combination settings, supporting its potential use in combination-based therapeutic strategies.

AD-O51 has completed formal preclinical development, including safety assessment demonstrating a favorable safety profile and an adequate therapeutic window.

Abstracts of Short Presentations

Biniecki Award Presentation

Targeting Cholinergic and GABAergic Neurotransmission: A Novel Therapeutic Strategy for Alzheimer's Disease

Dawid Panek

Jagiellonian University Medical College, Faculty of Pharmacy

Alzheimer's disease is a progressive neurodegenerative disorder characterized by cognitive decline, memory impairment, and disturbances in neurotransmission. Increasing evidence suggests that, apart from cholinergic dysfunction, alterations in GABAergic neurotransmission—particularly involving γ-aminobutyric acid transporter 3 (GAT-3)—may contribute significantly to the pathogenesis of the disease. 1 Therefore, simultaneous modulation of both systems may represent a promising therapeutic strategy.

The aim of this study was to design, synthesize, and evaluate novel multi-target-directed ligands acting as dual inhibitors of butyrylcholinesterase (BuChE) and GAT-3. Starting from an in-house library of BuChE inhibitors, we identified a hit compound displaying balanced inhibitory activity toward both targets. 2 Structural optimization led to the development of a new series of compounds with improved dual-target profiles.

Among the synthesized molecules, compound JT-3 emerged as the most promising candidate, exhibiting potent inhibition of hBuChE (IC 50 = 0.21 μM) together with inhibitory activity against GAT3 (corresponding mGAT4 subtype: IC 50 = 7.7 μM) comparable with reference inhibitors. ADME-tox studies demonstrated favorable metabolic stability and acceptable pharmacokinetic properties, including brain penetration after intraperitoneal and intragastric administration. Acute toxicity studies established a maximum tolerated dose of 10 mg/kg. *In vivo* studies confirmed procognitive activity in scopolamine-induced memory impairment models, including the passive avoidance task and Barnes maze test. Additionally, JT-3 demonstrated anxiolytic-like effects in behavioral assays.

The obtained results indicate that dual modulation of cholinergic and GABAergic neurotransmission constitutes a promising direction for the development of novel therapeutics for Alzheimer's disease. Compound JT-3 represents an attractive lead structure for further optimization and preclinical investigation.

Acknowledgements: This work was supported by the National Science Centre, Poland Grant

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1 Wu Z. et. al. Nature Communications 5 (2014) 1 – 13.

2 Pasieka A et. al. Eur. J. Med. Chem. 218 (2021) 113397

3 Panek D. et al. Angew Chem Int Ed Engl 64, 6 (2025) e202420510

Abstract No. SP01

From Concept to Successful Phase II Clinical Trials: CPL'36, a Best-in-Class PDE10A Inhibitor for the Treatment of Schizophrenia and L-DOPA-Induced Dyskinesia

Mikołaj Matloka[1]

[1] Celon Pharma SA, Preclinical Development Department

Phosphodiesterase 10A (PDE10A) is highly enriched in medium spiny neurons of the striatum, where it regulates intracellular levels of cyclic nucleotides, thereby modulating the activity of the direct and indirect pathways of the basal ganglia—a structure critically involved in the pathophysiology of multiple psychiatric and neurological disorders, including schizophrenia and L-DOPA–induced dyskinesia (LID).

The development of PDE10A inhibitors has historically been hindered by the clinical failure of several compounds, highlighting limitations in the canonical translation of preclinical pharmacology into effective clinical trial design. CPL'36 is a novel, second-generation PDE10A inhibitor that has progressed from concept to clinical evaluation, demonstrating superior antipsychotic and antidyskinetic efficacy across multiple preclinical models. Moreover, CPL'36 is characterized by unique properties, including a distinct kinetic interaction profile and robust in vivo behavioral effects. These findings support a mechanistic hypothesis underlying its differentiated pharmacological profile. Unlike earlier PDE10A inhibitors, CPL'36 selectively enhances signaling in the indirect over the direct striatal pathway, effectively acting as a functional "fuse." Its kinetic and pathway-selective properties appear to confer an improved therapeutic window, which—together with appropriate target engagement levels—translates into clinically meaningful efficacy across both schizophrenia and L-DOPA–related symptom domains.

To date, CPL'36 is the only PDE10A inhibitor to have successfully completed Phase II clinical trials in both schizophrenia and L-DOPA–induced dyskinesia associated with Parkinson's disease.

Abstract No. 002

IRX4-derived micropeptide IRX4_PEP1 drives Wnt/ß-catenin-mediated stemness and taxane resistance in prostate cancer

Jyotsna Batra[1,2], Achala Fernando[1,2]

[1]BOND UNIVERSITY LIMITED, Health Sciences and Medicine
2Queensland University of Technology, Center for Genomics and Personalised Health

Background & unmet need: Prostate cancer (PCa) progression and acquired resistance to docetaxel remain major clinical challenges, particularly in aggressive disease where cancer stem-like programs and pro-survival signalling limit durable responses. While GWAS implicate IRX4 in PCa susceptibility, the functional effectors downstream of the locus are not fully defined. Target/biological hypothesis: We identified a 78–amino acid IRX4-derived micropeptide (IRX4_PEP1) translated from a short open reading frame (sORF) initiated at an alternative start site within the IRX4 gene. We hypothesised that IRX4_PEP1 acts as an intracellular protein–protein interaction (PPI) hub that rewires oncogenic signalling and therapy response.

Materials and Methods: IRX4_PEP1 function was characterised in PCa models using proliferation/migration/invasion assays, pathway-level analyses, stem cell enrichment assays, and immunoprecipitation-based interactome mapping. Translational relevance was assessed by evaluating IRX4_PEP1 expression in PCa patient tissues and correlating expression with disease features and pathway enrichment.

Results: IRX4_PEP1 promoted PCa proliferation, migration and invasion, and interacted with HNRNPK (hnRNP K). Mechanistically, IRX4_PEP1 also interacted with CTNNB1 (ß-catenin) and was associated with dysregulated Wnt/ß-catenin signalling, increased stemness markers/stem-like cell enrichment, and docetaxel resistance. Clinically, IRX4_PEP1 expression was elevated in PCa tissues and correlated with aggressive disease, alongside positive correlations with CTNNB1/HNRNPK levels and ssGSEA enrichment of WNT/CTNNB1 signalling.

Drug discovery impact: IRX4_PEP1 represents a previously underexplored target class (oncogenic micropeptides) with dual potential as (i) a therapeutic vulnerability (via inhibition of IRX4_PEP1 function or its PPIs with ß-catenin/HNRNPK) and (ii) a predictive/stratification biomarker for Wnt-driven, docetaxel-resistant PCa.

Reference:

Fernando A. et al. DOI: 10.1038/s43856-024-00613-9 (PMID: 39487222).

Abstract No. 003

Semi-Solid Extrusion 3D Printing Enhances Epidermal Menthol Accumulation from PVA-Based Transdermal Films: Ex Vivo Comparative Study

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[2]Lithuanian University of Health Sciences, Kaunas, Lithuania, Institute of Pharmaceutical Technologies, Faculty of Pharmacy

Background and Aim: Menthol, the bioactive terpene alcohol of Mentha piperita L., exerts topical analgesia through epidermal TRPM8 receptor activation. Semi-solid extrusion 3D printing enables precise layer-by-layer construction of polyvinyl alcohol (PVA) matrices, potentially creating more homogeneous drug distribution than conventional solvent casting. This study aimed to evaluate whether 3D printing enhances epidermal menthol accumulation from PVA-based transdermal films.

Materials and Methods: PVA-based films (PVA 10%, glycerol 3%) containing menthol (5% w/w), benzocaine (5% w/w), and capsaicin (0–1% w/w) were manufactured by semi-solid extrusion 3D printing (n=12) and solvent casting (n=12) using matched formulations. Ex vivo permeation studies used human abdominal skin from 10 Caucasian female donors (30–55 years) in flow-through diffusion cells at 32°C over 24 hours (ethics: Kaunas Regional Biomedical Research Ethics Committee, BE-2-42). Menthol was quantified by validated GC/FID (Shimadzu GC-2010 Plus; R^2=0.99996).

Statistics: Mann-Whitney U test; Cohen's d effect size. Results: 3D printing produced 4.4-fold higher epidermal menthol accumulation versus solvent casting (22.0 vs 5.0 µg/mL; p=0.0004; Cohen's d=1.79 — very large effect size). The 3D printed film concentration substantially exceeded the therapeutic threshold for TRPM8 activation, whereas casting-derived levels were at the minimal effective level. The epidermal-to-dermal ratio was markedly higher for 3D printing (0.230 vs 0.066), indicating preferential epidermal drug retention — the pharmacokinetically ideal profile for topical analgesia. Manufacturing precision was superior for 3D printing: thickness uniformity CV 7.3% vs 19.7% for casting (2.7-fold improvement); drug content RSD 4.7% vs 9.5%.

Conclusions: Semi-solid extrusion 3D printing significantly enhances epidermal menthol accumulation from PVA films, creating a therapeutic drug reservoir through structurally homogeneous matrix construction. Superior manufacturing precision and ideal epidermal retention profile establish 3D printing as a promising platform for natural terpene-based transdermal formulation development.

Abstract No. 004

Repurposing disulfiram as a modulator of opioid-induced hyperalgesia: evidence for disulfide-dependent enhancement of Gi/o protein signaling

Zuzanna Żelażewska[1], Sandra Siedlecka[1], Iraj Alipourfard[1], Anna Leśniak[1]

[1]Medical University of Warsaw, Department of Pharmacotherapy and Pharmaceutical Care, Faculty of Pharmacy

Objectives: Opioids remain indispensable for the management of severe pain; however, their prolonged use is associated with adverse outcomes, including opioid-induced hyperalgesia, dependence, and withdrawal. Disulfiram, an FDA-approved drug for alcohol use disorder, has recently been reported to attenuate several opioid-related adverse effects, although the underlying molecular mechanisms remain poorly understood. In this study, we investigated whether disulfiram modulates opioid signaling by altering Gi/o protein activity.

Materials and Methods: The ability of disulfiram and its major metabolites—diethyldithiocarbamate (DTC), S-methyl-N,N-diethylthiocarbamate sulfone (DETC-sulfone), and S-methyl-N,N-diethylthiocarbamate sulfoxide (DETC-sulfoxide)—to activate Gi/o proteins was assessed in rat hypothalamic homogenates using the [^{35}S]GTPγS binding assay. To examine the involvement of thiol-dependent processes, experiments were conducted in the presence of the reducing agent dithiothreitol (DTT). Modulation of Gi/o activity under μ- and δ-opioid receptor stimulation was evaluated using a homologous [^{35}S]GTPγS displacement assay with saturating concentrations of DAMGO, deltorphin II, or morphine. Downstream signaling was further examined by measuring intracellular cAMP levels in SH-SY5Y cells using the cAMP-Glo assay.

Results: Only disulfiram significantly enhanced Gi/o protein activation by accelerating GDP/GTP exchange through disulfide bond formation. In addition, disulfiram, but not DETC-sulfone or DETC-sulfoxide, increased the number of receptor-coupled Gi/o activation sites following opioid receptor stimulation, suggesting stabilization of the GDP-bound state of the G protein. Under conditions of constitutive Gi/o signaling, disulfiram reduced basal activity in the presence of μ- and δ-opioid receptor agonists. Consistent with these observations, both disulfiram and its metabolites potentiated morphine-induced inhibition of cAMP production.

Conclusions: These findings demonstrate that disulfiram enhances Gi/o protein signaling through a disulfide bridge formation and modulates constitutive Gi/o activity during μ- and δ-opioid receptor stimulation. The results provide mechanistic support for the potential repurposing of disulfiram as a pharmacological modulator of opioid-induced hyperalgesia.

Keywords: disulfiram, Gi/o protein, morphine

Abstract No. 005

Targeting Human Adenylate Kinase 4 (AK4) by Natural Prenylated Phenolics: Structure-Activity Relationship and Conformational Insights

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[3]Nicolaus Copernicus University in Toruń, Department of Biochemistry, Faculty of Biological and Veterinary Sciences

Objectives: Mitochondrial metabolic reprogramming is a hallmark of cancer progression, with adenylate kinase 4 (AK4) emerging as a critical, yet pharmacologically challenging, regulator. AK4 is significantly upregulated in various types of cancer, promoting epithelial-mesenchymal transition and chemoresistance. While inhibitors for the cytosolic isoenzyme AK1 have been previously reported, mitochondrial AK4 remains poorly explored, with no potent small-molecule modulators described in the literature to date. This study aims to characterize a focused collection of natural prenylated phenolics from Morus alba as the first natural inhibitors of human AK4. To assess the broader impact of these compounds, we evaluated their inhibitory potential against AK1 and AK2, representing both short-type (AK1) and long-type (AK2 and AK4) LID domain architectures. These structural motifs are crucial for the catalytic cycle and conformational dynamics.

Materials and Methods: Recombinant human AK1, AK2, and AK4 were produced and purified using in-house optimized protocols. These proteins were used in enzymatic assays to determine IC50 values and evaluate redox sensitivity, particularly for redox-sensitive AK4. To investigate potential ligand-induced structural changes and protein stability, biophysical techniques were employed. Finally, molecular docking was performed to explore the structural basis of ligand-enzyme interactions.

Results: We identified several prenylated Diels-Alder adducts as highly potent inhibitors, achieving over 95% enzymatic inhibition at 50 μM for AK4, with significant activity also observed against AK1 and AK2. SAR analysis revealed a correlation between molecular complexity and potency. While bulky Diels-Alder adducts were highly effective, simpler prenylated flavonoids like morusin showed markedly lower inhibitory efficacy. Biophysical and docking data indicate that these inhibitors may constrain the enzyme's architecture, thereby modulating LID domain flexibility to impede the catalytic cycle.

Conclusions: By demonstrating strong inhibition across multiple adenylate kinase isoenzymes, we identify a universal inhibitory scaffold that provides a robust basis for targeting the AK phosphotransfer dynamics to leverage metabolic vulnerabilities in cancer.

Acknowledgements: This research was funded by the Polish National Science Centre, grant no. 2023/51/B/NZ7/03056.

Abstract No. 006

Transforming Early Drug Discovery Through Cambridge Structural Database-Driven Structural Knowledge and Computational Analytics

Rupesh Chikhale[1], Bojana Popovic[1]

[1]Cambridge Crystallographic Data Centre, Cambridge, United Kingdom, Discovery Science

Objectives: The rapid and reliable generation of high-quality structural insights is essential for accelerating early drug discovery. This work showcases how the Cambridge Structural Database (CSD) and its knowledge based computational tools enable medicinal chemists to derive experimentally informed, structurally grounded hypotheses that enhance target assessment, hit identification, and lead optimization. By leveraging over one million curated small molecules crystal structures, CSD tools provide a robust foundation for predictive modelling, interaction analysis, and scaffold exploration.

Materials and Methods: CSD knowledge libraries capture intrinsic intramolecular geometries, preferred intermolecular interaction motifs, and 3D conformational preferences derived from crystallographic evidence. Tools such as SuperStar and Fragment Hotspot Maps support early evaluation of pocket energetics and ligandability. Additionally, cavity analysis and structural comparison methods facilitate target prioritization by identifying conserved or differentiating features relevant to selectivity design.

Results: In hit identification, CSD-CrossMiner enables pharmacophore-based mining across protein–ligand complexes and small molecule structures, allowing researchers to uncover scaffold–target relationships and identify new chemotypes. Case studies illustrate how this approach has linked spirobarbituric dihydrofurans to aldose reductase and supported scaffold hopping across diverse receptor families, including nAChRs and EP2 receptor agonists. Furthermore, the GOLD molecular docking platform, support hit identification and ligand optimization.

Conclusions: Collectively, these tools demonstrate how crystallographic data driven methods can significantly enhance the reliability and efficiency of early discovery pipelines. The integration of CSD derived structural insights reduces design risk, accelerates the identification of high-quality starting points, and informs downstream optimisation strategies. By embedding experimentally validated structural information into everyday computational workflows, CSD resources empower medicinal chemists to make better supported decisions, shorten design cycles, and progress candidates more efficiently from concept to development.

Keywords: Structural Database, Molecular docking, Pharmacophore Modelling.

Abstracts of Poster Presentations

Abstract PP No. 001

Advanced Liposomal Nanocarriers for Annamycin Delivery

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Background: Anthracyclines that inhibit Topo II are among the most potent cytotoxic agents used in oncology. Annamycin (2′-iodo-3′-hydroxy-4′-epi-4-demethoxy-doxorubicin) (ANN) is a highly lipophilic anthracycline with reduced cardiotoxicity and resistance to MDR efflux. Encapsulation of ANN in liposomes further lowers systemic toxicity while improving plasma stability, bioavailability, and cellular uptake. However, conventional liposomal formulations consist of heterogeneous, multilamellar vesicles in the micrometer size range, which are rapidly cleared by the reticuloendothelial system. To address these limitations, we developed pegylated, long-circulating, unilamellar nanoliposomes with significantly improved properties.

Materials and Methods: Liposomes were produced using thin lipid film hydration and extrusion techniques. ANN was incorporated either within the lipid bilayer or actively loaded into the aqueous core via an ion-gradient–driven process. Various lipid compositions were evaluated (SPC, DMPC, DMPG, DSPG). Formulations were systematically optimized with respect to lipid composition and drug-to-lipid weight ratio, based on stability parameters (aggregation, precipitation, drug crystallization, and encapsulation efficiency). Particle size distribution, polydispersity, and colloidal stability were assessed using dynamic light scattering (DLS).

Results: This optimization process led to the identification of highly stable liposomal nanoparticles with diameters below 150 nm and encapsulation efficiencies exceeding 95%. Favorable drug-to-lipid weight ratios of up to 1:20 were achieved without compromising formulation stability. These optimized nanoliposomal formulations demonstrate properties suitable for advancement into preclinical and clinical evaluation.

Conclusions: In summary, the developed long-circulating ANN-loaded nanoliposomes exhibit characteristics consistent with enhanced permeability and retention (EPR)–mediated tumor accumulation. These formulations offer a promising platform for combination treatment strategies, with the potential to enhance therapeutic synergy while overcoming MDR-associated resistance.

This project was co-financed from the European Regional Development Fund under the Smart Growth Operational Program 2014-2020. Project is implemented as part of the NCRD: Proposal No 6/1.1.1/2020 – “Fast track. Priority I: Support for conducting R&D works by enterprises, Sub-measure 1.1.1. Industrial research projects and Experimental development work projects carried out by companies. Contract No: POIR.01.01.01-00-1913/20-00.

Abstract PP No. 002

Therapeutic Potential of Berubicin in Primary and Metastatic Brain Tumors

Radosław Borowski[1], Angelika Kaczynska[1], Marcin Mielecki[1], Mariusz Olejniczak[1], Beata Pajak-Tarnacka[1,2]

[1]WPD Pharmaceuticals
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Objectives: Brain metastases (BM) represent the most frequent complication of systemic cancers and occur approximately ten times more often than primary brain tumors. It is estimated that up to 40% of patients with systemic malignancies will eventually develop BM. Unfortunately, secondary brain tumors are associated with a very poor prognosis, with an average survival of only 8 months. Current standard therapies, including surgical resection and radiotherapy, provide limited benefit, and there is no universally effective chemotherapy for BM. Berubicin (BER) is a novel chemotherapeutic agent with potential to address this unmet medical need. BER is a 4'-O-benzyllated derivative of doxorubicin (DOXO), demonstrating 2–3 times higher potency than DOXO and a unique ability to cross the blood-brain barrier. Clinical trials have shown BER to be effective in treating primary brain tumors in adults. In this study, we explored the potential of BER as a chemotherapeutic option for the treatment of brain metastases.

Materials and Methods: To evaluate BER's efficacy, we employed cell lines representing brain metastatic sites from multiple cancer types: DU145 (prostate cancer), COLO792 (melanoma), MA-MEL-45A (melanoma), COLO668 (lung cancer), and MDA-MB-361 (breast cancer). We assessed cell viability using MTS assays, cell proliferation via BrdU incorporation, and induction of apoptosis following BER treatment.

Results: BER treatment led to a dose- and time-dependent reduction in cell viability and proliferation across all tested cell lines, as determined by MTS and BrdU assays. The calculated IC50 values for BER were 14.14 nM (DU145), 84.12 nM (COLO792), 47.89 nM (MA-MEL-45A), and 26.02 nM (COLO668), all significantly lower than those observed with DOXO. Apoptosis was confirmed as the primary mechanism of cancer cell elimination.

Conclusions: BER effectively inhibits proliferation and viability of brain-metastatic cancer cells, independent of their tissue of origin. These findings suggest that BER is a promising candidate for the development of therapies targeting secondary brain tumors.

This project was co-financed by the European Union from the European Regional Development Fund under the Smart Growth Operational Program 2014-2020, Sectoral Programme InnoNeuroPharm, Priority Axis I: Support R&D carried out by enterprises, Measure 1.2: Sectoral R&D Programmes, implemented under National Center for Research and Development.

Abstract PP No. 003

Cytotoxic Effects of IL-13RA2-Directed Immunotoxins in Cancer Cells

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Objectives: Interleukin-13 receptor alpha 2 (IL-13RA2) is a membrane-bound protein overexpressed in more than 75% of gliomas while being absent in normal brain tissue. Beyond gliomas, IL-13RA2 is also upregulated in several other cancers, including melanoma, ovarian, and pancreatic tumors, making it an attractive target for cytotoxic therapies. In this study, we evaluated the anticancer potential of two immunotoxins, WPD 101a.1 and WPD 101a.2, which combine an optimized IL-13 ligand with a modified bacterial toxin, in in vitro models of glioblastoma, breast cancer, and prostate cancer.

Materials and Methods: We utilized cancer cell lines representing glioblastoma (U-251, LN-229, SNB-19), breast cancer (MDA-MB-231, T47D), and prostate cancer (PC-3). IL-13RA2 expression was assessed using immunocytochemistry. Following treatment with the immunotoxins, we measured cell viability (MTS assay), protein synthesis (SRB assay), proliferation (BrdU incorporation), apoptosis (Hoechst 33342 staining), and the ability to form colonies (clonogenic assay).

Results: Both WPD 101a.1 and WPD 101a.2 significantly reduced cell viability and clonogenic potential in a dose- and time-dependent manner in cells with moderate to high IL-13RA2 expression, primarily through induction of apoptosis. In contrast, their anticancer effects were considerably lower in cells expressing low levels of IL-13RA2.

Conclusions: Our findings demonstrate that WPD 101a.1 and WPD 101a.2 exhibit potent anti-tumor activity across multiple in vitro cancer models, with efficacy directly correlated to receptor density on the cell surface. These results support the potential of both immunotoxins as promising candidates for targeted cancer therapy.

This project was co-financed by the European Union from the European Regional Development Fund under the Smart Growth Operational Program 2014-2020. Project is implemented as part of the National Center for Research and Development: Proposal No 5/1.1.1/2017 - "Fast track", Priority I: Support for conducting R&D works by Medium and Small companies and Microenterprises, Sub-measure 1.1.1. Industrial research projects and Experimental development work projects carried out by companies. Contract No: POIR.01.01.01-00-0912/17-00.

Abstract PP No. 004

WPD-103-($^{177}Lu^{3+}$): A Novel Targeted Theranostic Platform for IL-13Rα2-Positive Tumors

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Background: WPD-103-($^{177}Lu^{3+}$) is a novel theranostic agent designed to selectively target the IL-13Rα2, a tumor-associated antigen, enabling patient stratification, precise diagnostics, and targeted molecular radiotherapy. This study describes the development and characterization of WPD-103-($^{177}Lu^{3+}$), encompassing biomanufacturing, chemical modification, radiometal complexation, stability assessment, and cellular internalization.

Materials and Methods: A scalable biomanufacturing process was established to produce WPD-103 protein using T7-driven bacterial expression in inclusion bodies, followed by dialysis-based refolding and hexahistidine tag removal. Lysine-directed modification selected for DOTA conjugation. Radiolabeling with $^{177}Lu^{3+}$ was optimized by buffer composition, pH, temp., reaction time, and stabilizing agents. Product quality was assessed using MS, radiochemical purity analysis, and stability testing in PBS and human serum. Cellular uptake and internalization were evaluated in IL-13Rα2-positive U-251 glioma cells using fluorescently and radioactively labeled constructs.

Results: Highly pure (>95%) WPD-103 protein was successfully produced, with refolding efficiencies of up to 9%, yielding sufficient material for downstream development. Lysine-directed DOTA conjugation resulted in a well-defined product population, with an intensity-weighted average of 2.45 DOTA moieties per protein molecule (range 1–5) and a total intensity-weighted Mw of 14,137 Da. Optimized $^{177}Lu^{3+}$ complexation achieved reproducible radiochemical purity exceeding 90%. The radiolabeled conjugate remained stable (>90%) for 24 h in PBS and human serum at both RT and 37°C. In vitro assays confirmed efficient and complete internalization of both fluorescently labeled and radiolabeled WPD-103 by U-251 glioma cells.

Conclusions: The favorable radiochemical stability and target-specific cellular internalization support WPD-103-($^{177}Lu^{3+}$) as a promising theranostic candidate. These results justify further biodistribution studies in mouse CDX models and continued preclinical evaluation toward translational development.

The studies were conducted as part of Project No. KPOD.07.07-IW.07-0275/24 entitled “Development of Technology and Proof-of-Concept Studies of the Radiopharmaceutical WPD-103 for Theranostic Applications in Targeted Oncology Therapies”, co-financed by the National Recovery and Resilience Plan, Component D: Efficiency, accessibility and quality of the healthcare system, Investment D3.1.1: Comprehensive development of research in medical and health sciences.

WPD401: A Next-Generation Multireceptor-Targeted Cytotoxic Conjugate with Broad Antitumor Activity

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Background: Despite significant advances in oncology, lung cancer, pancreatic cancer, colorectal cancer, melanoma, and ovarian cancer remain among the most common and deadly malignancies worldwide. These tumors continue to represent major unmet medical needs due to late diagnosis, high heterogeneity, and frequent resistance to available therapies. WPD401 is a multivalent targeted cytotoxic conjugate originally developed for glioblastoma (GBM), designed to simultaneously engage four tumor-associated receptors: IL-13RA2, EphA2, EphB2, and EphA3, frequently overexpressed in solid tumors. With WPD401 entering a Phase I clinical trial this year, we investigated whether its receptor-targeted mechanism could be effective against other cancer types.

Materials and Methods: A panel of cancer cell lines representing lung cancer, pancreatic cancer, colorectal cancer, melanoma, and ovarian cancer was selected based on literature and ProteinAtlas.org evidence of receptor expression. Cells were treated with increasing concentrations of WPD401. After 72 hours, cell viability was assessed using the MTS assay, and cell proliferation was measured via BrdU incorporation.

Results: Cancer cell lines displayed varying sensitivity to WPD401 treatment. Importantly, several lines within pancreatic cancer, melanoma, lung cancer, and colorectal cancer panels exhibited exceptionally high sensitivity, with half-maximal inhibitory concentration (IC_{50}) values in the low picomolar range, demonstrating potent cytotoxic activity. Sensitivity correlated with the level of receptor expression in the tested cell lines.

Conclusions: These results indicate that WPD401 has strong cytotoxic effects beyond GBM, highlighting its potential as a versatile candidate for targeted therapy across multiple solid tumor types. The findings support further preclinical and translational evaluation of WPD401 for the treatment of aggressive cancers with high unmet medical needs.

The studies were conducted as part of Project No. KPOD.07.07-IW.07-0283/24 entitled “Development of the WPD-401 molecule in the context of new indications for targeted therapy”, co-financed by the National Recovery and Resilience Plan, Component D: Efficiency, accessibility and quality of the healthcare system, Investment D3.1.1: Comprehensive development of research in medical and health sciences.

Culture of tumoroids inside double emulsion droplets produced using a microfluidics-based bioprinter

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Objectives: Microfluidic droplet-based bioprinting offers several advantages such as high-precision control over the contents of each droplet including cells, molecules, drugs and bioinks; ease of their compartmentalization and high repeatability. In this work we present an automated setup capable of printing chains of double emulsion aqueous core-droplets onto substrates under external aqueous media.

Materials and Methods: For the double emulsion droplets, the inner phase composition per 100 µl is shown in the table below. The shell phase is NOVEC 7500 + 3 % w/w PFPE-PEG-PFPE surfactant and the external phase used was PBS and cell culture media. The substrate is a plastic container coated with NOVEC 1720. The microfluidic chip is displaced using a XYZ stage with an UV lamp with wavelength of 385 nm pointing towards the needle tip for immediate crosslinking. Gel reagents w/o RGD (µl) With RGD (µl) 10 x CB (pH 7.2) w/o Phenol Red 8.00 8.00 Water 17.00 12.50 N-Dextran 16.67 18.33 RGD Peptide 0.00 2.50 Cell suspension (K562 cells-IMDEM) 20.00 20.00 Crosslinking by CD-Link or PEG-Link 25.00 25.00 AgaFloat 10.00 10.00 LAP 3.33 3.67 Methods The droplets are encapsulated using a microfluidic T-junction micromilled in a polycarbonate chip. The generated droplets are then directed towards a substrate through a 25G needle immersed in an external aqueous media. The needle-substrate distance is adjusted so the droplets wet the substrate as soon as they are extruded. In parallel, the UV lamp is crosslinking the extruded droplets with an intensity of 1350 mW/cm2.

Results: We show that the Dextran droplets can be printed onto a substrate forming stable chains of droplets. The cells inside droplets with RGD peptide aggregate faster than cells in droplets without RGD.

Conclusions: In this present a method to print chains of double emulsion droplets containing cells that aggregate into spheroids. In the future, we expect to improve the hydrogel and external phase formulation to allow for faster cell aggregation and spheroid formation. The next step will be to perform drug screening on those spheroids.

Abstract PP No. 007

Membrane-Driven Modulation of Insulin Aggregation States: Impact of GM3-Containing Lipid Rafts and Zn^{2+} on Insulin-Membrane Interactions

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Insulin, a cornerstone biopharmaceutical in diabetes therapy, can form insoluble fibrillary deposits at sites of repeated injections and is prone to aggregation during handling and formulation. Since membrane interfaces may promote or modulate amyloid–protein interactions, we investigated how raft-like membrane composition enriched with ganglioside GM3 (implicated in insulin resistance) and Zn^{2+}-driven insulin hexamerization affect insulin–membrane interactions across insulin aggregation states.

Biomimetic lipid rafts were prepared as DOPC/sphingomyelin/cholesterol (DOPC/SM/Chol) monolayers and supported bilayers, either without GM3 or with 25 mol% GM3. Insulin species from monomers to fibrils were verified by Thioflavin T fluorescence. Interactions at the lipid interface were quantified using the Langmuir technique and followed over time by ATR-FTIR on immobilized bilayers. Parallel experiments were performed in the presence of Zn^{2+} to assess zinc–insulin complex effects. Monomeric insulin partially incorporated into the interfacial, hydrophilic region without detectable acyl-chain reorganization. Aggregated insulin did not insert and interacted predominantly with the membrane surface. GM3 significantly altered insulin–membrane interactions for both monomeric and aggregated insulin, and this modulation persisted in the presence of Zn^{2+}. Zinc–insulin hexamers were unable to penetrate the membrane and instead accumulated at the surface, inducing changes in lipid molecular orientation. Insulin–interface interactions depend on aggregation state and membrane composition. GM3-containing raft-like membranes modulate both monomeric and aggregated insulin binding, while Zn^{2+}-mediated hexamerization favors surface adsorption over insertion. These findings provide mechanistic insight relevant to controlling insulin aggregation and interfacial behavior.

Abstract PP No. 008

Physical Stabilisation of Gellan Gum by Musa sapientum and Functionalisation with Cucurbita maxima for Accelerated Wound Healing

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Objectives: The objective was to create effective, eco-friendly, and sustainable wound dressings by physically stabilizing gellan gum (GG) with Musa sapientum (banana peel/MS) powder and functionalizing it with Cucurbita maxima (pumpkin) pulp extract, without the need for chemical crosslinking agents.

Materials and Methods: Biocomposite sponges were fabricated using a sequential synthesis strategy. GG was mixed with Musa sapientum powder, followed by the incorporation of Cucurbita maxima pulp extract at concentrations of 1%, 10%, and 20% (denoted as GG-MS@P1, GG-MS@P2, and GG-MS@P3). The materials were frozen and lyophilized to produce porous sponges. Structural characterization was performed using ATR-FTIR spectroscopy, and surface morphology was examined by SEM. The sponges underwent analysis, including mechanical testing, TGA, swelling, biodegradation, porosity, WVTR, protein adsorption, and in vitro assays. In vivo efficacy was evaluated using a murine excisional wound model.

