

54th Conference

Synthesis and Analysis of Drugs

54. Konferencia

SYNTÉZA A ANALÝZA LIEČIV



Bratislava

September 17 & 18

2026

54th Conference
SYNTHESIS AND ANALYSIS OF DRUGS
2026

Faculty of Pharmacy
Comenius University Bratislava

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Dear Researchers

It is our great pleasure to welcome you to the 54th conference Synthesis and Analysis of Drugs, hosted under the auspices of Faculty of Pharmacy, Comenius University in Bratislava. It is an established international conference dedicated to all aspects of pharmaceutical chemistry and analysis. The conference is held alternately in the Czech and in the Slovak Republic and cherishes a long tradition dating back to 1971.

This year, the conference is hosting about 70 participants from Slovakia, the Czech Republic, Austria, Hungary, Bulgaria, and Poland. We hope that it will provide a platform to gather and disseminate the latest knowledge in pharmaceutical chemistry and related disciplines. We would particularly like to encourage the junior scientists and students to profit from the knowledge and the know-how offered by experienced experts in this current and exciting scientific field. We are grateful to all contributors for presenting their valuable research.

*Doc. Ing. Ladislav Habala, Dr.rer.nat.
on behalf of the Organizing Committee*

Undergraduate students are welcome to attend the conference presentations, their admission to all lectures is free.

CONFERENCE PROGRAM

All lectures take place in Multimedia Room

Thursday September 17			
Arrival, registration of participants 10:00 –			
Conference opening	13:00		Dean's Speech
SESSION 1 <i>Session chairs: J. Valentová D. Nádaská</i>	13:20	PL1	ARYL GUANYLHYDRAZONES AS PRIVILEGED SUBSTITUTIONS IN MEDICINAL CHEMISTRY: SYNTHETICALLY ACCESSIBLE, FLEXIBLE AND BIOCOMPATIBLE MOIETIES PROMOTING BINDING TO PROTEIN AND RNA TARGETS IN DRUG DISCOVERY (<i>P. Seneci, Università degli Studi di Milano, IT</i>)
	14:00	PL2	AMINOPEPTIDASE N AS A POTENTIAL DRUG TARGET (<i>P. Mladěnka, Charles University Hradec Králové, CZ</i>)
	14:40	SL1	ISOTHERMAL MICROCALORIMETRY AS A MODERN TOOL FOR INVESTIGATING THE ANTIBACTERIAL ACTIVITY OF NATURAL PRODUCTS (<i>S. Bittner Fialová, Comenius University Bratislava, SK</i>)
	14:55	SL2	EFFECT OF NUCIFERINE ON THE TOLL-LIKE RECEPTOR 4 SIGNALING PATHWAY IN AN INFLAMMATORY MODEL ESTABLISHED IN THE THP-1 CELL LINE (<i>M. Bohunčák, Comenius University Bratislava, SK</i>)
Coffee break	15:10 -15:30		
SESSION 2 <i>Session chairs: S. Bittner Fialová R. Šandrik</i>	15:30	PL3	PHARMACEUTICALS AS ENVIRONMENTAL POLLUTANTS (<i>R. Ješinová, Masaryk University Brno, CZ</i>)
	16:10	PL4	ACHIEVEMENTS IN ELECTROCHEMICAL SENSORS FOR THE JANUS KINASE INHIBITORS DETERMINATION AT CLINICALLY RELEVANT LEVELS (<i>J. Labuda, Comenius University Bratislava, SK</i>)
	16:50	SL3	DESIGN OF A MOBILE APP FOR MEDICATION ADHERENCE MONITORING WITH REDCAP/MYCAP INTEGRATION (<i>T. Fazekaš, Comenius University Bratislava, SK</i>)
	17:05	SL4	ORDERED MESOPOROUS SILICA PREPARED WITH BIODEGRADABLE GEMINI SURFACTANTS AS TEMPLATES FOR ENVIRONMENTAL APPLICATIONS (<i>S. Kurbonov, HUN-REN Centre for Energy Research, Budapest, HU</i>)
Coffee break	17:20 – 17:40		
Poster session	17:40 – 18:30	Hallway 1 st floor	
Welcome evening	19:30	Multimedia Room	

Friday September 18

SESSION 3 <i>Session chairs: J. Zitko K. Oláhová</i>	8:30	PL5	LYOTROPIC LIPID NANOCARRIERS FOR SMALL-MOLECULE CORONAVIRUS INHIBITORS (<i>M. Klacsová, Comenius University Bratislava, SK</i>)
	9:10	PL6	ALBUMIN AS A DRUG CARRIER: TARGETED PLATINUM(IV) PRODRUGS FOR ENHANCED TUMOR DELIVERY (<i>Ch. Kowol, University of Vienna, AT</i>)
	9:50	SLX	ANALYTICAL SOLUTIONS FOR PHARMACEUTICAL RESEARCH: FROM SYNTHESIS TO CHARACTERIZATION (<i>R. Landl, Anton Paar Slovakia s.r.o., SK</i>)
Coffee break	10:00 -10:20		
SESSION 4 <i>Session chairs: M. Klacsová M. Bohunčák</i>	10:20	PL7	AMINOPEPTIDASE N: A MOONLIGHTING ENZYME (<i>O. Farsa, Masaryk University Brno, CZ</i>)
	11:00	PL8	3-BENZAMIDOPYRAZINE-2-CARBOXAMIDES AS PROLYL-tRNA SYNTHETASE INHIBITORS: FROM HUMAN TO MYCOBACTERIAL TARGETS (<i>J. Zitko, Charles University Hradec Králové, CZ</i>)
	11:40	SLX	COPPER(II) COMPLEXES OF METHOXYBENZYLAMINE SCHIFF BASES AND REDUCED SCHIFF BASES – SYNTHESIS, STRUCTURE AND BIOLOGICAL ACTIVITIES (<i>L. Lintnerová, Comenius University Bratislava, SK</i>)
Luncheon	11:50 – 13:00		
SESSION 5 <i>Session chairs: L. Lintnerová I. Bondar</i>	13:00	SL5	OPTIMIZING CAPILLARY ZONE ELECTROPHORESIS FOR MONOCLONAL ANTIBODY ANALYSIS THROUGH SAMPLE PRETREATMENT (<i>J. Havlíková, Comenius University Bratislava, SK</i>)
	13:15	SL6	WHAT IS REALLY IN PRODUCTS DECLARING ANDARINE? EVIDENCE FROM HPLC–HRMS PROFILING (<i>K. Slíž, Comenius University Bratislava, SK</i>)
	13:30	SL7	DECIPHERING THE BINDING MECHANISM OF NON-STEROIDAL ANTI-INFLAMMATORY DRUGS WITH BOVINE SERUM ALBUMIN THROUGH INTEGRATED THERMODYNAMIC, SPECTROSCOPIC, AND COMPUTATIONAL APPROACHES (<i>H. Lal, Comenius University Bratislava, SK</i>)
	13:45	SL8	SYNTHESIS AND ANALYSIS OF MONONUCLEAR 3D COMPLEXES BASED ON PYRIDINE DERIVATIVES AND CHELATING LIGANDS (<i>R. Bajcura, Comenius University Bratislava, SK</i>)
Closing of the conference	14:00		

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PL2 Mladěnka P.: CAN FLAVONOIDS SERVE AS A SOURCE OF NOVEL DRUGS?

PL3 Ješinová R., Lamaczová A.: PHARMACEUTICALS AS ENVIRONMENTAL POLLUTANTS

PL4 Labuda J., Mikuš P.: ACHIEVEMENTS IN ELECTROCHEMICAL SENSORS FOR THE JANUS KINASE INHIBITORS DETERMINATION AT CLINICALLY RELEVANT LEVELS

PL5 Klacsová M., Búcsi A., Hubčík L., Guil J.C.M., Uhríková D.: LYOTROPIC LIPID NANOCARRIERS FOR SMALL-MOLECULE CORONAVIRUS INHIBITORS

PL6 Kowol Ch.: ALBUMIN AS A DRUG CARRIER: TARGETED PLATINUM(IV) PRODRUGS FOR ENHANCED TUMOR DELIVERY

PL7 Farsa O., Uher T., Murgašová V.: AMINOPEPTIDASE N: A MOONLIGHTING ENZYME

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SL2 Bohunčák M., Vyletelová V., Pašková L.: EFFECT OF NUCIFERINE ON THE TOLL-LIKE RECEPTOR 4 SIGNALING PATHWAY IN AN INFLAMMATORY MODEL ESTABLISHED IN THE THP-1 CELL LINE

SL3 Garajová A., Schnorrerova P., Schnorrerova V., Fazekas T.: DESIGN OF A MOBILE APP FOR MEDICATION ADHERENCE MONITORING WITH REDCAP/MYCAP INTEGRATION

SL4 Kurbonov S., Pisárčik M., Lukáč M., Czigány Z., Kovács Z., Tolnai I., Kriechbaum M., Ryukhtin V., Petrenko V., Avdeev M. V., Tian Q., Lacrămă A.-M., Almásy L.: ORDERED MESOPOROUS SILICA PREPARED WITH BIODEGRADABLE GEMINI SURFACTANTS AS TEMPLATES FOR ENVIRONMENTAL APPLICATIONS

SL5 Havlikova J., Strempekova N., Ponteš A., Maráková K., Mikuš P.: OPTIMIZING CAPILLARY ZONE ELECTROPHORESIS FOR MONOCLONAL ANTIBODY ANALYSIS THROUGH SAMPLE PRETREATMENT

SL6 Slíž K., Mikuš P.: WHAT IS REALLY IN PRODUCTS DECLARING ANDARINE? EVIDENCE FROM HPLC–HRMS PROFILING

SL7 Lal H., Pisárčik M.: DECIPHERING THE BINDING MECHANISM OF NON-STEROIDAL ANTI-INFLAMMATORY DRUGS WITH BOVINE SERUM ALBUMIN THROUGH INTEGRATED THERMODYNAMIC, SPECTROSCOPIC, AND COMPUTATIONAL APPROACHES

SL8 Bajcura R., Valentová J., Moncol' J., Samoľová E., Zalibera M., Rajnák C.: SYNTHESIS AND ANALYSIS OF MONONUCLEAR 3D COMPLEXES BASED ON PYRIDINE DERIVATIVES AND CHELATING LIGANDS

SL9 Lintnerová L., Herich P., Svitková B., Valentová J.: COPPER(II) COMPLEXES OF METHOXYBENZYLAMINE SCHIFF BASES AND REDUCED SCHIFF BASES – SYNTHESIS, STRUCTURE AND BIOLOGICAL ACTIVITIES

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PLENARY LECTURES

PL1

ARYL GUANYLHYDRAZONES AS PRIVILEGED SUBSTITUTIONS IN MEDICINAL CHEMISTRY: SYNTHETICALLY ACCESSIBLE, FLEXIBLE AND BIOCOMPATIBLE MOIETIES PROMOTING BINDING TO PROTEIN AND RNA TARGETS IN DRUG DISCOVERY

Pierfausto Seneci

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Aryl guanylhyazones represent a privileged substitution for biologically active molecules, as they establish molecular interactions by adapting to multiple binding sites, due to their positive charge and their tautomeric forms; and facilitate penetration through biological membranes, due to a low pKa and to a significant portion of neutral molecules at physiological pH. My lecture will survey a few meaningful pharmaceutical applications of aryl guanylhyazones, describing the rational design and synthesis, in vitro testing and in vivo evaluation of several leads binding to protein and RNA targets against CNS diseases.

Keywords: CNS diseases, drug discovery, pharmaceutical chemistry, protein and RNA targets, aryl guanylhyazones

PL2

CAN FLAVONOIDS SERVE AS A SOURCE OF NOVEL DRUGS?

Přemysl Mladěnka

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Flavonoids are known for their very large spectrum of biological effects, particularly including cardiovascular positive effects. They encompass traditionally reported antioxidant effects but also antiplatelet, anti-inflammatory and vasodilatory activities. On one hand, it should be emphasized that most of these effects were observed in high and hence biologically unachievable or hardly achievable concentrations. On the other hand, flavonoids given orally were reported to exert cardiovascular positive effects in vivo as well. This paradoxical situation can be explained by their small phenolic metabolites formed by microbiota, which are generally well absorbed. This lecture will critically summarize the known data on flavonoids, their small phenolic metabolites and close derivatives, which seem to have a large potential for development of novel cardiovascular drugs. An important example is 4-methylcatechol, which was already shown to possess strong antiplatelet potential not only in healthy controls but also in patients suffering from hypercholesterolemia and diabetes mellitus, as well as to decrease blood pressure in spontaneously hypertensive rats.

Acknowledgements

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Keywords: flavonoid, phenolic, cardiovascular, platelet, blood pressure

PHARMACEUTICALS AS ENVIRONMENTAL POLLUTANTS

Radka Ješinová, Adéla Lamaczová

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Pharmaceutical residues constitute an important group of environmental micropollutants that have been continuously detected in municipal wastewater, surface waters, and other environmental compartments as a consequence of their widespread use. Conventional wastewater treatment plants were not originally designed to remove pharmaceutical compounds; therefore, many biologically active substances persist in the aquatic environment at concentrations capable of affecting non-target organisms. Frequently detected compounds include antidepressants, antiepileptics, antibiotics, non-steroidal anti-inflammatory drugs, and antihypertensive agents. Their occurrence in aquatic ecosystems raises concerns regarding long-term ecotoxicological effects and the promotion of antimicrobial resistance. [1]

The growing importance of this issue is reflected in the adoption of Directive (EU) 2024/3019 of the European Parliament and of the Council concerning urban wastewater treatment. The revised directive introduces the mandatory implementation of advanced quaternary treatment processes specifically targeting the removal of micropollutants and establishes an Extended Producer Responsibility (EPR) scheme for human medicinal products and cosmetic products. The directive foresees the application of advanced treatment technologies, particularly ozonation, activated carbon adsorption, and membrane-based processes. The performance of these technologies will be assessed using selected indicator pharmaceuticals and predefined removal efficiency criteria. [1]

This contribution summarizes the main objectives of the new legislation and discusses its expected impact on the pharmaceutical sector, wastewater management, and environmental analysis. Particular attention is paid to the environmental risks associated with the persistence of pharmaceuticals, their metabolites, and transformation products, as well as their potential ecotoxicological effects on aquatic organisms. The role of modern analytical techniques in the monitoring of pharmaceutical residues in environmental matrices is also highlighted. The implementation of the new regulatory framework represents a significant opportunity for the advancement of environmental analysis, ecotoxicology, and the concept of green pharmacy, aimed at reducing the environmental footprint of pharmaceuticals throughout their entire life cycle.