Results: ATR-FTIR spectra confirmed intermolecular interactions between pumpkin extract components and the GG-MS matrix. SEM images revealed a disordered and interconnected porous microstructure, with MS-modified sponges exhibiting larger pore sizes and more uniform surface morphology than pure GG. The incorporation of MS significantly improved structural cohesion and thermal resistance. Increasing pumpkin extract resulted in a progressive increase in porosity (up to 90.3% for GG-MS@P3) and enhanced mechanical properties. GG-MS@P3 demonstrated remarkable pH-responsive swelling, the highest antioxidant activity (84% inhibition), and improved adsorption of human serum albumin and fibrinogen. The sponges showed a marked bacteriostatic effect against MRSA but not against Pseudomonas aeruginosa or Candida albicans. In vivo studies confirmed that GG-MS@P3 accelerated wound healing, achieving 71.33% wound closure by Day 14. All formulations were non-hemolytic and highly biocompatible.

Conclusions: This study demonstrates that plant-derived physical reinforcement enables tunable biointeractions and improved wound closure. The GG-MS@P3 sponge represents a promising, eco-friendly, and multifunctional platform that valorizes agricultural by-products in line with circular economy principles.

Keywords: Biocomposite Sponges; Wound Dressing; Physical Stabilisation

Abstract PP No. 009

Design, synthesis, and biological evaluation of Small-Molecule TMPRSS2 Inhibitors as potential candidate drugs for the treatment of COVID-19

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Transmembrane protease, serine 2 (TMPRSS2) has been recognized as a key host cell factor for viral entry and pathogenesis of SARS-CoV-2. In particular, TMPRSS2 proteolytically processes the SARS-CoV-2 Spike (S) protein, enabling virus–host membrane fusion and infection of the airways. Inhibiting TMPRSS2 blocks the entry and replication of SARS-CoV-2 in the viral host cell, making it an attractive target for antiviral drug development.

In this study, we introduce a comprehensive drug discovery workflow that integrates bioisosterism, in silico virtual screening, and cellular activity profiling to identify potent TMPRSS2 inhibitors. From the screened set, 200 compounds exceeded 50% inhibition, and 27 compounds showed sub-micromolar to low-micromolar potency ($IC_{50} \leq 5.0 \pm 0.5$ μM). The consistent dose–response behaviour and distinct inhibition curves are consistent with a direct interaction with the catalytic domain, and no cytotoxicity was observed in DMSO-matched controls. To translate enzymatic inhibition into a relevant functional phenotype, we evaluated selected hits in a SARS-CoV-2 Spike-dependent cell fusion assay (InvivoGen), using 293-hMyD88-Spike donor cells and hACE2-TMPRSS2 acceptor cells. The assay is based on MyD88 transfer from donor to acceptor cells, which activates an NF-κB–SEAP inducible reporter in acceptor cells; cell fusion is quantified from coculture supernatants using QUANTI-Blue™ (SEAP detection). In this functional assay, the reference inhibitor Camostat produced 35% ± 5% inhibition of cell fusion, which we treat as the maximal benchmark activity under these conditions. Notably, eight compounds achieved Camostat-comparable activity. While viral variants may omit TMPRSS2 dependency, this host protease could be a biologically validated target for future pandemics and infections resulting from epithelial viral entry. These novel compounds could be used to identify serine protease inhibitors with promising antiviral or anticancer potential.

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Abstract PP No. 010

The granatane-triazole hybrid molecules as dual acetylcholinesterase and ß-secretase 1 inhibitors

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Objectives: The multifactorial nature of Alzheimer's disease (AD) means that there are many potential therapeutic targets. The biggest challenge in discovering effective multi-target compounds for treating a disease as complex as AD is identifying the key and best combination of biological targets. Among the proposed mechanisms underlying Alzheimer's disease cholinesterases, especially acetylcholinesterase (AChE), remain important biological targets for therapeutic intervention. ß-secretase (BACE1), which initiates Aß production, has also become an attractive therapeutic target, as ß-amyloid aggregation is still considered one of the key factors in the pathogenesis of AD, and its inhibition may influence disease progression.In this study, we report the synthesis, biological evaluation, and computational analysis of multifunctional ligands exhibiting inhibitory activity against both AChE and BACE1.

Materials and Methods: 1,2,3-triazole compounds and granatone derivatives were obtained in a five-step synthesis. The AChE inhibitory activity was evaluated using Ellman's colorimetric method, while BACE1 inhibitory activity was assessed using a fluorescence resonance energy transfer (FRET) assay. Molecular docking studies with AChE and BACE1 were performed to investigate the binding modes and interaction profiles of the synthesized ligands.

Results: The synthesised ligands show potential for the development of anti-Alzheimer's disease agents. Compounds 1D and 2E(CF_3) shown in Figure 1 exhibited the most promising profiles.

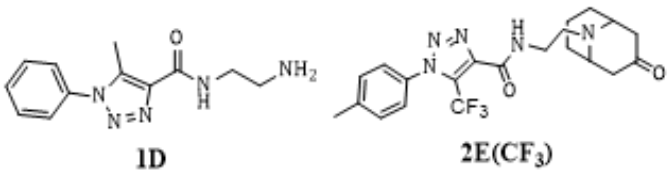

Fig.1.

Docking and molecular dynamics analyses suggest that triazoles interact with the target enzymes through mechanisms distinct from those of donepezil and quercetin, likely due to their greater conformational flexibility and ability to adopt multiple binding modes. In addition, the compounds satisfy major drug-likeness criteria and show a high predicted ability to cross the blood–brain barrier.

Conclusions: All obtained compounds were active against selected enzymes, especially compounds 1D and 2E(CF3) represent promising candidates for further investigation in Alzheimer's disease therapy.

Keywords: Alzheimer Disease; AChE inhibitors; BACE1 inhibitors.

Abstract PP No. 011

The dual DNA binders/DHFR inhibitors bearing arylidene-hydrazinyl-1,3-thiazole scaffold as novel potential anticancer agents

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Objectives: Thiazole heterocycles are of great interest due to their broad polypharmacological potential, including anticancer activity. Arylidene-hydrazinyl-thiazoles represent a promising structure capable of DNA intercalation and DNA damage induction. The aim of this study was to synthesize small-molecule anticancer agents targeting DNA that combine direct DNA binding with non-classical antifolate activity for breast cancer therapy. The designed compounds contain an arylidene-hydrazinyl-1,3-thiazole scaffold expected to exhibit dual DNA-binding and antifolate properties.

Materials and Methods: Fourteen new arylidene-hydrazinyl-1,3-thiazoles were synthesized and evaluated for dual DNA minor-groove binding and in vitro hDHFR inhibition. Presented on figure 1 compounds 5 and 11 showed dual activity and were further analyzed by molecular docking and molecular dynamics to investigate their binding mode and stability in the DHFR active site.

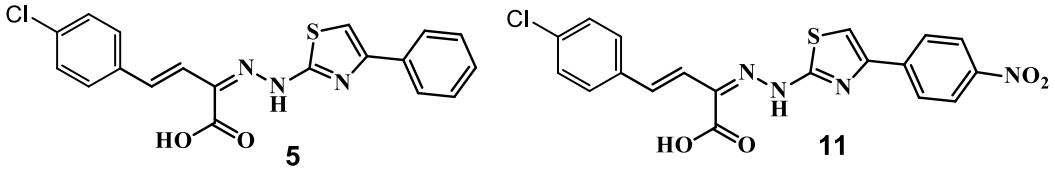

Fig.1.

Their anti-breast cancer activity was assessed in MCF-7 cells using methotrexate (MTX) as a reference. Cytotoxicity toward normal skin fibroblasts, apoptosis induction, and wound-healing assays were also evaluated. Finally, in silico ADMET analysis was performed to assess their potential as drug leads.

Result: Both selected compounds demonstrated a stabilizing effect on hDHFR as revealed from molecular dynamics results. Anti-breast cancer evaluation confirmed the potent activity of them against MCF-7 cells with least toxicity on normal skin fibroblasts compared to MTX. Both compounds showed greater potency and selectivity than MTX based on IC_{50} values.

Conclusions: The biological results, together with the predicted pharmacokinetic and ADMET parameters, indicate favorable drug-like properties of these ligands and support their potential for further optimization as breast cancer-targeted therapeutics.

Keywords: Thiazoles; DHFR inhibitors; DNA binders.

Abstract PP No. 012 **Spotlight Presentation (PSP2)**

Haemoglobin as a Functional Modulator of Chlorin e6 Binding, Release, and Photodynamic Properties in Magnetic Chitosan Nanocarriers

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Objectives: The aim of this study was to develop and evaluate haemoglobin (Hb)-functionalised chitosan-coated magnetic nanoparticles as drug delivery systems for photodynamic therapy (PDT). The work focused on determining how Hb binding and the conjugation route influence chlorin e6 loading, release behaviour, singlet oxygen generation, and photodynamic activity.

Materials and Methods: Magnetic nanoparticles (Fe_3O_4) were coated with chitosan and further modification with long-distance amino groups to check protein binding. Hb was bonded to the nanoparticle surface via either amino or carboxyl groups. The obtained nanocarriers were characterised using ATR-FTIR spectroscopy, X-ray diffraction (XRD), dynamic light scattering (DLS), SEM/STEM microscopy, BET surface area analysis, and thermogravimetric analysis. Chlorin e6 was incorporated into the system through either physical adsorption or chemical bonding. Drug loading efficiency, release kinetics, and singlet oxygen production were evaluated using spectroscopic methods. Photodynamic activity was assessed in vitro using HeLa cancer cells, and therapeutic efficiency was expressed as EC_{50} values.

Results: Hb binding increase the drug loading efficiency and influenced chlorin e6 release profiles. Hb-modified systems reduced the initial burst release and enabled more controlled drug release over 24 hours. In addition, haemoglobin increased singlet oxygen generation compared with Hb-free nanoparticles. In vitro studies confirmed better photodynamic activity of Hb-functionalised nanoparticles. EC_{50} values decreased from approximately 0.25–0.35 mg/mL for Hb-free carriers to 0.09–0.12 mg/mL for systems with physically adsorbed chlorin e6 and to 0.05–0.10 mg/mL for chemically bound chlorin e6.

Conclusions: Haemoglobin significantly improves the performance of chitosan-based magnetic nanocarriers designed for photodynamic therapy. Its presence enhances chlorin e6 loading, stabilises release profiles, and increases singlet oxygen generation, resulting in improved photodynamic efficacy in cancer cells. These findings demonstrate the potential of Hb-functionalised magnetic nanoparticles as advanced nanocarriers for more efficient PDT applications.

Abstract PP No. 013

Training of State-of-the-Art Deep Learning Models with and Without Ambiguous Regions for Nuclei Instance Segmentation

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Supervised deep learning (DL) is the most common approach for nuclei instance segmentation and requires annotated data for training. While manual nuclei annotations are still considered the gold standard, they are subject to variability. We investigated how these “ambiguous regions”, where even experts are uncertain about the precise nuclei boundaries, influence the performance and generalizability of DL-based instance segmentation models. We used the NuInsSeg dataset, which not only provides annotations for nuclei instances, but also includes manual annotations of ambiguous regions. We evaluated the impact of ambiguous regions on nuclei instance segmentation performance using three state-of-the-art models: HoVer-Net, HoVer-NeXt, and CellViT++.

Models were trained and tested, with and without ambiguous regions. Experiments were conducted via five-fold cross-validation on NuInsSeg and Panoptic Quality (PQ) was used as performance metric. Our results show that training without ambiguous regions significantly decreased PQ on the NuInsSeg dataset, with HoVer-NeXt, HoVer-Net, and CellViT++ showing a decrease of 7.9% ($p < 0.001$), 3.6% ($p < 0.001$), and 4.8% ($p < 0.001$), respectively. Removing ambiguous regions during testing, however, improved PQ with HoVer-NeXt, HoVer-Net, and CellViT++, showing performance improvements of 3% ($p < 0.01$), 1.9%, and 2.6% ($p < 0.01$), respectively. We conclude that for annotation protocols, ambiguous regions should be retained in annotations. Training with these regions forces the model to learn robust features that generalize to real world images. During inference, removing ambiguous areas from the evaluation pipeline had a positive impact on performance in most cases.

Abstract PP No. 014 **Spotlight Presentation (PSP 4)**

Novel Synthetic Retinoids Inhibit Proliferation and Interfere with the Migration of Patient-Derived High-Grade Serous Ovarian Cancer Cells

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Objectives: High-grade serous ovarian cancer (HGSOC) is the most lethal gynaecological malignancy, with a five-year survival rate below 30%. Most patients present with advanced metastatic disease and frequently relapse, partly due to cancer stem cells (CSCs). These are a chemotherapeutic resistant sub-population of cells that is capable of self-renewal. CSCs in HGSOC are characterised by the expression of CD44 and/or CD133 protein. For ovarian cancer, there has been an increasing interest in blocking the synthesis of all-trans retinoic acid (ATRA) to prevent cell growth. Solomargine prevents ATRA synthesis and inhibited the growth of an ovarian cancer cell line in mouse xenografts. Here, we have use four novel synthetic retinoids (NSRs) that are structurally different to target retinoic acid receptors.

Materials and Methods: A panel of patient-derived primary HGSOC cell lines was used to reflect clinical heterogeneity. The expression of CD44 and CD133 was assessed by RT-qPCR, immunohistochemistry and flow cytometry. Cells were treated for 96 hours with increasing concentrations of the three NSRs, and standard therapeutics (carboplatin and olaparib). Cellular confluency was monitored as a marker for cellular proliferation every 2 hours using the Incucyte® live-cell imaging system.

Results: For two cell lines, CD44 was highly expressed, whereas CD133 expression was comparatively low at both mRNA and protein levels. The NSRs and carboplatin induced a CSC CD44+CD133+ phenotype in both cell lines. The NSRs reduced cell proliferation in all HGSOC models in a dose-dependent manner, with EC50 values ranging from ~7-14 µM. One NSR produced predominantly cytostatic effects, while another demonstrated cytotoxic activity. For the two most sensitive cell lines, the NSRs also reduced cancer cell migration in a dose-dependent manner, showing greater inhibitory effects than carboplatin. However, co-treatment with carboplatin did not demonstrate any synergistic effects, and proliferation was not significantly further reduced compared with NSR monotherapy.

Conclusion: We have identified NSRs that suppress the proliferation and migration in patient-derived HGSOC models enriched for CSC-like features. Targeting RAR signalling may represent a promising therapeutic strategy in HGSOC. This project is funded by the European Union under grant agreement number 101119427 (MSCA-DN “eRaDicate”).

Key Words: Cancer Stem Cells; High Grade Serous Ovarian Cancer; Retinoic Acid Receptors.

Abstract PP No. 015 **Spotlight Presentation (PSP 7)**

Targeting Retinoic Acid Receptor Gamma in Prostate Cancer

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Prostate cancer (PCa) is frequently diagnosed in elderly males and remains a major cause of cancer related death. Androgen deprivation therapy remains the gold-standard treatment for most patients. However, in many cases, an aggressive and metastatic hormone independent form eventually develops, for which treatment options remain limited. This highlights the need for novel therapeutic strategies, including targeting potential new players such as retinoic acid receptor gamma (RARγ). For in vitro assays, two androgen-sensitive PCa cell lines (LNCaP and 22Rv1) were tested. Cells were treated with the RARγ antagonist AGN205728, and cell viability was assessed using a resazurin assay. Cell death and cell cycle were analysed by Annexin V and propidium iodide staining followed by flow cytometry. Data were confirmed by RNA sequencing. Combinatory effect with Enzalutamide was evaluated through synergy finder tool. For 3D models, three in-house established PCa organoid lines harbouring PTEN, PTEN/TP53, and PTEN/STAT3 alterations were used to investigate the effects of AGN205728 in a three-dimensional context. CellTiter-glo 3D assay was used to assess proliferation reduction. A reduction in cell viability was observed in the concentration range of 5–10μM across both cell lines after 72h of treatment with AGN205728. Further analyses revealed a dose-dependent increase in apoptosis, with an accumulation of cells in the G1 phase and a reduction in the G2/S population. These findings were supported by RNA sequencing of both cell lines after 72 h of treatment. Moreover, the combination of AGN205728 with Enzalutamide resulted in a synergistic effect, further enhancing the antiproliferative response. In the 3D models, RARγ expression was assessed at protein and mRNA level. AGN205728 treatment significantly reduced organoid size and viability after 72h of treatment with and IC50 ranging from 30 to 50μM. These findings suggest that pharmacological inhibition of RARγ reduces prostate cancer cell growth and survival in both 2D and 3D models. Importantly, the observed effects in organoid models carrying clinically relevant genetic alterations support the potential of RARγ as a promising therapeutic target in prostate cancer. Further studies are needed to elucidate the underlying molecular mechanisms and to evaluate the efficacy of RARγ targeting in in vivo models.

The project is supported by the eRaDicate doctoral training network funded by the European Union MSCA-DN. Grant agreement: 101119427

Keywords: prostate cancer, retinoic acid receptor gamma, targeted therapy

Abstract PP No. 016

Ligand-Dependent Structural and Functional Investigation of the Human Vitamin D Receptor

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Objectives: The human Vitamin D receptor (VDR) is a ligand-activated nuclear receptor that regulates gene expression involved in calcium homeostasis, immune responses, and cancer-related pathways. These functions depend on ligand binding, receptor conformation, and interdomain communications. We aim to study that how ligand binding influences VDR structure and function by combining in vitro protein experiments with integrative computational analyses and structural investigation of new Vitamin D analogs.

Materials and Methods: Recombinant expression of the VDR ligand-binding domain (LBD) was established in E. coli. Purification protocols are being optimized to improve protein stability and yield. Purified protein is being prepared for crystallization trials and to generate complexes with new vitamin D analogs for structural characterization using X-ray crystallography and Micro ED. Expression of full-length VDR is also being optimized in mammalian and insect cell lines. A computational workflow is also being developed to study ligand-dependent structural regulation of VDR-LBD. Suitable sequences and structural templates were selected, different docking and molecular dynamics (MD) simulations software were evaluated, and standard procedures for receptor and ligand preparation were established.

Results: Our results show that soluble recombinant expression of the VDR LBD can be achieved under defined bacterial expression conditions. Initial purification yielded sufficient protein for preliminary crystallization trials. Our analyses identified suitable starting models using available VDR sequences and experimentally resolved LBD crystal structures from the Protein Data Bank (PDB) complemented by predicted structures from AlphaFold. Evaluation of candidate tools for docking and MD simulations including BoltzDock, SwissDock, AutoDock Vina, and GROMACS with ChimeraX-based preparation workflows resulted in a preliminary and reproducible computational pipeline for VDR LBD complexes with new ligand analogs. Structural insights from these analyses support interpretation of transcriptomic datasets related to VDR activation and ligand-dependent gene regulation.

Conclusion: This study combines experimental and computational approaches to address limitations in understanding ligand dependent VDR regulation. While recombinant expression of VDR remains challenging, ongoing optimization and the established computational framework provide a basis for ligand-dependent structural and functional analyses with novel vitamin D ligands.

Abstract PP No. 017 **Spotlight Presentation (PSP 8)**

Retinoic acid receptor gamma antagonism impairs stemness and promotes differentiation in Acute myeloid leukemia

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Objectives: Acute myeloid leukemia (AML) is an aggressive hematologic malignancy with an overall survival rate of about 30%. AML is characterized by an accumulation of immature myeloid cells in the bone marrow and the peripheral blood. This uncontrolled accumulation rapidly impairs normal hematopoiesis, leading to anemia, infections, and bleeding. Moreover, AML relapses are mainly due to the persistence of leukemic stem cells (LSCs). The retinoic acid receptors α (RARα) and γ (RARγ) are transcriptional factors with opposing roles in hematopoiesis. They represent druggable targets for modulating the balance between differentiation and stemness. Here we hypothesized that antagonizing RARγ using AGN205728, could sensitize the AML cells to all-trans retinoic acid (ATRA), to block stemness maintenance and to induce cell differentiation.

Material and methods: Using RT-qPCR we analyzed the relative expression of RARA and RARG in leukemic cell lines and bone marrow mononuclear cells (BMMNCs) from healthy donors and AML patients. BMMNCs and a human leukemia-initiating model, the TEX cells, were used to test the combination treatments: AGN205728 and ATRA. The effect on the balance between stemness (CD34+/CD38-) and differentiation (CD11b+/CD14+) was assessed using flow cytometry. Cell viability after antagonist treatment was evaluated using an MTT assay. Furthermore, the subcellular localization of RARγ in the AML cell line was assessed by confocal microscopy.

Results: No significant differences in RARA and RARG expression were observed between healthy donors and leukemic patients. The antagonization of RARγ in TEX cells using AGN205728 resulted in a decreased proportion of hematopoietic stem and progenitor cells (HSPCs; $CD34^+/CD38^-$), without inducing immediate toxicity. The combination of AGN205728 with ATRA was tested on BMMNCs from seven patients which showed heterogeneous responses. Nevertheless, three patient samples showed a marked increase in differentiated $CD11b^+/CD14^+$ cells following combination treatment.

Conclusion: In the TEX cells, we showed that RARγ antagonization using AGN205728 reduces the percentage of HSPCs, without affecting the cell viability. In primary cells, AGN205728 in combination with ATRA showed heterogeneous responses which require validation in a larger cohort. This finding demonstrates a therapeutic potential of RARγ antagonist AGN205728 to sensitize AML cells to ATRA and eradicate LSCs.

Keywords: Nuclear receptors, RARγ antagonism, leukemic stem cells

Abstract PP No. 018 **Spotlight Presentation (PSP 9)**

From Friedreich Ataxia to Leukaemia: Omaveloxolone Cooperates with Vitamin D Derivatives to Enhance Differentiation and Growth Arrest of Acute Myeloid Leukaemia Cells

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Acute myeloid leukaemia (AML) is an aggressive blood cancer with a poor prognosis, particularly for older patients who are often unfit for intensive chemotherapy. Low-toxic differentiation therapy is limited to one rare AML subtype, acute promyelocytic leukaemia (APL). Previous work from our laboratory showed that 1α,25-dihydroxyvitamin D (1,25D) or its synthetic analogues, e.g., PRI-5202, combined with electrophilic agents that activate Nrf2, the major transcriptional regulator of antioxidant and cytoprotective pathways, synergistically enhanced myeloid differentiation and growth arrest in non-APL AML cells. Omaveloxolone (RTA-408), a semisynthetic Nrf2-activating triterpenoid that has been recently approved for the treatment of Friedreich's ataxia, reportedly exerts cytotoxicity to lymphoblastic leukaemia and multiple myeloma cells in vitro at clinically achievable nanomolar concentrations.

Objectives: We investigated whether RTA-408 combines the cytotoxic activity with enhancing 1,25D- and PRI-5202-driven differentiation in non-APL AML cells.

Materials and Methods: Human HL60, MOLM-13 and KG-1a AML cell lines were used as in vitro models. Myeloid differentiation was assessed by measuring the cell surface expression of the monocytic marker CD14 and the general myeloid marker CD11b. Cell viability and growth were determined by the intracellular ATP assay and trypan blue exclusion assay, cell cycle distribution by propidium iodide DNA staining, apoptosis by annexin V/7-AAD staining and protein expression by western blotting.

Results: RTA-408 killed AML cells by inducing apoptosis in a dose-, time-, and cell type-dependent manner (IC50 = 50-120 nM). At lower concentrations (≤100 nM), it had almost no effect on the expression of myeloid markers, while markedly enhancing cell differentiation induced by minimally effective concentrations of 1,25D or PRI-5202 (0.25-2.5 nM). This was accompanied by inhibition of cell growth, accumulation of the G1 cell population, and upregulation of the Vitamin D Receptor expression. At higher concentrations found in patients' plasma (100-500 nM), RTA-408 cooperated with PRI-5202 to induce apoptotic cell death. Notably, the remaining cells displayed full CD14/CD11b positivity and G1 cell cycle arrest.

Conclusions: RTA-408 synergizes with 1,25D and PRI-5202 to enhance myeloid differentiation while exerting cytotoxicity at pharmacological concentrations. These data support its potential role in differentiation-based therapeutic approaches for AML.

Abstract PP No. 019

Design, Synthesis, and Biological Evaluation of Side-Chain- and A-Ring-Modified Vitamin D Analogues

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Objectives: The toxic calcemic side effects and rapid metabolic degradation of the natural hormone 1,25D3 limit its clinical use and necessitate the development of highly active, metabolically stable, and low-calcemic analogues. This study aimed to investigate vitamin D analogues modified in the side chain and triene system, and to evaluate the impact of structural modifications on their biological activity.

Materials and Methods: Vitamin D analogues bearing modified and branched side chains, namely PRI-1927, PRI-1937, and PRI-1938, were designed and synthesized. Coupling of the side-chain ketone with a vitamin D C-22 benzothiazole sulfone via a modified Julia olefination afforded mixtures of 22-cis and 22-trans olefins. PRI-1927 possesses a natural 5,6-cis (5Z) configuration, whereas PRI-1937 and PRI-1938 contain 5,6-trans (5E) triene systems.

Results: PRI-1927 and PRI-1937 exhibited increased stability toward the vitamin D-metabolizing enzyme CYP24A1 compared with 1,25D3. PRI-1938, featuring a 5,6-trans triene system and a 22,24-all-trans side-chain geometry, showed significantly reduced metabolic conversion by cytochrome P450 3A4. Molecular modeling indicated that PRI-1938 adopts a highly stable conformation within the CYP24A1 active site, stabilized by hydrogen-bonding and hydrophobic interactions. The affinities of PRI-1938 and PRI-1927 for VDR, as measured by the fluorescence polarization (FP)-based competition assay, were comparable to that of 1,25D3, whereas the affinity of PRI-1927 was substantially reduced. The potency of PRI-1927 and PRI-1938 in inducing monocytic differentiation of HL-60 cells were compared to that of 1,25D3; however, they were lower than those of the previously reported analogues PRI-1906 and PRI-5202.

Conclusions: Modifications of the side-chain and triene system of vitamin D improve metabolic stability and support the further development of PRI-1938 as a promising vitamin D analogue with anticancer potential.

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Reference: Żolek, T.; Kadam, M.; Kutner, A.; et al. Biomolecules 2025, 15, 1222.

Key words: vitamin D analogues, molecular modeling, biological activity

Abstract PP No. 020 **Spotlight Presentation (PSP 15)**

Targeting Retinoic Acid and Vitamin D Receptors to Enhance Chemotherapy Outcomes in Triple-Negative Breast Cancer

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Introduction: Treatment resistance and tumor relapse represent major challenges faced by patients with breast cancer, a worldwide leading cause of female cancer mortality. Among its subtypes, triple-negative breast cancer (TNBC) is considered the most aggressive and is associated with poorer prognosis and limited therapeutic options. Even with continuous progress in treatment and ongoing research efforts, the high rates of incidence, mortality and relapse in breast cancer emphasize the need for more effective treatments. Cancer stem cells (CSCs) are great contributors to this burden. The vitamin D receptor (VDR) and retinoic acid receptor (RAR) signaling pathways are critical for CSC maintenance and function, affecting tumor progression, response to therapy and regrowth of the tumor mass. Thus, the present study assessed the effects of RAR and VDR ligands, administered alone or in combination with standard chemotherapy, across different TNBC models.

Materials and Methods: Three TNBC cell lines (MDA-MB-231, MDA-MB-436, and HCC1937) were selected to investigate the effects of RAR and VDR ligands. The cells were treated either with a VDR ligand (P) and an RAR ligand (A), or in combination with conventional chemotherapy, doxorubicin (DOX) and paclitaxel (PTX). The experiments employed both two-dimensional (2D) monolayer cultures and three-dimensional (3D) spheroid models to more accurately mimic tumor behavior and intercellular interactions. Cell viability/proliferation was assessed using MTT assays in 2D cultures (after 72 h of treatment), and CellTiter-Glo® assays in 3D cultures (after 96 h of treatment).

Results: In 2D cultures, treatment with ligands A and P alone induced significant antiproliferative/cytotoxic effects. In addition, both VDR and RAR ligands combined with DOX and PTX significantly ($p < 0.01$) reduced viability in all three cell lines. In 3D cultures, our results demonstrated a tendency towards greater cytotoxic effects in combinatorial treatments compared to chemotherapy and the ligands alone.

Conclusions: The trends observed in spheroid models reinforce the potential translational relevance of this approach, suggesting that targeting VDR and/or RAR pathways alongside chemotherapy may improve therapeutic efficacy. In summary, these findings position ligands A and P as strong candidates for combination therapy, offering renewed therapeutic prospects for patients with triple-negative breast cancer.

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Abstract PP No. 021 **Spotlight Presentation (PSP 16)**

Decoupled Physical and Chemical Stability in Vitamin D_2 and D_3 Amorphous Solid Dispersions Prepared by Spray Drying and Ball Milling

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Objectives: The formation of amorphous solid dispersions (ASDs) is used to improve bioavailability of drugs by enhancing their solubility. This study investigates how the preparation method (spray drying, SD, and ball milling, BM) and structural differences between vitamin D_3 (VD_3) and vitamin D_2 (VD_2) influence the physical and chemical stability of ASDs.

Materials and Methods: ASDs of VD_3 and VD_2 were prepared at a drug loading of 50% using either Soluplus® (SP) or polyvinylpyrrolidone K-30 (PVP) as polymers. Each VD-polymer system was processed via SD and BM. To mitigate degradation during BM, nitrogen flushing, cooling, addition of 5% tocopheryl polyethylene glycol 1000 succinate, and their combination were evaluated. Solid state properties were analysed using powder X-ray diffraction and differential scanning calorimetry. Chemical stability was quantified by high-performance liquid chromatography. Physical stability was assessed after storage at 25 °C and 70% relative humidity (RH).

Results: All VD_3 systems were fully amorphous and remained physically stable under humid conditions. In contrast, VD_2 systems showed polymer- and process-dependent solid state nature. SD VD_2-SP exhibited residual crystallinity, and SD VD_2-PVP crystallised after humidity exposure. The corresponding BM samples remained amorphous after preparation and for three weeks at 70% RH, indicating improved physical stability compared with SD samples. Chemically, VD_2 systems were generally more stable than those containing VD_3, which contrasts with their behaviour in the neat crystalline form where VD_3 is more stable. BM induced more degradation than SD, likely due to mechanical and thermal stress. However, VD_3 content in BM VD_3-SP was significantly improved from 46% to 78% retained VD content by applying cooling and antioxidant strategies.

Conclusions: In ASDs containing VD, physical and chemical stability are decoupled. The additional double bond and methyl group in VD_2 have implications for physical and chemical stability. Additionally, our study shows that BM is a viable solvent-free approach for producing physically stable ASDs, but it requires further optimisation to minimise degradation.

Keywords: Amorphous Solid Dispersions, Stability, Vitamin D

Abstract PP No. 022

Mechanistic Insight into Retinoic Acid Receptor-γ Regulation in Prostate Cancer

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Objectives: The retinoic acid receptors (RARs) α, β, and γ (RARs) are type II nuclear receptors that modulate cell proliferation and differentiation via transcriptional control of gene expression. RARγ plays a role in prostate development and inhibition of its activity has been shown to reduce the growth of prostate cancer (PCa) cells. The mechanism by which this occurs is not well understood. Through analysis of transcriptional regulatory dynamics, we aim to define how RARγ controls gene expression and how this regulates PCa progression. We examined the effect of antagonism of RARγ on gene expression and how associated pathways were influenced.

Material and Methods: Experiments were performed using three PCa cell lines, PC3, 22RV1 and LNCaP. The effects of the RARγ antagonist AGN205728, the PPARγ agonist pioglitazone, and the THRβ antagonist NH3 on cell viability were evaluated using MTT assays. RNA sequencing was performed for PC3 cells post AGN205728 treatment. Differentially expressed genes (DEGs) after 6 and 30 hours of RARγ antagonist treatment were subjected to gene ontology (GO) and pathway enrichment analysis. RT-qPCR and Nile red staining was performed for quantification of RNA expression and lipid accumulation, respectively.

Results: Treatment of the PCa cell lines with RARγ antagonist treatment reduced cell viability. RNA sequencing revealed that the other type II nuclear receptors VDR and PPARγ were downregulated post treatment whereas PPARβ/δ and THRβ were upregulated post treatment. GO analysis indicated effects on lipid and cholesterol metabolic processes, which aligns with the function of PPAR and THRβ. The two latter receptors are known to be involved in PCa progression. Lipid accumulation increased in all three cells lines after RARγ antagonism.

Conclusions: Results indicate that RARγ inhibition regulates the expression of other type II nuclear receptors, some of which (e.g. THRβ and PPARγ) are known to be important for PCa progression. Our results further imply that RARγ antagonism disrupts metabolic processes associated with these receptors.

Keywords: Retinoic acid receptors, Type II nuclear receptors, Prostate cancer

Abstract PP No. 023 **Spotlight Presentation (PSP 18)**

In silico analysis of vitamin D_3 analogues within the heterodimer VDR-RXRα complex using AlphaFold3 and multi-algorithm docking

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Objectives: The active form of vitamin D_3 exerts its pleiotropic effects by binding to the heterodimeric complex formed by the vitamin D receptor (VDR) and retinoid X receptor α (RXRα), which subsequently interacts with specific DNA sequences. Although numerous side-chain modified vitamin D_3 analogues with improved therapeutic profiles have been developed, the precise structural and mechanistic basis of their interaction with the complete VDR-RXRα assembly remains largely unexplored. The aim of this study was to investigate the molecular recognition patterns and binding mechanisms of selected novel vitamin D_3 analogues within the full VDR-RXRα complex using state-of-the-art computational approaches.

Materials and Methods: The VDR-RXRα heterodimeric complex was generated using AlphaFold3. A series of structurally diverse vitamin D_3 analogues was docked into the ligand-binding domain using two independent algorithms (GOLD and CDOCKER) to compare docking performance and binding consistency. Structural optimization and energy calculations were performed in BIOVIA Discovery Studio. Binding free energies were estimated from averaged docking poses, and detailed interaction profiles (hydrogen bonds, hydrophobic contacts) were analyzed to assess ligand-induced receptor dynamics.

Results: Docking results showed consistent ligand orientation toward helix H12 - the key structural element responsible for receptor activation - across most analogues. Significant differences in total energy, interaction patterns, and receptor flexibility were observed between the analogues, indicating that even subtle side-chain modifications can markedly influence binding affinity and conformational stabilization of the VDR-RXRα complex.

Conclusions: This study demonstrates that AlphaFold3-generated models of the complete VDR-RXRα complex, combined with multi-algorithm molecular docking, provide valuable insights into the molecular recognition of vitamin D analogues. The results highlight how targeted structural modifications can modulate receptor activation and support the rational, structure-based design of new vitamin D_3 derivatives with enhanced therapeutic potential. Funding: This work was funded by the EU HORIZON-MSCA-2022-DN “eRaDicate” Project Number 101119427.

Keywords: Vitamin D analogues, VDR-RXRα complex, molecular docking

Abstract PP No. 024 **Spotlight Presentation (PSP 19)**

AlphaFold-guided multi-algorithm docking of side-chain modified vitamin D_2 analogues into the VDR-RXRα heterodimer

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Objectives: Vitamin D_2 exerts its biological effects through binding to the vitamin D receptor (VDR) as part of the VDR-RXRα heterodimer, a ligand-activated transcription factor complex that regulates calcium-phosphate homeostasis, immune function, and cell differentiation. Despite extensive structural studies, the molecular determinants of ligand selectivity and receptor activation by vitamin D_2 metabolites and its analogues within the full VDR-RXRα heterodimer remain incompletely understood. This study aimed to characterize the binding interactions of the active metabolite of vitamin D_2 and selected analogues with the VDR-RXRα heterodimer and to predict their relative binding affinities and potential functional consequences.

Materials and Methods: An integrative computational approach combining structure-based drug design and molecular docking was employed. The structure of the VDR-RXRα heterodimer was obtained from AlphaFold. Three-dimensional structures of the active vitamin D_2 metabolite and its side-chain modified analogues were generated and energy-minimized. Molecular docking simulations were performed using two independent algorithms - GOLD and CDOCKER - to evaluate ligand orientation and binding within the VDR-RXRα heterodimer, assess shape complementarity within the binding pocket, and compare docking performance. Detailed interaction analysis included identification of conventional and carbon hydrogen bonds, hydrophobic contacts, and key amino acid residues involved in ligand stabilization and receptor activation.

Results and Conclusions: Docking results revealed that side-chain modifications significantly influence ligand orientation and interaction patterns within the VDR-RXRα heterodimer. Both GOLD and CDOCKER algorithms consistently showed that several analogues exhibited enhanced interactions with critical residues compared to the native active metabolite of vitamin D_2, suggesting improved binding affinity. These structural differences were found to modulate receptor conformation, potentially affecting coactivator recruitment. The study demonstrates that the combination of AlphaFold-generated VDR-RXR heterodimer models with multi-algorithm molecular docking is a powerful tool for predicting structure-activity relationships of vitamin D analogues and provides a rational basis for the design of new compounds with optimized therapeutic profiles. Keywords: vitamin D_2, VDR, molecular docking, GOLD, structure-activity relationship

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Abstract PP No. 025 **Spotlight Presentation (PSP 17)**

Rational design of molecularly imprinted polymer for sensitive and selective electrochemical detection of imatinib: synergy between computational modeling and electrochemistry

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Objectives: Imatinib is a cornerstone of targeted therapy for chronic myeloid leukemia, yet its selective and rapid determination in complex biological matrices remains challenging. This study aimed to develop a highly selective molecularly imprinted polymer (MIP) film for the electrochemical detection of imatinib using differential pulse voltammetry (DPV) and cyclic voltammetry (CV). Molecular modeling was employed before synthesis to rationally select the optimal functional monomer and monomer-template stoichiometry in order to enhance the efficiency and selectivity of the resulting MIP-based sensor. The analytical parameters of the devised chemosensor were established.

Materials and Methods: The geometries of imatinib, carbazole-based functional monomers, and potential interferents were optimized using density functional theory (DFT) at the B3LYP/6-311G(d,p) level (Gaussian 16). Pre-polymerization complexes were investigated by molecular mechanics and molecular dynamics simulations in Discovery Studio (BIOVIA), taking solvent effects into account. Subsequently, the carbazole functional and cross-linking monomers were synthesized. The MIP and control non-imprinted (NIP) films were electrodeposited potentiodynamically onto a gold electrode surface. All voltametric measurements were performed with a Biologic SP-300 potentiostat.

Results: The MIP-modified electrode exhibited a wide linear response to imatinib in the range of 10-200 μM in organic medium, with an imprinting factor of 3.9. The sensor demonstrated excellent selectivity towards structurally related interferents. Molecular modeling studies revealed strong and specific interactions between imatinib and the selected functional monomers, confirming the formation of stable, complementary binding sites. Theoretical predictions of selectivity were in good agreement with experimental results.

Conclusions: This work presents a computationally guided development of molecularly imprinted polymer platform that enables fast, cost-effective, and highly selective electrochemical detection of imatinib. The integration of advanced molecular modeling with voltametric analysis provides deep insight into the recognition mechanism and offers a powerful strategy for the rational design of next-generation MIP-based sensors. This approach holds significant promise for therapeutic drug monitoring and the development of intelligent polymer materials in clinical diagnostics.

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Abstract PP No. 026 **Spotlight Presentation (PSP 3)**

Phase Behavior and Kinetic Stability of Amorphous mixture of two Antifungal APIs

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Objectives: Co-amorphous drug systems are increasingly explored to improve the dissolution and bioavailability of poorly soluble active pharmaceutical ingredients (APIs). In this study, binary mixtures of the antifungal azoles fluconazole (FLZ) and econazole (ECZ) were investigated to evaluate their phase behavior and physical stability and to assess their potential for co-administration and enhanced dissolution of ECZ.

Materials and Methods: Differential scanning calorimetry (DSC), dielectric spectroscopy, and optical microscopy were used to investigate the equilibrium and out-of-equilibrium binary phase diagram of FLZ–ECZ mixtures. Thermal properties, glass transition temperatures (Tg), molecular mobility, and long-term physical stability of amorphous systems were analyzed over a wide composition range.

Results: The crystalline APIs form a eutectic phase diagram with a eutectic temperature of 351.1 ± 0.5 K and a eutectic molar fraction of FLZ of 0.22 ± 0.01. While amorphous FLZ readily crystallizes, amorphous ECZ remains kinetically stable for several weeks as a supercooled liquid and shows faster dissolution in water compared with crystalline ECZ. The Tg of the mixtures increases linearly with composition, rising by ~4 K for every 10% increase in FLZ fraction. Dielectric spectroscopy reveals a single structural and Johari-Goldstein relaxation at all compositions, indicating homogeneous amorphous mixtures. Equimolar and FLZ-rich mixtures phase-separate over time, with FLZ recrystallizing into rod-like crystallites within an amorphous ECZ matrix. In contrast, mixtures at the eutectic composition remain stable for six months above Tg and at least ten months below Tg. ECZ-rich mixtures (≥90%) show the highest stability, remaining amorphous for over 14 months at room temperature.

Conclusions: The stability of FLZ–ECZ co-amorphous systems strongly depends on composition. High ECZ content enhances kinetic stability due to reduced supersaturation, lower thermodynamic driving force for crystallization, and dilution of FLZ. These findings improve the understanding of stability in binary amorphous mixtures and support their potential in antifungal drug formulations. Keywords co-amorphous systems; glass transition; molecular mobility

Abstract PP No. 027

COMPARISON OF OVERWEIGHT AND OBESITY PREVALENCE AMONG YOUNG ADULTS IN 2013-2014 AND 2025.

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Objectives: The study aimed to compare the prevalence of overweight and obesity between two groups of young adults using anthropometric measurements and body composition analysis.

Materials and Methods: Two independent groups of pharmacy students from the Medical University of Warsaw were examined: one group in 2013-2014 (n = 217; 188 women and 29 men) and one group in 2025 (n = 115; 88 women and 27 men). All participants were aged 19–25 years. Anthropometric measurements were performed in accordance with the World Health Organization's recommendations and the guidelines for pharmacists on basic anthropometric measurements. Body mass index (BMI) was calculated. Additionally, body composition parameters were assessed using bioelectrical impedance analysis (BIA), using a professional Tanita SC-330P analyzer to allow for a more comprehensive comparison between the study groups.

Results: Students examined in 2025 exhibited significantly higher body weight, BMI, and waist circumference than those examined in 2013-2014. The prevalence of being overweight and obese was also higher in the 2025 group – Figure 1. While mean BMI values remained within the normal range in both groups, participants from the more recent study period had a higher body fat percentage and a higher metabolic age.

Conclusions: These differences align with global epidemiological trends indicating an increase in body weight and adiposity. The results highlight the need to monitor anthropometric and body composition parameters in young populations and the need to implement preventive measures.

Keywords: overweight, obesity, bioimpedance, young adults.

Abstract PP No. 028

Synthesis and characterisation of N-noropiate derivatives containing amine or amide functionality in their side chain

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Objectives: Substitution of the basic nitrogen in poppy alkaloids can lead to a variety of changes in their biological behaviour. Recently, the interest for derivatives containing one or more nitrogens in their substituent on this site got higher, as related compounds containing these proved to be effective agonists of the opioid receptors. We aimed to synthetize various amine and amide containing noropiates to test thier physichochemical properties and biological effects.

Materials and Methods: The reagents and solvents were purchased from Sigma-Aldrich (St. Louis, MO, USA) Alfa Aesar (Haverhill, MA, USA) and TCI Europe (Zwijndrecht, Belgium) and used without further purification. 1H and 13C-NMR spectra were recorded on a Varian Mercury Plus (, Varian, Inc., Palo Alto, CA, USA,) in DMSO-d6 or CDCl3 solutions; δ is given in ppm referenced to the residual solvent signal. 1H and 13C-NMR signals were assigned on the basis of one- and two-dimensional homo- and heteronuclear experiments. Melting points were determined on a SRS OptiMelt MPA100 apparatus (Stanford Research Systems, Sunnyvale, California, USA) and are uncorrected. Mass spectra were recorded on a Waters Xevo G2 XS-QTo system. Reaction progress was followed by thin-layer chromatography on commercial silica gel plates (Merck silica gel F254 on aluminum sheets, Darmstadt, Germany) using different mobile phases. For column chromatography, Kieselgel 60 (particle size 0.040–0.063 mm, VWR Chemicals, Radnor, PA, USA) was employed.

Results: 33 derivatives were snythetized in total, using norcodeine, dihydronorcodeine, normorphine, and noroxymorphone as starting materials. The goal of the selected substituents were the incoporation of different electron densities, and basicites into the nitrogen containing substituent. The compounds were fully characterised using NMR spectroscopy, and mass spectrometry methods. Purities were found to be over 95% percent, according to HPLC with UV-VIS detection. Some of these derivatives were subjected to different physichochemical tests, unearthing a range of interesting properties.

Conclusions: Nitrogen containing substituents in the semisynthetic morphine derivatives proved to be a rewarding field so far, with 33 compounds already produced, and around 50 more planned to be synthetized. Their characteristics also proved to be advantageous.

Abstract PP No. 029 **Spotlight Presentation (PSP 5)**

Assessment of Membrane Integrity and Membrane–Acceptor Compatibility in PAMPA Systems

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Objectives: Parallel Artificial Membrane Permeability Assay (PAMPA) is a widely used in vitro technique for modeling passive drug transport and is extensively applied in early-stage drug development due to its high-throughput capability. However, key experimental parameters - membrane composition, acceptor phase, and sink conditions - show wide variability. Therefore, this study aimed (i) to develop a robust membrane integrity test applicable across commonly used PAMPA membranes and acceptor phases, and (ii) to evaluate the compatibility of different membrane compositions with various acceptor media.

Materials and Methods: Experiments were performed using Millipore PAMPA plates with six membrane compositions: dodecane; phosphatidylcholine (PC)-containing mixtures with or without cholesterol, lecithin containing membrane and BBB lipid. Five acceptor media were investigated: PBS (pH7.4), PBS containing 20% of HPβCD, HEPES buffer with 1% SLS, and biorelevant media (FaSSIF, FeSSIF). Membrane integrity was assessed using Lucifer Yellow and calcein as non-permeating markers, while Brilliant cresyl blue (BCB) evaluated membrane functionality; benzalkonium chloride served as a positive control. The assembled system was incubated at 37 °C for 5 h. Following incubation, membranes were evaluated visually, and compound permeation was quantified by UV spectroscopy.

Results: During the measurements dodecane in all circumstances shows a lower permeability for BCB, indicating limited transport capacity. Membranes containing only PC showed disruption when HEPES buffer with 1% SLS was used as the acceptor phase. This effect was mitigated by the presence of cholesterol, which improved membrane stability. Furthermore, notable differences were observed in membrane-acceptor compatibility across biorelevant media, highlighting that both membrane composition and acceptor phase critically influence permeability through the membrane.

Conclusions: Calcein proved to be a reliable and practical alternative to Lucifer Yellow for membrane integrity tests. The study provides a systematic evaluation of membrane-acceptor compatibility, particularly under biorelevant conditions, offering valuable guidance for PAMPA method optimization. Overall, the findings underscore that appropriate selection of both membrane composition and acceptor medium is essential for ensuring experimental robustness, reproducibility, and physiological relevance in permeability studies.

Abstract PP No. 030 **Spotlight Presentation (PSP 6)**

Influence of Pharmaceutical Polymers on the Solubility-Permeability Interplay of Curcumin

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Objectives: Curcumin is a poorly water-soluble active pharmaceutical ingredient with limited oral bioavailability. Solubility enhancement is a key strategy to improve oral absorption; however, increasing apparent solubility often leads to reduced membrane permeability. Therefore, understanding the solubility-permeability interplay is essential for formulation design and early biopharmaceutical optimization. This study aimed to investigate how pharmaceutical polymers influence solubility and passive permeability. A further objective was to compare excipient performance within and across polymer families and to evaluate the relationship between solubility improvement and permeability reduction.

Materials and methods: Measurements were performed in the presence of PEGs, poloxamers, PVPs, PVPVA copolymer. Experiments were carried out at 37°C. The polymers were added in 5m/V% to pH2 Britton Robinson buffer solutions. Thermodinamic solubility was measured with the saturation shake flask method, while kinetic solubility was determined using the cosolvent-shift method. Permeability studies were conducted using the Parallel Artificial Membrane Permeability Assay (PAMPA).

Results: All polymers increased the solubility, the extent of improvement depending on polymer type and molecular weight. Higher molecular-weight variants generally produced greater solubility enhancement within each polymer class. PEGs showed the smallest, while PVPVA and several PVP polymers produced the largest effect. While kinetic solubility values are considerably higher compared to thermodinamic values, the extent of polymer-induced enhancement followed very similar trends. Permeability decreased in all cases where solubility improved, confirming a solubility–permeability trade-off. However, the gain in solubility exceeded the loss in permeability, suggesting a favorable biopharmaceutical effect. Kinetic solubility data correlated more strongly with permeability than thermodynamic data.

Conclusions: Polymer selection and molecular weight play critical roles in optimizing formulation strategies for poorly soluble drugs. Although solubility enhancement was consistently accompanied by reduced permeability, in most cases the overall balance remained advantageous. The stronger association between kinetic solubility and permeability indicates that kinetic measurements may be more suitable than equilibrium methods for early formulation screening and assessing solubility-permeability interplay.

Abstract PP No. 031

pH-Controlled Glycoprotein Binding in Boronic Acid-Functionalized Starch Magnetic Nanomaterials

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Objectives: Selective recognition and isolation of glycoproteins remain challenging due to the structural heterogeneity of glycans and their complex interactions with synthetic materials. Due to their critical role as biomarkers in inflammation, infection, and cancer, there is a growing need for efficient and controllable enrichment strategies. This study aimed to develop starch-based magnetic nanomaterials functionalized with phenylboronic acid for pH-responsive and reversible glycoprotein binding.

Materials and Methods: Dialdehyde starch (DAS) and carboxymethyl starch (CMS) were functionalized with phenylboronic acid to obtain DAS-PBA and CMS-PBA derivatives. These polymers were used to coat magnetite nanoparticles, forming magnetic nanocomposites. The materials were characterized using spectroscopic, microscopic, and thermal methods. The accessibility of boronic acid groups was evaluated using an Alizarin Red S (ARS) assay. Binding performance was investigated using α-1-acid glycoprotein (AGP) as a model system.

Results: All nanomaterials exhibited pH-dependent binding behavior typical of boronic acid–diol interactions. Enhanced AGP binding was observed under alkaline conditions, while partial release under mildly acidic conditions confirmed reversible interaction. CMS-PBA-based nanoparticles showed superior binding capacity compared to DAS-PBA systems, likely due to improved accessibility of boronic acid functionalities.

Conclusions: The developed boronic acid-functionalized starch-based magnetic nanomaterials provide an effective, tunable platform for selective, reversible capture of glycoproteins. Their pH-responsive behavior, combined with magnetic separability and biocompatible design, highlights their potential for advanced glycoprotein enrichment and bioanalytical applications under mild conditions.

Abstract PP No. 032 **Spotlight Presentation (PSP 10)**

Novel thienopyrimidine-based dually acting 5-HT3/5-HT6 receptor antagonists with cytoprotective effect in human astrocytes

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Objectives: Growing evidence implicates glial dysfunction as a central contributor to the pathomechanisms of neuropsychiatric and neurodegenerative disorders. In parallel, serotonergic signaling has emerged as an important regulator of glial physiology. Although antagonists of 5-HT3 and 5-HT6 receptors are well known for their beneficial effects in preclinical models of cognitive decline, recent studies indicate that selective inhibition of these receptors also mitigates toxin-induced damage in vitro, revealing promising glioprotective mechanisms.[1,2] Motivated by these findings, we investigated whether dual 5-HT3/5-HT6 receptor antagonism could provide additive benefits in enhancing glial resilience. Herein, we present a series of novel thienopyrimidine-based dual antagonists, supported by preliminary biological data.

Materials and Methods: A series of 25 compounds was designed through a scaffold-hopping approach and synthesized using multistep procedures involving mechanochemical and microwave-assisted techniques. Affinity for 5-HT3 and 5-HT6 receptors, as well as selectivity over other GPCRs (e.g., 5-HT1A, 5-HT2A, D2) were evaluated in radioligand binding studies. Antagonist activity at the 5-HT3 receptor was assessed in Ca2+ flux assay using CHO-K1 cells. Effect on 5-HT6 receptor-mediated Gs signaling pathway was evaluated in NG108-15 cells using BRET method. Metabolic stability was determined in vitro using rat liver microsome assay. Glioprotective effects were assessed in P10251-IM human astrocytes exposed to 6-hydroxydopamine or menadione.

Results: The study identified compound PZ-2671, which exhibits balanced affinity for 5-HT3 and 5-HT6 receptors (Ki = 40 and 21 nM, respectively) and high selectivity over other serotonergic and dopaminergic receptors. Functional profiling showed that PZ-2671 behaves as a neutral antagonist of 5-HT6 receptor-mediated Gs signaling and as an antagonist at 5-HT3 receptors. The compound exhibited good in vitro metabolic stability and exerted glioprotective effects, preventing toxin-induced damage in astrocytes at 0.25 μM.

Conclusions: These findings support further evaluation of PZ-2671 to elucidate its glioprotective potential in advanced models of neuropsychiatric and neurodegenerative disorders. Acknowledgments National Science Centre, Poland (grant no. 2021/43/B/NZ7/02855). References 1. Rahimian, R., et al. Eur. J. Clin. Invest. 2013, 43(10), 1039-1051. 2. Drop, M., et al. Eur. J. Med. Chem. 2024, 275, 116615.

Keywords: thienopyrimidine, dual 5-HT3/5-HT6 receptor antagonists, glioprotection

Abstract PP No. 033 **Spotlight Presentation (PSP 11)**

Novel Tiagabine-Based Modulators of the Cholinergic-GABAergic Axis: Design, Synthesis, and Biological Evaluation

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Objectives: This study aimed to develop novel tiagabine-derived modulators of the cholinergic–GABAergic system based on a previously identified chemotype enabling selective GAT3 inhibition. Structural optimization focused on replacing the 2,2-diphenylethyl moiety with heteroaryl fragments (2,2-bis(3-methylthiophen-2-yl)ethyl and 2,2-di(thiophen-2-yl)ethyl) to improve biological activity and ADME-Tox properties. The amine fragment was modified with non-aromatic substituents. Based on earlier findings, the (2R,3S) configuration was retained to preserve high GAT3 inhibitory activity.

Materials and Methods: The designed compounds were synthesized using standard organic synthesis procedures and characterized by appropriate analytical methods. Inhibition of the human GABA transporter subtype 3 (hGAT3) was evaluated in a cell-based assay measuring inhibition of hGAT3-dependent GABA uptake in stable COS-7 cells. Butyrylcholinesterase (BuChE) inhibitory activity was determined using the spectrophotometric Ellman assay. Cytotoxicity was assessed in BV-2 mouse microglial cells, HepG2 human hepatocellular carcinoma cells, and HT-22 mouse hippocampal neuronal cells. Selected ADME parameters were also evaluated, including blood–brain barrier permeability using the PAMPA-BBB assay, human serum albumin binding (%HSA), and potential interactions with major cytochrome P450 isoforms.

Results: A total of 18 tiagabine-derived compounds were synthesized and biologically evaluated. Four derivatives demonstrated dual activity toward both cholinergic and GABAergic systems through inhibition of BuChE and GAT3, and were selected for further ADME-Tox studies. Among them, compound DAW-WS-25 emerged as the most promising candidate, exhibiting balanced inhibitory activity toward BuChE (IC_{50} = 0.576 μM; selective over AChE) and GAT3 (IC_{50} = 7.22 μM), comparable to the reference inhibitor (S)-SNAP-5114. The compound showed IC_{50} values of 11 μM in BV-2 cells, 29 μM in HepG2 cells, and 926 μM in HT-22 cells. High plasma protein binding was observed (%HSA = 97.6%).

Conclusions: Compound DAW-WS-25 was identified as a promising multitarget ligand modulating both cholinergic and GABAergic neurotransmission via BuChE and GAT3 inhibition. Its balanced biological activity and favorable in vitro ADME-Tox profile indicate that this derivative represents a valuable lead in the search for new therapeutic options for neurodegenerative diseases.

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Abstract PP No. 034

Different types of substituents of the pyrazolo[1,5-a]pyrimidine core of CPL302415, PI3K inhibitor, and their influence on PI3K inhibition efficacy.

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Objectives: CPL302415 is a highly potent inhibitor of PI3K (Phosphoinositide 3-kinase). The structure is based on the pyrazolo[1,5-a] pyrimidine core, featuring a morpholine at position 7., 2-(difluoromethyl) benzimidazole at position 5., and N-tert-butylamine at position 2. This preclinical candidate for SLE (Systemic Lupus Erythematosus) exhibits great activity (PI3Kδ IC50 = 0.02 μM), favorable selectivity against other PI3K isoforms (PI3Kα/δ=79; PI3Kβ/δ=1415; PI3Kγ/δ=939), and advantageous other properties. In this work, we compared a series of compounds with different types of substituents in the third position of pyrzolo[1,5-a]pyrimidine core of CPL302415 with the reference inhibitor CPL 302415 and evaluated the impact of these modifications on PI3K inhibitory activity.

Materials and Methods: Docking procedure of the structures in the PI3Kδ protein (PDB:2WXP) was prepared using the Auto-DockVina program. All compounds were synthesized in a multistep synthesis. The experiments were carried out using the ADP-Glo Kinase Assay kit (Promega).

Results: We synthesized a library of pyrazolo[1,5-a] pyrimidine derivatives that may be novel, highly potent inhibitors of the kinase activity of PI3Kδ (potentially exhibiting improved activity and/or selectivity relative to CPL302415). Structural variation in substituents resulted in differentiated inhibitory potency against PI3K. The observed activity is comparable to CPL302415 (PI3Kδ IC_{50} ranging from 8 to 48 nM). The primary distinction among the evaluated compounds is in their selectivity profiles toward PI3Kδ relative to other PI3K isoforms. Alterations in critical interactions of structures with amino acid residues in the ATP-binding site correlate with a reduced inhibitory potential of the synthesized derivatives.

Conclusions: Among all obtained structures, CPL302415 demonstrated the highest potency of all obtained molecules. Modifications at the third position of the pyrazolo[1,5-a]pyrimidine core affect the compounds' selectivity profile. Despite these variations, CPL302415 remains the most promising candidate for further preclinical and clinical development.

Acknowledgements: This work was supported by The National Centre for Research and Development (POIR.01.02.00-00-0085/18). Keywords PI3Kδ inhibitors, CPL302415, pyrazolo[1,5-a]pyrimidine

Abstract PP No. 035

Chitosan-based films crosslinked with hemoglobin and bovine serum albumin enriched with BODIPY for potential application in PDT

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Objectives: The presented research focuses on obtaining and characterizing chitosan films crosslinked with bovine hemoglobin (Hb) and bovine serum albumin (BSA), enriched with BODIPY compounds, as potential materials for photodynamic therapy (PDT).

Materials and Methods: Chitosan-based biomaterials were prepared by crosslinking chitosan with natural proteins – bovine hemoglobin (Hb) and bovine serum albumin (BSA), which were functionalized with BODIPY-type photosensitizers. The obtained new biopolymer materials were comprehensively characterized in terms of structure using spectroscopic techniques (ATR-FTIR, UV-VIS, fluorescence) and surface morphology (SEM, AFM). In addition, their mechanical properties, thermal stability, swelling ability, biodegradation rate, and wettability were evaluated. The release profile of the photosensitizers and material interactions with model proteins were also investigated.

Results: Crosslinking chitosan with Hb and BSA improved its mechanical and physicochemical properties while enabling the release of BODIPY. The materials exhibited sufficient thermal stability, favorable bioadhesive properties, biodegradability, and a suitable swelling ratio. Moreover, all materials demonstrated a relatively rough surface and hydrophilicity, which is desirable for biomedical applications.

Conclusions: The tested properties of the obtained materials indicate that the proposed chitosan–protein–BODIPY composites may be considered as a promising carrier for photosensitizers. These systems warrant further investigation toward potential applications in photodynamic cancer therapy.

keywords: biopolymers, BODIPY, photodynamic therapy (PDT).

Abstract PP No. 036 **Spotlight Presentation (PSP 12)**

Integrated computational and experimental study of novel selective MMP-13 inhibitors

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Objectives: Matrix metalloproteinase 13 (MMP-13) has been identified as an important molecular target due to its involvement in the progression of osteoarthritis and rheumatoid arthritis. Moreover, its pathological activity in the tumour microenvironment promotes cancer cells migration and metastasis. Therefore, the development of MMP-13 inhibitors represents a promising strategy for various chronic diseases. In this study, we aimed to explore the inhibitory potential of a series of novel non-zinc-chelating compounds toward MMP-13. Based on our previous research, we optimized a series of pyridine-3-carboxamide derivatives by introducing an oxadiazole ring, to enhance key interactions with amino acid residues in the enzyme binding pocket. The newly designed compounds were first assessed computationally to predict their affinity toward the target enzyme. Subsequently, their inhibitory activity was evaluated experimentally using a commercially available MMP-13 fluorometric assay kit.

Materials and methods: Computational studies included molecular docking performed with AutoDock Tool 1.5.7 using a modified AutoDockZN force field to improve modelling of ligand-zinc interaction. Molecular dynamics simulations were carried out using GROMACS 2024, with 100 ns simulations performed for complexes of the designed compounds with MMP-13 and MMP-8 enzymes. Ligand binding affinities were estimated using the gmx_MMPBSA tool to calculate binding free energies of the obtained complexes. The inhibitory activities of the designed compounds toward MMP-13 and MMP-8 were experimentally evaluated using Abcam fluorometric inhibitor screening assay kits. Additionally, ADMET properties were predicted using the Ersilia platform based on machine learning models.

Results: All designed compounds demonstrated strong affinity toward MMP-13, with significantly lower affinity for MMP-8, indicating promising selectivity. The computational and fluorometric results were consistent, indicating that lower binding free energy correlates with stronger inhibitory activity.

Conclusion: The combined computational and experimental approach confirmed the potential of the designed compounds as selective MMP-13 inhibitors. The agreement between in silico predictions and experimental data supports the reliability of the applied drug design strategy and provides a basis for further optimization of this compound series.

Abstract PP No. 037

Influence of extraction method on the inhibitory activity of fruit extracts against adenosine-metabolizing enzymes

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Objectives: To investigate the impact of different extraction methods on the enzymatic inhibitory activity of fruit extracts from Cornus mas (CM), Hippophae rhamnoides (HR), and Chaenomeles japonica (CJ) against key enzymes of adenosine metabolism: CD73 and adenosine deaminase (ADA), and to assess the relationship between phytochemical composition and biological activity.

Materials and Methods: Extracts were obtained using traditional aqueous extraction and supercritical fluid extraction (SFE) with CO2, with and without ethanol as a co-solvent. Enzymatic activity was determined using spectrophotometric assays adapted to a 96-well microplate format. The inhibitory effect was expressed as percentage inhibition and IC_{50} values where applicable. The phytochemical composition of the extracts was further characterized using HPLC-MS.

Results: The extraction method had effect on enzymatic inhibition profiles. Traditional extracts demonstrated the highest inhibitory activity toward both ADA and CD73. Among the tested samples, CJ and HR traditional extracts showed the strongest ADA inhibition. CD73 inhibition was generally less pronounced. In contrast, SFE extracts obtained without co-solvent exhibited minimal inhibitory activity and did not reach 50% inhibition. The addition of ethanol moderately improved their activity, although it remained significantly lower than that of traditional extracts. HPLC-MS analysis of traditional extracts showed that CJ was particularly rich in procyanidin B2, epicatechin, and caffeic acid, whereas HR was dominated by isorhamnetin glycosides. In contrast, CM extracts contained mainly iridoid compounds such as loganic acid and cornuside. In SFE and SFE/ethanol extracts, phenolic compounds such as epicatechin, quercetin, trans-ferulic acid, and chlorogenic acid were detected at relatively low concentrations, with higher levels generally observed in ethanol-modified extracts compared to SFE alone.

Conclusions: The extraction method is a critical determinant of the biological activity of plant-derived extracts. Traditional extraction proved more effective in recovering phenolic compounds associated with strong enzymatic inhibition, whereas SFE, particularly without co-solvent, yielded extracts with limited bioactivity.