[1] Directive (EU) 2024/3019 of the European Parliament and of the Council of 27 November 2024 concerning urban wastewater treatment (recast). *Official Journal of the European Union*, 2024.

Keywords: pharmaceutical micropollutants; quaternary wastewater treatment; pharmaceutical ecotoxicology; environmental analysis; wastewater treatment directive

**ACHIEVEMENTS IN ELECTROCHEMICAL SENSORS FOR THE JANUS KINASE
INHIBITORS DETERMINATION AT CLINICALLY RELEVANT LEVELS**

Ján Labuda, Peter Mikuš

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Janus kinase inhibitors (JAKis) represent a relatively new class of drugs approved for the treatment of inflammatory and autoimmune diseases. Together with their determination in multicomponent biological matrices by conventional methods of liquid chromatography–mass spectrometry (LC-MS), and in simpler (e.g., pharmaceutical) matrices also by fluorescence and UV-Vis spectrometry, sensitive electrochemical sensors and procedures have been recently developed as a promising alternative. The objective of the work is to address both current trends and future potential in electrochemical sensing of JAKis directed to a real-time point-of-care analysis and therapeutic drug monitoring.

The results obtained for JAKis such as baricitinib, ruxolitinib, tofacitinib, and upadacitinib demonstrated successful utilization of conventional bare electrodes and, particularly for situations requiring increased selectivity, tailored sensors based on chemically modified electrodes incorporating nanomaterials and their composites as powerful catalysts as well as molecularly imprinted polymers. Extraordinarily low detection limits obtained in pharmaceutical and biological matrices reached the values of 10^{-9} M to 10^{-12} M (ng to pg/mL) matching clinically relevant drug levels. Achievements in the electrochemical sensing of JAKis are compared with those of conventional LC–MS and spectrometric assays. Advantages and disadvantages of electrochemical approaches for the JAKis assay in clinical settings are critically evaluated.

Acknowledgments

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Keywords: electrochemical sensing, inflammatory and autoimmune diseases, Janus kinase inhibitors, nanomaterials, pharmaceutical and biological analysis

PL5

LYOTROPIC LIPID NANOCARRIERS FOR SMALL-MOLECULE CORONAVIRUS INHIBITORS

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Small-molecule coronavirus (CoV) inhibitors act by targeting specific proteins essential for viral replication, predominantly PLpro and 3CLpro cysteine proteases, which contain highly conserved domains encoded by the CoV genome. 3CLpro is successfully targeted by clinically approved drugs like Paxlovid (nirmatrelvir), while there are currently no approved PLpro-specific inhibitors. Promising non-covalent scaffolds (e.g. GRL0617 derivatives) were advanced into early-phase clinical trials.

Despite their therapeutic potential, pure antivirals face major delivery challenges, mainly poor solubility and low bioavailability. Therefore, the development of effective drug carriers is crucial to ensure their efficacy. Suitable carriers would maximize drug encapsulation, improve metabolic stability, and overcome hydrophobic barriers to enhance cellular uptake. In our preliminary study, antiviral activity of the GC376 inhibitor on VERO-E6 cells infected with SARS-CoV-2 was enhanced by its incorporation into lipid vesicles. This finding supports the rationale for the use of lyotropic nanocarriers as antiviral delivery systems.

We performed a series of physicochemical experiments to investigate the interaction of small-molecule CoV inhibitors (GC376, 13b-K, Calpain inhibitor XII, N3, and GRL0617) with model lipid systems of varying composition. A complex mechanism of interaction was observed, determined by different factors, such as rigidity and curvature of the lipid system, as well as the lipophilicity and charge of the drug molecule. Phosphatidylethanolamine-based (PE) systems and systems with saturated acyl chains display limited capacity for antiviral delivery due to bulk crystallization at a drug-to-lipid molar ratio exceeding 0.1. Hereby, they induce the formation of mesophases with cubic symmetry. Beyond guiding the rational design of lyotropic lipid nanocarriers, this knowledge is crucial to elucidate the inevitable interactions of the nanocarrier with both the viral lipid envelope and the host cell membrane.

Acknowledgments

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Keywords: cysteine protease inhibitors, drug-lipid interactions, drug delivery systems

**ALBUMIN AS A DRUG CARRIER: TARGETED PLATINUM(IV) PRODRUGS FOR
ENHANCED TUMOR DELIVERY**

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Despite recent advances, cancer remains a leading cause of death, with current therapies limited by toxicity and resistance. Tumors differ metabolically from healthy tissues, for example by increased uptake and catabolism of plasma proteins such as albumin. Albumin-drug conjugates exploit this difference and additionally benefit from the enhanced permeability and retention (EPR), similar to liposomal formulations. The clinical progress of Abraxane (albumin–paclitaxel nanoparticles) and Aldoxorubicin (albumin-binding doxorubicin) underscores albumin's value as a drug carrier [1].

In the last years, we have developed albumin-targeted platinum(IV) prodrugs bearing a thiol-specific maleimide moiety [2]. Upon intravenous administration, they rapidly and selectively bind endogenous albumin, yielding higher circulating drug levels, prolonged half-life, and greater tumor accumulation. In vivo, these agents show markedly improved anticancer activity compared to the clinically approved free drug. More recently, we enhanced this platform by introducing a second bioactive group e.g. approved anticancer agents and resistance or immune modulators to further increase efficacy and overcome resistance [3].

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Keywords: drug delivery, anticancer, albumin, platinum

PL7

AMINOPEPTIDASE N: A MOONLIGHTING ENZYME

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The term “moonlighting” in the field of proteins refers to the fact that a protein has two or more distinct functions independent of each other. Aminopeptidase N, E.C.3.4.11.2, is a broad-specificity zinc metallopeptidase with many functions. Inhibition of its proteolytic function can trigger the amino acid deprivation response (AADR), a stress response characterized by upregulation of amino acid transporters and synthetic enzymes, activation of stress-related pathways such as nuclear factor kB (NFkB) and other pro-apoptotic regulators, which leads to cancer cell death by apoptosis. That is why APN inhibitors, such as bestatin or tosedostat, can work as anticancer drugs. The APN protein also serves as a receptor for tumor-homing peptides containing an NGR (= Asn-Gly-Arg) motif in their sequence. This insight enables the enhancement of anticancer drug activity through its conjugation with an NGR peptide, or to construct diagnostic agents by binding fluorescent compounds, or chelated radioisotopes, or MRI-detectable metals (⁶⁸Ga) by the same type of conjugation. [1]. APN protein also plays the role of an entrance receptor for some coronaviruses, such as HCoV-229E, which circulate in the human population where they are responsible for a significant part of the common colds, and also in some porcine coronaviruses, such as transmissible gastroenteritis virus (TGEV), respiratory coronavirus (PRCV), epidemic diarrhea virus (PEDV), deltacoronavirus (PDCoV), and feline infectious peritonitis virus (FIPV), which can be fatal not only for domestic cats [1].

Within the past almost three decades, we have prepared several structurally different series of potential APN inhibitors. Some of them reached interesting values of inhibitory activity and were also successfully tested for anti-proliferation activity in some types of cancer cell lines. We also published some QSAR studies with APN inhibitors prepared by us and other authors. This lecture aims to give a brief overview of APN and its inhibitors in general, as well as in the research at our department.

[1] Farsa O, Uher T. Amino peptidase N: a multifunctional and promising target in medicinal chemistry. RSC Adv. 2025;15(32):26455-26472.

Keywords: amino peptidase N, inhibitors, antitumor activity, coronaviruses, tumor diagnostics

3-BENZAMIDOPYRAZINE-2-CARBOXAMIDES AS PROLYL-tRNA SYNTHETASE INHIBITORS: FROM HUMAN TO MYCOBACTERIAL TARGETS

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Aminoacyl-tRNA synthetases (aaRSs) are essential components of protein synthesis and represent attractive antimicrobial targets, particularly where structural divergence between microbial and human homologues enables selective inhibition. Adachi et al. reported 3-aminopyrazine-2-carboxamides as potent inhibitors of human cytosolic prolyl-tRNA synthetase (hcProRS), binding to the ATP site in a proline-dependent manner [1]. We investigated whether this chemotype could be redirected toward mycobacterial ProRS (mtProRS). Two successive series of 3-benzamidopyrazine-2-carboxamides established key structure-activity relationships and yielded compounds active against virulent and multidrug-resistant *M. tuberculosis* strains, while maintaining low mammalian cytotoxicity [2,3]. Computational modeling supported mtProRS as the molecular target and informed further structural optimization [2,3].

Direct structural validation was subsequently obtained from high-resolution crystal structures of mtProRS in complex with six inhibitors [4]. The compounds occupy the ATP-binding pocket and induce local conformational rearrangements that disfavor proline binding. Comparison with human cytosolic ProRS revealed differences in active-site architecture, electrostatics, and conformational flexibility that provide a structural basis for species-selective inhibition [4]. These studies define the progression of 3-benzamidopyrazine-2-carboxamides from inhibitors of human ProRS toward a selective antimycobacterial chemotype targeting mtProRS.

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Keywords: antimycobacterial agents, *Mycobacterium tuberculosis*, prolyl-tRNA synthetase, pyrazinamide, structure-based drug design

SHORT TALKS

SL1

ISOTHERMAL MICROCALORIMETRY AS A MODERN TOOL FOR INVESTIGATING THE ANTIBACTERIAL ACTIVITY OF NATURAL PRODUCTS

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The global rise of antimicrobial resistance has intensified the search for novel antibacterial agents, with plant-derived natural products representing one of the most promising sources of structurally diverse bioactive compounds. Many phytochemicals exhibit multitarget mechanisms of action. The complexity of these biological effects requires analytical approaches that extend beyond conventional antimicrobial susceptibility testing [1]. This lecture provides a critical overview of contemporary methodologies for evaluating the antibacterial activity of natural products, emphasizing how conventional susceptibility assays can be complemented by advanced techniques that offer deeper insight into bacterial physiology. Standard methods, including disk diffusion and broth microdilution, remain indispensable for antimicrobial screening and determination of minimum inhibitory concentrations (MICs) [2]. However, their endpoint-based nature limits the characterization of the dynamic metabolic responses of bacterial populations. Isothermal microcalorimetry (IMC) is an innovative, highly sensitive, and non-destructive approach for real-time monitoring of microbial metabolic activity. By continuously measuring heat production, IMC enables kinetic analysis of bacterial growth and antimicrobial responses without labels or invasive sample preparation, providing quantitative information that complements conventional susceptibility assays [3]. Recent studies on *Origanum vulgare* extracts illustrate how IMC analysis complemented broth microdilution testing and provided a more comprehensive evaluation of antibacterial activity against methicillin-susceptible and methicillin-resistant *Staphylococcus aureus* [4]. By integrating conventional microbiological methods with real-time metabolic monitoring, IMC represents a valuable addition to the antibacterial research toolbox. Its ability to reveal subtle physiological responses to natural products offers new opportunities for the discovery, characterization, and rational development of plant-derived antibacterial agents in the fight against antimicrobial resistance.

Acknowledgments

This work was supported by grant VEGA 1/0170/24.

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Keywords: isothermal microcalorimetry, natural products, antibacterial activity, antimicrobial resistance, antimicrobial susceptibility testing

SL2

EFFECT OF NUCIFERINE ON THE TOLL-LIKE RECEPTOR 4 SIGNALING PATHWAY IN AN INFLAMMATORY MODEL ESTABLISHED IN THE THP-1 CELL LINE

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Introduction and aim: The Toll Like Receptor 4 (TLR4) signaling pathway plays a crucial role in the development of inflammatory diseases, and modulation of this pathway is a potential strategy for influencing these pathologies. TLR4 is activated by lipopolysaccharide (LPS) originated from bacteria; however, it can also be activated by molecules released from injured tissues, including fibronectin hyaluronan and fatty acids [1]. Nuciferine (NCF), the bioactive alkaloid isolated from lotus leaves (*Nelumbo nucifera*), possesses anti-inflammatory and antioxidant properties [2]. Our aim was to determine the effectiveness of NCF in modulating the TLR4 pathway in an *in vitro* inflammatory model using the THP-1 monocyte cell line.

Methods: The inflammatory model was established in the THP-1 monocytes differentiated with phorbol-12-myristate-13-acetate (PMA) for 72 hours. Following differentiation, cells were treated with NCF for 2 hours and subsequently stimulated with the LPS for 4 hours. Changes in gene expression were evaluated by the qRT-PCR method.

Result and Discussion: In the inflammatory model, NCF treatment resulted in decreased gene expression of TLR4 receptor and both subunits of the Nuclear Factor κ B (NF- κ B) transcription factor (p50 and p65). Furthermore, the expression of pro-inflammatory cytokines, including interleukin 6 and 1β , was reduced. These findings suggest that NCF is capable of modulating TLR4-mediated inflammatory signaling pathway.

Acknowledgments

The study was supported by FaF/2/2026 and VEGA 2/0126/23.

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Keywords: TLR4 pathway, inflammation, nuciferine, THP-1 cells

DESIGN OF A MOBILE APP FOR MEDICATION ADHERENCE MONITORING WITH REDCAP/MYCAP INTEGRATION

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Objectives: This study aimed to design and evaluate a mobile tool to support medication adherence in the REDCap/MyCap environment (RC/MC). The tool was developed to enable centralized, secure, and longitudinal collection of medication adherence data for future clinical and pharmaceutical research.

Background: Digital technologies are helping patients with chronic diseases improve medication adherence and become more involved in the management of their treatment. However, their successful implementation depends on usability, patient acceptance, and compatibility with routine clinical practice. The RC/MC environment offers an opportunity to rapidly develop mobile research tools while ensuring secure and standardized data collection [1,2]

Methods: Based on previous findings on medication adherence and technology acceptance, we designed a specific workflow for the mobile application in the RC/MC environment. We emphasized that the confirmation of medication usage was intuitive and did not impose a burden on patients. The technical feasibility and usability of the system were subsequently evaluated in a pilot study.

Results: A mobile medication adherence tool was successfully implemented within the RC/MC environment. The system enabled automated reminders, patient confirmation of medication intake, and centralized collection of data in the RC/MC platform. Pilot testing demonstrated the feasibility and usability of the solution and identified opportunities for further optimization of the reminder workflow and patient engagement.

Limitations: This study was conducted as a pilot project with a limited number of participants and a short follow-up period. Further studies involving larger and more diverse patient populations and longer follow-up are needed to evaluate the system's performance and applicability in routine clinical practice.