Acknowledgments: The study was financially supported by the Medical University of Warsaw (grant no. 6/F/MB/N/24)

Keywords: Adenosine metabolism, Supercritical fluid extraction, Polyphenols

Abstract PP No. 038 **Spotlight Presentation (PSP 13)**

Development and Bioequivalence of a Novel, Titanium Dioxide- and Lactose-Free Dapagliflozin TZF film-coated tablets Formulation: A "Generic+" Approach

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The primary objective of this project was to develop a novel, immediate-release, oral, film-coated tablet formulation of dapagliflozin (5 mg and 10 mg) that is pharmaceutically and biologically equivalent to the reference drug product (Forxiga).

To address contemporary formulation challenges and enhance patient compliance, a "generic+" approach was adopted. Specifically, the novel formulation was designed to be free of titanium dioxide, aligning with recent European Medicines Agency (EMA) safety recommendations, and lactose-free to ensure better tolerability and acceptability for lactose-intolerant patients. The development process involved a comprehensive characterization of the active pharmaceutical ingredient (API, Dapagliflozin propylene glycol monohydrate) utilizing among others Scanning Electron Microscopy (SEM), Particle Size Distribution (PSD) analysis, and X-ray Powder Diffraction (XRPD). Simultaneously, the reference drug product was subjected to thorough physicochemical profiling, which included the evaluation of tablet dimensions, weight, hardness, disintegration time, and dissolution profiles, alongside Raman mapping and XRPD. Finally, the biological equivalence of the newly developed formulation was evaluated through an in vivo bioequivalence study.

Rigorous physicochemical profiling of the API and the reference product enabled the successful design of a novel film-coated tablet. The "generic+" product exhibited physical properties and in vitro dissolution profiles highly comparable to the reference drug. Notably, change in a composition did not compromise quality or stability of generic product. Furthermore, the in vivo bioequivalence study confirmed that the new formulation demonstrates a pharmacokinetic profile highly similar to the reference product, fulfilling strict biological equivalence criteria. A comprehensive analytical approach facilitated the successful development of a novel dapagliflozin formulation. The resulting immediate-release tablets are pharmaceutically and biologically equivalent to the reference product. By implementing the "generic+" concept—excluding titanium dioxide and lactose—the new drug product offers a modern, safe, and patient-centric alternative for conditions indicated for dapagliflozin.

Abstract PP No. 039 **Spotlight Presentation (PSP 14)**

A Small Structural Change with a Big Impact: Hydrogen Bond Donor Reduction in the Optimization of DAW-AP-218

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Objectives: The aim of this study was to improve the physicochemical profile of a lead compound in order to enhance its ADME–Tox properties, particularly by reducing off-target interactions and cytotoxicity. The design strategy was based on the previously described [1] dual cholinergic and GABAergic modulator DAW-JT-3, which served as the starting scaffold for further optimization. A rational structural modification was introduced by reducing the number of HBD, to the design of a novel analogue, DAW-AP-218. Subsequently, in vitro and in vivo studies were performed to evaluate the impact of this modification and to characterize the pharmacological and pharmacokinetic properties of the new compound.

Materials and Methods: DAW-AP-218 was synthesized using standard organic chemistry methods. In vitro activity was assessed against key targets relevant to its mechanism of action, including butyrylcholinesterase (BuChE) and GABA transporters. Selected ADME parameters were determined. Off-target interactions were screened using a panel of 44 molecular targets (Eurofins Scientific). In vivo studies included pharmacokinetic, toxicological, and pharmacodynamic assessments. Behavioral models were used to evaluate cognitive, anxiolytic-like, and antidepressant-like effects.

Results: Reduction of a single hydrogen bond donor resulted in significant changes in biological activity and the ADME–Tox profile. BuChE inhibitory activity was improved, while inhibitory activity against GABA transporters decreased markedly. DAW-AP-218 also showed reduced cytotoxicity (neurotoxicity and immunotoxicity) compared with DAW-JT-3. Importantly, DAW-AP-218 demonstrated an improved safety profile in antitarget screening, with no significant interactions detected, whereas DAW-JT-3 showed notable off-target effects. In vivo studies confirmed pro-cognitive, anxiolytic-like, and antidepressant-like activity of the new analogue.

Conclusions: The conducted studies led to the development of a promising drug candidate for the treatment of neurodegenerative disorders. The results demonstrate that even a subtle modification, substantially improve physicochemical and ADME–Tox properties while reducing cytotoxicity and off-target interactions. These findings highlight the importance of precise structural optimization in medicinal chemistry.

Acknowledgements: This work was supported by the National Science Centre, Poland Grant No. 2021/41/B/NZ7/02825 [1] Panek D. et. al. Angew Chem Int Ed Engl. 3, 64, (2025), e202420510

Abstract PP No. 040

Curcuminoid chalcone derivatives – synthesis and biological activity profile

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Objectives: The objective of this study was to synthesize a series of curcuminoid chalcones and their derivatives through structural modifications using classical, microwave-, and ultrasonic-assisted organic synthesis. Their biological activity was assessed for antioxidant, anti-inflammatory, anticholinergic, anticancer, and neuroprotective effects. Preliminary molecular docking research using in silico methods was conducted.

Materials and Methods: The synthesis of curcuminoid chalcones and their derivatives using various aromatic ketones and aromatic aldehydes was investigated via the Claisen-Schmidt reaction under appropriate conditions. Moreover, a method for the synthesis of direct-type curcuminoid chalcone hybrids containing a nonsteroidal anti-inflammatory drug fragment via the Steglich esterification reaction was developed, and the stability of the resulting hybrids was determined. The cytotoxicity of the obtained chalcone compounds was assessed using the resazurin reduction assay, LDH assay, MTT assay, and SRB assay. The cytotoxicity of selected compounds was also assessed in combination with ultrasound (sonodynamic therapy). In biological studies, qRT-PCR, the HORAC method, the modified Elman method, flow cytometry-based methods, and Western blot were also used. Antitumor activity was assessed against the CACO-2, SCC-25, FaDu, MeWo, and A375 cell lines. The HT-22 cell line was used to assess neuroprotective activity.

Results: The results of this study were the synthesis of new derivatives with favorable pharmacological potential. SAR analysis showed that the presence of hydroxyl and methoxyl on at least one aromatic ring of the molecule significantly shaped biological activity. Moreover, in silico screening studies showed results correlating with data from in vitro experiments. The results provide a solid foundation and inspiration for continued chemical and pharmacological research. The observed biological properties of the obtained derivatives indicate their potential for further development as drug candidates targeting multifactorial conditions, including neurodegenerative diseases and cancer.

Conclusions: The results of the conducted research, both synthetic and biological, proved that the topic of so-called curcuminoid chalcones and the proposed research strategy constitute a promising and developmental direction in the search for new drugs in the treatment of diseases of complex etiology.

Keywords: curcuminoid chalcones, Claisen-Schmidt condensation, biological activity Research

Abstract PP No. 041

Surface-supported polymer microparticles decorated with selenium nanoparticles as potential drug carriers.

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Objectives: This study aimed to develop polymer microparticle-selenium nanoparticles composites and evaluate their in vitro efficacy as drug delivery vehicles for the treatment of triple-negative breast cancer.

Materials and methods: Thin polycaprolactone (PCL) films with selenium nanoparticle (SeNP) deposits were prepared on a solid substrate, and then melted while submerged in polar aqueous solvent, which induced dewetting and microparticle formation. Morphology and elemental composition were characterized by SEM, EDS and Raman spectroscopy. To test efficacy as a drug delivery vehicle, doxorubicin (DOX) was incorporated, and its loading was confirmed by fluorescence spectroscopy. Subsequently, the resulting drug delivery systems were tested using MTT cytotoxic assays on triple-negative breast cancer cell line, MDA-MB-231.

Results: The successful incorporation of SeNPs on the microparticle surface was confirmed by Raman spectroscopy and EDS mapping. The DOX-loaded SeNP-PCL microparticles composites demonstrated significant cytotoxic effect against triple-negative breast cancer cells. Notably, the presence of SeNPs was found to enhance the overall cytotoxic effect.

Conclusions: The engineered SeNP-PCL microparticles represent a novel and effective platform for drug delivery.

Keywords: breast cancer, nanoparticles, doxorubicin

Abstract PP No. 042

Development of new nitrosation procedures – stress testing for the APIs to verify their stability with respect to the risk of forming NDSRI impurities.

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Objective: The worldwide crisis caused by carcinogenic nitrosamines and nitroso-APIs (NDSRIs) in medicinal products since 2018 has prompted analytical scientists to develop new approaches to predict these impurities. Following the initial detection of carcinogenic nitrosamines in valsartan-containing medicinal products, regulatory authorities worldwide requested that marketing authorisation holders conduct risk assessments regarding nitrosamines in their products. Despite these assessments, new nitrosamines continue to be identified. We found that the quality control tests described in international pharmacopoeias may be insufficient for detecting new NDSRIs. Therefore, it is crucial to develop a nitrosation method to predict the risk of contamination associated with nitrosamine active substances in medicinal products.

Materials and Methods: The ROXY electrochemical reactor cell (EC) from Antec was used to generate nitroso-API impurities. Measurements were made at different potentials and using excess amounts of nitrosating agent. In addition to the electrochemical approach, a conventional chemical synthesis of the nitroso derivatives was carried out in a flask using nitrites and acid. Identification of the resulting products was made using LC-QTOF Maxis 4G (Bruker).

Results: Atenolol was selected for the initial stress tests towards NDSRIs formation. EC-MS analyses were performed at: 0.5, 0.8, 1.0, and 1.5 V. Conventional NDSRI synthesis was carried out at: 25 °C and 60 °C. Following EC-LC-QTOF analysis, N-nitrosoatenolol and the oxidised form of N-nitrosoatenolol were identified. Their structures were determined from fragmentation spectra obtained using a high-resolution QTOF spectrometer.

Conclusions: A novel nitrosation methodology to predict the risk of NDSRIs formation in medicinal substances was presented. The generation of NDSRIs using EC–MS and chemical synthesis has confirmed that these approaches are well-suited for studies in the field of drug stability, particularly for the investigation of drug-related impurities. Our research contributes to expanding knowledge about nitrosamines, especially unexplored NDSRIs, and will enable better risk analysis, thereby contributing to ensuring the safety of therapies for patients.

Acknowledgements: The project was financially supported by the Polish Ministry of Health: subvention No.10/2026.

Keywords: EC-MS, Quadrupole Time-of-Flight (QTOF) Mass Spectrometry, Nitrosamine Drug Substance-Related Impurities (NDSRIs)

Abstract PP No.043

Qualitative and quantitative analysis of supplements containing soy isoflavones using HPLC and NMR methods

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Objectives: Soybean (Glycine max (L.) Merr.) from the Fabaceae family is one of the richest natural sources of isoflavones, particularly genistin and daidzin. These compounds belong to the group of phytoestrogens, which exert estrogen-like effects in the human body. Numerous studies have demonstrated that isoflavones present in soy products may help regulate hormonal balance in women, reduce cholesterol levels, prevent osteoporosis, and exhibit anticancer properties. The aim of this study was to determine the isoflavone content in commercially available soybean-based preparations and to compare the obtained results with the declared content on the product label.

Materials and Methods: The study material consisted of six dietary supplements available on the Polish market. Extracts of each preparation were obtained using 96% ethanol. Isoflavone content was determined by high-performance liquid chromatography with diode-array detection (HPLC-DAD). The analytical results were compared with labeled content. In addition, qualitative analysis was performed using 1H and 13C nuclear magnetic resonance (NMR) spectroscopy.

Results: The isoflavone content and compositional profiles varied significantly among the analyzed products. The predominant compounds were the isoflavone glycosides: daidzin and genistin, with daidzin at the highest concentration. Variable amounts of glycitin were also detected, indicating differences in the soybean extracts used in the supplements. This compositional diversity was further reflected in the NMR spectra of the analyzed products. In most cases, the total isoflavone content, expressed as genistein, differed from the label-declared amount.

Conclusions: Soybean-based dietary supplements available on the Polish market show considerable variability in isoflavone composition and content. These findings underscore the need for improved quality control and standardization of isoflavone-containing supplements.

Keywords: isoflavones, HPLC-DAD, NMR

Abstract PP No. 044

Analysis of immunoreactivity and expression of E-cadherin and p38 MAPK in prostate cancer

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Objectives: Prostate cancer is one of the most prevalent malignancies among men, requiring a better understanding of the molecular mechanisms underlying tumor progression. Among the key regulatory pathways, the p38 MAPK signaling cascade and cell adhesion molecules such as E-cadherin play a critical roles in maintaining cellular homeostasis and epithelial integrity. The aim of this study was to evaluate the expression of p38 and E-cadherin in prostate cancer compared with benign prostatic hyperplasia (BPH).

Materials and Methods: The study was conducted using tissue samples from 20 patients diagnosed with BPH and 20 with prostate cancer. Expression levels of the analyzed proteins were determined using immunohistochemistry and qRT-PCR.

Results: The results revealed significantly decreased expression of p38 and E-cadherin in prostate cancer tissues compared with BPH. Reduced p38 activity may impair oxidative stress-response and differentiation processes, while loss of E-cadherin disrupts cell adhesion and promotes a more invasive phenotype.

Conclusions: These findings suggest a potential functional link between p38 MAPK signaling and epithelial integrity in prostate cancer. Concomitant downregulation of p38 and E-cadherin may contribute to tumor progression by facilitating loss of differentiation and increased cell motility. In conclusion, alterations in p38 and E-cadherin expression may represent an important molecular axis involved in prostate cancer development and may represent potential targets for future therapeutic strategies.

Abstract PP No. 045

In Silico Investigation of the Mechanism and Structural Dynamics of CamA Methyltransferase

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Objectives: Bacterial infections are an increasing challenge due to rising antibiotic resistance, highlighting the need for novel therapeutic targets. One promising target is the CamA methyltransferase from Clostridioides difficile, an enzyme essential for spore formation and biofilm development. This study aims to elucidate the catalytic mechanism of CamA and characterize its structural dynamics to support the rational design of potential inhibitors.

Materials and Methods: A combination of in silico approaches was employed. Molecular dynamics (MD) simulations were performed to investigate the behavior of CamA under an physiological conditions and to analyze the flexibility of the SAM-binding loop in the presence of different ligands. Sequence analysis of adenine methyltransferases was conducted to identify structural differences. Additionally, quantum chemical calculations were applied to model the catalytic reaction pathway and estimate the associated energy barriers.

Results: Sequence analysis revealed that CamA possesses a significantly longer SAM-binding loop compared to other adenine methyltransferases, which may contribute to its lower affinity for the cofactor relative to the reaction product, S-adenosylhomocysteine. MD simulations demonstrated that the dynamics of this loop are ligand-dependent. Quantum chemical calculations showed that enzyme conformations derived from MD simulations are favorable for methylation, with energy barriers comparable to experimental values. Furthermore, the second step of the reaction, deprotonation of methylated adenine, is not feasible via an enzymatic mechanism involving a glutamine residue. Instead, it likely occurs with the participation of water molecules after dissociation of the reaction product.

Conclusions: The study provides detailed insight into the catalytic mechanism and structural dynamics of CamA methyltransferase. The findings indicate that the deprotonation step is not enzyme-driven but likely occurs in a water-mediated manner following product dissociation. Additionally, CamA is characterized by a uniquely extended SAM-binding loop, whose conformational dynamics are strongly dependent on the bound ligand. These insights may support the rational design of CamA inhibitors as potential adjunct therapies against Clostridioides difficile infections.

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Abstract PP No. 046 **Spotlight Presentation (PSP 20)**

Sustainable Mechanochemical Synthesis of Selected Atypical Antipsychotic Drugs

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Objectives: Mechanochemistry has recently been recognized as an efficient method for the synthesis of biologically active compounds, offering a sustainable alternative to classical in-solution processes. Continuing our efforts on the development of greener chemical routes for obtaining APIs [1], we applied a solid-state approach for the synthesis of clinically used atypical antipsychotics selected from different chemical groups (i.e., risperidone, amisulpride, cariprazine), in a gram scale.

Materials and Methods: All reactions were performed in a vibratory ball-mill operating at 30 Hz using 35 and 50 mL stainless steel or PTFE jars charged with one stainless steel ball. Selected antipsychotic drugs were obtained using three different synthesis pathways involving N-alkylation of commercially available alicyclic amines, reductive amination, one pot-two-step Boc-deprotection/amine acylation or amide-bond formation.

Results: The developed mechanochemical procedures enabled the preparation of selected antipsychotic drugs in high yields (>70%) and in a fast manner (60-180 min), while limiting of the use of organic solvents as well as the formation of byproducts. Moreover, most of mechanochemically synthesized drugs were isolated in high purities (>95%) after simple washing with water, without the need for column chromatography purification. The advantages of the mechanochemical approach were further confirmed by the assessment using commonly applied green chemistry metrics E-factor and Chlortox [2]. The Differential Scanning Calorimetry (DSC) was employed to confirm the solid-state structure of mechanochemically synthesized drugs and to compare them with commercially available APIs.

Conclusions: These results confirmed the suitability of mechanochemistry as a sustainable and efficient method for the synthesis of APIs. It is worth noting, that the simplicity of our “green procedures” offers potential for scaling-up using twin-extruder approach, facilitating further industrial applications.

Acknowledgments: The project was financially supported by the JUMC Statutory Activity N42/DBS/000390.

References:

[1] Canale V. et al. ACS Sustain. Chem. Eng. 2023, 45, 16156.

[2] Nowak P.M. et al. Green Chemistry 2021 27, 1102.

Keywords: Green chemistry, mechanochemistry, antipsychotic drugs

Abstract PP No. 047 **Spotlight Presentation (PSP 21)**

Mechanosynthesis: a Sustainable Way to Obtain Antipoxviral Drug Tecovirimat on a Multigram Scale

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Objective: Smallpox was declared eradicated in 1980 yet concerns over its potential misuse as a biological weapon persist. Related orthopoxviruses, such as mpox, are gaining global importance, with rising infection rates in Africa underscoring the need for effective antiviral drugs. Tecovirimat, approved by the FDA (2018) and EMA (2022), is the first targeted therapy for smallpox and mpox [1]. This study aimed to develop environmentally friendly, mechanochemical synthetic routes for tecovirimat, minimizing the dependence on high-temperature conditions and hazardous organic solvents typically used in conventional solution-based approaches [2,3].

Materials and Methods: We optimized reaction parameters for mechanochemical Diels–Alder cycloaddition, imide cyclization, and hydrazide formation. Key variables included milling time, milling load, reagent ratios, milling additives, and catalysts. Two alternative synthetic pathways were established and assessed for efficiency and environmental impact. Workup and purification steps were simplified to reduce solvent consumption.

Results: The optimized mechanochemical methods enabled the synthesis of intermediates and the final compound under solvent-free or nearly solvent-free conditions, providing high-purity products with satisfactory yields. Both synthetic strategies successfully produced tecovirimat more rapidly and with less complex purification than conventional solution-based techniques, while also eliminating the requirement for elevated temperatures and strictly anhydrous conditions.

Conclusions: Our results demonstrate that mechanochemical synthesis is a promising environmentally friendly alternative for preparing tecovirimat. The proposed methods lower the ecological impact while preserving synthetic effectiveness, supporting the broader applicability of mechanochemistry in the development of antiviral compounds.

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References:

[1] Almehmadi, M., et al. Viruses, 2022, 14, 1870

[2] Bonku, E. M., et al. Organic & Biomolecular Chemistry, 2025, 23, 239

[3] Szafrański, P.W., et al. International Journal of Molecular Sciences, 2026, 27, 61

Abstract PP No. 048

Manufacturing Quality Assessment of PVA-Based Pharmaceutical Films: Microstructural Analysis and USP Compliance Evaluation

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Background and Aim: USP General Chapter <905> mandates coefficient of variation (CV) below 6% for content uniformity in pharmaceutical films. Systematic quality comparisons between semi-solid extrusion 3D printing and conventional manufacturing methods for PVA-based films remain limited. This study aimed to evaluate manufacturing consistency, microstructural characteristics, and USP <905> compliance across three manufacturing methods.

Methods: PVA-based films containing menthol (5%), benzocaine (5%), and capsaicin (0–1%) were manufactured by 3D printing (n=12), solvent casting (n=8), and robotic arm-assisted electrospinning (n=13) using matched formulations. Manufacturing precision was assessed by CV analysis per USP guidance. Surface morphology was characterized by scanning electron microscopy (SEM). Thickness uniformity and moisture content were determined per USP <731>. Statistics: one-way ANOVA, Tukey's post-hoc test, Cohen's d.

Results: Electrospinning demonstrated superior mechanical precision (CV: 13.36%), followed by 3D printing (CV: 21.29%) and casting (CV: 42.63%; $F(2,13)=3.869$, $p=0.048$). 3D printing achieved best thickness uniformity (CV: 7.3% vs 19.7% for casting; 2.7-fold improvement). SEM revealed distinct microstructures: casting exhibited severe surface roughness and internal porosity from menthol volatility; 3D printing showed smooth surfaces with controlled internal architecture; electrospinning produced characteristic nanofibrous layered structure (film thickness 398 µm). Only two conditions achieved USP <905> compliance (CV<6%): 3D printing with capsaicin (CV: 4.39%) and electrospinning at optimized volume. Capsaicin addition reduced CV from 51.86% to 4.39% ($p<0.001$) — an 11.8-fold improvement.

Conclusions: Manufacturing method significantly impacts pharmaceutical film quality. 3D printing offers exceptional dimensional control and achieves USP compliance through formulation optimization. SEM confirms direct correlation between surface microstructure and manufacturing precision.

Abstract PP No. 049

GC/FID Method Development and Validation for Menthol Quantification in PVA-Based Pharmaceutical Films: Application to Content Uniformity Assessment

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Background and Aim: Accurate quantification of menthol in PVA-based pharmaceutical films is essential for content uniformity testing per USP . Validated analytical methods for menthol in PVA film matrices remain limited. This study aimed to develop and validate a GC/FID method for menthol quantification and apply it to content uniformity assessment across three manufacturing methods. Methods: A GC/FID method was developed on a Shimadzu GC-2010 Plus.

Extraction: film samples dissolved in water–methanol (1:1 v/v), ultrasonicated 10 min, membrane-filtered. Analytical conditions: injection volume 1 µL; helium carrier gas 1.26 mL/min; injector 260°C; FID 315°C; split ratio 1:60; oven programme 50–315°C within 23.53 min; Restek Rxi-5ms column (30 m × 0.25 mm × 0.25 µm). Calibration range: 0.00078–0.4 µg/mL. Method applied to PVA films from 3D printing, solvent casting, and electrospinning with blank controls.

Results: The method demonstrated outstanding linearity ($Y=169748x+68.2398$; $R^2=0.99996$) across the full calibration range. Menthol retention time was 7.451 min with clear separation from matrix peaks. Blank PVA films produced no interfering peaks, confirming selectivity. All three manufacturing methods yielded well-resolved menthol peaks confirming successful extraction. Applied to content uniformity: 3D printing achieved CV 4.7% vs casting CV 9.5%. With capsaicin optimization, 3D printing reached USP -compliant CV of 4.39%.

Conclusions: The validated GC/FID method provides a robust, sensitive, and selective platform for routine menthol quantification in PVA pharmaceutical films. The simple aqueous–methanolic extraction combined with optimized chromatographic conditions enables reliable content uniformity assessment across all tested manufacturing technologies.

Abstract PP No. 050 **Spotlight Presentation (PSP 22)**

Capsaicin as a Stabilizing Agent for Volatile Terpene-Containing Pharmaceutical Films: Achieving USP Compliance Through Formulation Optimization

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Background and Aim: Menthol, a volatile cyclic terpene from Mentha piperita L., poses significant manufacturing challenges in semi-solid extrusion 3D printing of PVA-based films due to its high vapour pressure, resulting in poor content uniformity (CV>>6%) that precludes USP compliance. Capsaicin (Capsicum annuum L.) was investigated as a natural formulation excipient for menthol stabilization.

Methods: PVA-based films containing menthol (5% w/w) with or without capsaicin (0–1% w/w) were manufactured by semi-solid extrusion 3D printing using matched base formulations (PVA 10%, glycerol 3%, ethanol/water). Manufacturing precision was assessed by CV analysis per USP guidance. Film thickness was determined per USP . Statistical significance: one-way ANOVA with Tukey's post-hoc test; effect size by Cohen's d. Menthol content quantified by validated GC/FID (R^2=0.99996).

Results: Formulation optimization with capsaicin achieved USP -compliant precision (CV: 4.39%), representing a dramatic improvement over the unoptimized menthol-only formulation (CV: 51.86%; p compliance. The stabilization phenomenon warrants mechanistic investigation (FTIR, DSC) and has broader implications for co-formulation of volatile and non-volatile natural compounds in transdermal drug delivery systems.

Abstract PP No. 051 **Spotlight Presentation (PSP 23)**

Fungal polysaccharides used as a component of multilayer biomaterials

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Fungal polysaccharides, alpha- and beta-glucans, chitin and chitosan, and mannose-rich polymers show tunable charge density, high hydrophilicity, and structural versatility, thus enabling them to be exploited in the LbL (layer-by-layer) approach to design multilayer biomaterials. In this work, polysaccharides were extracted from Pleurotus ostreatus and Pleurotus djamor species, and applied to regulate the behavior of fungal polysaccharides under controlled physicochemical conditions that determine the effects of pH, ionic strength, and zeta potential on film growth kinetics and interlayer electrostatic interactions. Multilayers were obtained by alternating deposition at pH ranging from 3.0 to 6.5 and ionic strength from 10 to 150 mM NaCl, so that polymer shapes, charge compensation, and film compactness could be modulated. Stability tests in a buffered environment showed improved hydration resistance of beta-glucan-containing layers and improved mechanical integrity of chitosan-rich architectures. The cytocompatibility of these structures and possible immunomodulatory activity in ß-glucan-enriched outer layers was also confirmed by biological assays.

The results emphasize the capacity of fungal polysaccharides to form structurally stable, stimuli-responsive LbL layers and their application in novel biomedical surface engineering such as drug delivery interfaces and implant coatings.

Key words: fungal polysaccharides, extraction, beta – glucans, glucan-chitin complex, LbL

Abstract PP No. 052

Laccase-catalyzed degradation of selected pharmaceutical active compounds in aqueous medium

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Objectives: Due to insufficient wastewater treatment, pharmaceutical active compounds (PhACs) constitute a significant type of contamination in surface waters. One of the methods for removing PhACs is their enzymatic degradation using laccase. This oxidoreductase, primarily produced by white-rot fungi, is capable of transforming various compounds, including PhACs. The aim of the study was to conduct a quantitative (degradation efficiency) and qualitative (transformation products) evaluation of the degradation potential of laccase toward 9 selected PhACs: clomipramine, citalopram, erythromycin, fluoxetine, mianserin, mycophenolic acid, paroxetine, sertraline, and venlafaxine, in the absence or presence of a laccase-mediated system comprising: 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt, acetosyringone, phenothiazine, sinapinic acid, trimethylphenol, vanillin, and violuric acid.

Materials and Methods: Reaction mixtures containing acetate buffer (pH 4.5, 50 mM), laccase (0.1 or 10 mg/mL), PhACs (100 ng/mL or 10 µg/mL for each PhAC), and, where applicable, additional mediator (0.01 or 1 mM) were shaken at 25°C and 120 rpm for 48 h. Samples were collected at 0, 4, 24, and 48 h of reaction. The reaction was stopped by mixing the sample of reaction mixture into acetonitrile at a v/v ratio of 2:5. Quantitative analysis was performed using liquid chromatography (LC) coupled with low resolution mass spectrometry (MS), whereas qualitative analysis was carried out using LC coupled with high resolution MS.

Results: In the absence of mediators, laccase degraded clomipramine, mianserin, and mycophenolic acid most efficiently. Paroxetine and erythromycin were degraded only at higher concentrations of laccase. Citalopram, fluoxetine, sertraline, and venlafaxine were resistant to laccase action. Degradation of clomipramine, mianserin, and mycophenolic acid was qualitatively analyzed, and products of oxidation (hydroxyl or carbonyl groups), as well as deacylation and decyclization, were identified. The presence of a mediator in the enzymatic reaction mixture enhanced the degradation efficiency. The most effective mediator was sinapinic acid, enabling degradation efficiency above 50% for each PhAC considered in the study.

Conclusions: The obtained results confirm the ability of laccase to degrade PhACs, whereas mediators increase the efficiency of the process. Hence, laccase constitutes a promising method for the removal of PhACs from wastewater.

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Abstract PP No. 53

Optimizing ring-opening polymerization with ethyl vanillate-magnesium catalysts for the precision synthesis of biomedical polyesters

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Objectives: Ring-opening polymerization (ROP) is one of the principal methods for producing biodegradable polyesters, enabling synthesis of polymers with precisely tailored properties. Typically, the ROP of cyclic esters such as L-lactide or ε-caprolactone proceeds through coordination mechanisms employing organometallic catalysts such as tin(II) 2-ethylhexanoate, which is highly effective yet limited by certain shortcomings. The toxicity of tin is still an arguable point in the context of biomedical applications, with the FDA imposing a 60-ppm concentration limit for parenteral administration. This underscores the demand for novel and indisputably non-toxic alternatives. Herein, we introduce an innovative aryloxymagnesium catalytic system featuring ligands derived from vanillic acid (VA), specifically ethyl vanillate (VanEt). Renowned for its antioxidative activity, VA also exhibits antimicrobial, hepatoprotective, cardioprotective, and antineoplastic features. Paired with magnesium, which is a vital biological macroelement, proposed catalyst holds promise as a transformative advance in the synthesis of polymers for biomedical applications.

Materials and Methods: In the initial stage of the study, catalytic systems were synthesized in the reactions of VanEt with di-n-buthylmagnesium ((n-Bu)2Mg), using two distinct molar ratios ((VanEt/(n-Bu)2Mg) 1:1 and 2:1. Subsequently, a response surface methodology (RSM) was employed to optimize the ROP using VanEt-Mg catalysts and different monomers, systematically varying catalyst amount, temperature and reaction time. Obtained polymeric products were thoroughly characterized using different spectroscopic techniques (1H, 13C NMR spectroscopy, MALDI-TOF mass spectrometry) and Size Exclusion Chromatography with Multi-Angle Light Scattering (SEC-MALS).

Results: The VanEt-Mg catalysts displayed high activity in ROP, achieving monomer conversions of over 95% within 72 h under optimized bulk conditions and yielding molecular weights considered suitable for prospective drug carrier applications. Statistical analysis of RSM data revealed significant effects of catalyst loading, temperature and reaction time on the ROP of L-lactide and ε-caprolactone, confirming reliable optimization.

Conclusions: The studies conducted to date demonstrate that VanEt-Mg based catalytic systems provide a robust alternative to conventional catalysts, enabling biodegradable polyesters for advanced drug delivery applications.

Abstract PP No. 054

pH-Sensitive Polyurethane Hydrogels for Controlled Local Delivery of 5-Fluorouracil and Paclitaxel for Pancreatic Cancer Therapy

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Pancreatic ductal adenocarcinoma (PDAC) remains one of the most lethal malignancies, largely due to late diagnosis, poor drug penetration into tumor tissue, and systemic toxicity of chemotherapeutic agents. 5-Fluorouracil (5-FU) and paclitaxel (PTX) are widely used in pancreatic cancer therapy; however, their clinical effectiveness is limited by rapid clearance, low bioavailability, and severe systemic side effects. Therefore, the development of localized and high-controlled drug delivery systems capable of sustained 5-FU and PTX release is of significant interest. In this study, biodegradable and pH-responsive polyurethane (PU) hydrogels were developed as implantable drug delivery systems for controlled release of 5-FU and PTX. The hydrogels were synthesized using hexamethylene diisocyanate, copolymers of ε-caprolactone, rac-lactide, and poly(ethylene glycol) (CL-LA-PEG), as well as PEG-block-poly(propylene glycol)-block-PEG, 1,4-butanediol, and L-glutamine. The CL-LA-PEG copolymers were prepared via ring-opening polymerization employing a $ZnEt_2$/ethyl-3,4-dihydroxybenzoate catalytic system. The resulting hydrogels were characterized in terms of swelling behavior, mechanical properties, cytocompatibility, hydrolytic degradation, and in vitro drug release. The developed hydrogels exhibited high swelling capacity and mechanical parameters within the range typical for soft biological tissues, indicating their suitability for biomedical implantation. Cytotoxicity studies confirmed that the materials were non-toxic toward epithelial cells. Drug release tests demonstrated sustained and controlled release of drugs over approximately 8–11 days at physiological pH (7.4) and 5–8 days at alkaline pH (8.5). Kinetic profile analysis indicated that drug release followed predominantly near-zero-order kinetics and diffusion-controlled mechanisms. These results demonstrate that the developed pH-sensitive polyurethane hydrogels represent promising implantable carriers for localized and controlled delivery of 5-FU and PTX. Such systems may potentially enhance therapeutic efficacy while reducing systemic toxicity in the treatment of pancreatic cancer.