Conclusions: Our study demonstrates how previous research about technology acceptance can be translated into a smartphone-based solution built on the RC/MC platform. The platform provides a feasible environment for developing mobile tools for medication adherence monitoring. The proposed solution enables secure, centralized and longitudinal collection of patient-reported data and may support future clinical research and medication adherence interventions.

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Keywords: medication adherence, digital health, mobile applications, REDCap, patient-reported data, telemedicine

SL4

ORDERED MESOPOROUS SILICA PREPARED WITH BIODEGRADABLE GEMINI SURFACTANTS AS TEMPLATES FOR ENVIRONMENTAL APPLICATIONS

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Mesoporous silica sieves have been prepared through sol–gel synthesis using diester gemini surfactants as pore templates, aiming to obtain new materials with potential use for water remediation. A series of mesoporous spherical silica particles of submicron size have been prepared in an alkali-catalyzed reaction, using a tetraethyl orthosilicate precursor and bis-quaternary ammonium gemini surfactants with diester spacers of varied lengths as pore-forming agents. The effect of the spacer length on the particle morphology was studied using nitrogen porosimetry, small-angle X-ray scattering (SAXS), ultra-small-angle neutron scattering, scanning, and transmission electron microscopy (SEM, TEM). The results revealed that for all spacer lengths, a long-range hexagonal pore ordering developed in the materials. The silica particles were nearly spherical, with sizes below 1 micrometer, and a weak dependence of the mean particle size on the spacer length could be observed. The template removal procedure had a strong influence on the porosity: calcination caused a moderate shrinkage of the pores while retaining the hexagonal structure, whereas treatment with acidified ethanol resulted in only partial removal of the surfactants; however, the hexagonal structure was severely destroyed. The applicability of the obtained calcined materials as adsorbents for heavy metal ions from water was studied with the

example of Pb(II). A high sorption capacity of 110 mg/g was obtained in batch experiments, at pH 5 and 4 h contact time.

Keywords: sol–gel, MCM-41, SAXS, Pb(II), gemini surfactant

SL5

OPTIMIZING CAPILLARY ZONE ELECTROPHORESIS FOR MONOCLONAL ANTIBODY ANALYSIS THROUGH SAMPLE PRETREATMENT

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Monoclonal antibodies (mAbs) are biopharmaceuticals that have transformed the treatment of many diseases in recent years. These proteins have a molecular weight of approximately 150 kDa and are highly heterogeneous. Analytical methods are therefore required to monitor structural heterogeneity and stability, since degradation and changes in post-translational modifications may affect product quality and efficacy [1]. The development of improved analytical tools for mAb characterization remains highly important. Liquid chromatography coupled to mass spectrometry (LC-MS) is a standard platform for mAb analysis, but in recent years capillary electrophoresis has emerged as an attractive alternative separation technique. In particular, capillary zone electrophoresis-mass spectrometry (CZE-MS) offers a fast and efficient approach for mAb analysis. In this study, we explore the potential of sample pretreatment to optimize and improve currently available CZE-MS methods for mAb analysis.

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Keywords: monoclonal antibodies, capillary electrophoresis, sample preconcentration

SL6

WHAT IS REALLY IN PRODUCTS DECLARING ANDARINE? EVIDENCE FROM HPLC–HRMS PROFILING

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Selective androgen receptor modulators (SARMs) are experimental drugs intended to produce testosterone-like effects in muscle and bone. Despite unresolved safety concerns, particularly hepatotoxicity and cardiovascular risk, they are readily available online, often as oral products packaged to resemble dietary supplements and labelled “for research purposes only” or “not for human consumption”. World Anti-Doping Agency (WADA) testing figures recorded 863 adverse analytical findings for SARMs worldwide between 2011 and 2024, including 473 between 2020 and 2024.

We assessed three commercially available products labelled as containing the SARM andarine [1]. A high-performance liquid chromatography–high-resolution mass spectrometry (HPLC–HRMS) method was developed, validated, and applied to their analysis. Quadrupole time-of-flight (QTOF) detection in MS and MS/MS modes enabled identification based on accurate masses of precursor and product ions and comparison with reference standards. Neither andarine nor any other drug was detected in two products. The third contained less andarine than declared, together with two undeclared metabolic modulators, SR9009 and GW501516.

These findings show that products marketed as containing SARMs may differ substantially from their labels, leaving consumers uncertain about the identity and amounts of drugs to which they are exposed. This may increase the risk of adverse health effects. The issue extends beyond elite sport because SARMs are also used recreationally and promoted to young people through social media. In an internet survey of 343 self-identified SARM users, 98.5% were male, 72.3% were aged 18–29 years, and more than half reported side effects, including mood swings, decreased testicular size, and acne [2]. Stronger market surveillance, regulatory enforcement, and targeted risk communication are therefore needed to protect public health.

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Keywords: HPLC–HRMS, SARMs, andarine, metabolic modulators, doping

SL7

DECIPHERING THE BINDING MECHANISM OF NON-STEROIDAL ANTI-INFLAMMATORY DRUGS WITH BOVINE SERUM ALBUMIN THROUGH INTEGRATED THERMODYNAMIC, SPECTROSCOPIC, AND COMPUTATIONAL APPROACHES

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Serum albumin is a highly adaptable transport protein that plays a crucial role in the distribution and pharmacokinetics of a wide range of endogenous and exogenous molecules, particularly hydrophobic therapeutic agents. Most pharmaceutical compounds interact with two primary binding regions located in subdomains IIA and IIIA of albumin, commonly referred to as Sudlow's sites. Understanding these interactions is essential, as the binding behavior of drugs to albumin can significantly influence their solubility, bioavailability, therapeutic activity, tissue distribution, and elimination. In the present study, we investigated the molecular interactions of two widely used non-steroidal anti-inflammatory drugs (NSAIDs), ibuprofen (IBF) and naproxen (NPX), with bovine serum albumin (BSA) through an integrated experimental and computational approach [1]. The binding mechanism was characterized using isothermal titration calorimetry (ITC), fluorescence, UV-visible absorption, circular dichroism (CD) spectroscopy, dynamic light scattering (DLS), and molecular docking studies.

Both IBF and NPX showed strong affinity toward BSA with a binding stoichiometry of approximately 1:1. Thermodynamic analysis revealed that the complex formation was driven by favorable enthalpic and entropic contributions, suggesting the involvement of hydrogen bonding, van der Waals interactions, and hydrophobic forces in stabilizing the drug-protein complexes [2]. Fluorescence spectroscopy (for IBF) and UV-visible absorption studies further confirmed the formation of stable ground-state BSA-NSAID complexes. DLS measurements indicated an increase in the hydrodynamic radius of BSA upon drug binding, reflecting alterations in the protein environment. Furthermore, CD spectroscopy revealed a reduction in the α -helical content of BSA, suggesting subtle conformational rearrangements induced by NSAID interaction.

Overall, this comprehensive investigation provides deeper insights into the thermodynamic and structural aspects governing BSA-NSAID interactions. The findings enhance our understanding of drug-protein binding behavior and may contribute to the rational design and optimization of therapeutic molecules with improved pharmacological profiles.

Acknowledgements

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Keywords: bovine serum albumins (BSA), NSAIDs, ITC, spectroscopic measurements, DLS

SL8

SYNTHESIS AND ANALYSIS OF MONONUCLEAR 3D COMPLEXES BASED ON PYRIDINE DERIVATIVES AND CHELATING LIGANDS

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New mononuclear Cu(II), Ni(II) and Fe(II) complexes were synthesized and structurally and spectroscopically characterized. The heteroleptic complexes were prepared by a one-pot synthesis using the chelating ligand hexafluoroacetylacetonate (*hfac*) and pyridine derivatives with general formula $[M^{II}(L^{1-3})_2(hfac)_2]$ {where M = Cu, Ni, Fe; L¹ = 4-benzylpyridine; L² = 4-ethylpyridine; L³ = 4-(4-chlorobenzyl)pyridine}. The single-crystal X-ray diffraction confirms that all complexes are hexacoordinate with the {MN₂O₄} donor set forming a distorted octahedron. Well-resolved X-band EPR data confirm an axial character of the spectra with well-resolved hyperfine $A(^{63,65}\text{Cu})$ ($I = 3/2$) and super-hyperfine $A(^{14}\text{N})$ interactions for Cu(II) complexes. The cyclic voltammetry of Cu(II) complexes revealed two electrochemically irreversible reduction processes and an irreversible oxidation process. The first reduction process is assigned to the single-electron reduction of the central Cu(II) to Cu(I). Furthermore, all complexes were investigated for their interactions with DNA and bovine serum albumin (BSA).

Acknowledgments

Slovak grant agency (VEGA-1/0661/24) is acknowledged for the financial support.

Keywords: Cu(II) complexes, DNA/BSA interactions, EPR, X-ray structure

SL9

COPPER(II) COMPLEXES OF METHOXYBENZYLAMINE SCHIFF BASES AND REDUCED SCHIFF BASES – SYNTHESIS, STRUCTURE AND BIOLOGICAL ACTIVITIES

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Methoxybenzylamine derived Schiff bases and reduced Schiff bases were prepared from salicylaldehyde and used in preparation of two series of copper(II) complexes. The *O,N*-bidentate ligands bound to the copper center were studied in solid form by X-ray crystallography and in solutions using EPR spectra at low temperatures showing the 2 : 1 or 2 : 2 ratio of ligand and copper [1]. The complexes were studied for their cytotoxic properties using two cancer cell lines (HepG2, A549) and non-cancerous fibroblasts but also by a model monitoring mitochondrial activity of *S. cerevisiae*. The copper complexes were highly active against the HepG2 cells, showing activities close to cisplatin, but poorly active against the lung cancer cell line A549. Moreover, DNA binding experiments using absorption titration and EB-displacement measurements revealed groove binding mode of the complexes. Plasmid DNA and subsequent gel electrophoresis method was used to determine DNA cleaving properties of the complexes. The Schiff base derivatives derived from 2- and 4-methoxybenzylamine revealed to be best active in all these experiments. Bovine serum albumin binding was performed to study the potential of transportation of the prepared complexes in the organisms. On the other hand, SOD-mimetic properties of the complexes were studied using a tetrazolium dye method (INT) [2], showing good activities for the reduced Schiff base series.

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Keywords: Schiff bases, copper complexes, interactions with DNA, cytotoxicity, X-ray crystallography

POSTERS

P1

CHEMICAL ELICITATION: A STRATEGY TO ENHANCE ANTIOXIDANT ACTIVITY AND POLYPHENOL PRODUCTION IN *SALVIA HISPANICA* L. CELL CULTURES

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Salvia hispanica L. (chia) is a valuable medicinal and functional plant known for its rich content of bioactive compounds, particularly polyphenols, flavonoids, and phenolic acids, which contribute to its antioxidant properties. However, the natural accumulation of these compounds is often low and variable, limiting their large-scale production. Plant *in vitro* cell cultures offer a controlled and sustainable system for the production of valuable secondary metabolites independent of environmental conditions [1].

Chemical elicitation is an effective strategy for enhancing secondary metabolite production in plant cell cultures. This study investigated the effect of methyl jasmonate (MeJ; 100 $\mu\text{mol}\cdot\text{L}^{-1}$) on polyphenol accumulation and antioxidant activity in *Salvia hispanica* L. suspension cultures during 24, 48, and 72 hours of elicitation.

The content of total hydroxycinnamic acid derivatives (THD), expressed as rosmarinic acid, increased significantly at all time points compared with non-elicited controls ($p < 0.001$). The most pronounced increase occurred between 24 and 48 hours, suggesting rapid activation of phenolic metabolism following elicitor treatment. In contrast, the total polyphenol content, expressed as gallic acid equivalents (GAE), showed no statistically significant differences between control and elicited samples at any time point during the experiment.

Antioxidant activity, determined using the DPPH assay, showed substantially lower SC_{50} values in elicited cultures at all monitored time points, indicating enhanced radical-scavenging capacity. The highest antioxidant activity was achieved after 72 hours of elicitation. This trend corresponded to increased THD levels, highlighting their contribution to the overall antioxidant potential.

These findings demonstrate that MeJ effectively stimulates the biosynthesis of specific phenolic compounds and enhances antioxidant activity in *S. hispanica* L. suspension cultures, highlighting its potential for improving the production of valuable bioactive compounds.

Acknowledgements

This study was supported by VEGA grant No. 1/0101/23.

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Keywords: *Salvia hispanica* L., methyljasmonate, elicitation, polyphenols, antioxidant activity

P2

QUALITY CONTROL OF TOCILIZUMAB BY SIZE EXCLUSION CHROMATOGRAPHY

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Tocilizumab is a recombinant humanised monoclonal antibody (mAb), that inhibits IL-6 receptor. It has been approved as a safe drug in treatment of autoimmune diseases, like reumatoid arthritis [1]. IL-6 is a molecule, that also plays a key role in respiratory infections caused by viruses and in cytokine release syndrome. In patients with COVID-19, high levels of cytokines are linked to a more severe course of the disease and a higher risk of death [2]. For this reason, tocilizumab is suitable for treatment of patients with severe COVID-19 disease. Since the current exclusively parenteral administration of tocilizumab presents therapeutic limits, research is focused on the development of new non-invasive alternatives, such as inhaled dosage forms.

Inhalation delivery route of drugs represents an effective non-invasive alternative to systemic administration, characterized by high bioavailability, directly in the target organ. Since the first pass effect is bypassed, the drug can be administered in a much smaller dose and, last but not least, the occurrence of side effects is eliminated.

Size exclusion chromatography (SEC) is a separation technique widely used for the characterization and quality control of therapeutic proteins. It separates biomolecules according to their hydrodynamic radius. The principle of separation is based on the size difference of the particles and does not depend on their chemical properties [3].

Here, simple SEC method have been developed for quality control and characterization of the mAb tocilizumab.

Acknowledgments

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Keywords: inhalation drug delivery, monoclonal antibody, size exclusion chromatography, tocilizumab

P3

INFLUENCE OF SALICYLIC ACID DERIVATIVES ON THE SYNTHESIS AND PROPERTIES OF GOLD NANOPARTICLES

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Gold nanoparticles (AuNPs) represent an important class of nanomaterials with significant potential applications in biomedicine, analytical chemistry, and catalysis. Their physicochemical properties are strongly influenced by the synthesis method and the nature of the reducing and stabilizing agents employed [1]. In this study, AuNPs were synthesized using various derivatives of salicylic acid (2-hydroxybenzoic acid) as reducing agents. The work aimed to evaluate how structural differences among the salicylic acid derivatives affect the size, polydispersity, and colloidal stability of the resulting nanoparticles.

Gold nanoparticles were prepared by the chemical reduction of tetrachloroauric acid in the presence of selected salicylic acid derivatives. The synthesized nanoparticles were characterized using dynamic light scattering (DLS), zeta potential measurements, and UV–Vis spectroscopy. The obtained results demonstrated a significant influence of the molecular structure of the reducing agent on the synthesis process and the resulting nanoparticle properties. Differences in particle size, size distribution, and zeta potential were observed, resulting in variations in colloidal stability among the prepared systems. UV–Vis spectroscopy confirmed the formation of gold nanoparticles through the characteristic localized surface plasmon resonance band [2].

The findings indicate that the physicochemical properties of gold nanoparticles can be effectively tailored by selecting appropriate salicylic acid derivatives, providing a simple and versatile approach for optimizing AuNPs for future applications in nanotechnology and biomedical fields.

Acknowledgments

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Keywords: gold nanoparticles, 2-hydroxybenzoic acid derivatives, chemical reduction, dynamic light scattering, zeta potential.

P4

SYNTHESIS OF NOVEL PEPTIDOMIMETIC INHIBITORS OF SARS-COV-2 CONTAINING KYNURENIC ACID AND NON-PROTEOGENIC AMINO ACID

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Although through the vaccination efforts the pandemic of COVID-19 was suppressed, but due to the emergence of new viral strains, mutations and possible drug resistance, the need to design and synthesise novel drugs is still present [2].

One of the possible strategies in treating COVID-19 infection is to inhibit viral proteases, where M^{pro} became more suitable target since unlike PL^{pro} it has no homolog in the human body, meaning that inhibitors of the main protease promise high selectivity as well as low toxicity [3].

Amongst the most potent inhibitors of the active site are peptidomimetics, substances mimicking peptide substrates, while expressing similar or improved pharmacological properties, such as selectivity, metabolic stability or bioavailability, achieved by cyclisation of linear peptide, replacing natural amino acids for non-proteogenic ones, or by introduction of heterocyclic ring into the molecule. [1, 3].

This work aims to prepare and optimise the synthesis of novel heterocyclic peptidomimetic inhibitors efficient against COVID-19. Structures of the inhibitors were modelled in collaboration with the Faculty of Pharmacy and contain three distinct parts - heterocyclic moiety, non-proteogenic amino acid with alkyl side chain and quisqualic acid moiety. Here, we optimised the synthesis of kynurenic acid, formed via aza-Michael addition followed by intramolecular Friedel-Crafts acylation. We also implemented green chemistry techniques – ball milling and microwave irradiation. The amide derivatives were synthesized using peptide coupling reagents, where the most efficient was third-generation uronium reagent COMU.

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Keywords: COVID-19, peptidomimetics, kynurenic acid, Friedel-Crafts acylation, peptide coupling

P5

EFFECT OF BOCEPREVIR ON LATERAL PRESSURE MONITORED IN HYDROPHOBIC CORE OF LIPOSOMES

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Liposomes are important drug delivery vehicles that improve drug efficacy, bioavailability, allow control of drug release, and decrease premature degradation. Boceprevir is a lipophilic M^{PRO} inhibitor previously used as a Hepatitis C NS3 protease inhibitor, known as VictrelisTM, later withdrawn from the market. It was also studied during the COVID-19 pandemic as a possible repurposed drug. The interaction of boceprevir with several phospholipids was studied with DSC and X-ray scattering and revealed varying degrees of influence on the physicochemical properties of the lipids, depending on the structure of the lipid [1]. Lateral pressure experiments in lipids measure the minor modifications in the lipid bilayer, such as the response of the fluorescence spectra of fluorescence probes (Pyr4PC: 1,2-bis-pyrenebutanoyl-phosphatidylcholine; Pyr10PC: 1,2-bispyrenedecanoyl-phosphatidylcholine) on changes in the lipid bilayer. The two pyrene groups of fluorescence probes in appropriate distance and conformation form excimers, and the ratio of the intensities of excimer and monomer fluorescence is a measure of the lateral pressure [2]. These probes are located at different depths in the bilayer and provide information about the position of antiviral incorporation. DMPC (1,2-dimyristoyl-sn-glycero-3-phosphocholine) and POPE (1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanolamine), used in the experiments, differ in polar head structure and in saturation of acyl chains, while exhibiting similar gel-to-liquid crystalline phase transition temperatures.

The experiments proved changes in the lateral pressure for both lipids on the boceprevir concentration to a different extent.

Acknowledgements

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Keywords: lateral pressure, antiviral, lipid, drug delivery

P6

MULTIFUNCTIONAL PHENYL-LINKED BIS-INDOLE SCAFFOLDS WITH ANTICANCER, ANTIOXIDANT, AND ANTI-AMYLOID PROPERTIES

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Indole is a widely occurring heterocycle in natural and synthetic bioactive molecules, and recent studies highlight bis-indoles as promising multifunctional scaffolds [1]. We designed and synthesized nature-inspired bis-indoles with a phenyl linker, focusing on bis-indole dithiourea and diurea. Cyclization of bis-indole dithiourea with bromine or methyl bromoacetate afforded analogues of 1-methoxyspirobrassinol methyl ether and thiazolidin-4-one, while TFA-induced cascade rearrangement of the spirocyclic bis-indole yielded a cyclobassinin-type product. Biological evaluation revealed selective inhibition of A549 lung cancer cell proliferation by the bis-indole diurea and apoptosis induction in Caco-2 and HCT116 colon cancer cells by the bis-spiroindole. The thiazino derivative showed strong antioxidant activity in ABTS and DPPH assays. Nile Red fluorescence, CD spectroscopy, and AFM demonstrated that the bis-indole dithiourea and thiazino derivatives effectively inhibited insulin fibril formation, whereas the bis-indole diurea was the most efficient disruptor of preformed fibrils.

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Keywords: phytoalexins, bis-indoles, anticancer, antioxidant and anti-amyloid effects

P7

***IN VITRO* AND *IN SILICO* SCREENING OF NATURAL SMALL MOLECULAR COMPOUNDS FOR INHIBITION OF TRUNCATED TAU AGGREGATION**

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Soluble oligomers—not relatively inert mature filaments—are thought to be the toxic agents of several neurodegenerative diseases. Oligomers can propagate between neurons and could be

isolated by *in vivo* microdialysis from brain interstitial fluid [1]. We have prepared models of truncated tau dimers by molecular dynamics simulations that are used as targets for *in silico* docking of natural small-molecule compounds [2].

High throughput *in vitro* tau aggregation assay was established using truncated tau fragments tau297-391 (dGAE) and tau321-391 to test the efficacy of docked compounds. The affinity and specificity of compounds for binding to truncated tau was measured using biophysical methods - surface plasmon resonance, biolayer interferometry and microscale thermophoresis.

Acknowledgements

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Keywords: tau protein, aggregation, small molecule, docking, kinetics

P8

INFLUENCE OF BUFFER COMPOSITION ON THE NEBULIZATION PERFORMANCE AND PULMONARY DISTRIBUTION OF TOCILIZUMAB SOLUTIONS

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Tocilizumab is a monoclonal antibody currently approved for parenteral administration in the treatment of inflammatory diseases and COVID-19. Pulmonary delivery of therapeutic antibodies represents a promising approach for local treatment of respiratory diseases while potentially reducing systemic exposure. However, aerosolization performance and deposition behavior of protein therapeutics are strongly influenced by formulation parameters, particularly buffer system composition. This study aimed to evaluate the effect of buffer composition on the nebulization performance and *in vitro* pulmonary deposition profile of tocilizumab formulations intended for inhalation delivery.

Four tocilizumab formulations (2 mg/mL) were investigated: RoActemra and Tyenne diluted in LC-MS grade water, and both products after buffer exchange into a proline-based system (100 mM L-proline, pH 5.8). Buffer exchange was performed using Zeba™ Spin Desalting Columns

(MWCO 7K). Nebulization experiments were conducted using a Glass Twin Impinger apparatus under an airflow rate of 60 ± 5 L/min for 60 s. Samples were collected from individual apparatus compartments, concentrated using Amicon[®] centrifugal filters (MWCO 10K), and analyzed by HPLC-UV system equipped with Xtimate[®] Bio SEC 300 column (5 μ m, 4.6×300 mm; Welch Materials, USA). Separation was performed using 25 mM phosphate buffer (pH 6.8) containing 250 mM NaCl with an analysis time of 18 minutes.

The results demonstrated a significant effect of buffer composition on the nebulization behavior and stability of tocilizumab formulations. Proline-based formulations showed more favorable characteristics, with no visible aggregate formation observed. RoActemra in proline buffer system exhibited the most suitable aerosol transport and deposition profile, with the drug fraction reaching both upper and lower impingement chambers. In contrast, Tyenne showed increased deposition in the mouthpiece region, which was also observed for the aqueous Tyenne formulation. All aqueous formulations demonstrated an increased tendency towards aggregate formation.

These findings highlight the importance of formulation optimization for maintaining protein stability and improving aerosol performance of tocilizumab formulations intended for pulmonary delivery.

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Keywords: glass twin impinger, nebulization, proline buffer system, size exclusion chromatography, tocilizumab

P9

MOLECULAR DOCKING OF ANTIMYCOBACTERIALLY ACTIVE COMPOUNDS INTO FOUR *MYCOBACTERIUM TUBERCULOSIS* POTENTIAL TARGET PROTEINS

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Tuberculosis (TB) remains a critical global health concern, exacerbated by the increasing prevalence of drug-resistant strains, necessitating the development of novel antitubercular agents [1]. This study employs computational molecular docking to evaluate a series of synthetic compounds based on a benzofuran/benzothiophene core structure as potential inhibitors of four essential *Mycobacterium tuberculosis* enzymes: Enoyl-ACP reductase (InhA), Polyketide synthase 13 (Pks13), Salicylate synthase (MbtI), and DNA gyrase B (GyrB).

Molecular docking simulations were performed using the Glide software with standard precision (SP). To enhance the accuracy of the binding affinity predictions, the MM-GBSA (Molecular Mechanics-Generalized Born Surface Area) method was utilized, incorporating the VSGB solvation model and the OPLS4 force field. The computational protocols were validated by re-

docking co-crystallized inhibitors for Pks13, MbtI, and GyrB, yielding satisfactory RMSD values, while InhA was studied in the presence of the NAD cofactor.

The docking analysis identified several promising candidates within the synthesized library. Notable compounds, such as DN-35B, DN-38B, and DN-41B, demonstrated significant binding free energies (ranging from -60.98 to -88.90 kcal/mol) across the target enzymes. Structural analysis of the docking poses revealed stable interactions within the enzyme binding pockets, primarily mediated by hydrophobic contacts and hydrogen bonding, which contribute to the calculated thermodynamic stability of the complexes.

These computational findings highlight the potential of the benzofuran/benzothiophene-based compounds as lead inhibitors for antitubercular research. The high binding affinities and stable interaction profiles observed suggest that these structures are suitable candidates for further experimental investigation.

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Keywords: molecular docking, *Mycobacterium tuberculosis*, benzofuran/benzothiophene derivatives

P10

FLUORINE- AND PHOSPHORUS-CONTAINING LIGANDS AS MODIFIERS OF BIOACTIVITY IN METAL COMPLEXES

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Schiff bases represent an important class of ligands, yielding metal complexes with great structural variability. In many cases, the ligands themselves exhibit various types of biological activity, and complexation with metals often potentiates this activity. Modification of the physicochemical properties—and thus the biological effect—can be achieved by introducing suitable substituents into the ligand molecule.

One of the most effective methods of modification is the use of fluorine-containing substituents. As part of our work, we prepared various reduced fluorine-containing Schiff bases, as well as their copper and zinc complexes. The cytotoxic activity of the products was determined *in vitro* using the MTT assay, effectively reducing cell viability in the studied cell lines. The metal complexes generally exhibited higher cytotoxic activity compared to the free ligands, in some cases higher than that of cisplatin. As part of the assessment of biological activity, interactions with serum albumin (BSA) and DNA (ct-DNA) were also evaluated. The molecules under investigation represent promising structures for the development of new anticancer agents [1, 2].

An innovative approach to the design of cytotoxic compounds involves the introduction of substituents that enable these molecules to target mitochondria. To this end, we have successfully modified the salicylaldehyde molecule by attaching a triphenylphosphonium group. This precursor will subsequently be used to prepare Schiff bases and their metal complexes.

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Keywords: metal complexes, cytotoxicity, cancer, mitochondria targeting

P11

CHEMICAL DIVERSITY OF ESSENTIAL OILS IN IRANIAN *MENTHA SPICATA* POPULATIONS

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The present study evaluated the chemical composition and variability of essential oils (EOs) obtained from eight *Mentha spicata* populations collected across different geographical regions of Iran to identify chemotypic diversity. Essential oils were isolated from aerial plant parts by hydrodistillation and analyzed using gas chromatography–mass spectrometry (GC–MS). Multivariate statistical analysis, including principal component analysis (PCA), was applied to assess similarities and differences among the samples based on their chemical profiles. Oxygenated monoterpenes represented the predominant class of compounds, with carvone, D-limonene, and 1,8-cineole identified as the major constituents. PCA explained the total variance and demonstrated distinct clustering of the analyzed populations, reflecting considerable chemical heterogeneity. Three major chemotypes were distinguished: a carvone–limonene type, a mixed carvone/carvone oxide type, and a 1,8-cineole-rich type. The observed variation in essential oil composition

highlights the substantial chemical diversity within Iranian *M. spicata* populations. It suggests the combined influence of genetic and environmental factors on secondary metabolite production. The identified chemotypes represent valuable genetic resources and may support the selection of plant material for pharmaceutical, food, cosmetic, and aromatic applications, as well as future breeding and cultivation programs.