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Abstract PP No. 055

Determination of elemental impurities using the Inductively Coupled Plasma Mass Spectrometry method in an active pharmaceutical ingredient used in the pharmacotherapy of neuropsychiatric disorders

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Objectives: ICH Guideline Q3D EMA/CHMP/ICH/353369/2013 requires the manufacturer of a drug product to establish a strategy for controlling elemental impurities. This is an integral part of the quality assessment of the drug product and the active substance, based on a risk analysis in accordance with ICH Guideline Q9 Quality Risk Management. During method development, the relationship between sample weight, dilution factor and instrumental limit of quantification was carefully evaluated for the selected panel of elemental impurities. The API micronization process is part of the product manufacturing process, and the purpose of the method is also to verify whether the micronization process introduces contaminants or not (part of the risk analysis). A typical sample weight for solid drug substances ensuring representativeness and complete digestion in the available microwave mineralization system is in the range of 0.05 - 0.5 g. The selected sample weight of approximately 0.3 g and final dilution to 50 mL are consistent with common pharmaceutical ICP-MS practice and published digestion volumes (25 - 100 mL).

Materials and Methods: ICP-MS (7900, Agilent Technologies) was used to determine the content of elemental contaminants (Cd, Pb, As, Hg, Co, V, Ni, Li, Sb, Ba, Mo, Cu, Sn, and Cr) in the active ingredient. Closed-system microwave digestion (Speedwave Xpert, Berghof) was used to prepare the test samples, standards solutions from Supelco was used to prepared stock solution and ISTD solution.

Results: Due to ICH requirements PDE for inhalation drug product was determined. Calculation according to Option 3 Inhalation PDE were made taking into account the Maximum Daily Dose amounting to 0.06921 g. Based on these information LOQ level was calculated – 0.17 [µg/g] (for Ni 1.7 [µg/g]). All obtained results were scaled to a MDD and compared with the PDE. None of the results exceeded LOQ level.

Conclusions: The Permissible Daily Exposure (PDE), expressed in µg/day, is a key parameter used to assess the quality of a medicinal product in terms of its metallic impurity content, as defined by ICH Q3D. The results obtained for individual elements are less than 30% of the PDE, no additional testing of the medicinal substance is required.

Key words: ICP-MS, validation, elemental impurities

Abstract PP No. 056 **Spotlight Presentation (PSP 24)**

Encapsulation Efficiency Assay for RNA-LNPs – application to stability assessment of formulations

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Objectives: Lipid nanoparticles (LNPs) are widely as a delivery systems for mRNA-based therapeutics and represent a critical component of modern vaccines and advanced medicinal products LNP formulations typically consist of: ionizable cationic lipids (which play the most important role in encapsulating and delivering nucleic acids), phospholipids, cholesterol and polyethylene glycol (PEG)-lipids - enable efficient encapsulation and protection of mRNA from degradation. Encapsulation efficiency is a key quality attribute, directly impacting product stability, bioavailability and therapeutic performance. The aim of the study was to evaluate encapsulation efficiency in different LNP formulations during preliminary stability studies.

Materials and Methods: Encapsulation efficiency was determined using Quant-iT Ribogreen RNA reagent (Thermo Fischer Scientific), a fluorescent dye used in the detection and quantification of RNA and DNA. Fluorescence measurements were performed using a GloMax fluorometer (Promega). An 8-point calibration curve was prepared, and mRNA concentration was verified using a NanoDrop spectrophotometer. Free mRNA was quantified in TE buffer, while total mRNA was measured after disruption of LNPs with Triton-containing buffer. Encapsulation efficiency was calculated based on the ratio of free to total mRNA.

Results: Six LNP formulations containing a cryoprotectant were stored at -20°C for 9 months. Initial encapsulation efficiency ranged from 79 to 97%, with total mRNA content between 231 – 286 µg/mL. After storage, encapsulation efficiency remained stable (82–97%), while mRNA content ranged from 215 to 267 µg/mL. The maximum decrease in mRNA content was 16%, whereas encapsulation efficiency varied by no more than 3 percentage points.

Conclusions: The results demonstrate that the evaluated LNP formulations maintain stable encapsulation efficiency and acceptable mRNA content during long-term storage at -20°C. The RiboGreen-based assay proved to be a suitable and reliable method for assessing encapsulation efficiency in stability studies of mRNA-LNP formulations.

Acknowledgements: Project supported by co-financed by the Medical Research Agency, Poland (grant No. 2021/ABM/05/00005).

Key words: encapsulation, ribogreen, mRNA

Abstract PP No. 057 **Spotlight Presentation (PSP 25)**

Pharmacokinetics of dual JAK/ROCK Inhibitor CPL'116 and its metabolite M3 in rheumatoid arthritis patients

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Objectives: CPL'116 (formerly CPL409116) is a dual inhibitor of Janus (JAK) and Rho (ROCK) [1]. JAK inhibition reduces the immune response and inflammation, ROCK inhibition promotes tissue regeneration. This synergic mechanism makes CPL'116promising candidate for treating inflammatory diseases, and their complications like cardiovascular disease or pulmonary fibrosis. Following results in healthy participants [2], CPL'116 was administered twice daily. The pharmacokinetics of CPL'116 and its M3 metabolite were evaluated in a Phase II trial in patients with rheumatoid arthritis [3].

Materials and Methods: CPL'116 concentrations in human plasma were measured by validated LC-MS/MS method. The clinical part was conducted in accordance with Good Clinical Practice (GCP), while the bioanalytical part followed Good Laboratory Practice (GLP) principles. Pharmacokinetic parameters were calculated by non-compartmental analysis using WinNonlin 8.4 and R 4.1.3.

Results: Dose-dependent trends in exposure (Cmax, AUC(0–6h)) were observed, with dose proportionality in the 60–240 mg dose range. Accumulation of CPL'116 was limited across all dose levels. The median time to reach peak concentration (tmax) was 1.75–2 h across days and doses. On Day 85 the mean half-life (t1/2) was 5.3–6.2 h. Metabolite-to-parent ratio was 16–36% for Cmax and 18–52% for AUC(0–6h).

Conclusion: The pharmacokinetics of CPL'116 in patients support a twice daily dosing. Promising results of Phase II study confirmed CPL'116 therapeutic potential for rheumatoid arthritis and possibly other autoimmune conditions.

Acknowledgements: Project supported by the European Regional Development Fund through the National Center for Research and Development, Poland (POIR.01.01.01-00-0382/16). The authors would like to thank the patients who participated in the study and the clinical teams.

References:

[1] Dulak-Lis M, et al. A novel JAK/ROCK inhibitor, CPL'116, demonstrates potent efficacy in the mouse model of systemic lupus erythematosus. J Pharmacol Sci. 2021 Apr;145(4):340–8. doi 10.1016/j.jphs.2021.02.002.

[2] Rudzki PJ, et al. First-in-human study of CPL'116 - a dual JAK/ROCK inhibitor - in healthy subjects. Front Pharmacol. 2025;16:1583723. doi: 10.3389/fphar.2025.1583723.

[3] Wieczorek M, et al. Dual JAK and ROCK inhibition with CPL'116 in patients with rheumatoid arthritis with inadequate response to methotrexate: a randomised, double-blind, placebo-controlled, phase 2 trial. Lancet Rheumatol. 2025;7(9):e629-e641. doi: 10.1016/S2665-9913(25)00060-8

Abstract PP No. 058

In-study performance of LC-MS/MS method for the determination of ticagrelor in human plasma

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Objectives: Ticagrelor is the first oral adenosine diphosphate-induced platelet aggregation inhibitor, indicated for coadministration with acetylsalicylic acid in patients with acute coronary syndromes or a history of myocardial infarction with high risk of cardiovascular events. A novel bioanalytical method for determination of ticagrelor in human plasma was developed at Bioanalytical Laboratory of Celon Pharma S.A. The method was fully validated according ICH M10 [1] and applied to analysis of samples from clinical study.

Materials and Methods: Plasma samples were collected during bioequivalence study in healthy volunteers [2]. Ticagrelor plasma concentrations were determined using QTrap 6500+ mass spectrometer (Sciex) coupled to 1290 Infinity II liquid chromatograph (Agilent Technologies). Samples were prepared by protein precipitation with acetonitrile. The chromatographic separation was achieved using Aeris Widepore XB-C8 column (100 x 4.6 mm, 3.6µm, Phenomenex). Positive electrospray ionization (ESI) was used to monitor ticagrelor ions at m/z 523 > 495 and the isotope-labeled internal standard ions (2H7-ticagrelor) at m/z 530 > 502.

Results: Calibration range 5-1200 ng/mL was adequate to characterize ticagrelor concentrations in clinical plasma samples. The lowest recorded Cmax was 182.1 ng/mL resulting in LLOQ = 2.7% Cmax. The highest measured concentration was 1378.6 ng/mL was below dilution QC (DQC2 = 2000 ng/mL). Accepted bioanalytical runs met the criteria set for calibration curve and QCs. Acceptance criteria were met in 122 out of 124 (98%) incurred samples reanalyzed. The long-term stability for ticagrelor was confirmed for at least 57 days and it covered the storage time of the study samples (37 days).

Conclusions: All in-study method performance criteria set by ICH M10 guideline [1] were met. Reliable measurement of ticagrelor concentrations enabled assessment of its pharmacokinetics and evaluation of bioequivalence. ACKNOWLEDGEMENTS: Project supported by the Medical Research Agency, Poland (grant No. 2022/ABM/04/00001). The authors thank Irena Kita and Justyna Czajkowska for quality assurance monitoring and Sylwia Kotańska for technical assistance.

References:

[1] ICH guideline M10 on bioanalytical method validation and study sample analysis, EMA/CHMP/ICH/172948/2019

[2] Kaza M. et al. Relative bioavailability of ticagrelor from hard capsules and film-coated tablets in healthy Caucasian volunteers. Acta Pol. Pharm. 2025, 82(1), 37-48.

Abstract PP No. 059

Pharmacokinetics of the FGFR inhibitor CPL'110 in patients with solid tumors

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Objectives: CPL'110 (formerly CPL304110) is a novel fibroblast growth factor receptor (FGFR1-3) inhibitor under development as a candidate anticancer drug [1-2]. Its safety and preliminary efficacy were evaluated in a Phase I clinical trial (NCT04149691) in patients with advanced solid tumors. Evaluation of CPL'110 pharmacokinetics was one of the secondary endpoints of the study.

Materials and Methods: CPL'110 in human plasma was quantified by LC-MS/MS method validated in accordance with European Medicines Agency (EMA) guideline [3]. The multi-center clinical study was conducted in line with Good Clinical Practice (GCP)and the Declaration of Helsinki. Bioanalysis was performed in line with Good Laboratory Practice (GLP) principles. A non-compartmental analysis using WinNonlin 8.4 and R 4.1.3 was applied to evaluate CPL'110 pharmacokinetics after oral administration of different doses.

Results: The blood sampling schedule and the appropriate bioanalytical method enabled a reliable analysis of pharmacokinetics. An increase in exposure (Cmax, partial AUCs) was observed with increasing dose. Variability in exposure between patients and between visits can be partially explained by the nature and progression of the disease, as well as by high inter-individual variability. The median time to reach maximum concentration (tmax) was 1–4 h across cohorts and visits. Cmin indicates that the steady state was reached after 1 week.

Conclusions: The pharmacokinetic data recorded provide a basis for the planning of further clinical trials.

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References:

[1] Yamani A, Zdżalik-Bielecka D, Lipner J, et al. Discovery and optimization of novel pyrazole-benzimidazole CPL304110, as a potent and selective inhibitor of fibroblast growth factor receptors FGFR (1-3). Eur J Med Chem. 2021; 210: 112990. doi: 10.1016/j.ejmech.2020.112990.

[2] Popiel D, Stańczak A, Skupińska M, et al. Preclinical characterization of CPL304110 as a potent and selective inhibitor of fibroblast growth factor receptors 1, 2, and 3 for gastric, bladder, and squamous cell lung cancer. Front Oncol. 2024; 13: 1293728. doi: 10.3389/fonc.2023.1293728.

[3] European Medicines Agency. Guideline on bioanalytical method validation (EMEA/CHMP/EWP/192217/2009 Rev.1 Corr. 2**), 21 July 2011.

Abstract PP No. 060 **Spotlight Presentation (PSP 26)**

Electromagnetic Field-Assisted Enhancement of Transdermal Delivery of Nonsteroidal Anti-Inflammatory Drugs

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Objectives: The aim of this study was to evaluate the influence of different electromagnetic field (EMF) configurations on the physicochemical properties, transdermal permeability, and skin accumulation of selected nonsteroidal anti-inflammatory drugs (NSAIDs). Although NSAIDs are widely used for the treatment of pain and inflammatory conditions, their transdermal delivery is limited by the barrier properties of the stratum corneum. Therefore, electromagnetic fields were investigated as a non-invasive strategy to enhance the transport of active pharmaceutical ingredients across the skin and improve the effectiveness of transdermal drug delivery systems.

Materials and Methods: Selected NSAIDs were exposed for 8 hours to different EMF configurations, including oscillating, pulsed, static (positive and negative polarity), and rotating magnetic fields. Samples not exposed to electromagnetic fields served as controls. The influence of EMF exposure on physicochemical properties was evaluated using FTIR spectroscopy, X-ray diffraction, and thermal analysis, together with measurements of aqueous solubility and lipophilicity.

Results: Exposure to electromagnetic fields did not cause degradation or structural changes in the studied compounds. However, EMF treatment altered crystal organisation, thermal parameters, solubility, and partition coefficient. Pulsed and rotating magnetic fields significantly enhanced transdermal permeability and drug transport, whereas static magnetic fields with negative polarity reduced permeation and increased dermal retention, indicating that the effect strongly depends on EMF configuration.

Conclusion: Electromagnetic field exposure represents a promising non-invasive approach for modulating the transdermal transport of NSAIDs. Pulsed and rotating magnetic fields were identified as the most effective conditions for enhancing skin permeability without affecting the chemical integrity of the active substances. These findings support the potential application of EMF-assisted strategies in the development of advanced transdermal drug delivery systems for anti-inflammatory therapy. Keywords: nonsteroidal anti-inflammatory drugs; electromagnetic field; transdermal drug delivery

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Abstract PP No. 061 **Spotlight Presentation (PSP 28)**

From Monolayers to Bilayers: Multitechnique Characterization of Anti-Inflammatory Drug Interactions with Model Intestinal Membranes

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Most drugs administered to humans affect the organization and surface properties of biological membranes, which may alter cellular functions. The epithelial membrane of the small intestine constitutes the first barrier during drug absorption and is therefore a critical target for studying drug–membrane interactions. Although Janus kinase inhibitors (JAKs) are widely used as anti-inflammatory agents, their molecular-level interactions with membrane lipids remain insufficiently understood.

The objective of this study was to elucidate how selected JAK inhibitors interact with model intestinal membranes and how these interactions evolve from simple monolayers to more complex bilayer systems. Multicomponent lipid systems mimicking the intestinal epithelial membrane (PC/SM/PE/PS/PI) were employed. The investigation followed a stepwise approach, starting with Langmuir monolayers and extending to supported lipid bilayers. Selected JAK inhibitors were incorporated into these model membranes and analyzed using a combination of complementary techniques, including surface pressure–area isotherms, polarization-modulation infrared reflection–absorption spectroscopy (PM-IRRAS), electrochemical methods (capacitance measurements), and atomic force microscopy (AFM). Monolayer studies revealed that JAK inhibitors significantly influence lipid packing, phase behavior, and interfacial properties, with effects depending on the chemical structure and lipophilicity of the drugs. In bilayer systems, these interactions translated into measurable changes in membrane integrity, including bilayer fluidization and, in some cases, defect healing. Spectroscopic data indicated drug-induced rearrangements within lipid headgroup regions, while AFM imaging confirmed alterations in bilayer morphology. Electrochemical measurements further supported these findings by revealing changes in membrane capacitance associated with drug incorporation.

The results demonstrate that interactions between JAK inhibitors and lipid membranes are strongly governed by drug structure and lipophilicity, which determine their preferred membrane localization and affinity for specific lipid components. The combined multitechnique approach provides comprehensive insight into drug-induced molecular rearrangements in model intestinal membranes. Such understanding is essential for optimizing JAK-based therapies, improving drug efficacy, and minimizing adverse membrane-related side effects.

Abstract PP No. 062 **Spotlight Presentation (PSP 29)**

Enhancing Antimicrobial Efficacy Against Methicilin Resistant Staphylococcocus Aureus: Synergistic Combinations of a Novel Lipooligourea with Approved Antibiotics

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The widespread misuse of antibiotics has accelerated the emergence of numerous multidrug-resistant strains of pathogenic bacteria. As a result, the search for entirely new, safe, and stable compounds has become a critical area of research – compounds that could either replace ineffective antibiotics or be used in combination with other drugs. We wanted to evaluate a efficacy of a novel antimicrobial agent, lipooligourea, against methicillin-resistant Staphylococcus aureus (MRSA) using Langmuir monolayer models of the bacterial membrane and then by expanding our research to microbiological assays, including the search for possible synergistic drug interactions. To characterize the interactions between the lipooligourea and the S. aureus model monolayers, we employed the Langmuir trough technique combined with Brewster angle microscopy (BAM). The same approach was used to study systems assessing the impact of vancomycin on the monolayer, both alone and in combination with the lipooligourea. The biological activity of the lipooligourea was evaluated using minimum inhibitory concentration (MIC) assays and checkerboard tests conducted on three clinical MRSA strains in combination with selected antibiotics: cloxacillin, vancomycin, amikacin and azithromycin. Combining the results obtained from the Langmuir method and BAM imaging, we concluded that the lipooligourea is capable of disrupting model lipid systems containing lipoteichoic acid (LTA) and promotes the formation of aggregates on the surface of the studied monolayers. For vancomycin, we observed a tendency for incorporation into the forming monolayer, which influenced the process of lipid domain formation during compression. The combined application of vancomycin and the lipooligourea revealed complex interactions between the compounds, resulting in a monolayer with fundamentally altered properties. Microbiological studies determined the lipooligourea's MIC values for the tested strains, which ranged from 2.5 to 5.0 μg/ml. The lipooligourea demonstrated favorable interactions with the tested antibiotics, exhibiting both additive and synergistic effects. Notably, the combination of the lipooligourea with cloxacillin resulted in an approximately 500-fold reduction in the required concentration of cloxacillin compared to its standalone MIC. We have confirmed that lipooligourea is a promising candidate for an antimicrobial agent against MRSA strains and demonstrates significant potential to be utilized in combination of other antibiotics that have become ineffective due to resistance.

Abstract PP No. 063 **Spotlight Presentation (PSP 30)**

Macroalgal Bioactives Extracted via Supercritical CO_2 for Green Synthesis of ZnO Nanoparticles for Biomedical Applications

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Objective: Zinc oxide nanoparticles (ZnO NPs) have many promising applications in biomedicine and pharmacy. Green methods of synthesis, with the use of natural products, have become very popular in the last decades due to their numerous advantages. They are eco-friendly, less expensive, and allow to receive the NPs with well-defined size and morphology. Macroalgae can be used for both intra- and extracellular biosynthesis of NPs due to the presence of bioactive compounds such as pigments, polysaccharides, polyphenols, lipids, and antioxidants that act as biocompatible reductants and offer several functional groups, which play important roles in the formation and stabilization of NPs.

Materials and Methods: Extracts were produced with the use of Supercritical CO2 Extraction with methanol as co-solvent. Extracts were prepared from six different macroalgae. To optimize the method, the effects of temperature (40-60°C), pressure (200-400 bar), and extraction time (0.5-2 h) on the extraction process were investigated. The obtained extracts were analyzed using rapid screening methods to determine which bioactive compounds were successfully extracted under the given conditions. In the next step, the total polyphenol content and antioxidant activity of the extracts were evaluated using UV–Vis spectroscopy. Selected extracts were further subjected to phytochemical profiling using LC–MS QTOF. The extracts with the highest polyphenol content were then used for ZnO NPs biosynthesis.

Results: The highest polyphenol content was obtained under different conditions depending on the macroalgae species used. For the green algae, the optimal conditions were 40°C, 200 bar, and an extraction time of 1 h; for the brown algae, 60°C, 200 bar, and 1 h; and for the red algae, 60°C, 400 bar, and 0.5 h. The polyphenol content obtained under these conditions was approximately 50-65 mg GA/L (gallic acid equivalent per liter of extract).

Conclusions: The obtained algal extracts contain a high levels of phytochemicals and polyphenols, making them effective reducing and stabilizing agents in the biosynthesis of ZnO NPs. Nanoparticles obtained from extracts of different algae may exhibit distinct physicochemical properties that warrant further investigation.

Acknowledgements: The study was supported by the National Science Centre, Poland, as part of the MINIATURA 9 competition for single scientific activities, project No. 2025/09/X/ST11/00505. Keywords: supercritical CO2 extraction, macroalgae, nanoparticle biosynthesis

Abstract PP No. 064 **Spotlight Presentation (PSP 31)**

Novel dimethylamino-substituted pyrrolidine-2,5-diones with antiseizure and antinociceptive activity

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Objectives: Drug-resistant epilepsy (DRE) affects ~30% of patients[1] and remains a serious clinical challenge, highlighting the urgent need to identify and validate novel molecular targets such as the excitatory amino acid transporter 2 (EAAT2). Following our discovery of (R)-7[(R)-AS-1], a first-in-class EAAT2 positive allosteric modulator (PAM)2, we aimed to optimize its physicochemical and pharmacokinetic properties. We hypothesized that introducing a dimethylamino moiety into the pyrrolidine-2,5-dione scaffold would enable the formation of highly water-soluble salts. This strategy was intended to facilitate salt formation and potentially improve the overall pharmacokinetic profile.

Materials and Methods: The target compounds and their corresponding hydrochloride salts were synthesized using a multi-step approach. Structures and purities were confirmed via NMR, UPLC-MS, and SFC analyses. In vitro EAAT2 activation was assessed in transfected COS-7 cells. In vitro ADME-Tox properties were assessed using standard methods described in literature2. In vivo antiseizure and analgesic efficacy were evaluated in mice (i.p.). Acute motor impairment was screened via the rotarod test.

Results: The most potent compound, (R)-AS-103 (hydrochloride salt), demonstrated moderate enhancement of glutamate uptake in EAAT2-expressing COS-7 cells. In vivo, it provided robust protection in MES (ED50=74.4 mg/kg) and 6 Hz, 32 mA (ED50=28.2 mg/kg) models. Notably, motor impairment in the rotarod test was observed only at higher doses (TD50 = 271.06 mg/kg). (R)-AS-103 was also effective in the MES seizure threshold test and revealed potent efficacy in formalin-induced tonic pain, capsaicin-induced pain, oxaliplatin- and streptozotocin-induced peripheral neuropathy. Moreover, in vitro ADME-Tox data proved favourable drug-like properties of (R)-AS-103.

Conclusion: The introduction of a dimethylamino moiety into the pyrrolidine-2,5-dione scaffold preserved the EAAT2 PAM profile of the compounds while enabling the formation of highly water-soluble salts. Overall, these findings highlight dimethylamino-substituted pyrrolidine-2,5-diones as promising novel chemical class of EAAT2 modulators for further development as antiseizure agents with potential additional applications in pain management. The studies were supported by the National Science Centre, Poland grant UMO-2022/45/B/NZ7/00598

[1] Chen Z. et al. JAMA Neurol. 75 (2018) 279-286. [2] Abram M. et al. J. Med. Chem. 65 (2022) 11703-11725.

Abstract PP No. 065 **Spotlight Presentation (PSP 32)**

Design, Synthesis, and In Silico Evaluation of Quinoline Derivatives as Potential Topoisomerase I and Cyclooxygenase 2 Inhibitors

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Objectives: Cancer mortality continues to rise worldwide, and this trend is expected to persist in the coming decade. Conventional therapies targeting a single molecular pathway are becoming progressively less effective due to resistance and the biological complexity of tumors. A multi-target strategy may help overcome these limitations. This study aims to design structures capable of simultaneously inhibiting topoisomerase I and cyclooxygenase-2. Inhibition of topoisomerase disrupts genomic integrity and induces apoptosis. Meanwhile, COX-2 inhibition weakens the tumor-promoting microenvironment.

Materials and methods: Quinoline derivatives were synthesized by the Friedländer annulation. The potential activity of these compounds was evaluated through molecular modeling targeting the topo I and COX-2. Electronic structure calculations were performed to assess their reactivity. Molecular dynamic simulations were used to evaluate the stability of ligand-target interactions. Finally, AMDET predictions were carried out to estimate drug-likeness and pharmacokinetic properties.

Results: Docking studies confirmed the ability of the compounds to form stable complexes with both targets. The most promising compound showed good affinity towards topo I, stabilized by π–π stacking interactions with DNA, as well as additional hydrogen bonding with key amino acid residue (ARG364). Binding analysis revealed that the studied compound occupies the hydrophobic channel of COX-2, forming multiple π–alkyl interactions. Additional hydrogen bonding interaction with ARG121 contributes to ligand stabilization. Analysis of electronic parameters revealed that the designed compounds exhibit a moderate HOMO–LUMO energy gap (3.6–4.8 eV) and high electrophilicity indices. Molecular dynamics simulations confirmed stable ligand–target complexes. ADMET predictions indicated favorable drug-likeness properties.

Conclusions: The obtained results indicate that quinoline-based derivatives represent promising multi-target candidates with anticancer potential. Their ability to interact with both proteins suggests a synergistic mechanism of action, and further optimization and experimental validation are required.

Acknowledgments: This research was funded by Wroclaw Medical University, grant number SUBD.D290.26.002. Created using resources provided by Wroclaw Centre for Networking and Supercomputing.

Key words: quinoline-derivatives, topoisomerase I; COX-2 Research category: Medicinal Chemistry

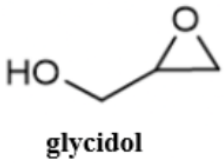
Abstract PP No. 066 **Spotlight Presentation (PSP 33)**

Determination of Glycidol in Non-Opioid Medicinal Products by Gas Chromatography Coupled with Mass Spectrometry

Anna Konefał[1], Maria Jedynak[1], Marta Zezula[1]

[1]Łukasiewicz Research Network – Industrial Chemistry Institute, Pharmaceutical Analysis Laboratory

The aim of this study was to develop and verify a gas chromatography coupled with mass spectrometry method (GC-MS) for the determination of glycidol, a genotoxic impurity, in a medicinal products. A commonly used BSTFA derivatization was found insufficiently selective due to co-derivatization of impurities, causing peak overlaps and ambiguous glycidol determination. Therefore, an alternative derivatization using p-toluenesulfonyl reagent was applied, based on literature where glycidol was determined in e-liquid samples from electronic cigarettes [1]. The method utilized glycidol-d5 as an internal standard to improve control of the derivatization reaction and enhance accuracy and precision. The required glycidol limit was 5 ppm according to the European Pharmacopoeia [2]. Linearity, selectivity, sensitivity and injection repeatability were evaluated. The detection limit (DL) and quantitation limit (QL) were established at 0.25 ppm and 0.50 ppm, respectively. Recovery studies were performed on samples spiked with the analyte reference solution to confirm method accuracy. The developed GC-MS method proved suitable for quantitative glycidol determination in pharmaceutical products, ensuring the safety and quality control.



Recovery studies were performed on samples spiked with the analyte reference solution to confirm method accuracy. The developed GC-MS method proved suitable for quantitative glycidol determination in pharmaceutical products, ensuring the safety and quality control.

***Fig. 1** Structure of the glycidol.*

Bibliography:

[1] Cook D.K., Stump B., Fraley N., Humphries K., Gillman I.G., Jameson B., "Determination of glycidol in e-liquid and aerosol samples from ENDS products by GC-MS," Juul Labs Science, Tobacco Science Research Conference, 2023, Abstract 69, p. 76

[2] European Pharmacopoeia (Ph. Eur.) 11th Edition, Monograph no. 1535

Keywords: glycidol, pharmaceutical products, GC-MS Research Category: Pharmaceutical Analysis

Abstract PP No. 067

The determination of residual dimethylformamide and ethanol by HS GC in products with K2 vitamin

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[2]VITASYNTH sp. z o.o.

The aim of the study was to develop and verify the method for the determination of residual solvents at very low level (<30 ppm) in products containing K2 vitamin (menaquinone-7). Fig. 1 shows the structure of Menaquinone-7. Both solvents are approved for pharmaceutical manufacturing. According to Q3C guideline [1], they are classified as class 3 (ethanol, 5000 ppm limit) and class 2 (DMF, 880 ppm limit).

During the method development, the focus was on determination of DMF which, due to its physicochemical properties (boiling point of 153°C), is problematic for HS-GC analysis. High-boiling 1-methyl-2-pyrrolidone NMP was used as a solvent for samples. The developed method was tested for selectivity, sensitivity, precision and accuracy. The detection limit (DL) and quantitation limit (QL) were estimated based on signal-to-noise ratio and they are as follows: DL=0.5 µg/mL, QL= 2.0 µg/mL for ethanol and DL 2.2 µg/mL, QL=6.6 µg/mL for dimethylformamide.

The developed methodology allowed for confirmation the quality of the investigated products containing K2 vitamin. The absence of these solvents in the investigated samples was proved.

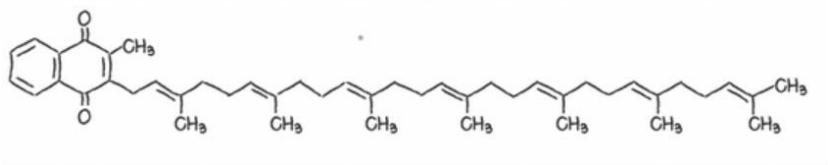

***Fig. 1** Menaquinone-7*

Acknowledgementss: The studies were performed on commission of Vitasynth sp. z o.o.

Bibliography:

[1] ICH Q3D Elemental impurities - Scientific guideline

[2] K. Keerthi, IJRSB Vol 13 ISS 6, June 2015

Keywords: residue solvents, pharmaceutical products (vitamin K2), HS GC

Abstract PP No. 068 **Spotlight Presentation (PSP41)**

Simultaneous LC-MS Analysis of Structurally Diverse Antivirals and Their Metabolites in Human Plasma

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Objectives: Antiviral pharmacotherapy remains a cornerstone in managing emerging viral infections. Liquid chromatographic techniques coupled to mass spectrometry (LC-MS) are the most robust techniques for antiviral quantification, allowing simultaneous detection, high sensitivity, and selectivity. However, the accurate quantification of these compounds in clinical settings is hindered by the vast structural diversity and different hydrophilicity between prodrugs and their active metabolites [1]. This work presents the development and optimization of a robust LC-MS method for the simultaneous determination of six key antivirals and their primary active metabolites in human plasma.

Materials and methods: Plasma samples were spiked with the antivirals to achieve concentrations matching blood levels under therapeutic ranges. A mixture of internal standards was employed to ensure high precision and account for matrix effects and recovery. The analytical workflow was optimized (extraction protocol, separation, and detection) for a reliable quantification of all compounds over the selected concentration ranges. Various extraction methods were explored, and the separation was achieved using an Agilent 1260 Infinity System liquid chromatograph coupled to a SCIEX QTRAP 4000 triple quadrupole mass spectrometer (QqQ MS) detector.

Results: Using the protein precipitation method, linearity domains matching the reported plasmatic concentrations were obtained for eight out of nine compounds, with the lowest limit of quantification (LLOQ) reaching 5% of the Cmax and the upper limit of quantification reaching 130% of Cmax. Particular focus was directed toward the quantification of the remdesivir metabolite (GS-441524), necessitating tailored ionization and fragmentation parameters to ensure compatibility within the multi-drug mixture. To enhance the detection of the hydrophilic remdesivir metabolite, specialized plasma extraction protocols were developed to minimize matrix interference.