Acknowledgments

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Keywords: *Mentha*, Iran, chemotypes, GC-MS

P12

MODULATORY EFFECTS OF KN-62 ON STRESS, METABOLIC ACTIVITY, AND IL-1 β RESPONSE IN UVC-EXPOSED THP-1 CELLS

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Oxidative stress is a critical factor in the pathogenesis of inflammatory and degenerative diseases, with the purinergic P₂X₇ receptor implicated in stress-induced cellular responses. KN-62, an inhibitor of P₂X₇-associated signalling, has been reported to partially and concentration-dependently reduce IL-1 β production [1]. In this study, the effects of KN-62 on redox homeostasis, energy metabolism, and inflammatory signalling were investigated in THP-1 cells exposed to UVC-induced stress. Cells were treated with low (64 J/m²) and high (150 J/m²) doses of UVC radiation and with KN-62 at 20 nM, corresponding approximately to reported IC₅₀ values for IL-1 β inhibition [1]. Selected markers of oxidative metabolism, antioxidant defense, cellular energetics, and inflammatory response, including IL-1 β production, were assessed. Low-dose UVC induced an adaptive response characterized by increased metabolic activity, enhanced antioxidant defenses, and moderate inflammatory signalling. In contrast, high-dose UVC resulted in impaired metabolic function, dysregulated redox responses, and reduced protein content, indicating progression toward cellular damage. KN-62 enhanced redox activity while suppressing IL-1 β production, suggesting partial dissociation of oxidative and inflammatory responses under basal conditions. Under combined exposure, KN-62 maintained a compensated, redox-active, and inflammation-attenuated phenotype at low UVC dose. In contrast, at high doses it was associated with impaired metabolic responses and a paradoxical increase in IL-1 β , consistent with damage-associated signalling. Overall, KN-62 acts as a modulator of cellular responses to oxidative stress rather than a cytoprotective agent, with effects that depend on the intensity of the stress and cellular energy status.

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Keywords: purinergic P₂X₇R, THP-1 cells, stressor, KN-62, IL-1 β

P13

NMR AND THEORETICAL STUDY OF IOTA AND KAPPA CARRAGEENANS

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A detailed understanding of the molecular structure of sulphated polysaccharides is essential for elucidating the relationship between their physicochemical properties and biological functions. Among these compounds, carrageenans are a family of sulphated galactans derived from red algae that have attracted considerable attention due to their diverse applications in food, pharmaceutical, and biomedical fields [1-2]. In particular, κ -carrageenan and ι -carrageenan differ in the number and distribution of sulphate groups, resulting in distinct conformational behaviour, gelation properties, and intermolecular interactions. Characterising the structural consequences of these sulphation patterns at the molecular level remains crucial for understanding their functional properties [3]. The integration of density functional theory (DFT) with high-resolution NMR spectroscopy has emerged as a powerful approach for investigating the structural dynamics, solvation characteristics, and protein-recognition mechanisms of glycosaminoglycans [4,5]. In this contribution, density functional theory (DFT) calculations were combined with high-resolution NMR spectroscopy to investigate the structural and electronic characteristics of κ - and ι -carrageenan model compounds. Optimised molecular geometries, together with NMR proton–proton and proton–carbon spin–spin coupling constants, were calculated and compared with experimental NMR data. The results demonstrate that the degree and position of sulphation significantly influence local molecular geometry, electronic charge distribution, and the magnitude of indirect spin–spin coupling constants. Furthermore, the calculations reveal the important role of hydrogen-bonding networks and electrostatic interactions in stabilising specific conformations and shaping the first hydration shell. These solvent-mediated effects contribute substantially to the three-dimensional organisation of the polysaccharide chains. The combined DFT–NMR approach provides valuable molecular-level insight into the structural features governing the behaviour of κ - and ι -carrageenans and advances our understanding of structure–property relationships in sulphated polysaccharides.

Acknowledgments

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Keywords: carrageenans, NMR, DFT, molecular structure, sulphation pattern

P14

ANTICANCER POTENTIAL OF Cu(II) AND Zn(II) COMPLEXES WITH SALEN-TYPE SCHIFF BASE LIGANDS AGAINST COLORECTAL AND HEPATOCELLULAR CARCINOMA: AN IN VITRO VALIDATION

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Development of new metal-based anticancer drugs is a growing field of research aimed at overcoming the resistance and toxicity associated with platinum-based chemotherapies. Metallodrugs have been reported to exhibit antibacterial, antifungal, antiviral, anti-inflammatory, enzyme-inhibitory and anticancer activities [1–2]. The biological and pharmacological activity of metal complexes depends on structure-activity relationships, in which the central metal ion and organic ligands determine solubility, lipophilicity, and interactions with biomolecules (DNA, proteins, enzymes).

Copper and zinc complexes, as an alternative to platinum-based drugs, display notable anticancer activity while causing fewer side effects than cisplatin. Complexes of the salen-type ligands represent a promising group of compounds in medical research. Colorectal and liver cancer are among the most frequently diagnosed types of cancer worldwide. Therefore, the aim of this study was to examine the antitumor effect of Schiff base ligands derived from 1,2-cyclohexanediamine and fluorine-substituted benzaldehydes and their Cu(II) and Zn(II) complexes on two model cancer cell lines [3,4]. A series of compounds was examined for their anticancer activity against human colorectal adenocarcinoma (HT-29) and hepatocellular carcinoma (HepG2) cell lines in in vitro conditions. In addition, the effect of the most active compound on the cell cycle was investigated. Experimental results revealed that, compared to cisplatin, the Cu(II) and Zn(II) complexes can significantly inhibit the viability of both HT-29 and HepG2 cancer cells, making them a promising alternative to cisplatin.

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Keywords: anticancer activity; salen-type ligands; Cu(II), Zn(II) complexes

P15

ANTICANCER ACTIVITY AND MODE OF ACTION OF COPPER(II) COMPLEXES WITH SUBSTITUTED QUINAZOLINONES

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Coordination compounds represent an important chemical platform for drug development. By combining various metal centres with organic ligands, it is possible to create compounds with tuneable properties and novel mechanisms of action. Quinazolinones represent a privileged scaffold in medicinal chemistry, exhibiting a wide range of biological activities. These compounds have been reported to possess antimicrobial, antiviral, anti-inflammatory and anticancer activities. Quinazolinone-based Schiff bases possessing azomethine group are used in many fields [1]. They are versatile structures with a significant capability to form chelates with various metal ions. A number of transition-metal complexes (Cu, Ni, Zn, Co) have been investigated for their anticancer activity in order to develop new effective therapeutics with the ability to overcome the limitations of platinum complexes [2–3]. Recently, the versatility of transition metal complexes with quinazolinones has been investigated in terms of their spectroscopic properties, coordination geometry, and biological activity [4]. This contribution is focused on the study of 2,3-substituted quinazolinone ligands and their Cu(II) complexes. A series of compounds was evaluated for their *in vitro* cytotoxic activity against the human breast (MCF-7) and lung (A549) cancer cell lines in order to determine their biological efficacy. All compounds were evaluated for their antioxidant properties and their ability to induce the formation of intracellular reactive oxygen species (ROS) in cancer cells. In addition, the mechanism underlying cell death was also investigated.

Acknowledgments

This work was financially supported by the Slovak grant agency VEGA project 2/0042/26.

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Keywords: Cu(II) complexes, quinazolinones, anticancer activity, cell cycle, apoptosis

P16

EFFECT OF OXIDATION ON THE SURFACE TENSION LOWERING ABILITY OF PULMONARY SURFACTANT

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Pulmonary surfactant is a highly specialized lipid–protein complex that is essential to reduce alveolar surface tension and maintain normal respiratory mechanics. Exogenous surfactant preparations such as poractant alfa (Por α) may undergo autooxidation during storage or handling, and also after application in the lungs, potentially compromising their biophysical performance. We investigated time-dependent autooxidative changes in Por α exposed to air during incubation at 37°C for a 14-day period. Phospholipid oxidation was quantified by measuring conjugated diene absorbance at 233 nm, which showed a continuous and pronounced increase throughout the 14-day period.

Surface activity was evaluated using a pulsating bubble surfactometer as the minimum surface tension ($\gamma_{\min}$) reached during a pulsation after a 5-minute equilibration period. Fresh Por α rapidly achieved $\gamma_{\min}$ values of ~0–2 mN/m, typical for a fully functional surfactant. Similarly, the Por α oxidized for 1–2 days was able to reach $\gamma_{\min} < 2$ mN/m. After 3–4 days, $\gamma_{\min}$ values exceeded 2 mN/m but remained below 5 mN/m, indicating partial functional impairment. Prolonged oxidation caused a pronounced deterioration of surface activity: $\gamma_{\min}$ increased to ~15 mN/m after 5 days and exceeded 25 mN/m after 14 days.

The thermotropic properties of oxidized Por α were examined by differential scanning calorimetry (DSC). Fresh Por α and samples oxidized for 1–2 days exhibited a single broad phase transition at ~27.5°C. Por α oxidized for 3–4 days showed formation of a second phase with a slightly elevated phase transition. On day 7, the whole mixture showed a significant increase in phase transition temperature, which exceeded 44°C on day 14.

These findings demonstrate a clear time-dependent decrease in surfactant performance, with a transition from preserved function to severe dysfunction occurring between 4 and 7 days of oxidation accompanied by pronounced changes in the fluidity and packing of the surfactant.

Acknowledgement

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Keywords: pulmonary surfactant, oxidation, surface tension, differential scanning calorimetry

P17

**OPTIMALIZATION OF CHROMATOGRAPHIC CONDITIONS FOR
DETERMINATION OF CANNABIDIOL IN PHARMACEUTICAL AND FOOD
SAMPLES**

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More than 12,000 years, plants of the genus *Cannabis* spp. have been used for textile production or as a plant suitable for consumption. Their medicinal properties were first described as early as 3000 BC (for nausea, malaria, rheumatism, etc.). Recently, the substances from the plant are used in the treatment of chronic pain, multiple sclerosis, drug-resistant epilepsy and chemotherapy-related nausea and vomiting [2]. HPLC methods appear to be the most suitable for analysing the constituents of *Cannabis*, as they do not result in the decarboxylation of the components. Despite the many studies describing the composition of the active constituents in *Cannabis* plants, or analysing medicines containing non-psychoactive substances derived from the *Cannabis* plant, there is no single, validated method for their analytical determination [1].

As part of our research, we focused on optimising an HPLC-UV analytical method for the detection and quantification of cannabidiol. Here, the YOUNG IN Chromass (Anyang-si, Korea) chromatography system coupled with UV detector ($\lambda=228$ nm) was used. Isocratic chromatography separation mode was used with the starting of mobile phases A:B (24 :76). The mobile phase A represents water solution of 20 mM ammonium formate with 0.1% formic acid and the phase B was composed of acetonitrile. The separation of individual substances was performed on a 2.6 μ m Kinetex[®] C18 chromatography column. Optimization of this method was performed under three different conditions: a) by varying the ratio of mobile phases (15:85; 20:80; 30:70; 40:60); b) by varying the composition of mobile phase A; and c) by varying the temperature (30°C, 50°C). The most significant changes relating to the detection of cannabidiol were observed when the ratio of the mobile phases was altered. These changes involved both a shift in detection times and alterations to the size and improved separation of the individual peaks.

Acknowledgments

This work was supported by the grant VEGA 1/0302/2024, FaF/1/2026 and GVRFaFUK/1/2026.

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Keywords: cannabidiol, HPLC-UV, optimisation

P18

LIPOPHILICITY ASSESSMENT OF POTENTIAL ANTIMICROBIAL DRUGS USING REVERSED-PHASE HPLC AND COMPUTATIONAL PREDICTORS

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The lipophilicity of twenty newly synthesized substances with potential antimicrobial activity was determined using the RP-HPLC method and calculated using several interactive tools (iLOGP, xLOGP3, WLOGP, MLOGPS, SILICOS IT logp and Consensus). Chromatographic measurements were performed on a Dionex UltiMate 3000 Series UHPLC System using a Symmetry® C18 5 μ m, 4.6 x 250 mm column. 10 μ L of a methanolic solution of the substance with an approximate concentration of 0.01 mg/mL was injected. The flow rate of the mobile phase was 1 mL/min. The column temperature was maintained at 40 °C. A wavelength close to the absorption maximum of the studied substances (270 nm) was chosen for detection. The analysis was performed in five methanol/water mobile phases with volume ratios of 95:5, 90:10, 85:15, 80:20 and 75:25. A methanolic solution of potassium iodide was a dead time marker. Based on the retention times of the studied substances (t_R) and dead time (t_0), the logarithms of the capacity factors $\log k = \log((t_R - t_0)/t_0)$ were calculated. The linear dependence of the $\log k$ values on the methanol content in the mobile phase was extrapolated to zero methanol content in the mobile phase. Thus, the $\log k_w$ value was obtained, which is used as a lipophilicity parameter corresponding to the aqueous environment. Subsequently, all the experimentally determined $\log k_w$ values were correlated with the in silico generated logarithmic values of partition coefficients ($\log P$) for an octan-1-ol/water partition system, and the relationship between lipophilicity and structure of the studied substances was evaluated.

Acknowledgments

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Keywords: lipophilicity, RP-HPLC, computational predictors, antimicrobial activity

PHYSICOCHEMICAL AND BIOLOGICAL SCREENING OF POLYMER BASES FOR MEDICATED OROMUCOSAL FILMS

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Oromucosal delivery is advantageous for drugs susceptible to gastrointestinal degradation and hepatic first-pass metabolism but is limited by short residence time in the oral cavity. Therefore, polymer-based mucoadhesive dosage forms are being developed to prolong retention in the oral cavity [1, 2].

This study aimed to screen selected polymer bases for the development of mucoadhesive oromucosal films. Gelatin (bloom 200) was used as a primary film-forming polymer, combined with functional mucoadhesive polymers, namely chitosan (CHIT), hydroxypropyl methylcellulose (HPMC), and sodium carboxymethyl cellulose (NaCMC). The films were prepared by solvent-casting. The prepared films were evaluated for basic physicochemical characteristics (uniformity of mass [3], tensile strength [4], swelling index [5]) and biocompatibility (pH, irritation potential by the Hen's Egg – Chorioallantoic Membrane test [6]). Finally, mucoadhesivity was measured by texturometry [7] as a mucoadhesion strength on an *ex vivo* model (porcine buccal mucosa at 37 °C with 2% mucine in PBS 6.8).

The results showed that NaCMC was the most suitable mucoadhesive polymer for medicated oromucosal films. The prepared films exhibited a physiologically acceptable pH (6.24 ± 0.04) and were evaluated as non-irritating. They possess good technological properties (elasticity, uniformity of mass) and the highest swelling ratio ($922.55 \% \pm 4.13 \%$ after 3 hours) among the studied formulations. They also achieved the highest mucoadhesion strength values ($0.059 \text{ N} \pm 0.002 \text{ N}$). This finding can be explained by its ability to form strong hydrogen bonds with mucin through its hydroxyl and ether groups [8, 9].