Conclusions: These results underscore the critical role of optimized extraction protocols in achieving simultaneous, high-sensitivity detection of structurally diverse antivirals, providing a reliable tool for therapeutic drug monitoring and clinical research.

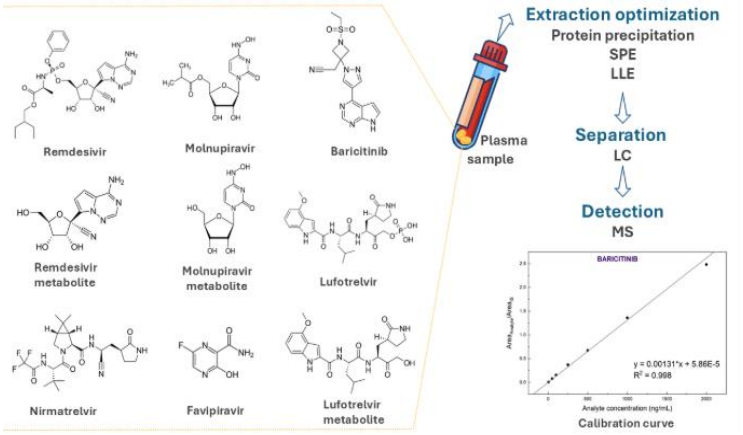

Figure 1. The structures of the analyzed compounds and the analytical workflow.

Acknowledgements: This study was funded by the NCN OPUS25 2023/49/B/NZ7/02718 2024-2028 project.

[1] Khalil HA., Hassanein NA, El-Yazbi AF, RSC Adv., 2023, 13, 13224

Abstract PP No. 069

Discovery of novel AK4 inhibitors by high-throughput screening of natural compounds

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Objectives: Human adenylate kinase 4 (AK4) is a mitochondrial matrix enzyme that acts as a stress-responsive protein involved in the regulation of energy metabolism, mitochondrial homeostasis, and cell survival. In cancer, AK4 has been implicated in metabolic reprogramming toward glycolysis, epithelial–mesenchymal transition, tumor progression, and metastasis. In lung adenocarcinoma, AK4 overexpression is associated with disease progression and poor prognosis. Although increasing evidence highlights its significance, AK4 remains undruggable, and no selective inhibitors have been identified to date. This study aimed to identify AK4 inhibitors through high-throughput screening (HTS) of natural compounds, providing a foundation for AK4-targeted drug discovery.

Materials and Methods: A natural compound library (1709 compounds) was screened against AK4 using a commercially available luminescence-based assay suitable for high-throughput screening and optimized for AK4 (ADP-Glo Kinase Assay, Promega), with all compounds tested at a concentration of 50 μM. Identified inhibitor candidates (hits) were validated by high-performance liquid chromatography (HPLC). For selected modulators, molecular docking analysis was performed to predict potential ligand–protein binding orientations. In addition, their anticancer activity was evaluated in four genetically distinct non-small cell lung cancer (NSCLC) cell lines.

Results: The primary screening process identified multiple compounds that inhibit AK4. HPLC validation confirmed the inhibitory activity of selected hits and eliminated false-positive results. Several chemical classes, including polyphenols, quinone-like compounds, redox-active agents, and prenylated phenolic derivatives, reduced AK4 activity by approximately 75% or more at 50 μM. Additionally, selected compounds demonstrated variable anticancer activity across non-small cell lung cancer (NSCLC) cell lines.

Conclusions: This study presents the first high-throughput identification of AK4 inhibitors from a natural compound library. The validated hits address an important gap in the pharmacological targeting of AK4. These compounds represent promising structural scaffolds for further optimization and the development of selective AK4 inhibitors with potential therapeutic relevance, particularly in lung cancer.

Acknowledgements: This research was funded by the Polish National Science Centre, grant no. 2023/51/B/NZ7/03056

Keywords: adenylate kinase, AK4, inhibitor discovery, screening

Abstract PP No. 070 **Spotlight Presentation (PSP 34)**

Impact of Selective Serotonin Reuptake Inhibitors on Doxorubicin-Induced Senescence

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Objectives: Most chemotherapeutic approaches induce irreversible growth arrest known as therapy-induced senescence (TIS). TIS markers have been reported in both cancer and non-malignant cells in patients with triple-negative breast cancer. Increasing evidence suggests that senescent cells contribute to therapy resistance and disease progression, making them targets for senolytic strategies. This study aimed to investigate the effects of selected selective serotonin reuptake inhibitors (SSRIs) on doxorubicin-induced senescence in vitro.

Materials and Methods: Experiments were performed in MDA-MB-231 cells. TIS was induced with doxorubicin (Dox, 75 nM) for 6 days, followed by 5-day exposure to SSRIs (escitalopram or sertraline). The induction of senescence was confirmed based on cell morphology, SA-β-gal activity, and p21 expression. Senescence levels and cell growth were quantified using SA-β-gal and MTT assays, respectively. IC_{50} values were determined, and the enolytic index (SI) was calculated as the ratio of IC_{50} in untreated to Dox-treated cells. Apoptosis was analyzed by Annexin V-FITC/PI staining.

Results: Doxorubicin treatment induced a senescent phenotype in MDA-MB-231 cells, as reflected by morphological changes, increased SA-β-gal activity, and elevated p21 expression. Subsequent treatment with SSRIs resulted in a concentration-dependent reduction in cell growth, which was more pronounced in combination with Dox than with either agent alone. The senolytic index indicated that senescent cells were more sensitive to treatment, with SI values of approximately 2 for escitalopram and 4 for sertraline. In addition, SSRI treatment reduced SA-β-gal activity in Dox-treated cells by around 40% and did not induce senescence when used alone. Both SSRIs also promoted apoptotic features in senescent cells and led to a marked decrease in p21 levels.

Conclusions: Taken together, these findings suggest that SSRIs may selectively affect therapy-induced senescent breast cancer cells and exhibit senolytic-like activity in this model. This supports further investigation of their potential use in combination strategies aimed at targeting senescent cells in cancer.

Keywords: therapy-induced senescence, triple-negative breast cancer, senolytics, SSRIs, doxorubicin

Abstract PP No. 071

Effects of Cornus mas and Hippophaë rhamnoides Fruit Extracts on Biochemical Parameters Associated with Metabolic Syndrome in Rats

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[2]Medical University of Warsaw, Department of Biochemistry and Pharmacogenomics
[3]Medical University of Warsaw, Centre for Preclinical Research
[4]Prof. Waclaw Dabrowski Institute of Agricultural and Food Biotechnology - State Research Institute, Department of Food Safety and Chemical Analysis
[5]Medical University of Warsaw, Department of Toxicology and Bromatology

Objectives: Cornus mas (CM) and Hippophaë rhamnoides (HR) are traditionally used medicinal plants rich in anthocyanins and flavonoids, respectively, which may help prevent metabolic syndrome [1,2]. Therefore, the objective of this study was to determine the impact of plant extracts from the fruits of CM (70% ethanolic extract) and HR (aqueous extract) on biochemical parameters associated with metabolic syndrome, in rats fed a high-fat diet (HFD).

Materials and Methods: Male Sprague-Dawley rats were divided into groups: control (normal diet, n=12; HFD, n=4) and experimental receiving CM or HR extracts at doses of 100 or 500 mg/kg b.w. (all on HFD, n=8 per group). The extracts have been standardized for their content of selected compounds. Blood samples were collected at baseline and after 8 weeks. Serum concentrations of glucose, triglycerides (TG), total cholesterol, HDL, and LDL were measured.

Results: Studies have shown that the administration of CM extracts to rats at doses of 100 and 500 mg/kg b.w. led to a reduction in LDL and TG levels, accompanied by an increase in glucose concentration. HR extract at 100 mg/kg b.w., increased TG and glucose levels, whereas 500 mg/kg b.w. decreased TG and glucose levels, with a slight rise in LDL. All experimental groups showed reduced total cholesterol and HDL. However, the smallest decrease in HDL was observed in rats receiving the HR extract at 500 mg/kg b.w.

Conclusions: CM extracts demonstrate lipid-lowering potential. HR extracts exhibit a dose-dependent effect, with higher doses potentially improving selected biochemical parameters. These effects might be explained to a greater extent by the presence of CM anthocyanins rather than HR flavonoids.

Acknowledgements: This study was supported by the National Science Centre (grant no.UMO-2023/51/B/NZ9/00305). Keywords: high-fat diet, Cornelian cherry, sea buckthorn

References:

[1] Frumuzachi O, et al. Nutrients. 2024, 16(13), 2173.

[2] Geng Y, et al. Phytother Res. 2022, 36(11), 4101-4114.

Abstract PP No. 072 **Spotlight Presentation (PSP 35)**

SELECTIVE LASER SINTERING IN PHARMACEUTICS: A PATH TOWARD PERSONALIZED MEDICINE

Magdalena Urbanowicz[1], Patrycja Chmielewska[1], Anna Mocarska[1], Karolina Pioruńska[1], Marta Łaszcz[1], Ewelina Baran[2], Piotr Kulinowski[2]

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[2]Pedagogical University of Cracow, Institute of Technology

Objectives: The growing interest in personalized drug delivery systems has promoted the application of Selective Laser Sintering (SLS) in pharmaceutical manufacturing. This study evaluated the feasibility of SLS for the fabrication of pharmaceutical tablets, focusing on the characterization of the active pharmaceutical ingredient (API), the polymeric matrix, their physical mixtures (PM), and the resulting printed dosage forms. To minimize material waste, the potential for powder recycling during the SLS process was investigated. After each printing cycle, the remaining powder was reintroduced into the printer and used to produce subsequent batches of tablets over five consecutive cycles (prints 1–5). Recycled powders and the resulting tablets were evaluated for physicochemical stability, and dissolution profiles were determined to assess the effect of powder reuse on drug release

Materials and Methods: 2.1. Materials Met - Hubei Hongyuan Pharmaceutical Technology Co., Ltd., Fengshan, China; PA12 - Sintratec AG, Brugg, Switzerland. 2.2. DSC Mettler-Toledo system DSC 3+ with IntraCooler, STARe software. 2.2. HPLC analysis Shimadzu, LabSolution software, Phenomenex Gemini 5 µm, 250 × 10 mm column. 2.3. NMR VNMRS-500 spectrometer, Z-SPEC Nalorac IDG500-5HT probe. 2.4 IR-ATR Mettler Toledo Nicolet iS5 2.5. XRPD Bruker D6 Phaser 3.

Results: 5 series of printlets have been successfully printed. DSC profiles of fresh and recycled PM demonstrated consistent thermal behaviour, with no observable shifts in melting points, melting enthalpy and crystallinity changes, or the appearance of new thermal events. Only IR indicates minor differences between the powder after 1 printing cycle. HPLC and NMR indicate no appearance of new impurities in reuse powders and printed tablets.

Conclusions: Performed studies suggest that SLS printing may be a useful tool for pharmaceutical applications. The PM is stable under the studied conditions, and its recycling for up to the 5th cycle is possible.

Acknowledgements: Research financed by 2022/45/B/NZ7/04081 from the National Science Center.

Abstract PP No. 073

The effect of magnetic fields on skin barrier permeability and the in vitro pharmacokinetic profile of ibuprofen

Anna Muzykiewicz-Szymańska[1], Karolina Zyburtowicz-Ćwiartka[2], Anna Nowak[1], Katarzyna Piotrowska[3], Łukasz Kucharski[1], Maciej Konopacki[4], Rafał Rakoczy[4], Paula Ossowicz-Rupniewska[2]

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Objectives: Ibuprofen, despite its widespread use as a nonsteroidal anti-inflammatory drug (NSAID), exhibits limited aqueous solubility and low permeability across the skin barrier, limiting its efficacy in transdermal administration. The aim of this study was to evaluate the effect of various electromagnetic field (EMF) modalities as an innovative, noninvasive method of enhancing ibuprofen transport across the skin barrier.

Material and Methods: In vitro permeation studies were conducted using Franz diffusion cells, using porcine skin. A 1% solution of ibuprofen (in 70% ethanol) was exposed to electromagnetic fields (EMF) of varying intensity and frequency. The following EMFs were used in the studies: oscillatory (OMF), pulsed (PMF), static (SMF), and rotating (RMF). The process was monitored for 8 hours, and the drug concentration in the acceptor fluid was analyzed using HPLC. Key pharmacokinetic parameters were determined: cumulative amount permeated (Q8h), steady-state flux (JSS), permeability coefficient (KP), and lag time (LT).

Results: The use of magnetic fields significantly affected the kinetics of ibuprofen transport. The highest effectiveness was observed for a rotating magnetic field with a frequency of 10 Hz (RMF 10 Hz), where the cumulative drug mass after 8 hours was 358.8 ± 33.5 µg, which represents a more than two-fold increase compared to the control group (155.8 ± 8.4 µg). This configuration also achieved the highest JSS (127.4 ± 25.5 µg/cm^2·h) and KP (12.7×10^{-3} cm/h). Significant improvement in permeation was also observed with the use of a pulsed field (PMF 10/10), which was associated with a shortened diffusion lag time. The positively polarized SMFs significantly inhibited transepidermal ibuprofen transport.

Conclusions: Electromagnetic fields, particularly rotating (RMF 10 Hz) and pulsed (PMF) electromagnetic fields, are an effective tool for modifying the permeability of the skin barrier to ibuprofen. The mechanism of enhanced permeation likely results from the fields' interaction with the lipid structure of the stratum corneum, increasing its fluidity and reducing the resistance of the epidermal barrier. These results indicate the significant potential of magnetic field-assisted systems for optimizing the transdermal delivery of NSAIDs.

This research was funded by National Science Centre [Project NCN OPUS 25 no. UMO 2023/49/B/ST8/00605].

Keywords: Electromagnetic Field, Nonsteroidal anti-inflammatory drugs, Transdermal drug delivery

Abstract PP No. 074

Impact of Electromagnetic Fields on the Selective Accumulation of Naproxen in the Skin

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Electromagnetobiology is a rapidly developing scientific discipline that studies the effects of electromagnetic fields (EMF) on biological systems. In the field of transdermal therapeutic systems (TDDS), although research often prioritizes systemic flow, the local accumulation of active ingredients (APIs) in the skin layers is crucial for sustained-release kinetics and the treatment of inflammation. The stratum corneum (SC) is the main barrier not only to penetration into the circulation but also to the formation of a stable drug reservoir. Naproxen (NAP), a nonsteroidal anti-inflammatory drug (NSAID) commonly used for local pain management, is an ideal model for studying these processes due to its favorable physicochemical properties. The primary objective of this study was to assess how different magnetic field configurations—oscillatory (OMF), pulsed (PMF), static (SMF), and rotating (RMF)—modulate the quantitative accumulation of naproxen in the skin matrix. This study utilized a porcine skin model and 1% ethanol solutions of naproxen. Samples were exposed to OMF (45–65 Hz), PMF (65 Hz, with varying pulse durations), SMF (positive and negative polarity, 5–25 V), and RMF (10–50 Hz). After exposure, quantitative tissue accumulation was determined using high-performance liquid chromatography (HPLC).

The results showed significant differences in skin accumulation compared to the control (3671.02 µg/g skin). OMF fields generally reduced accumulation (to 2693.31 µg/g at 55 Hz), suggesting that although permeability increases, the drug rapidly passes through the tissue without forming a stable reservoir. PMF fields provided the most consistent levels of deposition. Static magnetic fields (SMFs) demonstrated a particularly strong voltage dependence: low voltage (+5 V) minimized retention (2401.16 µg/g), while high voltage (+25 V) led to high accumulation (3509.31 µg/g), effectively trapping the drug within skin structures. Rotating magnetic fields (RMFs) resulted in the lowest retention (1972.93 µg/g at 30 Hz) – Figure 1. By adjusting the field parameters, it is possible to maximize the dermal reservoir for local action or minimize retention for rapid systemic absorption.

This research was supported by the National Science Centre (Poland) under the OPUS 25 grant no. UMO-2023/49/B/ST8/00605.

Abstract PP No. 075 **Spotlight Presentation (PSP 36)**

Immunostimulatory properties of selected compounds from Chelidonium majus latex

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[1]Adam Mickiewicz University, Poznań, Poland, Faculty of Biology, Institute of Experimental Biology, Department of Molecular Virology

Chelidonium majus L. is a medicinal latex-bearing plant from the family Papaveraceae, with antiviral, proapoptotic and cytotoxic activities. Latex is a milky emulsion produced by the plant which exudes after mechanical disruption of plant tissue. It is composed of different biologically active compounds, mainly benzylisoquinoline alkaloids and proteins, like potentially antiviral and anticancer major latex protein (MLP) and glycine-rich protein (GRP). Antiviral and antitumor effect of C. majus latex and its components could be also stimulated by the immune response of the host.

Objectives: The goal of the study was to compare the effects of different concentrations of CmMLP and alkaloids from C. majus latex, as well as their combinations on cellular immune response.

Materials and Methods: The research utilized the recombinant major latex protein CmMLP produced in prokaryotic system and three selected alkaloids. Experiments were performed on a murine macrophage cell line RAW 264.7, and cytotoxity of the compounds was evaluated using the WST-1 assay. The level of the proinflammatory cytokines – TNFα and IL-6 was measured using ELISA. NO production was analyzed by its accumulation in a cell culture medium and determined with Griess reagent. In order to assess the activity of the tested substances under both functional and inflammatory conditions, a subset of the cell population was exposed to lipopolysaccharide (LPS).

Results: Results showed that CmMLP exhibit anti-inflammatory effects by reducing IL-6 levels, and when combined with low doses of chelidonine this effect was pronounced. A moderate immunostimulatory effect was observed for the combination of CmMLP with low concentrations of berberine, while berberine alone exhibited a strong stimulatory effects. In contrast, a low concentration of CmMLP under inflammatory conditions demonstrated a moderate anti-inflammatory effect. The results suggest that both the CmMLP protein structure and the type of alkaloid influence the biological activity of these combinations, which may be important in the context of their immunomodulatory properties.

Conclusions: These findings could be the basis for the understanding of immunostimulatory mechanisms of C. majus latex as well as their potential use as possible immunomodulators in various conditions.

Acknowledgements: Research was funded by the National Science Centre, Poland (grant number 2019/35/B/NZ9/03851).

Keywords: latex, immunomodulation, major latex protein, alkaloids

Abstract PP No. 076 **Spotlight Presentation (PSP 37)**

Search for GPR18 agonists among alkyl and cycloalkyl derivatives of theophylline

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Objectives: The GPR18 receptor, which belongs to the cannabinoid receptor family, is found primarily in tissues and cells associated with the immune system (e.g. the spleen, thymus, peripheral blood leukocytes and lymph nodes) and in cancer cells. It is believed that agonists of this receptor modulate the immune system [1]. We have recently described a new class of GPR18 agonists with a theophylline core [2]. This group includes the compound PSB-KK1415, currently the most potent known GPR18 agonist (EC50 of 19 nM). Continuing our research in this area, we designed a series of compounds based on PSB-KK1415. We focused on modifying the substituent at position 7 using an alkyl or cycloalkyl group.

Materials and Methods: The compounds were synthesised in three steps, starting from theophylline. The potency of the compounds against the human GPR18 receptor was assessed using a ß-arrestin recruitment assay, and EC50 values were calculated. Subsequently, for the most potent compounds, selectivity towards the cannabinoid-like receptor GPR55 and adenosine receptors was assessed. Furthermore, the toxicity of the most potent compounds to neuronal, hepatic and microglial cells was tested after 72 hours of incubation using the MTS assay.

Results: All compounds exhibited activity at the GPR18 receptor in the nanomolar concentration range (EC50 < 1 µM). The strongest activity was exhibited by compounds with a cycloalkylmethyl substituent (i.e. cyclopropyl, cyclobutyl and cyclopentyl) with an EC50 < 100 nM. None of the compounds activated the human GPR55 receptor above 50% at a concentration of 10 µM. All compounds exhibited affinity for adenosine receptors at high concentrations, in the micromolar range. The tested compounds showed moderate toxicity to SH-SY5Y and HepG2 cells, with IC50 values well above 10 µM. In contrast, all compounds exhibited toxicity towards microglial cell lines with IC_{50} < 5 µM.

Conclusions: The modifications introduced resulted in new GPR18 agonists with reduced molecular weight compared to the lead structure PSB-KK1415, including compounds with high capacity to stimulate this receptor, and moderate toxicity, particularly towards neuroblastoma and hepatic cells.

Acknowledgements: This research was in part funded by the National Science Centre, Poland, grant based on decision No DEC-2021/43/B/NZ7/01938.

[1] Honkisz-Orzechowska E., et al. Molecules 29 (2024) 1258.

[2] Mahardhika A.B. et al. J Med Chem. 67 (2024) 9896-9926.

Abstract PP No. 077 **Spotlight Presentation (PSP 38)**

Physicochemical and Computational Analysis of Potential Interactions Between Cisplatin and Proton Pump Inhibitors

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Cisplatin is still a mainstay of chemotherapy for several cancers; however, its pharmacokinetics may be affected by drugs administered concomitantly, among which Proton Pump Inhibitors (PPIs). PPIs are often given with Cisplatin to treat nausea and other gastrointestinal side effects. These inhibitors have heterocyclic rings containing nitrogen atoms, which can possibly serve as additional coordination sites for platinum(II) centers. As our previous studies show, Cisplatin and its analogues can interact with various compounds that resemble nucleobases in structure, namely those containing a nitrogen or sulfur atom with a lone pair of electrons in the heterocyclic ring, and each PPI has two such atoms. In the present work, probable interactions were investigated by means of DFT calculations (B3LYP/6-31G(d,p) with LanL2DZ and MN15/def2-TZVP levels) and confirmed by UV-Vis spectroscopic analysis. Calculations were performed using density functional theory (DFT), using two levels of approximation: B3LYP/6-31G(d,p), in which the atomic orbitals of platinum were described using the LanL2DZ basis set, which contains the relativistic effective core potentials necessary for heavy elements, and MN15/def2-TZVP. The theoretical calculations show that Cisplatin can establish stable coordination complexes with PPIs, even though the computed affinity for nucleobases of DNA is still higher. Although the spectroscopic evidence demonstrates the formation of Pt-PPI adducts in solution, the Dispersion Correction model indicates a minimal effect on drug availability in vitro. Additional biological analysis is required to establish the functional significance of these interactions in a cell.

Keywords: Cisplatin; Proton Pump Inhibitors; Density Functional Theory (DFT) and UV-Vis spectroscopy

Abstract PP No. 078

Identification of Novel Non-Competitive Inhibitors of Human Adenylate Kinase 1 via Virtual Screening and Enzymatic Validation

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Objectives: Purinergic signaling, involving ATP and adenosine is essential for many physiological processes and its dysregulation contributes to disease. Human adenylate kinase (hAK) maintains nucleotide balance, with hAK1 playing a key role, both intra- and extracellularly in regulating purinergic signaling and blood clotting. Altered AK1 activity is associated with hemolytic anemia, Duchenne muscular dystrophy, proliferative diabetic retinopathy and Alzheimer's disease. Only a limited number of hAK1 inhibitors are known, prompting the search for new compounds; therefore, virtual screening was applied to identify novel hAK1 inhibitors.

Materials and Methods: hAK1 activity was measured for 10 min at 37°C in 50mM Tris-HCl (pH 7.5) with 2mM $MgCl_2$ and 1 mM adenine nucleotides and analyzed by RP-HPLC on a Chromolith RP-18e column using a Shimadzu Prominence LC system. ADP and ATP were detected at 260nm based on standard retention times. Virtual screening involved preparing the hAK1 structure (PDB: 1Z83) in Maestro 13.8 and screening 64,960 compounds from the Enamine Kinase Library. Structures were generated with OpenBabel 3.1.1, and docking was performed using GOLD 2022.3.0 with ChemPLP scoring. Iterative redocking of top hits narrowed the set to several dozen ligands, from which 12 compounds were selected for experimental testing. Molecular docking with SwissDock was performed to explore the potential mechanism of hAK1 inhibition.

Results: The effects of 12 compounds on hAK1 activity were evaluated at 20 and 50μM in the direction of AMP and ATP synthesis. Most compounds showed only slight inhibition; however, two showed strong effects. Z599652496 showed IC_{50} values of 17.93μM for AMP and ATP synthesis and 13.69μM for ADP synthesis, while Z70808617 was less potent, with IC_{50} values of 40.51μM and 26.72μM. SwissDock analysis identified potential binding sites for both compounds near the entrance of the substrate-binding pocket, close to the LID domain. Since these sites are separate from the active site, the results suggest a non-competitive mechanism in which ligand binding may restrict LID domain mobility, reducing catalytic activity without directly blocking substrate binding.

Conclusions: The compounds, particularly Z599652496, showed strong inhibitory effects on hAK1 activity, providing a promising basis for the rational design of selective and effective hAK1 inhibitors.

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Keywords: human adenylate kinase 1, enzyme inhibition, virtual screening

Abstract PP No. 079

Development of new antiseizure and antinociceptive agents in a group of modified pyrrolidin-2-one derivatives

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Objectives: Epilepsy affects >70M people, with ~30% drug-resistant. Neuropathic pain affects 10%, yet 95%), and enantiomeric excess by chiral SFC (>97%). Structures were verified by NMR and UPLC–MS. In vitro activity was assessed via glutamate uptake in EAAT2-transfected COS-7 cells. In vivo activity was evaluated in mice (i.p.) using MES, 6 Hz, seizure thresholds (MES, 6 Hz, PTZ), and amygdala kindling. Motor impairment was assessed via rotarod. Antinociceptive and behavioral effects were evaluated in pain and CUMS models; ADME-Tox was determined using in vitro assays.

Results: (R)-AS-105 was the most potent EAAT2 modulator. It significantly enhanced glutamate uptake and showed robust antiseizure effects in MES and 6 Hz models (ED50=32.8 mg/kg). (R)-AS-105 increased seizure thresholds (MES, 6 Hz, PTZ) and inhibited PTZ-induced kindling. It demonstrated a favorable safety profile, with motor impairment only at higher doses (rotarod TD50=118.2 mg/kg). It exhibited potent antinociceptive activity and beneficial effects in the CUMS model, with favorable ADME-Tox properties.

Conclusion: Replacing the succinimide ring with a pyrrolidine-2-one scaffold maintains EAAT2 modulation and provides robust protection in multiple seizure models. (R)-AS-105 is a promising candidate for further development for drug-resistant epilepsy and neuropathic pain.

[1] CNS Drugs 35 (2021) 935.

[2] Nat. Rev. Dis. Primers 3 (2017) 17002.

Abstract PP No. 080

Targeting Human Adenylate Kinase 1: Benzoisoselenazolone Compounds as Novel Inhibitors

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Objectives: Adenylate kinases (AK, EC 2.7.4.3) are phosphotransferases that regulate cellular ATP levels by catalyzing the reversible reaction: $Mg^{2+}ATP + AMP \leftrightarrow Mg^{2+}ADP + ADP$. In humans, nine AK isoenzymes have been identified, differing in tissue distribution and subcellular localization. Among them, hAK1 is unique in being present both intracellularly and extracellularly, where it regulates purinergic signaling and ADP homeostasis—processes essential for, among others, blood clotting. Altered AK1 activity has been associated with various diseases, including hemolytic anemia, diabetic retinopathy, and Alzheimer's disease. Therefore, identifying effective inhibitors of hAK1 is of considerable interest. To date, only a limited number of inhibitor classes have been described, including dinucleoside polyphosphates, statins and their derivatives, and phenylcyanomethylenequinone oximes. Here, we report benzoisoselenazolone compounds as a new class of hAK1 inhibitors.

Materials and Methods: N-substituted benzoisoselenazolones were synthesized via the reaction of 2-(chloroseleno)benzoyl chloride—obtained through a multistep synthesis from anthranilic acid—with appropriate aliphatic or aromatic amines. Human AK1 activity was measured for 10 min at 37°C in 50 mM Tris-HCl (pH 7.5), 2 mM $MgCl_2$, and 1 mM adenine nucleotides, in the presence of the tested compounds, and analysed by RP-HPLC. ADP and ATP were detected at 260 nm based on retention times of standards. Twelve derivatives were screened at 1 µM and 20 µM using 1 mM ADP, and IC_{50} values were determined for selected compounds. To investigate the potential mechanism of hAK1 inhibition by the tested compounds, molecular docking was performed using the SwissDock server.

Results: All tested compounds inhibited hAK1 activity, although with varying potency. Nine compounds at 1 µM reduced enzyme activity by approximately 75% compared to the control. Notably, derivatives bearing N-substituted aromatic moieties exhibited higher inhibitory potency compared to their aliphatic counterparts. For the most effective inhibitors, IC_{50} values were determined, and molecular docking was performed to predict the inhibition mechanism.

Conclusions: Benzoisoselenazolones effectively inhibit hAK1 and represent a new class of hAK1 inhibitors. The obtained results provide a basis for the rational design of more potent next-generation hAK1 inhibitors.

Keywords: benzoisoselenazolones, adenylate kinase, inhibitors

Abstract PP No. 081

Enhancing Antibiotic Efficacy: A Small-Molecule Compound Active Against MRSA and Gram-Positive Pathogens in Diabetic Foot Infections

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Objectives: The global rise of antimicrobial resistance, particularly among ESKAPE pathogens, poses a major clinical challenge. Gram-positive bacteria such as Staphylococcus aureus, including MRSA strains, are frequently implicated in chronic and hard-to-heal infections, including diabetic foot infections. This study aims to characterize the antimicrobial activity and potential mechanism of action of a small-molecule compound with selective activity against Gram-positive bacteria.

Materials and Methods: Antimicrobial activity was assessed by MIC determination, time-kill assays, and growth curve analysis. Synergistic interactions were evaluated using checkerboard assays and confirmed by time-kill studies. Mechanistic studies included microscopy-based approaches and biochemical assays. Anti-biofilm activity as well as cytotoxicity towards human skin cells were also evaluated.

Results: The selected compounds exhibited selective activity against Gram-positive bacteria, including S. aureus and MRSA strains. Time-kill and growth curve analyses demonstrated a bacteriostatic mode of action. The compound showed synergistic activity with a ß-lactam antibiotic, resulting in enhanced antibacterial activity in combination. Mechanistic studies suggested that the compound does not act through direct disruption of the bacterial cell envelope or other classical antibacterial targets. Additionally, the compound showed anti-biofilm activity and low cytotoxicity in human cells.

Conclusions: The studied compound represents a promising candidate with selective activity against Gram-positive pathogens, including MRSA, and the ability to enhance antibiotic efficacy. Its distinct mode of action and anti-biofilm properties support further investigation, particularly in the context of chronic infections such as diabetic foot infections.

Acknowledgements: This work was supported by the National Science Centre (NCN), Poland, under the SONATA-19 grant no. 2023/51/D/NZ7/0259

Keywords: antimicrobial resistance, diabetic foot infections, antimicrobial agent

Abstract PP No. 082 **Spotlight Presentation (PSP 39)**

Preparation and structural analysis of salts and cocrystals of Pyrimethamine with Vanillic Acid and analogues

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The development of new solid forms of active pharmaceutical ingredients (APIs) represents an effectve strategy to overcome limitations such as poor solubility, low bioavailability, and increasing drug resistance. In this study, pyrimethamine—an antimalarial drug with reduced clinical efficacy due to resistance—was investigated as a model compound for crystal engineering through salt and cocrystal formation.

The aim of this work was the preparation and structural characterization of new crystalline forms of pyrimethamine with vanillic acid and its structurally related analogues, including vanillin, 3,4-dihydroxybenzoic acid, and 3,4-dimethoxybenzoic acid. Crystallization experiments were carried out using solution-based methods with various solvents (methanol, ethanol, n-propanol, and acetone) and different stoichiometric ratios.

The results demonstrate that subtle modifications in coformer structure and crystallization conditions significantly influence the resulting solid-state form and molecular packing. This study provides insight into the rational design of pharmaceutical solids and highlights the potential of vanillic acid derivatives as versatile coformers for tuning physicochemical properties of APIs.

Abstract PP No. 083 **Spotlight Presentation (PSP 40)**

Investigating the Complex Immunomodulatory Profile of Δ9-Tetrahydrocannabinol in a BV-2 Microglia Model of LPS-Induced Neuroinflammation

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Objectives: The primary objective of this study was to evaluate the anti-inflammatory properties of the natural cannabinoid Δ9-tetrahydrocannabinol (Δ9-THC) in a mouse in vitro model of neuroinflammation. Δ9-THC is characterized in literature as a full agonist at the GPR18 receptor, an orphan G protein-coupled receptor highly expressed in microglia [1,2]. The research aimed to determine if Δ9-THC could modulate microglial activation and the expression of inflammatory markers induced by lipopolysaccharide (LPS).