The findings of this study provide a basis for further optimisation and development of advanced buccal drug delivery systems with potential clinical applicability.

Acknowledgements

This work was supported by the projects FaF/19/2026 and KEGA 041UK-4/2024.

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Keywords: mucoadhesive films, gelatin-based systems, oromucosal drug delivery, polymers, texture analysis.

P20

RAPID ESTIMATION OF UNKNOWN ENZYME INHIBITORS USING EFFECT-DIRECTED HPTLC FINGERPRINTING: APPLICATION TO RHODODENDRON TOMENTOSUM EXTRACTS

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Effect-directed analysis comprises a range of biochemical, biological, and chemical assays, including enzyme inhibition, antimicrobial and cytotoxic activity testing, and radical-scavenging assays. The integration of high-performance thin-layer chromatography (HPTLC) with effect-

directed analysis through bioautography enables the direct correlation of chemical composition with biological activity in complex plant matrices. This approach provides a visual bioactivity fingerprint and allows rapid screening of crude extracts with minimal sample preparation [1]. The aim of this study was to evaluate the inhibitory activity of *Rhododendron tomentosum* Harmaja dry extracts against acetylcholinesterase (AChE), butyrylcholinesterase (BChE), and tyrosinase using HPTLC-bioautographic method. Dry extracts were prepared from *Rhododendron tomentosum* Harmaja shoots collected in the Rivne region, Ukraine. HPTLC analysis was performed using a CAMAG system (Muttens, Switzerland) equipped with an Automated TLC Sampler (ATS4), Automatic Developing Chamber (ADC3), Derivatizer, TLC Visualizer, TLC Scanner 4. The HPTLC-bioautographic AChE/BChE inhibition assay, tyrosinase inhibition assays were performed according to the protocol described in the CAMAG application note [2]. For phytochemical characterization, the chromatograms were derivatized by piezoelectric spraying with Natural Product Reagent and anisaldehyde–sulfuric acid reagent. Neochlorogenic acid, chlorogenic acid, ferulic acid, trans-ferulic acid, catechin, epicatechin, hyperoside, isoquercitrin, quercitrin, avicularin, oleanolic acid and ursolic acid were identified by comparing their R_f values with those of authentic reference standards. The AChE/BChE inhibition assay, performed using Ellman's derivatization method, revealed that oleanolic acid and ursolic acid exhibited inhibitory activity. In addition to these identified compounds, several unidentified bioactive zones exhibiting enzyme inhibitory activity were detected, predominantly in the non-polar mobile phase system. The tyrosinase inhibition assay also revealed several active zones, indicating the presence of tyrosinase inhibitors in the extracts. These findings demonstrate that *Rhododendron tomentosum* Harmaja is a promising source of bioactive constituents with acetylcholinesterase-, butyrylcholinesterase-, and tyrosinase-inhibitory activities.

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Keywords: HPTLC-bioautography, effect-directed analysis, acetylcholinesterase, tyrosinase

P21

THERMODYNAMIC AND STRUCTURAL PROFILING OF DIVALENT CATION-INDUCED DNA COMPACTION FOR PHARMACEUTICAL FORMULATIONS

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Non-viral gene delivery relies on condensing DNA into stable, compact nanoparticles. While divalent cations such as Ca^{2+} and Mg^{2+} are common physiological modulators, their thermodynamic and structural effects on DNA remain incompletely characterized under formulation-relevant conditions [1]. In this work, we used complementary biophysical methods.

We combined ITC, dynamic light scattering, circular dichroism and UV-Vis spectroscopy to map these interactions across a wide range of molar ratios.

Our results show that calcium and magnesium modulate DNA organization through different modes of interaction [2]. ITC revealed distinct binding thermodynamics that correlated with changes in hydrodynamic diameter and spectroscopic evidence of DNA conformational changes. Ca^{2+} promotes more efficient DNA compaction consistent with stronger ion-mediated interactions, whereas Mg^{2+} acts primarily as a stabilizer, enhancing the thermal integrity of the double helix at higher concentrations. Across all analytical methods, we observed a critical reorganization threshold at a characteristic cation-to-phosphate ratio.

These findings support the rational design of size-controlled DNA complexes. Our results clarify the energetic and structural effects of each cation on DNA and may help optimize the stability and performance of biopharmaceutical formulations.

Acknowledgements

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Keywords: DNA, divalent cations, compaction, isothermal titration calorimetry, non-viral gene delivery

P22

NEW APPROACHES TO THE DETECTION OF LICHEN ACIDS BASED ON THE FORMATION OF CHARGE-TRANSFER COMPLEXES

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Lichen acids constitute an important group of structurally diverse secondary metabolites produced by lichens. Their reliable identification is essential for chemotaxonomic studies [1] and for selecting compounds with potential applications, such as reducing agents in the green synthesis of gold nanoparticles [2]. This study evaluated the potential of 7,7,8,8-tetracyanoquinodimethane (TCNQ) as a novel detection reagent for thin-layer chromatography (TLC) of lichen acids.

The method is based on the formation of charge-transfer complexes between TCNQ and the analyzed compounds. Following chromatographic separation, these interactions produce characteristic color development of spots. The intensity and nature of the color response depend on the electronic properties of the molecules, allowing discrimination of compounds according to their ability to form donor–acceptor interactions.

More than 30 lichen acids representing the major structural classes of lichen secondary metabolites were analyzed by TLC. The relationship between chemical structure, particularly the substitution pattern of the aromatic ring, phenolic hydroxyl groups, other electron-donating or electron-withdrawing substituents, and the intensity of the color response after spraying with TCNQ was investigated. The results revealed a strong dependence of charge-transfer complex formation on molecular structure, with several groups of lichen acids exhibiting characteristic and reproducible color reactions.

The proposed detection reagent provides a simple, rapid, and sensitive method for visualizing lichen acids on TLC plates. Besides facilitating identification, it offers insight into the electronic properties of compounds, making it a useful screening tool for selecting substances with higher reducing potential for subsequent studies.

Acknowledgements

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Keywords: 7,7,8,8-tetracyanoquinodimethane (TCNQ), depsides, depsidones, thin-layer chromatography (TLC)

P23

CONSENSUS DOCKING-GUIDED VIRTUAL SCREENING, SYNTHESIS, AND IN VITRO EVALUATION OF NEW ACETYLCHOLINESTERASE INHIBITORS

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A series of novel pyrrole-based compounds was designed as potential acetylcholinesterase (AChE) inhibitors using structure-based virtual screening. An in-house database of more than 10,000 pyrrole derivatives was constructed and subjected to docking with GOLD 5.3 (CCDC) and Glide (Schrödinger). The resulting poses were rescored by MM/GBSA and the AI-based DiffDock protocol. Ten prioritized structures were selected for synthesis via Paal-Knorr condensation under

both conventional and microwave irradiation (Milestone FlexiWave), which afforded shorter reaction times and higher isolated yields. The new compounds were fully characterized by NMR, IR, and mass spectrometry, and their AChE inhibitory activity was evaluated using Ellman's spectrophotometric assay. Seven of the ten synthesized pyrroles displayed potent inhibition ($IC_{50} = 0.75\text{--}102.57\ \mu\text{M}$), whereas three compounds were inactive. The *in vitro* results correlated well with the *in silico* predictions, supporting the applied consensus docking and rescoring workflow as a reliable strategy for the discovery of new AChE inhibitors.

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Keywords: virtual screening, AChE inhibitors, microwave-assisted synthesis, pyrrole derivatives, structure-based drug design

P24

CLINICAL MONITORING OF JAK INHIBITOR LEVELS IN THE BLOOD DERIVATIVES OF PATIENTS TREATED FOR INFLAMMATORY BOWEL DISEASE BY FDA VALIDATED $\mu\text{SPE-HPLC-MS/MS}$ METHOD

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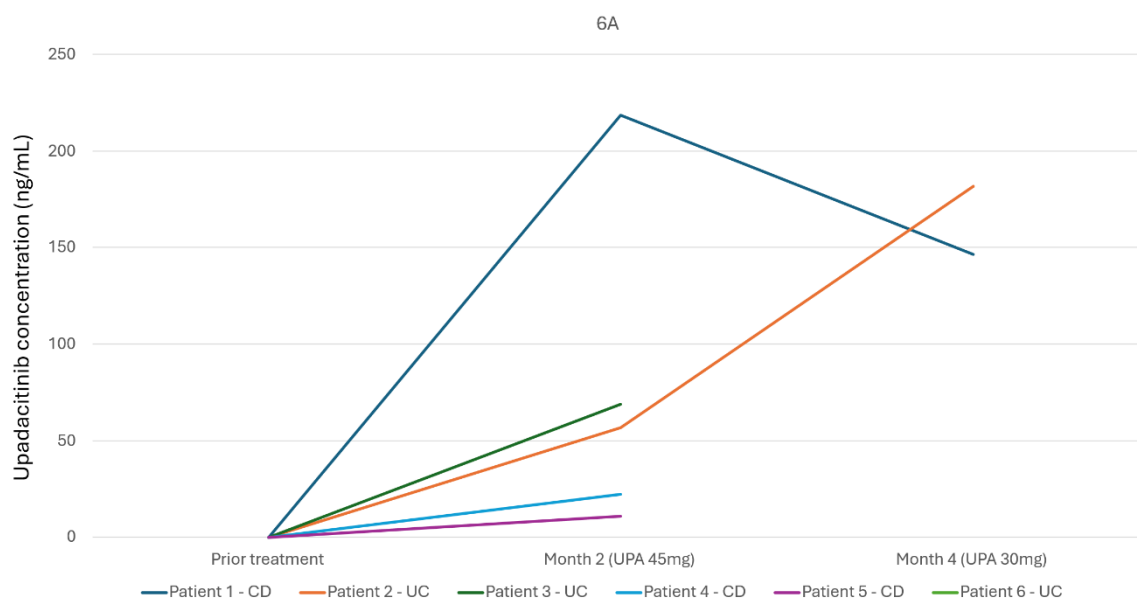
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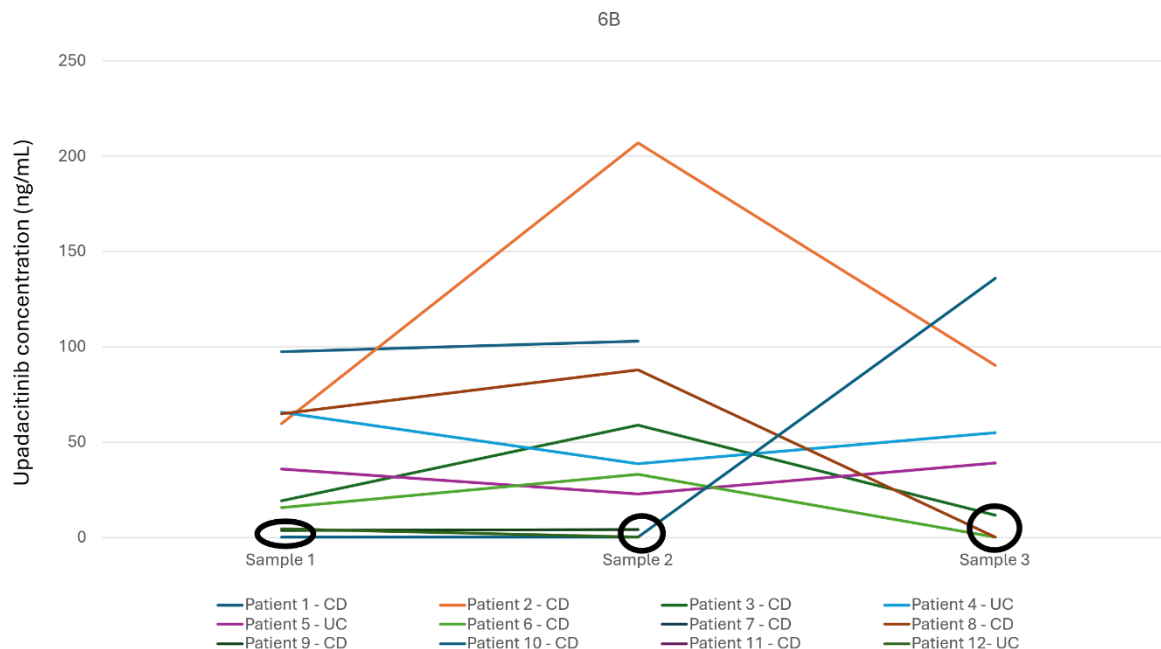
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Reliable monitoring of concentration levels of Janus kinase inhibitors (JAKis) is of current interest in analytical and gastroenterological research as the resulting findings could contribute to a future concept for the optimization of the efficacy and safety of the JAKis treatment of inflammatory bowel disease (IBD) [4,5]. Three JAKis, namely upadacitinib (UPA), tofacitinib (TOFA) and filgotinib (FILGO) were approved by Food and Drug Administration (FDA) and the European Medicines Agency (EMA) for the treatment of ulcerative colitis and Crohn's disease in the US and EU, respectively [1-3]. In this work, the first validated methodological protocol for reliable and high-throughput monitoring of clinically relevant levels of the entire group of JAKs approved for

IBD therapy was developed for application in real-world clinical settings. The validated HPLC-ESI-QQQ MS method, following the microelution SPE sample pretreatment, demonstrated adequate selectivity, sensitivity, linearity, accuracy, precision, recovery, carryover, and stability, meeting FDA criteria for biomedical analysis, i.e., for the quantification of TOFA, FILGO and UPA in human serum. The methodical advantages were further demonstrated by a concentration-matched verification (comparing concentration-matched serum standards with the corresponding clinical samples) when monitoring TOFA, UPA and FILGO concentration levels in the serum of patients treated for IBD. These performance characteristics enable reliable assessment of clinically relevant JAK inhibitor concentrations and support its application in routine exposure monitoring and future pharmacokinetic investigations. Outputs of this blind pilot clinical study approved practical use of the elaborated methodical protocol and can be briefly summarized as follows: (i) reliable detection of negative samples of all JAKis - the method prevents any false-positive analytical signal from the sample matrix, (ii) reliable detection of positive samples of all JAKis - the method allows monitoring clinically relevant JAKis levels and lower/higher intraindividual differences in serial measurements in patients on stable maintenance therapy (examples in illustrative Figure).