Materials and Methods: The study utilized the BV-2 mouse microglial cell line. A non-toxic working concentration for Δ9-THC was determined with MTS assay. Neuroinflammation was induced by treating cells with 1 µg/ml LPS. The anti-inflammatory potential of Δ9-THC (20 µM) was initially screened by measuring nitric oxide (NO) levels using the Griess assay. Subsequently, the mRNA expression levels of pro-inflammatory (IL-1ß, IL-6, TNF-α) and anti-inflammatory (IL-4, IL-10, TGF-ß) cytokines were analyzed after 24 hours of co-treatment using RT-qPCR.

Results: Δ9-THC exhibited moderate toxicity in BV-2 cells with a calculated of 61.70 µM, while 20 µM was confirmed as a safe concentration that did not affect viability. In the Griess assay, co-treatment with 20 µM Δ9-THC significantly reduced the production of NO compared to cells treated with LPS alone. Gene expression analysis revealed that Δ9-THC significantly lowered the mRNA levels of the pro-inflammatory cytokines IL-1ß and TNF-α in LPS-activated cells. Analysis of anti-inflammatory markers showed that Δ9-THC treatment led to an increase in IL-10 and TGF-ß mRNA levels. Furthermore, Δ9-THC significantly restored the expression of IL-4, which had been suppressed by LPS treatment.

Conclusions: Δ9-THC demonstrates an immunomodulatory profile in activated BV-2 microglia. While it effectively suppresses NO production and key pro-inflammatory cytokines such as IL-1ß and TNF-α, it also enhances the expression of anti-inflammatory markers. The stimulatory effect on IL-6 suggests that Δ9-THC may operate through diverse signalling pathways that differ from other GPR18 ligands. These findings indicate that Δ9-THC could potentially influence the transition from a pro-inflammatory to a pro-resolution phenotype in neuroinflammatory conditions.

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[1] Alexander et al., Br J Pharmacol,178 (2021) pp. 27–156.

[2] Honkisz-Orzechowska E., et al. Molecules 29 (2024) 1258.

Abstract PP No. 84

High-Throughput Approach to Cell-Based Permeability Assays

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Objectives: Understanding compound permeability is a key component of drug discovery, as it strongly influences oral absorption, systemic bioavailability, and overall pharmacokinetic and pharmacodynamic behavior. Poor permeability is a common cause of inadequate drug exposure, which can lead to therapeutic failure despite favorable in vitro potency. Therefore, early evaluation of permeability and P-gp substrate assessment enables the prioritization of drug candidates with favorable absorption properties, thereby reducing late-stage attrition and supporting the development of safe and efficacious therapeutics. To enable efficient assessment of large compound libraries, robust reliable high-throughput screening (HTS) methods are essential. In this study, we aimed to develop and optimize an HTS-based permeability assay using a Tecan automated platform. The goal was to establish a robust and scalable workflow, suitable for early-stage drug discovery screening.

Materials and Methods: In this study, Millicell (Millipore) cell culture insert plates were used in a 96-well format, employing the MDCKII-MDR1 cell line and control compounds (amprenavir, diclofenac, talinolol, atenolol) both in the presence and absence of a specific P-gp inhibitor (elacridar). The assay was performed using an automated Tecan platform (Freedom EVO) with a script developed internally. Samples were analyzed by LC–MS/MS (Sciex API4500).

Results: The permeability test compounds (well-characterized drugs) were evaluated using the Tecan-based HTS assay, and the obtained results were consistent with literature data.

Conclusions: The HTS permeability assay was successfully validated using the MDCKII-MDR1 cell line, demonstrating reliable and reproducible results consistent with published literature data. Importantly, the method can be readily adapted to other commonly used permeability models, including MDCK-WT or Caco-2 cell lines, thereby broadening its applicability for compound permeability and P-gp substrate assessment. The scalability and compatibility with automated platforms, make this assay a valuable tool for early-stage drug discovery, facilitating rapid screening of large compound libraries and supporting informed selection of candidates with favorable absorption properties for both commercial and research applications.

Abstract PP No. 085

Proteomics at the core of multi-omics: Applications in drug safety and disease mechanisms

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Proteomics occupies a central position within the omics landscape, linking genomic and transcriptomic information to functional biological outcomes at the protein level. As proteins represent the primary effectors of cellular processes, proteomic technologies provide unique insights into both physiological and drug-induced molecular changes that cannot be fully captured by other omics approaches alone. The integration of proteomics with transcriptomics and metabolomics further enables a systems-level understanding of biological responses relevant to pharmacology and disease. In this presentation, we highlight the role of mass spectrometry-based proteomics as a core analytical platform in multi-omics strategies for drug safety assessment and biomedical research. We demonstrate how proteomics complements other omics layers by enabling the identification of protein abundance changes, post-translational modifications, and chemically induced alterations following drug exposure.

Selected case studies will illustrate the versatility of this approach. These include the detection of drug-induced protein modifications in the context of fluorine-containing pharmaceuticals, analyses of disease-associated alterations in protein expression profiles, and proteomic characterization of inflammatory pathways in dermatological conditions such as psoriasis. Together, these examples highlight how proteomics can uncover molecular mechanisms underlying both therapeutic effects and adverse responses. By positioning proteomics as a central hub within integrated omics workflows, this work underscores its critical contribution to modern pharmacology and translational research. The combination of multiple omics layers enhances mechanistic interpretation, supports biomarker discovery, and improves the predictive power of safety assessments. Continued advances in analytical technologies and data integration strategies are expected to further strengthen the role of multi-omics approaches in the development of safer and more effective therapies.

Abstract PP No. 086

Advancing drug dafety assessment through integrated electrochemical and multi-omics technologies

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The increasing structural complexity of modern pharmaceuticals, including the widespread incorporation of fluorine, necessitates advanced analytical strategies for comprehensive drug safety assessment. Conventional toxicological approaches often fail to capture early-stage molecular alterations and chemically induced modifications that may contribute to adverse drug effects. Therefore, the integration of complementary analytical technologies is essential for next-generation safety evaluation. In this presentation, we introduce an integrated framework combining electrochemical methods, mass spectrometry, and proteomics to investigate drug-induced molecular transformations and their biological consequences. Electrochemical techniques provide a controlled and reproducible platform for simulating redox-driven metabolic processes, including the activation and cleavage of stable chemical bonds such as carbon–fluorine (C–F). Recent studies have demonstrated that electrochemical reduction in aqueous media enables efficient and selective defluorination of pharmaceuticals, with real-time monitoring of fluoride ion release and precise identification of transformation products using high-resolution mass spectrometry. Mass spectrometry serves as a central analytical tool for structural characterization of drug-derived products, while proteomics extends this approach by enabling the identification of protein-level responses, including changes in expression and chemically induced modifications. This multi-layered strategy allows for the detection of subtle molecular perturbations that are not accessible through conventional assays but may be critical for understanding mechanisms of toxicity.

Selected case studies illustrate how the integration of electrochemical simulation with omics-based analyses provides insight into both chemical reactivity and downstream biological effects. By linking transformation pathways with proteomic alterations, this approach enhances mechanistic understanding and supports the identification of potential safety biomarkers. Overall, the combination of electrochemistry and omics technologies represents a powerful next-generation platform for drug safety assessment. The continued development of such integrative methodologies is expected to improve predictive toxicology, facilitate early risk identification, and contribute to the design of safer and more effective therapeutic agents.

Abstract PP No. 087

Biodegradable Polyurethane Hydrogels for Localized Delivery of Fluorodeoxyuridine for Pancreatic Cancer Treatment

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The effectiveness of pancreatic ductal adenocarcinoma (PDAC) chemotherapy is often limited by systemic toxicity and rapid drugs clearance, highlighting the need for localized drug delivery strategies. 5-Fluoro-2′-deoxyuridine, FUdR) is a potent thymidylate synthase inhibitor with strong antiproliferative activity against cancer cells. In this study, pH-responsive biodegradable polyurethane (PU) hydrogels were designed as implantable matrices for the controlled release of FUdR. The hydrogels were synthesized using an aliphatic diisocyanate and multiblock copolymers composed of ε-caprolactone (CL), rac-lactide (LA), and poly(ethylene glycol) (PEG), together with ethylene oxide and propylene oxide copolymers, diols acting as chain extenders, and a pH-responsive component. FUdR was incorporated into the obtained hydrogel network at a concentration of 5 wt.%.

The resulting materials were evaluated in terms of swelling behavior, mechanical properties, cytocompatibility, and in vitro drug release kinetics. The hydrogels demonstrated high water uptake and elasticity consistent with the requirements for soft tissue applications. Drug release experiments revealed a sustained and composition-dependent release of FUdR under physiological conditions. The drug release kinetics were strongly influenced by the hydrophobic–hydrophilic structure of the polymer network: hydrogels with higher CL content exhibited reduced swelling and slower drug diffusion. At physiological pH (7.4), FUdR release was prolonged up to approximately 10 days, while slightly faster release was observed under alkaline conditions (pH 8.5). According to the experimental data, after 7 days at pH 7.4 approximately 100%, 73.7%, and 63.6% of the drug were released from HPU-1-FUdR, HPU-2-FUdR, and HPU-3-FUdR hydrogels, respectively. Kinetic profiles analysis indicated that FUdR transport from the hydrogels was mainly diffusion-driven and could be described by the Higuchi model. The developed PU hydrogels therefore represent promising candidates for localized chemotherapy, enabling tunable and sustained delivery of FUdR from implantable biomaterials.

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Abstract PP No. 088

Pharmacokinetics of Trametinib in Juvenile Patients with Refractory Histiocytic Neoplasms without BRAF Mutation or Following Vemurafenib Failure

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Purpose: Pharmacokinetic (PK) data for trametinib in juvenile populations remain limited, hindering evidence-based dose optimization and clinical decision-making. Current dosing strategies are largely extrapolated from adult exposure data, with insufficient prospective PK studies in children and adolescents. The aim of this study was designed to evaluate trametinib plasma concentrations in juvenile patients with refractory histiocytic neoplasms lacking BRAF mutations or after failure of vemurafenib therapy.

Method: Subjects under 18 years of age were enrolled. Trametinib was administered orally once daily using age-adapted weight-based dosing (<6 years: 0.032 mg/kg; ≥6 years: 0.025 mg/kg; maximum 2 mg/day). Blood samples were collected at predefined time points (days 0, 1, 15, 22, 29, and every 3 months thereafter). Plasma concentrations were quantified using a validated analytical method compliant with Good Laboratory Practice and European Medicines Agency guidelines.

Results: Maximum plasma concentrations (Cmax) of trametinib in 10 evaluable patients ranged from 5.637 to 30.762 ng/mL, demonstrating substantial interindividual variability in drug exposure.

Conclusions: Time to maximum concentration (Tmax) in juvenile patients appears comparable to that reported in adults. The observed variability in Cmax highlights the heterogeneity of the study population and underscores the importance of individualized dosing strategies. These findings support the role of therapeutic drug monitoring and further PK-driven optimization of trametinib therapy in juvenile patients with refractory histiocytic disorders.

Abstract PP No. 089

Pharmacokinetics of Vemurafenib in Juvenile Patients with BRAF-Mutated Histiocytic Disorders: Implications for Individualized Dosing

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Purpose: Data on the pharmacokinetics of vemurafenib in the juvenile population are limited, which makes it difficult to optimize dosing and its modification during therapy. This highlights the need for individualized treatment, preferably based on therapeutic drug monitoring. Available studies suggest that pharmacokinetics in children are comparable to those observed in adults, allowing the adoption of a target steady-state concentration >42 µg/mL. The aim of this study was to assess plasma concentrations of vemurafenib in juvenile patients with a BRAF mutation.

Materials and Methods: Subjects under 18 years of age with histiocytic disorders and BRAF mutation were included. Vemurafenib was administered orally at an initial dose of 20 mg/kg/day (maximum 1920 mg/day) in two divided doses, with possible escalation to 30 and 40 mg/kg/day if clinical response was not observed. Plasma drug concentrations were measured on days 1, 8, 15, 22, and 29 of therapy, and subsequently approximately every 3 months. Dosing was adjusted according to body weight. Vemurafenib concentrations in plasma were determined using a validated method in accordance with Good Laboratory Practice and European Medicines Agency guidelines.

Results: Maximum plasma concentrations of vemurafenib in the 19 juvenile patients ranged from 30.89 to 69.59 µg/mL.

Conclusions: The observed high variability in pharmacokinetic parameters, resulting from the heterogeneity of the juvenile population, indicates the need for individualized therapy. A personalized approach, based on therapeutic drug monitoring and dose adjustment according to patient-specific characteristics, is essential for optimizing treatment outcomes.

Abstract PP No. 090

New Triazole Derivatives to Combat Resistance in Fungal Pathogens

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The 1,2,4- as well as 1,2,3- triazole derivatives are widely recognized for their broad spectrum of biological activities covering anticancer, antiviral, anti-inflammatory properties but first off all antifungal and antibacterial [1, 2]. Nevertheless, over the past few decades, the rapid emergence of resistance among fungal pathogens has significantly reduced the clinical efficacy of commonly used antifungal agents, including such triazole derivative as fluconazole [3]. This phenomenon highlights the urgent need to design new molecular scaffolds that can overcome resistance mechanisms and broaden the therapeutic spectrum. In our recent studies, we focused on the structural modification of fluconazole by introducing additional 1,2,3-triazole scaffold and other potentially beneficial structural motives. Applying the Cu mediated Huisgen 1,3-cycloaddition, we obtained series of hybrid molecules of new biological profile, potentially combining or even synergistically enhancing the properties of the parent molecules. These strategies offer a route toward the discovery of novel antifungal agents with improved efficacy and the ability to address the growing problem of resistance. Selected synthetic pathways to representative conjugates and their biological activity pattern will be presented.

Acknowledgement: OPUS UMO-2019/33/B/NZ7/01608 Keywords: fluconazole, triazole, Huisgen 1,3-cycloaddition, antifungal, antibacterial, pathogen resistance.

References:

[1] Kharb, R.; Sharma, P. C.; Yar, M. S. Pharmacological significance of triazole scaffold. Journal of enzyme inhibition and medicinal chemistry (2011) 26 1-21.

[2] Janowski, M.; Demchuk, Oleg M.; Wujec, M. Fluconazole analogs and derivatives: An overview of synthesis, chemical transformations, and biological activity. Molecules (2024) 29, 2855.

[3] Kainz, K.; Bauer, M.A.; Madeo, F.; Carmona-Gutierrez, D. Fungal Infections in Humans: The Silent Crisis. Microbial Cell (2020) 7, 143.

Abstract PP No. 091 **Spotlight Presentation (PSP 42)**

Comparative Structural Analysis of DYRK1A Inhibitors Reveals Diverse Modes of ATP-Pocket Recognition

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Objectives: Dual-specificity tyrosine phosphorylation-regulated kinase 1A (DYRK1A) is a key regulator of ß-cell function, and its inhibition has been shown to enhance insulin secretion and improve glucose homeostasis in diabetic models. Although numerous chemically diverse inhibitors of DYRK1A have been described, the structural basis underlying their binding diversity and functional effects remains incompletely understood. This study aimed to define common and distinct features of DYRK1A–ligand interactions across structurally unrelated inhibitors.

Materials and Methods: A panel of structurally and functionally diverse DYRK1A inhibitors was investigated, including Harmine, a gold-standard inhibitor promoting ß-cell proliferation; Gossypin, polyphenolic compound with anti-inflammatory and anti-cancer properties; AZ191, a selective ATP-competitive DYRK inhibitor; Abemaciclib, a targeted anticancer drug; and AC27, a moderate-potency DYRK1A inhibitor used as a structural reference. High-resolution crystal structures of DYRK1A in complex with these ligands were determined at 2.05–2.30 Å resolution. Binding modes within the ATP-binding pocket were analyzed with emphasis on hinge-region interactions, hydrogen bonding networks, water-mediated contacts, and hydrophobic pocket occupancy. Enzymatic inhibition of selected compounds was evaluated using ADP-Glo kinase assays.

Results: All compounds occupied the ATP-binding site of DYRK1A, engaging the hinge region through conserved interactions, particularly involving residues such as Leu241. However, significant diversity was observed in hydrogen bonding patterns and interaction networks. Harmine exhibited a canonical hinge-binding mode with limited additional contacts, whereas gossypin formed extended hydrogen bond networks, including water-mediated interactions with residues such as Lys188 and Asp307. AZ191 and abemaciclib exhibited both common and distinct interaction features within the ATP pocket, combining conserved hinge binding with additional contacts that may contribute to their selectivity.

Conclusions: This study demonstrates that chemically diverse scaffolds achieve DYRK1A inhibition through a combination of conserved hinge interactions and variable hydrogen bonding and solvent-mediated networks. These findings highlight the structural plasticity of the DYRK1A ATP pocket and provide a framework for rational design of selective kinase inhibitors.

Abstract PP No. 092

EXPLORING PSIDIUM CATTLEYANUM PHENOLIC-RICH EXTRACTS AS INNOVATIVE DERMOCOSMETIC INGREDIENTS

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Psidium cattleyanum Sabine (Family Myrtaceae), commonly referred to as strawberry guava or araçá, has long been used in traditional medicine to treat infections, gastrointestinal ailments, and inflammatory disorders. Despite its well-established ethnopharmacological background, its dermatological potential remains largely unexplored. This study aimed to characterize the phytochemical profile of P. cattleyanum leaves and fruits and to assess their in vitro biological activities, with particular focus on skin-related applications. Leaves and fruits collected from Santo Antão Island (Cape Verde) were extracted using methanol–acetone–water (3:1:1, v/v/v) and 70% ethanol, followed by fractionation. Total phenolic, flavonoid, and phenolic acid contents were quantified spectrophotometrically. UHPLC-DAD-ESI-IT-MS analysis led to the identification of 42 compounds. Antioxidant capacity was examined through DPPH˙ and ABTS˙+ assays. Anti-aging and skin-brightening potentials were investigated by evaluating collagenase, elastase, and tyrosinase inhibition. Antibacterial activity was tested against skin-associated pathogens, including Cutibacterium acnes, Staphylococcus aureus, and Staphylococcus epidermidis. Cytotoxicity was determined on GMK cells using the MTT assay. The methanol–acetone–water extract and its butanolic fraction exhibited the highest polyphenol content and the strongest antioxidant properties. Both butanolic and ethyl acetate fractions displayed the most notable inhibitory activity against collagenase, elastase, and tyrosinase. All tested extracts demonstrated bacteriostatic effects against acne-related bacteria and showed synergistic or neutral interactions with selected antibiotics, with no antagonistic outcomes. No significant cytotoxicity was observed at biologically active concentrations. In summary, P. cattleyanum leaf extracts are rich in bioactive phenolic compounds with remarkable antioxidant, anti-aging, and antimicrobial activities, supporting their potential as valuable ingredients in dermocosmetic and phytopharmaceutical formulations.

Acknowledgements: This research was funded by the Ministry of Science and Higher Education in Poland DS45 project of the Medical University of Lublin.

Key Words: Psidium, Myrtaceae, anti-aging, antioxidant, antibacterial.

Abstract PP No. 093 **Spotlight Presentation (PSP 43)**

Evaluation of α-Lipoic Acid for Skin Applications: Permeation, Biological Activity and Safety Assessment

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Objectives: α-Lipoic acid (ALA) is important for skin tissue and epidermal structure formation.

Materials and Methods: The aim of the study was the assessment of ALA in skin applications. Ex vivo permeation of α-lipoic acid from a carbomer hydrogel through Strat-M membrane was evaluated using a flow-through diffusion system (USP apparatus IV). The EpiDerm Skin Irritation Test (SIT) was performed to assess the safety of ALA for dermatological applications. Cytotoxicity on HaCaT keratinocytes was determined using the neutral red uptake assay. Antioxidant potential was assessed via DPPH assay. Inhibition of fungal and murine tyrosinase (from lysates of murine melanoma B16F10 cells) was tested spectrophotometrically. The anti-inflammatory potential of ALA was evaluated by assessing its effect on pro-inflammatory cytokine release in LPS-stimulated human neutrophils and THP-1-derived macrophages.

Results: A hydrogel containing α-lipoic acid (2.67% w/w) was prepared using carbomer (1.33% w/w) and propylene glycol (13.33% w/w). ALA permeation through the Strat-M membrane was confirmed by HPLC-DAD, with the highest concentration (0.501 mg/L) observed after 1 h, indicating burst release. At higher concentrations, ALA significantly decreased tissue viability in the SIT assay, reaching 15.5 ± 0.9% for the 7% formulation and 5.2 ± 0.3% for the 14% formulation. ALA inhibited tyrosinase in a concentration-dependent manner, reducing murine enzyme activity to 5.18 ± 2.17% at 1.0 mg/mL and fungal tyrosinase to 42.64 ± 0.77%. In THP-1-derived macrophages, ALA inhibited TNF-α, IL-6, and MCP-1 release, with the strongest effect for MCP-1. In neutrophils, it reduced TNF-α and IL-8 secretion dose-dependently, with no effect on IL-10.

Conclusions: The hydrogel formulation enabled effective release and permeation of α-lipoic acid. ALA exhibited antioxidant, anti-tyrosinase, and anti-inflammatory activity; however, at higher concentrations it reduced tissue viability, indicating that its concentration should be carefully optimized for safe topical applications.

Abstract PP No. 094

Disease-Specific Alterations in SAM, SAH, and the SAM/SAH Ratio: A Clinical Overview

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Objectives: S-adenosylmethionine (SAM) and S-adenosylhomocysteine (SAH) play a central role in methylation and one-carbon metabolism. Growing evidence indicates alterations in their concentrations across major disease groups.

Materials and Methods: The study involved a population of 750 individuals with obesity. Parameters such as age, body weight, BMI, glucose concentrations, and blood pressure were recorded, as previously reported (1). SAM and SAH concentrations were measured using the LC–MS/MS method. The results were then compared with data from published studies reporting SAM and SAH concentrations in metabolic, renal, hepatic, and neurodegenerative disorders. Disease-specific patterns were analyzed with a focus on methylation potential and clinical correlations.

Results: Our results show higher SAM concentrations in patients with class III obesity (167.5 ± 64.3 nM) compared to those with class I obesity (146.5 ± 54.6 nM; p = 0.02). Additionally, individuals with hypertension had higher SAM concentrations (164.9 ± 62.4 nM) compared to those with normal blood pressure (p = 0.007). These observations may be interpreted in light of prior reports showing that patients with steatotic liver disease exhibit elevated SAM (121 vs. 96 nM), which correlates with an increased risk of hepatocellular carcinoma. Renal dysfunction, including chronic kidney disease and hemodialysis, is characterized by elevated plasma SAH concentrations and reduced SAM/SAH ratios (0.36–1.48). Neurodegenerative diseases show compartment-specific alterations: Alzheimer's disease is associated with reduced SAM in the CSF and brain and lower SAM/SAH ratios (linked to the APOE4 genotype), while Parkinson's disease exhibits decreased whole-blood SAM. Autism spectrum disorder shows variable SAM and SAH alterations, indicating impaired methylation capacity. Some cancers, including liver and lung cancer, exhibit elevated SAM levels, suggesting diagnostic potential. Healthy reference ranges are broad (plasma SAM: 52–148 nM; SAH: 15–128 nM; SAM/SAH ratio: 1.5–10.0).

Conclusions: SAM, SAH, and the SAM/SAH ratio are altered in a disease-specific manner, influencing methylation potential and epigenetic regulation. Patterns of change differ by organ system and disease type, with implications for early diagnosis, risk stratification, and therapeutic targeting.

References:

[1] Wrzosek M, Ślusarczyk K. Methylenetetrahydrofolate Reductase C677T Gene Variant in Relation to Body Mass Index and Folate Concentration in a Polish Population. Biomedicines. 2022,10(12):3140.

Abstract PP No. 095 **Spotlight Presentation (PSP 44)**

Synergistic Application of In Situ Fourier Transform Infrared Spectroscopy, Microwave, and Ultraviolet Irradiation in the Optimized Synthesis of 1,5-Benzodiazepines

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Objectives: The objective of this study was to establish an evidence-based, sustainable framework for the synthesis of 1,5-benzodiazepine derivatives, primarily known as a group of CNS depressants, including anxiolytic, anticonvulsant, and sedative effects. We aimed to evaluate the synergistic effects of simultaneous microwave (MW) and ultraviolet (UV) irradiation, utilizing real-time Fourier Transform Infrared (FTIR) spectroscopy to monitor reaction kinetics, elucidate mechanistic pathways, and optimize catalytic efficiency.

Materials and Methods: The condensation of 1,2-phenylenediamine with various ketones (e.g., acetone, acetylacetone) was investigated. Screening identified scandium(III) triflate ($Sc(OTf)_3$) and molecular iodine (I_2) as optimal catalysts. Reactions were performed under conventional reflux, single-mode MW, and MW/UV dual-activation. Reaction progression was quantitatively tracked using an in situ ATR-FTIR probe, with standard addition and calibration curve methods to ensure quantitative accuracy across varying conditions and solvents.

Results: Under single-mode MW irradiation, the model condensation of acetone yielded 94.5% in 6.5 minutes with Sc(OTf)3 as catalyst; remarkably, simultaneous MW/UV further accelerated process, delivering 97% yield in only 4.5 minutes. Conversely, when sustainability and cost-efficiency were prioritized, I2 in neat ketone or methanol emerged as an effective alternative, achieving up to 93% yield in 8.5 minutes under dual activation. FTIR monitoring provided empirical evidence of catalyst-substrate coordination, notably the dynamic shift of the triflate $vsSO_3$ band. Furthermore, real-time analytics identified strict operational boundaries, revealing ATR probe fouling by insoluble scandium species at >3 mol% loading and oxidative diamine degradation at >5 mol% I2 loading.

Conclusions: The synergy of MW and UV with FTIR feedback represents a powerful multimodal approach to heterocyclic synthesis. This methodology transcends trial-and-error optimization, providing a highly tunable, evidence-based, and rational framework for the rapid, efficient, and sustainable production of pharmacologically relevant 1,5-benzodiazepines.

Acknowledgements: The work was funded by National Science Centre, Poland, under research project № 2024/53/N/NZ7/00617 „The novel analytical technique of evaluation and imaging of the progress of selected chemical reactions assisted by MW/UV/US using the in situ ATR-FTIR method in real time”.

Keywords: 1,5-Benzodiazepines; MW + UV Dual Activation; In Situ FTIR

Abstract PP No. 096 **Spotlight Presentation (PSP 45)**

Blocking is the Past, Degradation is the Future: A New Direction for the (R)evolution of Cyclooxygenase-2 Targeted Drug Design

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Objectives: Cyclooxygenase-2 (COX-2) is overexpressed in inflammatory conditions and various types of cancer. While non-steroidal anti-inflammatory drugs (NSAIDs) effectively inhibit its enzymatic activity, they leave the non-catalytic, pro-oncogenic functions of the protein intact. Proteolysis-targeting chimera (PROTAC) technology offers a shift in this classical pharmacological paradigm by inducing the proteasomal degradation of COX-2, thereby overcoming the inherent limitations of traditional inhibition [1]. The primary objective is to develop a library of COX-2 degraders based on various NSAIDs, utilizing optimized linkers and E3 ligase-recruiting ligands, including novel achiral cereblon (CRBN) ligands. This approach enables a systematic evaluation of COX-2 degradation impact on pro-oncogenic processes.

Materials and Methods: The scope of this study encompasses in silico design, organic synthesis and physicochemical assessment. The research workflow further includes the biophysical analysis of ternary complex formation, followed by a biological evaluation focusing on cytotoxicity, COX-1/COX-2 degradation profiles (ELISA), and the RT-qPCR-based expression analysis of pro-inflammatory cytokines.

Results: To date, a library of 18 PROTAC molecules and 28 novel achiral CRBN ligands has been synthesized and characterized. Preliminary biological assays led to the identification of KK-POM-69 - an ibuprofen and pomalidomide-based degrader featuring a 12-carbon alkyl linker. This lead compound demonstrated significant COX-2 degradation ($p < 0.05$) at a level of 25%.

Conclusions: This study provides a proof-of-concept for CRBN-dependent COX-2 degradation; however, the current degradation efficiency requires further optimization. The findings suggest that while long alkyl chains effectively mimic the natural substrate - arachidonic acid - within the COX-2 binding site, their high hydrophobicity and poor solubility limit their overall degradation potential. Future work will focus on linker modification and the implementation of hydrophilic achiral CRBN ligands, establishing a foundation for the development of a novel class of COX-2-targeted therapeutics.

Acknowledgements: Doctoral School Research Grant, financed by the statutory funds of Poznan University of Medical Sciences (Grant No. 176/2025/DGB).

References:

[1] Kossakowski K, Jurga M, Pawełczyk A. How to design PROTAC molecules? A practical guide to proteolysis-targeting chimeras. Farm Pol, 2025, 81(4), 195-211.

Keywords: proteolysis targeting chimeras (PROTAC), targeted protein degradation (TPD), nonsteroidal anti-inflammatory drugs (NSAIDs)

Abstract PP No. 097

Reusability of polyamide (PA12) in Selective Laser Sintering (SLS) of Drug Delivery Systems: A Technological and Safety Assessment

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Objectives: This study evaluates the suitability and reusability of biocompatible PA12 in SLS for manufacturing individualized pharmaceutical dosage forms, with emphasis on performance across multiple reuse cycles. As SLS surrenders substantial amount of unused powder, recycling is desirable, provided that performance and safety remain uncompromised. The objectives were: (i) to assess processing performance and rheological properties of thermally aged powder as determinants of printability, and (ii) to investigate toxicological risks associated with repeated reuse, including intestinal permeability of microplastics and cytotoxicity in macrophages and hepatocytes.

Materials and Methods: PA12 powder (150 mL) was analyzed using Granudrum over five thermal cycles (22–130 °C). Flow properties were determined by first avalanche angle (n=30) and cohesion index at 5–50 rpm. Intestinal permeability was evaluated using isolated rat intestinal segments in an organ bath, with histological analysis. Cytotoxicity was assessed in hepatocytes (24–48 h, six concentrations) and RAW macrophages.

Results: Flowability depended on temperature and reuse cycle. At 22 °C, flow angle increased progressively, indicating cumulative deterioration likely due to surface aging and increased cohesion. At elevated temperatures (100–130 °C), flow angle decreased, suggesting improved flow behavior. Measurements at ≥70 °C showed reduced inter-cycle variability, indicating stabilization under SLS-relevant conditions. PA12 microparticles were detected within intestinal tissue, confirming barrier penetration. Hepatocytes exhibited time- and concentration-dependent, non-linear responses, with highest consistency at 0.5–1 mg/mL and increased variability at lower concentrations after prolonged exposure. Macrophages showed mild, non-monotonic responses without clear cytotoxicity, with highest reproducibility at intermediate concentrations.

Conclusions: PA12 remains suitable for reuse in SLS across multiple cycles, although performance is strongly influenced by processing conditions. Elevated temperatures characteristic of SLS improve flow and reduce variability despite deterioration observed at ambient conditions. While intestinal uptake indicates potential biological interaction, cytotoxic effects were mild, non-linear, and concentration-dependent, with no evidence of acute toxicity.

Keywords: Selective Laser Sintering (SLS), Additive Manufacturing (AM), Polyamide PA12

Abstract PP No. 098

Patient-Oriented Formulation: Multistep Dry Processing Approach for Cariprazine Film-Coated Tablets

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Objectives: Cariprazine is an atypical antipsychotic acting primarily as a dopamine D3/D2 receptor partial agonist and is indicated in the EU for the treatment of schizophrenia in adults. Formulating a low-dose, high-potency active pharmaceutical ingredient (API) into a solid oral dosage form requires a robust strategy to ensure content uniformity and solid-state stability while maintaining patient-oriented drug formulation. The objective of this work was to develop an immediate-release, film-coated tablet of Cariprazine that delivers high compositional homogeneity and stability.

Materials and Methods: The drug product was manufactured using a direct compression process comprising the steps of weighing, multistep sieving and blending, compression, film coating and packaging. Formulation components were introduced into the blend at different stages, and repeated deagglomeration and sieving steps were applied to improve distribution of the low dose API and control blend uniformity. Blend homogeneity was additionally supported by the selection of a coarser API grade with a broader particle size distribution (D90: 45–75 µm). The crystalline form of Cariprazine hydrochloride (polymorphic form I) was monitored throughout processing.

Results: The developed formulation and process provided high homogeneity at the blend and the finished drug product. The stability studies under accelerated and long-term conditions showed no changes in the polymorphic form of Cariprazine hydrochloride. In contrast to capsule-based reference products requiring additional light protection, the use of a non-functional, titanium dioxide-free film coating, combined with Alu/Alu blister packaging, supports convenient handling and storage. The tablet dosage form avoids gelatin, offers improved mechanical robustness, and benefits from established, cost-effective manufacturing technology.

Conclusions: The presented formulation and manufacturing development strategy demonstrates that a low-dose, high-potency API such as Cariprazine hydrochloride can be successfully formulated into an immediate-release, film-coated tablet using a multistage dry process. Through careful process control including blending and targeted deagglomeration, excellent content uniformity was achieved, making it suitable for divisible tablet designs. The strategy provides patient focused solution, resulting in a dosage form well suited for individualized treatment and convenient pharmacotherapy.

Abstract PP No. 0099

BAKUCHIOL: RELEASE KINETICS STUDY OF POORLY WATER-SOLUBLE COMPOUND FROM A HYDROGEL

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Objectives: Bakuchiol and its derivatives (including metabolites formed via CYP metabolism) show therapeutic potential as topically applied alternatives to retinoids in the treatment of skin conditions such as acne and psoriasis. This study represents a preliminary attempt to evaluate the release kinetics of bakuchiol, a poorly water-soluble compound, from a semi-solid hydrogel formulation.

Materials and Methods: A carbomer-based hydrogel containing 1% bakuchiol was prepared. Release studies were carried out using a mixture of phosphate buffer solution (PBS) at pH 5.8 and 7.4 with ethanol (2:1, v/v). A semi-permeable cellulose membrane was used, and the system was maintained at 32.0 ± 0.5°C in water-jacketed vessels. Quantitative determination of released bakuchiol was performed using UV–Vis spectroscopy.

Results: Partial replacement of the aqueous medium (PBS) with ethanol enabled the evaluation of release kinetics for poorly water-soluble compounds such as bakuchiol. The results provide insight into the influence of pH and formulation composition on its bioavailability.

Conclusions: Although the European Pharmacopoeia does not require the determination of pharmaceutical availability for semi-solid dosage forms, such studies may serve as a valuable tool in the optimization of pharmaceutical and cosmetic formulations, particularly for poorly water-soluble substances.

Abstract PP No. 100

Influence of Gastrointestinal Conditions on the Release of Hypericin, Hyperforin, and Rutin from St. John's Wort Tablets

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Objectives: The aim of this study was to evaluate the influence of gastrointestinal conditions, including pH, gastric emptying time, and food intake, on the pharmaceutical availability of hypericin, hyperforin, and rutin released from St. John's wort tablets. The study addresses an important gap in understanding how physiological gastrointestinal conditions influence the release of active compounds from herbal medicinal products and may help optimize dosing recommendations and improve therapeutic efficacy.

Materials and Methods: Dissolution tests were performed using a USP II paddle apparatus at 37°C and 50 rpm with media simulating gastrointestinal fluids. Samples were collected manually every 15 minutes. Fasted-state experiments lasted up to 180 minutes and fed-state experiments up to 300 minutes. In fasted conditions, artificial gastric fluid at pH 2.0 or 4.0 was applied for 15, 30, or 60 minutes to simulate gastric residence time, followed by modified fasted-state simulated intestinal fluid. In fed conditions, milk was used as the initial medium with gradual pH reduction to simulate postprandial gastric changes, followed by transition to intestinal conditions. Quantitative analysis of hypericin, hyperforin, and rutin was conducted using validated LC-MS/MS. Content uniformity was verified in randomly selected tablets from each product.

Results: Quantitative analysis confirmed agreement between measured and declared contents of all analytes. In fasted conditions, pH had only a minor effect on release. Slightly higher release of hypericin and hyperforin was observed at pH 4.0 compared with pH 2.0, while rutin release slightly decreased. In fed conditions, increasing pH from 2.0 to 6.5 significantly enhanced release of hypericin and hyperforin, with no marked effect on rutin. Transition to intestinal conditions increased release of hypericin and hyperforin, regardless of gastric emptying time. Under fed conditions, rutin release increased markedly. Simulated food intake enhanced release of all compounds, with the strongest effect for hyperforin, moderate for rutin, and smallest for hypericin.

Conclusions: pH, gastric emptying time, and food intake significantly affect the pharmaceutical availability of hypericin, hyperforin, and rutin from St. John's wort tablets. These factors may contribute to variability in therapeutic efficacy.

Acknowledgements: This research was funded by Medical University of Warsaw, grant number WF5/1/F/MB/N/23 Keywords: St. John's wort; pharmaceutical availability;

Abstract PP No. 101

Toward Non-Invasive Diagnostics: Salivary Metabolomics in Pediatric Inflammatory Bowel Disease

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Objectives: Saliva is an increasingly recognized diagnostic matrix that enables non-invasive, low-burden, and cost-effective sampling compared with blood, while retaining the ability to reflect systemic physiology. Multiple clinically relevant analytes, including cortisol and some proteins like CRP are already quantifiable in saliva using established chromatographic and immunoassay-based methods. One of the approaches for identifying such biomarkers is metabolomics, defined as the comprehensive profiling of small-molecule metabolites in biological systems. Pediatric inflammatory bowel disease (IBD) is an increasingly prevalent and heterogeneous disorder that remains challenging to diagnose and monitor due to reliance on invasive procedures such as endoscopy and the limited specificity of available non-invasive tests. These limitations underscore the need for novel, accurate, and non-invasive biomarkers. Therefore, the aim of this study was to evaluate whether IBD is associated with a distinct salivary metabolomic fingerprint.

Materials and Methods: The study included 15 healthy controls and 20 pediatric patients with IBD. Stimulated saliva samples were collected in the morning using parafilm and analyzed using high-performance liquid chromatography coupled with high-resolution mass spectrometry (HPLC–HRMS; Orbitrap Q-Exactive Focus). Data were processed and analyzed using MetaboAnalyst.

Results: The results demonstrated that amino sugar and nucleotide sugar metabolism, the citrate cycle (TCA cycle), arginine and proline metabolism, taurine and hypotaurine metabolism, and alanine, aspartate and glutamate metabolism are significantly altered in saliva of pediatric IBD patients. Importantly, similar alterations have been reported in plasma in both pediatric and adult populations.

Conclusions: These findings indicate that saliva reflects systemic metabolic disturbances and may serve as a suitable, non-invasive matrix for metabolomic biomarker discovery, with potential applications in diagnosis, prognosis, and disease monitoring in IBD.

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Abstract PP No. 102

Saliva as a Non-Invasive Matrix for Metabolomic Profiling in Pediatric-Onset Multiple Sclerosis

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Objectives: Pediatric-onset multiple sclerosis (MS) is a chronic, immune-mediated demyelinating disorder of the central nervous system associated with early cognitive and physical disability, making timely diagnosis and monitoring crucial to limit long-term neurological damage [1]. Current biomarker research in MS has identified extensive metabolomic alterations in cerebrospinal fluid and serum, particularly in pathways related to energy, lipid, and amino acid metabolism; however, no robust, non-invasive biomarkers have yet achieved sufficient sensitivity and specificity for routine clinical use [2]. Evidence for salivary metabolomics in pediatric MS remains limited, with only isolated studies focusing on targeted protein biomarkers and no comprehensive characterization of salivary metabolic profiles in this population [3]. Saliva represents an attractive diagnostic matrix due to its non-invasive collection and its ability to reflect systemic metabolic and inflammatory states [4]. Therefore, the aim of this study was to evaluate whether MS is associated with a distinct salivary metabolomic fingerprint.

Materials and Methods: The study cohort comprised 15 healthy controls and 10 patients with MS. Stimulated saliva samples were collected in the morning using parafilm. Metabolomic profiling was performed using high-performance liquid chromatography coupled with high-resolution mass spectrometry (HPLC–HRMS; Orbitrap Q-Exactive Focus), and data processing and statistical analyses were conducted using MetaboAnalyst.

Results: Significant alterations in multiple metabolic pathways were observed in the saliva of MS patients, including pyrimidine metabolism, histidine metabolism, glutathione metabolism, and glycine, serine, and threonine metabolism. Importantly, these findings suggest that metabolic changes previously reported in blood are also reflected in saliva.

Conclusions: Saliva may represent a promising non-invasive diagnostic matrix in multiple sclerosis, with potential applications in biomarker discovery and disease monitoring. Project 2024/ABM/03/KPO/KPOD.07.07-IW.07-0192/24-00 financially supported by Medical Research Agency Poland and EU NextGenerationEU within National Recovery and Resilience Plan.

[1] Fisher, K., Cuascut, F., Rivera, V., & Hutton, G. (2020). Biomedicines, 8.

[2] Smusz, J. et al. (2025). International Journal of Molecular Sciences, 26.

[3] Ganelin-Cohen, E., Tartakovsky, E., Klepfish, E., Golderman, S., Rozenberg, A., & Kaplan, B. (2022). Frontiers in Immunology, 13.

[4] Zhao, X., et al.(2025). MedComm, 6.

Abstract PP No. 103 **Spotlight Presentation (PSP 46)**

Synthesis and Evaluation of Bioactive Zn-Fluorapatite Nanocrystals

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Introduction: The reconstruction of critical-sized bone defects remains a significant challenge in modern regenerative medicine. While hydroxyapatite is widely used, fluorapatite ($Ca_{10}(PO_4)_6F_2$) offers distinct advantages, including higher chemical stability and the ability to release fluoride ions that stimulate bone-forming cells. The biological functionality of these materials can be further enhanced by incorporating therapeutic ions like zinc (Zn^{2+}), which is known for its role in bone mineralization and its antibacterial properties. This study characterizes nanocrystalline Zn-doped fluorapatite as a bioactive precursor for multifunctional bone scaffolds.

Material and Methods: Nanocrystalline powders of pure and Zn-doped fluorapatite were synthesized using a wet precipitation method. Calcium nitrate and diammonium orthophosphate were used as primary precursors, with zinc nitrate added to achieve specific substitution levels. After aging and drying, structural integrity was verified using powder X-ray diffraction (PXRD). Chemical bonding and functional groups were analyzed via FTIR and Raman spectroscopy. Surface morphology was evaluated using scanning electron microscopy (SEM). The specific surface area was measured by the Brunauer Emmett Teller (BET) method.

Results and discussion: PXRD presented in Figure 1 confirmed a single-phase hexagonal apatite structure with no secondary impurities. Refinement of lattice parameters showed measurable shifts, confirming successful Zn^{2+} substitution, while the decreasing crystallite size suggests increased lattice distortion and restricted crystal growth. FTIR and Raman spectra validated high phase purity and characteristic phosphate groups. SEM imaging revealed needle-like nanocrystals with high surface area, as confirmed by BET measurements showing an increased specific surface area.

Conclusions: This research successfully demonstrates the synthesis of nanocrystalline Zn-doped fluorapatites. The structural and spectroscopic data confirm that zinc ions are effectively integrated into the fluorapatite lattice without compromising phase purity. These materials, in combination with natural polymers, may serve as promising candidates for 3D printing applications in biomedical engineering.

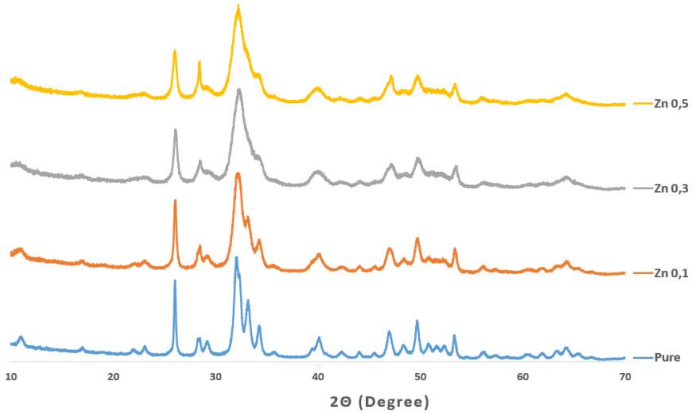

Figure 1. PXRD patterns of pure fluorapatite and Zinc-doped samples at varying molar concentrations (0.1, 0.3, and 0.5).

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Abstract PP No. 104

Cobalt-Doped Brushite as a Biologically Active Calcium Phosphate for Next-Generation Bone Substitute Materials

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Objectives: Calcium phosphate-based materials play a crucial role in medical applications due to their biocompatibility and osteoconductivity. Among various calcium phosphates, brushite (Dicalcium Phosphate Dihydrate: $CaHPO_4 \cdot 2H_2O$) has gained significant attention, primarily as a key component of injectable bone cements used to fill bone defects resulting from trauma or surgery. However, despite their broad use as bone scaffolds, they still lack intrinsic biological activity that could actively support bone regeneration process. To introduce the missing biological functionality, we incorporated cobalt ions (Co^{2+}) into the brushite lattice, aiming to develop pro-angiogenic biomaterials for bone regeneration while retaining their structural identity.

Materials and Methods: A series of cobalt-substituted brushite powders was synthesized by a controlled precipitation method, with Co^{2+} partially replacing Ca^{2+} at nominal molar substitution levels of 0.05, 0.1, 0.2 and 0.4. The powders were characterized by FT-IR, Raman, PXRD, SEM [Fig.1], and AAS, while biocompatibility was evaluated in vitro using MTT and NRU assays.

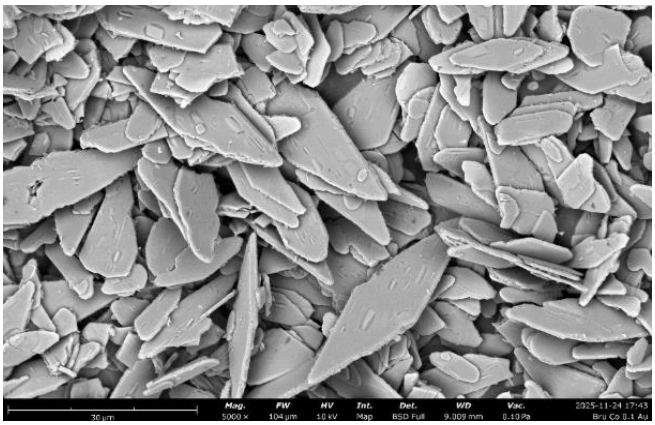

Fig. 1: Morphology of Co^{2+} doped brushite powder (Bru-Co 0.1) observed by SEM (5,000×, 10 kV)

Results: The analyses confirmed successful cobalt incorporation into the brushite structure and cobalt-related crystallographic changes while preserving the brushite phase. SEM showed the characteristic plate-like morphology of brushite crystals, while AAS verified the presence of cobalt in the doped powders. In vitro tests showed that cobalt incorporation did not reduce brushite biocompatibility and maintained high cell viability.

Conclusions: The results highlight cobalt-doped brushite as a highly promising next-generation bone substitute material. By combining the high resorbability and osteoconductive character of brushite with the pro-angiogenic activity of cobalt, this approach opens new opportunities for the development of functional calcium phosphate biomaterials designed to actively support and accelerate bone tissue regeneration.

Studies were supported by the National Science Center (Opus UMO-2023/51/B/NZ7/00555).

Abstract PP No. 105

Synthesis and Physicochemical Characterization of Co^{2+}/Cu^{2+}-Doped Brushite for 3D Bio-Printed Bone Scaffolds

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Objectives: Brushite, a bone substitution biomaterial is widely investigated calcium phosphate compound hence to its own unique properties like, high biocompatibility and injectability, osteoconductivity, and rapid resorption. Addition of bioactive entities to brushite like Co^{2+} ions and Cu^{2+} ions can accelerate angiogenesis of tissues, enhance antibacterial properties and improve proliferation of bone tissue cells, which could be significant improvement in bone repair and bone tissue engineering. Aim of the study was to obtain a series of different brushite powders enriched with different concentrations of cobalt and copper ions and provide reliable physicochemical characterisation of them, which would be important point in further research towards using them in 3D bio-printed bone scaffolds.

Materials and Methods: All brushite powders were obtained via wet chemical precipitation method, Co^{2+} and Cu^{2+} powders, all of them having general $Ca_{1-x}Cu_x/Co_xHPO_4 \cdot 2H_2O$ formula. Then all were appropriately tested and compared to pure synthesized brushite reference. Structural and chemical characterization was performed using powder X-ray diffraction (PXRD) and Fourier-transform infrared spectroscopy (FTIR). Scanning electron microscopy (SEM) showed morphology and microstructure of powders.

Results: FT-IR spectrum confirmed chemical identity of brushite and indicated lack of crystallinity loss in samples. Minor spectral changes suggested successful incorporation of cobalt and copper ions into the structure of brushite. PXRD diffractograms revealed specific brushite reflections, i.e. (020), (121) and (141) and thereby confirmed brushite as a main crystalline phase. Additionally lattice parameters like distances between unit cell vertices in a crystal lattice are decreasing with the increasing concentration of Co^{2+} and Cu^{2+} ions in samples. SEM images showed morphology of plate like brushite crystals and after measuring them suggested that with higher concentration of Co2+ ions and Cu^{2+} ions crystals tend to be smaller.

Conclusions: Cobalt and copper-doped brushite materials were successfully synthesized and physiochemically characterized with variety of methods. Results ensure the identity and stability of obtained materials and are promising factor for further research directed towards bone-regenerative biomaterial used in 3D bio-printing with potential antibacterial and proangiogenic functionality.

Studies were supported by the National Science Center (Opus UMO-2023/51/B/NZ7/00555)

Abstract PP No. 106

In search of novel strategies for treatment of schizophrenia: the development of PZ-2636 as dual 5-HT3/5-HT6 receptor antagonist with promising antipsychotic and pro-cognitive properties

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Objectives: Schizophrenia is a complex mental disorder characterized by positive and negative symptoms, along with cognitive impairment. Despite the wide range of available antipsychotic agents, clinical management remains far from optimal, highlighting the need for more efficacious therapies. Preclinical evidence in rodents indicates that the combination of 5-HT3 receptor antagonism and 5-HT6 receptor neutral antagonism effectively reverses PCP-induced hyperlocomotion, a predictive model of positive-like symptoms, exhibiting pro-cognitive properties in novel object recognition (NOR) task [1]. To further assess the therapeutic potential of this dual-target strategy as novel antipsychotic treatment, we designed, synthesized and biologically evaluated a series of novel dual 5-HT3/5-HT6 receptor antagonists.

Materials and Methods: A series of compounds was designed through a scaffold-hopping approach and synthesized using a multi-step mechanochemical protocol. Affinity for 5-HT3 and 5-HT6 receptors, as well as selectivity over other serotonin and dopamine receptors (e.g., 5-HT1A, 5-HT2A, D2), were evaluated in radioligand binding studies. Antagonist activity at the 5-HT3 receptor was assessed using Ca2+ flux assay in CHO-K1 cells, while an impact at 5-HT6 receptor-mediated Gs signaling pathway was evaluated in NG108-15 cells using BRET method. Pharmacokinetic profiling was performed in Wistar rats. Antipsychotic activity and pro-cognitive properties were evaluated in Sprague-Dawley rats using widely used preclinical models.

Results: The study identified PZ-2636, exhibiting balanced low nanomolar affinity for both 5-HT3 and 5-HT6 receptors, along with good selectivity over tested off-targets. It acted as 5-HT3 receptor antagonist and behaved as a neutral antagonist at 5-HT6 receptor-dependent Gs pathway. PZ-2636 showed adequate brain penetration in vivo (at 3 mg/kg, ig). Moreover, it reversed positive-like symptoms of schizophrenia in the PCP-induced hyperactivity test (at 1 and 3 mg/kg, ip) and exerted pro-cognitive activity in NOR task (at 1 mg/kg, ip), without affecting locomotor activity.

Conclusions: Collectively, these findings support further in vivo evaluation of PZ-2636 to elucidate its potential antipsychotic activity in advanced rodent models of schizophrenia.

Acknowledgments: The project is financed by National Science Centre, Poland (grant no. 2021/43/B/NZ7/02855).

References: [1] Zajdel, P.; et al. J. Med. Chem. 2021, 64, 13279–13298.

Keywords: dual 5-HT3/5-HT6 receptor antagonists, schizophrenia, pro-cognitive properties

Abstract PP No. 107

Growth response and biodegradation potential of erythromycin and clarithromycin by Schizophyllum commune

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Objectives: Erythromycin (ERY) and clarithromycin (CLA) are widely used macrolide antibiotics frequently detected in municipal and industrial wastewater, contributing to the spread of antibiotic resistance and posing environmental risks [13]. This study aimed to evaluate the tolerance, growth response, and biodegradation potential of the ligninolytic fungus Schizophyllum commune toward ERY and CLA for potential mycoremediation.

Materials and Methods: Experiments were conducted in triplicate using Sabouraud Dextrose Broth (SDB) supplemented with ERY or CLA lactobionates at concentrations ranging from 0.01 to 1000 mg/L. Cultures were incubated for 14 days at 26 °C in darkness with shaking. Biomass was harvested and quantified as dry weight. Growth response was expressed as the fungal tolerance ratio (FTR) [4] relative to controls. Residual antibiotics in the medium were measured by LC-MS and expressed relative to initial levels.

Results: S. commune exhibited a non-linear, concentration-dependent growth response to ERY. Growth was stimulated at low concentrations (0.01–1 mg/L), with biomass exceeding control levels by 40–50% (FTR >100%), and remained largely unaffected up to 100 mg/L. Inhibition occurred only at 1000 mg/L, where biomass decreased by ~61%, indicating a hormetic-like response. ERY concentration in the medium decreased substantially, reaching ~20% of the initial value at 1000 mg/L. In contrast, S. commune showed limited ability to remove CLA. The highest biodegradation efficiency (72%) was observed at 0.01 mg/L and declined with increasing CLA concentration, reaching only 10% at 10 mg/L. At 100 mg/L and above, fungal growth was inhibited, resulting in minimal removal.

Conclusions: S. commune shows strong growth capacity and effective removal of erythromycin, indicating its suitability for mycoremediation of ERY-contaminated environments. Its ability to degrade clarithromycin is limited and concentration-dependent, with higher removal under non-inhibitory conditions, reflecting the link between fungal growth and biodegradation efficiency. These findings highlight the compound-specific nature of fungal biodegradation and suggest that while S. commune is effective for ERY, its application for CLA removal may be restricted.

References:

[1] Schafhauser, B.H. et al. Environ Pollut 2018, 238, 440–451

[2] Adhikari, S. et al. iScience 2024, 27, 110789

[3] Wilkinson, J.L et al. PNAS 2022, 119, e2113947119 [4] Kasonga, T.K. et al. Front Microbiol 2021, 12

Abstract PP No. 108

Identification and characterization of polymorphic forms of PKL-021 - a very potent, orally available matrix metalloproteinase inhibitor

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Polymorphism is a critical solid-state property of active pharmaceutical ingredients (APIs) that can significantly influence their physicochemical behavior, including stability, solubility, and processability. The identification and characterization of polymorphic forms is therefore an essential part of early-stage drug development and supports informed selection of the most suitable solid form for further development.

In this study, different solid-state forms of PKL-021 - a very potent, orally available matrix metalloproteinase inhibitor were investigated and characterized using a range of analytical techniques, including powder X-ray diffraction (XRPD), differential scanning calorimetry (DSC), thermogravimetric analysis (TGA), and solid-state nuclear magnetic resonance (ssNMR). These methods enabled a comprehensive evaluation of crystallinity, thermal behavior, and structural differences between the observed forms. The results confirmed the presence of three polymorphic forms exhibiting distinct solid-state properties. Differences in crystal structure and solid-state processability were observed, indicating variability in their potential suitability for further pharmaceutical development. Overall, the obtained data provide a solid foundation for the selection of an optimal solid form of the API and support subsequent formulation and process development activities. This work highlights the importance of systematic solid-state characterization in ensuring robust and reproducible drug development pathways.

Acknowledgments: Project no. 2024/ABM/05/KPO/ KPOD.07.07-IW.07-0276/24 „Development of an oral formulation of a novel neuroprotective compound for a Phase II clinical trial to evaluate efficacy in patients in the acute phase of ischemic stroke” was co-financed by the European Union.

Keywords: Matrix metalloproteinases, PKL-021, polymorphism

Abstract PP No. 109

Nitrosodimethylamine in Metformin-contetaining products

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Objectives: The objective of research was to establish an analytical method for measuring the level of nitrosodimethylamine (NDMA) in drug products with metformin (MET). An additional goal was to examine how different factors (composition, manufacturing technology, packaging) can affect NDMA concentration in MET products. Metformin is a synthetic active pharmaceutical ingredient (API) with the molecular formula $C_4H_{11}N_5$. Metformin is widely used in the treatment of diabetic conditions, as type 2 diabetes and insulin resistance. Since December 2019, published test results have indicated that certain products with MET have shown exceedances of the permissible level of NDMA. NDMA in MET products can be generated from dimethylamine, which may be present as a residual starting material or as a degradation product. This occurs through a reaction with a nitrosating agent from excipients. Therefore, the level of NDMA must be monitored during product release and in stability testing.

Materials and methods:

- Gas chromatography with mass spectrometer, column DB-624, currier gas: He
- Column temperature: 40 to 240°C
- Injection mode: direct injection
- Reagents and standards: dichloromethane, carbonate buffer, sodium sulfate, NDMA, isotopically labeled NDMA
- Samples: MET, tablets with MET, linagliptin and other antidiabetic APIs,

Results: The analytical method developed by Adamed Pharma enables the determination of NDMA levels in MET products within the range of 4.8 to 57.6 ppb (vs. API). Multiple MET tablet formulations were analyzed. The results demonstrated several findings: NDMA content in MET products increases over time. Immediately after manufacturing, NDMA levels are close to the limit of quantification, after few months under elevated temperature and humidity, rise over the acceptance limit. Samples stored under normal conditions exhibited NDMA levels several times lower than kept in accelerated conditions. Tablets in aluminum blisters showed approx. 2.4 times lower NDMA levels compared to those in PVDC blisters after 3 months. In formulations without inhibitors, NDMA levels surpassed the acceptance limit after 3 months in accelerated conditions. The addition of an inhibitor slowed the increase of NDMA; after 6 months in accelerated conditions, NDMA content was below the acceptance limit.

Conclusions: Increasing the NDMA level in tablets with MET was observed in all tested samples. The grow rate depends on the composition of tablets, package and storge conditions.

Abstract PP No. 110

Physicochemical and Pharmaceutical Evaluation of Novel Furazidin Solid-State Form

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Objectives: Urinary tract infections (UTIs) have a significant impact on global health and patients' quality of life [1]. Furazidine (FUR), used to treat urinary tract infections, has poor aqueous solubility, which limits its bioavailability and efficacy [2]. The aim of this study is to increase the solubility of FUR by developing a new crystalline form with improved physicochemical properties.

Materials and Methods: N-(2-hydroxyethyl)nicotinamide (AM) was selected as the coformer due to its ability to form hydrogen bonds with FUR. The new crystalline form of FUR:AM was prepared using liquid-assisted grinding. The product was characterized using FT-IR spectroscopy, NMR spectroscopy, powder X-ray diffraction (PXRD), and thermal analysis methods (DSC and TG). Solubility was evaluated in water and buffers (pH 6.8 and 7.4) using UV-VIS spectroscopy.

Results: PXRD and FT-IR analyses of the FUR:AM compound revealed distinct shifts in the reflection and absorption bands, confirming the formation of a new crystalline phase rather than a physical mixture. These results were further confirmed by thermal analysis, while solubility studies showed a significant improvement in solubility of FUR:AM in water and buffer solutions compared to pure FUR.

Conclusions: We have succeeded in increasing the solubility of furazidine by obtaining a new crystalline form from AM. These promising results open up new possibilities for improving the therapeutic efficacy of FUR-based pharmaceutical preparations.

[1] Bermingham SL, Ashe JF. BJU International. 2012; 110, E830-E836. doi:10.1111/j.1464-410X.2012.11337.x

[2] Trimdale-Deksne A, Kons A, Orola L, et al. Cryst. Growth Des. 2023; 23, 2 930-945. doi: 10.1021/acs.cgd.2c01142

Keywords: furazidin, urinary tract infections, solubility

Abstract PP No. 111

Synthesis, characterization and biological evaluation of a tyrosinase inhibitor with antimelanoma potential

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Objectives: 4,4′-(Sulfanediyldimethanediyl)diphenol (IT) [1] was first isolated from Gastrodia elata Blume; however, isolation from plant material is inefficient and subject to variability depending on cultivation conditions. Chemical synthesis is therefore the most effective approach for obtaining IT. The compound is one of the most potent known tyrosinase inhibitors [2]. The aim of this study was to optimize the synthesis conditions of IT, perform its physicochemical and structural characterization, and evaluate the sensitivity of melanoma cell lines to this compound.

Materials and Methods: IT was synthesized from 4-hydroxybenzyl alcohol and sodium thiosulfate via Bunte salt intermediates at 60–80°C in the presence of various acids. The structure of IT was confirmed using X-ray diffraction and spectroscopic techniques. The biological activity of IT was assessed using the MTS assay on the MNT1 melanoma cell line. Additionally, molecular docking studies using the GOLD program were performed to investigate IT binding to tyrosinase and to provide insight into its molecular mode of action

Results: The results demonstrate that the synthesis of IT is more efficient when stronger acids than acetic acid are applied. Comprehensive structural analysis confirmed the identity of the obtained compound, while biological evaluation revealed that melanoma cells are sensitive to IT, indicating its significant bioactivity. Furthermore, molecular docking studies support the ability of IT to bind tyrosinase, highlighting its potential as a lead structure for the development of new tyrosinase inhibitors with possible application in melanoma treatment.

Conclusions: This study demonstrates that a simplified, acid-optimized synthesis yields structurally confirmed IT with significant tyrosinase-inhibitory and antimelanoma activity, highlighting its potential as a lead compound for melanoma treatment.

This work was financially supported by Medical University of Warsaw, grant number 20/F/MG/N/24.

Keywords: tyrosinase inhibitor, melanoma, crystal structure.

Abstract PP No. 112

Dose-controlled delivery of an anti-inflammatory agent via multiple emulsions for radiotherapy-induced skin damage

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Objectives: Radiotherapy-induced skin damage significantly impairs the skin's natural barrier function, potentially leading to unpredictable drug absorption and systemic side effects. This study evaluates the impact of irradiation-induced skin lesions on the transdermal transport of an anti-inflammatory agent and the potential of water-in-oil-in-water multiple emulsions to serve as a dose-controlled delivery system for drugs applied to compromised skin.

Materials and Methods: UV-irradiated (250 mJ/cm2) pig skin was used as a damaged-skin-mimicking barrier, with healthy porcine skin as the control. UV irradiation was used as a model of radiotherapy-induced damage, since both UV and X-ray radiation exert comparable biological effects. Multiple emulsions with diclofenac sodium (0.4% w/w) in the internal phase as a drug delivery system were prepared in a Couette-Taylor flow contactor. Transdermal drug transport was carried out using Franz diffusion cells. The drug released from the emulsion was transported through the skin into the receptor cell (PBS buffer, pH 7.4). The drug content in the buffer was analysed spectrophotometrically (276nm).

Results: Two multiple emulsions with internal drops volume packing of 0.38 were prepared: e1 (membrane/internal droplet diameter: 28.0/6.2μm) and e2 (11.2/3.2μm), with low polydispersity indexes (1.1, 1.6) and emulsion concentrations of 0.75 and 0.25, respectively. Transdermal drug delivery occurred more rapidly when emulsions with smaller droplets were used as the dose-controlled delivery system (e2). Also, the drug transport was significantly faster through damaged than healthy skin. After 50 hours, the drug concentration in the receptor phase was approx. twice as high in the irradiated system compared to a non-irradiated. The drug concentration reached steady state faster in the damaged skin variant (100h) than in the control (250h).

Conclusions: The study highlights that radiotherapy-induced skin damage necessitates precise dose adjustments of the topical drug. This can be addressed by using multiple emulsions as a programmable system for controlled drug delivery, owing to their complex structure and specific physicochemical characteristics. The research was financially supported by the Warsaw University of Technology under the IChem-2025 grant from the Scientific Council of Chemical Engineering.

Keywords: Multiple emulsions, drug delivery, radiotherapy-induced skin lesions