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Acknowledgement

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Keywords: therapeutic drug monitoring, high throughput analysis, Janus kinase inhibitor drugs, inflammatory bowel disease

P25

DEVELOPMENT OF AN HPLC-MS/MS METHOD FOR THE DETECTION OF BIOACTIVE COMPOUNDS IN FLAX (*LINUM USITATISSIMUM L.*)

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Flax (*Linum usitatissimum* L.) is widely appreciated for its abundance of bioactive compounds, including phenolic acids, which represent a significant group of natural antioxidants with potential therapeutic effects. Given the high complexity of plant matrices, there is a need for a sensitive and highly selective analytical method for their determination. High-performance liquid chromatography coupled with tandem mass spectrometry (HPLC-MS/MS) can meet these requirements.

The main goal of the study was to develop and optimize an HPLC-MS/MS method for the simultaneous determination of selected phenolic acids (*p*-coumaric, *o*-coumaric, salicylic, 4-hydroxybenzoic, 3,4-dihydroxybenzoic, ferulic, caffeic, cinnamic, chlorogenic, gallic). Experiments were performed in negative electrospray ionization mode (H-ESI⁻) using a triple-quadrupole mass spectrometer. Optimal MS parameters and characteristic product ions were determined for each analyte via direct infusion of certified analytical standards. Subsequently, HPLC conditions were optimized with a focus on chromatographic separation of analysed compounds.

Based on the obtained results, it is possible to state that HPLC-MS/MS enabled the simultaneous determination of individual phenolic acids and differentiation of isomers (*o*-coumaric vs *p*-coumaric, salicylic vs 4-hydroxybenzoic acid) based on their retention times and characteristic product ions. The developed method provides a reliable analytical tool and, following validation, will be further applied to profile phenolic acids in flax extracts.

Acknowledgements

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Keywords: *Linum usitatissimum* L., phenolic acids, HPLC-MS/MS

P26

PREPARATION AND CHARACTERIZATION OF GOLD NANOPARTICLES STABILIZED BY MODIFIED AMINO ACIDS FOR PHARMACEUTICAL APPLICATIONS

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Historically, gold compounds were used to treat progressive rheumatoid diseases, however, their clinical application was limited due to high dosing requirements and associated toxicities such as hepatotoxicity, nephrotoxicity, and bone marrow suppression [1]. This study aims to uncover the therapeutic potential of gold nanoparticles (AuNPs) as a safer and more efficient alternative. By significantly reducing the required therapeutic dose, AuNPs retained biological efficacy while

minimizing adverse effects [2]. The presented research examined the role of key physicochemical parameters and the synthesis method in influencing cytotoxicity and therapeutic outcomes. AuNPs were synthesized via chemical reduction of chloroauric acid using sodium borohydride. Stabilization was achieved using amino acids with varying molecular structure which was hydrophobically modified with alkyl chains of variable length. Hydrophobized amino acids served as surface-active stabilizers, improving nanoparticle dispersion and stability. AuNPs functionalized with amino acids were characterized utilising several experimental methods such as UV-VIS spectral analysis to confirm existence and stability of gold nanodispersions, dynamic light scattering for nanoparticle size determination, and zeta potential measurements to determine surface charge of AuNPs.

Acknowledgements

This research was supported by the APVV grants No. APVV-23-0349, APVV-SK-HU-24-0023 and FaF UK grant No. FAF/6/2026 and project CEEPUS No. RS-1113-09-2526.

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Keywords: gold nanoparticles, amino acids, surfactants

P27

EFFECT OF TEMPERATURE ON MICELLAR PROPERTIES AND THERMODYNAMICS OF BENZETONIUM CHLORIDE MICELLIZATION IN AQUEOUS SOLUTIONS

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The structure and physicochemical properties of drugs determine the non-specific mechanism of their action, as well as their behavior in the organism and transport of the drug in its effective form to the site of action. Some drugs with an amphiphilic structure - for example, quaternary ammonium salts - QAS, which also includes the drug - benzethonium chloride (known as Hyamine, abbreviation BZCl), with the chemical name: [N,N-dimethyl-N-2-[2-[4-(1,1,3,3-tetramethylbutyl)-phenoxy]ethoxy]ethyl]-benzenemethanaminium chloride], have the ability to form homogeneous or nonhomogeneous associates in solution - micelles.

The associates affect the form of drug application its biological effectiveness, the speed of onset, the duration of the effect and the mutual compatibility of the drugs.

BZCl is used in detergents, deodorants, topical antiseptics, spermicides, cosmetics, surface disinfectants, etc.

Important properties from the point of view of drug application are: (CMC) critical micellar concentration, which can be determined by monitoring the changes of the selected experimental quantity depending on the concentration of the substance in the solution, (β) - degree of counterion binding, describes the binding of ions of the opposite sign to the micelle in the solution, and thermodynamic parameters of micellization (changes in the standard molar Gibbs energy ($\Delta_m G^0$), enthalpy ($\Delta_m H^0$) and entropy of micellization ($\Delta_m S^0$).

The self-association of BZCl in solution has been analyzed as a function of temperature. The experimental results were determined using six experimental methods (conductometry, UV VIS spectrometry, spectrofluorimetry densitometry, potentiometry and NMR) in the temperature range 25-50 °C.

Conclusions: All methods used gave similar values of CMC. Temperature suppresses micellization. Mass action model was used for calculation of thermodynamic parameters of micellization. The process of micellization of the studied drug in an aqueous solution is spontaneous (negative and decreasing values of ($\Delta_m G^0$) and exothermic (decreasing values of the ($\Delta_m H^0$)).

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Keywords: benzethonium chloride (hyamine), critical micellar concentration, thermodynamic parameters of micellization

P28

RECENT TRENDS IN PURIFICATION AND ANALYSIS OF RNA AND DNA SAMPLES BY CAPILLARY ISOTACHOPHORESIS AND LIQUID CHROMATOGRAPHY

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Oligonucleotides are short molecules of DNA or RNA, that have an interesting application potential as biopharmaceuticals. Similarly, they are very often used in genetic testing, research, and forensic. The application of such molecules is typically accompanied with a complex sample pretreatment which is based on extensive extraction and purification procedures. Moreover, all of these pretreatment steps are bound with the quality control based on appropriate analytical methods and strategies.

Capillary isotachopheresis (CITP) is a very effective concentration electromigration analytical method which can be used for direct analysis of ions and ionizable substances. It is also often used to concentrate and purify various samples. Capillary isotachopheresis performed in the

hydrodynamically closed separation system arranged with a micropreparative valve represents a valuable tool for effective and robust concentration and purification of DNA and RNA samples. In combination with approaches based on liquid chromatography (implementation of novel stationary phases), such procedure facilitates unambiguous characterization and quality control of the mentioned biopolymers and biopharmaceuticals.

Here, we presented preparative capillary isotachopheresis to concentrate and purify the model mRNA and DNA samples with further liquid chromatography quality control. The obtained results represent a promising starting point for further investigation and open the possibility of that instrumentation to be adapted into the development of innovative biopharmaceuticals.

Acknowledgements

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Keywords: biopharmaceuticals, DNA, liquid chromatography, mRNA, preparative capillary isotachopheresis, quality control

P29

pH-DEPENDENT AGGREGATION OF ALKYLDIMETHYLAMINE OXIDES

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Alkyldimethylamine oxides are single-chain surfactants whose aggregation behaviour is strongly controlled by pH. Depending on solution acidity, they exist in the form of a mixture of nonionic and protonated molecules, resulting in self-assembled structures with properties that differ significantly from those of conventional nonionic or ionic surfactants. The most extensively studied member of this surfactant series is the analogue with twelve-carbon alkyl chain – dodecyldimethylamine oxide (DDAO). The studies have shown that its micellar properties such as critical micelle concentration, surface activity, and micelle morphology are sensitive to the degree of protonation [1]. This feature also affects its binding to charged biomacromolecules [2]. The strongest aggregation of DDAO is typically observed near equal proportions of protonated and non-protonated molecules [3]. The pH-dependent behaviour arises from a balance between electrostatic repulsion and attractive interactions between amine oxide molecules. At higher ionic strength, DDAO micelles may grow into elongated cylindrical structures [4]. Similar trends have been also reported for other alkyl chain lengths, indicating that pH-responsive aggregation is a general feature of the alkyldimethylamine oxide surfactant group [5].

The presented study provides novel experimental data of hydrodynamic diameter and zeta potential of micellar solutions of dodecyl- and tetradecyldimethylamine oxide as a function of pH over a broad range of pH values. Understanding the mechanism of pH-dependent surfactant protonation may facilitate the design of colloidal systems with tuneable properties for biological applications.

Acknowledgments

The presented research was funded by the Slovak Research and Development Agency grants APVV-23-0349 and APVV SK-HU-24-0023.

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Keywords: alkyltrimethylamine oxides, pH-responsive aggregation, protonation, micelles, self-assembly.

P30

EXPLORING DUAL INHIBITORS OF TAU KINASES AND TAU AGGREGATION – IN SILICO STUDIES

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During the development of Alzheimer's disease, tau protein undergoes hyperphosphorylation by various kinases and forms NFTs, which contribute to worsening of neurodegeneration. Current research shows that tau phosphorylating kinases can be druggable target in fight with Alzheimer's disease. [1]

In our work, we explored 6 of these kinases – Rho kinase (ROCK), DYRK1A, GSK3 β , CDK5, p38MAPK and MARK4, which phosphorylate tau protein at various amino acid residues. Our main focus was to determine whether known inhibitors of tau aggregation – small molecules, such as methylene blue, epigallocatechin gallate, homoprejudomycine, cyanidin and more – also inhibit mentioned kinases, making them dual inhibitors of tau aggregation. [1]

For docking 29 known tau aggregation inhibitors and 20 known kinase inhibitors, we used the Small-Molecule Discovery Suite software package (Schrödinger Inc., USA). We used advanced methods of molecular modelling and computer simulations based on molecular mechanics. Post docking evaluation included visually inspecting key interactions between ligands and amino acid residues in binding pocket of each kinase, such as hydrogen bonds, hydrophobic interactions or π - π interactions. Ligands were ranked based on the best binding energies expressed through GlideScore, and the more precise MM-GBSA binding free energy.

In silico studies, specifically MM-GBSA results, suggest that the most promising artificially synthesised dual inhibitors are anthracyclines, rhodanine derivatives and benzothiazoles. From natural occurring compounds, optimistic molecules include EGCG, rosmarinic acid and curcumin.

Acknowledgements

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Keywords: Tau kinases, Rho kinase (ROCK), DYRK1A, GSK3 β , CDK5

P31

EFFECT OF FORMULATION COMPOSITION ON THE QUALITY OF INDOMETHACIN MULTI-UNIT PELLETT SYSTEM TABLETS

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The compression of drug-loaded pellets into tablets remains a challenge, as formulation variables can significantly affect both mechanical integrity and drug release. The study aimed to formulate and evaluate MUPS tablets containing indomethacin (IND) and to investigate the influence of drug concentration and polyvinylpyrrolidone (PVP) on their technological and dissolution properties. Four MUPS tablet formulations were prepared from indomethacin-loaded pellets produced by the extrusion–spheronization technique. The formulations differed in indomethacin content and the presence or absence of PVP. The resulting tablets were evaluated for resistance to crushing, friability, disintegration, uniformity of mass, and bulk density. In addition, dissolution testing was performed in acidic medium to assess the effect of formulation composition on indomethacin release.

The excipients significantly influenced both mechanical and dissolution characteristics of the MUPS tablets. Formulation F2 (1% indomethacin, without PVP) exhibited the most favorable technological performance, showing the highest crushing strength combined with low friability. Tablets containing a higher concentration of indomethacin (1%) generally demonstrated superior mechanical properties compared with those containing 0.1% drug. The inclusion of PVP resulted in a slight reduction in mechanical strength but promoted drug release during dissolution testing. Formulation F2 demonstrated the best balance between mechanical resistance and tablet integrity.

Keywords: pellets, indomethacin, polyvinylpyrrolidone, extrusion–spheronization, compression, MUPS tablets

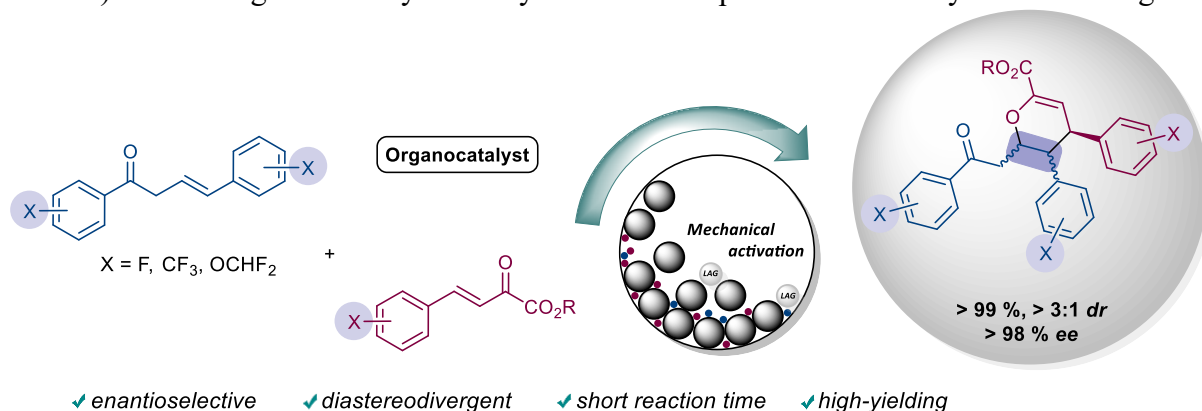
ENANTIOSELECTIVE SYNTHESIS OF FLUORINATED 3,4-DIHYDROPYRANS AS POTENTIALLY BIOACTIVE SCAFFOLDS

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Chiral 3,4-dihydropyrans are found in numerous natural products and biologically active compounds. Particular attention has been devoted to fluorinated derivatives, as the incorporation of fluorine can significantly influence their physicochemical and biological properties [1]. Among the synthetic approaches to these heterocyclic scaffolds, [4+2] cycloaddition reactions represent a particularly efficient strategy [2,3]. An asymmetric organocatalytic cycloaddition between fluorinated β,γ -unsaturated ketones and α -ketoesters was carried out under mechanochemical conditions using liquid-assisted grinding (LAG) in a ball mill. The reaction proceeded via dienolate activation and provided access to a series of 3,4-dihydropyran derivatives bearing three newly formed stereogenic centers. The developed methodology afforded the corresponding products in high yields (up to 99%), high diastereoselectivity ($>3:1$ *dr*) and high enantiomeric excess (up to 98% *ee*). The biological activity of the synthesized compounds is currently under investigation.



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Keywords: 3,4-dihydropyrans, [4+2] cycloaddition, organocatalysis, mechanochemistry

P33

MECHANOCHEMICAL ACCESS TO AXIALLY CHIRAL SCAFFOLDS RELEVANT TO DRUG DISCOVERY

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Over the past decade, mechanochemistry has emerged as an efficient and sustainable synthetic strategy for the preparation of structurally complex molecules. At the same time, axially chiral scaffolds have attracted increasing attention owing to their distinct stereochemical and structural properties. Utilizing organocatalytic Michael/aldol cascade with subsequent oxidation, axially chiral 1,2'-binaphthalene-3'-carbaldehydes were prepared under mechanochemical conditions [1]. Evaluation of milling parameters such as frequency or liquid-assisting agents led to optimum reaction conditions, which afforded products in high yields, and short times while preserving high enantiomeric purity.

The authors very gratefully acknowledge the financial support received from the Slovak Research and Development Agency under the Contract No. APVV-22-0133.

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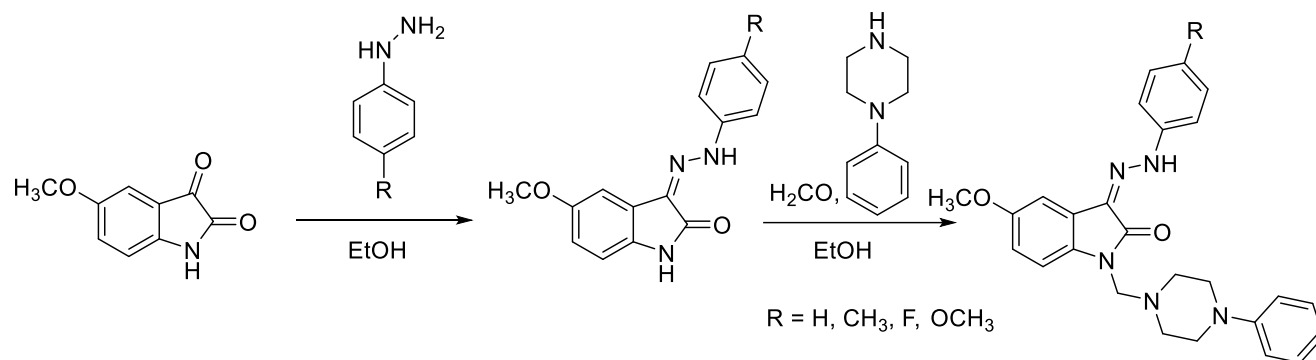
Keywords: organocatalysis, catalysis, atropisomerism, mechanochemistry.

P34

NOVEL ISATIN DERIVATIVES-POTENTIAL DRUGS

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The research is focused on the synthesis, characterization and study of the biological properties of isatin hydrazones as suitable candidates for new drugs. Isatin and its derivatives belong to the organic compounds used in chemical practice. They are used primarily in areas such as medicinal chemistry, in the field of nanotechnology, development of analytical reagents and dyes, and in the field of synthesis of heterocyclic compounds and stereoselective procedures. The high application potential of isatin and its derivatives, their occurrence and the occurrence of their metabolites in plants and in the human body have aroused the great interest of chemists, doctors and pharmacists in the study of their chemical reactivity. Isatin undergoes various modifications easily, therefore its derivatives belong to the class of privileged heterocyclic structures, on the basis of which biologically active substances of a wide spectrum of action have been proposed. [1][2] The aim of the work was the synthesis of new, not yet described in the literature, derivatives of 3-(phenylhydrazono)-5-methoxyisatin with a phenylpiperazine substituent in the N-1 position.[3]

Acknowledgments

The authors very gratefully acknowledge the financial support received from the Slovak Research and Development Agency under the Contract No. APVV-22-0133.

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Keywords: isatin, hydrazones, piperazine

P35

SYNTHESIS AND BIOLOGICAL SCREENING OF TRIAZINOINDOLE ALDOSE REDUCTASE INHIBITORS TARGETING PREVENTION OF DIABETIC COMPLICATIONS

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Diabetes mellitus is associated with chronic hyperglycemia, which activates several biochemical pathways involved in the development of secondary diabetic complications. One of the most important is the polyol pathway, whose first and rate-limiting enzyme, aldose reductase (ALR2), represents an attractive therapeutic target. Inhibition of ALR2 has the potential to reduce polyol pathway over-activation and alleviate diabetes-associated oxidative stress.

In our previous studies, we developed the thioxotriazinoindole aldose reductase inhibitor centirestat (CMTI), which exhibited potent inhibitory activity ($IC_{50} = 116$ nM), good selectivity ($S_F = 302$), and antioxidant properties. [1] Subsequent oxygen bioisosteric replacement led to the development of otirestat (OTI), displaying improved potency ($IC_{50} = 42$ nM) and markedly enhanced selectivity ($S_F > 2\,381$). [2]

To further optimize biological activity and selectivity, a series of fluorinated CMTI and OTI analogues bearing fluorine or fluorinated substituents on the indole ring was designed using *in silico* approaches, synthesized, and biologically evaluated. Altogether, seven CMTI and eight OTI derivatives were prepared and assessed for inhibitory activity against ALR2, selectivity toward the anti-target aldehyde reductase (ALR1), and for CMTI derivatives, antioxidant activity. Fluorination proved to be an effective strategy for improving enzyme inhibition and selectivity. The most promising compound (8-F)OTI, exhibited excellent inhibitory potency ($IC_{50} = 15$ nM) together with more than threefold higher selectivity ($S_F > 6\,667$) compared with the parent OTI. Molecular docking studies confirmed the experimental findings, indicating that substitution at position 8 provides the most favourable interactions within the ALR2 active site while simultaneously reducing stabilizing interactions with ALR1, thereby contributing to enhanced selectivity.

These results identify fluorination as an effective optimization strategy for triazinoindole-based aldose reductase inhibitors and highlight (8-F)OTI as a promising lead compound for further development.

Acknowledgments

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Keywords: diabetes mellitus, diabetic complications, aldose reductase inhibitors, fluorinated oxotriazinoindoles

BIOSYNTHESIS OF NANOPARTICLES USING EXTRACTS FROM *GANODERMA LUCIDUM*

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Over the past two decades, nanoparticles have become one of the most extensively studied areas in materials science, biotechnology, and pharmacy. Their physicochemical properties resulting from their large specific surface area, nanoscale dimensions, reactivity, optical properties, and ability to interact specifically with biological systems, have laid the foundation for innovative applications. A limiting factor in traditional nanoparticle synthesis is the use of chemical reducing agents, which can be toxic and problematic when applied in biological systems. These shortcomings have led to the rapid development of so-called “green synthesis” of nanoparticles, which utilizes metabolites from plants, fungi, algae, or microorganisms as natural reducing and stabilizing reagents. Among these biological sources, the *Ganoderma lucidum* mushroom has attracted particular attention. It is a wood-decaying mushroom used in traditional Chinese medicine and known for its rich chemical composition. Its extracts contain polysaccharides, triterpenoids, phenolic compounds, proteins, and other bioactive secondary metabolites that possess natural reducing properties and can also act as stabilizing reagents. Nanoparticles prepared from mushroom extracts are currently being studied primarily for their antibacterial, antioxidant, anti-inflammatory, antitumor, and antiviral effects [1-2].

Two aqueous extracts of *Ganoderma lucidum* mushrooms, prepared by maceration and reflux, were analysed. The course of Ag NPs synthesis was monitored using UV/Vis spectrophotometry. During the synthesis of Ag NPs, the *Ganoderma lucidum* extracts demonstrated a significant ability to reduce Ag⁺ ions to elemental silver. This process was accompanied by a characteristic change in the color of the reaction mixture and the appearance of an absorption band in the region of approximately 400–450 nm (maximum ~430 nm) of the visible spectrum. The extract prepared by reflux exhibited a more intense and time-stable absorption band compared to the extract prepared by maceration, which may indicate its higher reducing potential and more effective stabilization of the resulting nanoparticles. The reaction temperature had a significant effect on the course of Ag NPs synthesis. At 40 °C, the synthesis proceeded both qualitatively and quantitatively, particularly when using the extract obtained by reflux. In contrast, at the higher temperature of 80 °C, the synthesis was very rapid, but accompanied by a decrease in absorbance intensity, which can be attributed to the aggregation or sedimentation of the nanoparticles.

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Keywords: *Ganoderma lucidum*, maceration, reflux, nanoparticles of silver

P37

SYNTHESIS, CYTOTOXICITY AND TESTING OF BLOOD-BRAIN BARRIER PERMEABILITY OF NEW DERIVATIVES WITH ARYLOXYAMINOPROPANOL AND CARBAMATE PHARMACOPHORES

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The work focuses on the synthesis of new compounds whose chemical structure contains two pharmacophore groups, a carbamate group and an aryloxyaminopropanol group. The presence of these two chemical groups makes the synthesized derivatives attractive for therapeutic use, as they combine in a single molecule the properties of drugs used in the treatment of Alzheimer's disease and cardiovascular diseases.

Results from cytotoxicity testing of seven derivatives on endothelial cells isolated from rat brains showed that these derivatives exhibited minimal toxicity, with IC₅₀ values ranging from 1.000 to 1.445 mM. When testing the passage of ethyl{3-[2(*R,S*)-hydroxy-3-(4-benzylpiperidin-1-yl)propoxy]phenyl}carbamate through the blood-brain barrier in a model using cells isolated from rat brains, the endothelial permeability coefficient was found to be $P_e 108 \times 10^{-6}$ cm/s. Ethyl{3-[2(*R,S*)-hydroxy-3-(4-benzylpiperidin-1-yl)propoxy]phenyl} carbamate is classified as a central nervous system (CNS+) substance, meaning that it crosses the blood-brain barrier very well and is therefore a potentially suitable candidate for the treatment of central nervous system disorders. The future of pharmaceutical research lies not only in the synthesis of new molecules, but also in new ways of thinking about their use in the treatment of complex diseases.

Keywords: aryloxyaminopropanol, carbamate, synthesis, cytotoxicity, blood-brain barrier

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HPLC ANALYSIS OF CONTROLLED APIXABAN RELEASE FROM NANOPOROUS MSM-60

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Apixaban (APX) is an oral anticoagulant used for the treatment of non-valvular atrial fibrillation in patients at high risk of stroke and systemic embolism. However, APX has poor water solubility (40–50 µg/mL) and limited oral bioavailability (approximately 50 %) following administration of a single dose [1]. A promising strategy to overcome these restrictions is incorporation of drug molecules into porous carriers without compromising their pharmacological efficacy. This method optimizes drug delivery by ensuring targeted release and minimizing side effects. Mesoporous silica materials (MSMs) are promising candidates for the delivery of therapeutic agents due to their high surface area, tuneable pore sizes, and functionalization capabilities. Therefore, the aim of this study was to investigate the release behaviour of Apixaban from MSM-60 using HPLC analysis. The desorption conditions were optimized by evaluating key parameters, including pH, solution volume, and active substance concentration, in order to closely simulate physiological conditions and enable in vitro modelling of system behaviour. Drug release was studied in different medium volumes ranging from 50 mL to 2 L of distilled water at pH 2 and 7, representing gastric and physiological environments, respectively, to assess the influence of medium volume and pH on drug solubility and release behaviour. Apixaban release was quantified using a Dionex UltiMate 3000RS HPLC system with diode-array detection (DAD) and Chromeleon 7.2 software (Thermo Fisher Scientific, Germany). Separation was performed on a Kinetex PFP F5 column (150 × 4.6 mm, 2.6 µm) under isocratic conditions using a methanol–water mobile phase (65:35, v/v) at a flow rate of 0.8 mL/min. Detection was carried out at 280 nm with an injection volume of 20 µL. The results indicate that the mesoporous MSM-60 material enables controlled and sustained drug release, reducing burst release and prolonging therapeutic action.

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Keywords: Apixaban, HPLC-DAD, desorption

P39

THE EVALUATION OF MOLECULAR MECHANISM OF COPPER COMPLEXES AS ANTICANCER AGENTS

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Metal-based compounds offer a promising opportunity to develop new cancer treatments. These compounds with their unique properties and mechanisms of action have shown many advantages in the fight against cancer compared to other chemical compounds [1]. Copper complexes have emerged as promising candidates in the development of novel anticancer agents due to their unique redox properties, structural versatility, and ability to participate in biologically relevant

interactions. Unlike traditional platinum-based drugs, copper complexes can exploit intracellular redox cycling to generate reactive oxygen species (ROS), leading to oxidative stress, DNA damage, and apoptosis in cancer cells. These multifaceted pathways contribute to overcoming drug resistance, a major limitation of conventional chemotherapy [2].

The mechanism of anticancer effect was studied in novel Schiff bases copper(II) complexes with derived from 3-methoxybenzaldehyde and amino acid L-serine with heterocyclic N-donor ligands. The cytotoxicity of complexes was evaluated using the cancer cell lines: A549 (human lung carcinoma), HepG2 (hepatocyte carcinoma), and noncancer cells HEL. The complexes exhibited a more significant cytotoxic effect against anticancer cell lines compared to the cytotoxicity against normal cells. Subsequently, the mechanism of their antitumor activity was evaluated. The cytotoxic effect was observed at multiple levels within the cell. Genotoxicity was detected, including effects on nuclear DNA and chromosomes, as well as impacts on mitochondria and the cell cytoskeleton.

Acknowledgements

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Keywords: Schiff base, copper(II) complexes, cytotoxic activity

P40

STUDY OF PHYSICAL PROPERTIES OF SILVER NANOPARTICLES FUNCTIONALIZED BY AMINO ACIDS

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Silver nanoparticles (AgNPs) were prepared by chemical reduction from silver nitrate and stabilized using amino acids. Prior to their use as stabilizing agents, the amino acids were chemically modified (hydrophobized)¹ to form surfactant-capped AgNPs. The role of the amino acids in the synthesis of silver nanoparticles is twofold: to enhance their temporal (colloidal) stability and, more importantly, to expand the application potential of the synthesized nanoparticles toward biological use. Amino acid surfactant-modified AgNPs are expected to exhibit enhanced interaction with the intracellular environment, including cellular organelles and the nucleus itself. This approach enables the development of functionalized AgNPs with promising biological activities, particularly for antimicrobial and antineoplastic applications.² In our study, amino acid surfactant-modified AgNPs are characterized by multiple physical methods such as UV-VIS spectrometry, zeta potential measurements for surface charge determination, and dynamic light scattering for the determination of AgNPs hydrodynamic size.

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Keywords: silver nanoparticles, amino acid surfactants