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BOOK OF ABSTRACTS 51ST CONFERENCE SYNTHESIS AND ANALYSIS OF DRUGS (SAD 2023) POSTER SESSION

SCHIFF BASES AS POTENTIAL THERAPEUTIC AND ANTICANCER AGENTS

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Abstract

Basic thiosemicarbazone and semicarbazone derivatives of acetophenone with imine functional group belong to the group commonly called Schiff bases. Therapeutics and compounds belonging to this group are versatile pharmacophores with a significant capability of forming chelates with various metal ions. Such metal complexes play an important role in therapeutics due to their remarkable broad spectrum of biological activities.

Due to the above-mentioned complex formation, many Schiff bases appear as an important intermediate in a number of enzymatic reactions. One of the possible target enzymes is a neutral zinc-binding metalloenzyme aminopeptidase N (AP-N), also called membrane alanyl aminopeptidase. Potential inhibitors of this omnipresent enzyme may offer an effective and broad-spectrum therapy.

Through a three-step synthesis, it is possible to obtain three arene substitution isomers of basic thiosemicarbazone and semicarbazone derivatives of acetophenone. The initial step of the synthesis is the chloroacetylation of aminoacetophenone, followed by substitution with a secondary amine. Symmetrical secondary amines and heterocyclic amines with saturated heterocyclic skeleton were used for substitution. Synthesized compounds with the best half maximal inhibitory concentration against the enzyme AP-N underwent testing for inhibition of cell proliferation on the three different cell lines. A simple QSAR model describing the dependence between the inhibitory activity expressed as IC_{50} and the descriptors derived from the chemical structure was established.

Keywords

Schiff bases, basic acetophenone derivative, aminopeptidase N, metalloenzyme inhibitor

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THROMBOLYSIS IN VIVO

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Abstract This innovative experimental work focuses on the development of targeted thrombolytic combinations for the diagnosis and therapy of cerebral infarction, their preparation and testing in a rat animal model. The effect of the addition of plasminogen to the standard recombinant alteplase in the treatment of cerebral infarction was tested. A rat model of systemic embolism was used for this purpose. The design of the model is set to simulate as much as possible the course of thrombolysis in the human organism, and the implementation of preclinical research into clinical practice is realistic.

Keywords thromboembolism, micro-fluoroscopy, ischemic stroke, limb ischemia, alteplase

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Study of the protective effect of Monophosphoryl lipid A on UVC-irradiated THP-1 monocytes

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Abstract The experiment aims to observe the potential protective effect of monophosphoryl lipid A (MLA—vaccine adjuvant, enhancer of the immune response) at a 1.0 µg/ml concentration on cells exposed to stressors. We observed the effect of MLA on the non-irradiated and irradiated samples of the THP-1 cells (source: human leukemia monocytic cell line). The basis of the experiment was to study the effect of different doses of radiation on the vitality and biological activities of the THP-1 cells at values 64, 318, 636, and 954 J/m^2. Due to the expected weakened defensive capabilities of the THP-1 cells corresponding to samples after exposure to UVC in the doses, we selected a dose of UVC radiation of 64 J/m^2 to induce stress in the THP-1 cells in the main part of the experiment. The effect of bio-active MLA was followed on the samples without UVC irradiation, and the samples were exposed to UVC after one-hour pre-incubation. Thereafter, the samples were incubated for 18 more hours in the CO_2 atmosphere. The effects of MLA compounds on reductase activity, production of superoxide radicals, nonspecific immune response in case of phagocytosis, and the changes in the activity of cell antioxidant defense enzyme – catalase – were observed. Based on the results, finally, we can evaluate that MLA as a well-known TLR4 receptor agonist can protect the biological activities in cells irradiated with UVC radiation at a dose of 64 J/m^2.

Keywords MLA, UVC radiation, stressor, THP-1 cells, biological activities

Acknowledgements The study was supported by VEGA 1/0429/21.

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PHYTOCHEMICAL ANALYSIS OF THE WATER–ETHANOL ROOT EXTRACT OF *DIPSACUS FULLONUM* L.

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Abstract *Dipsacus fullonum L.*, a flowering plant from the family Caprifoliaceae, is used in traditional folk medicine, among others, as a supportive treatment for Lyme disease. Its antioxidant and antibacterial effects were confirmed experimentally in vitro. The aim of this work was to identify the secondary metabolites extracted in solution ethanol/water in a ratio of 70/30 (V/V), prepared via ultrasonication, from the root of *D. fullonum L.* growing in Slovakia. LC–MS was used for the identification of secondary metabolites and HPLC-DAD for their subsequent quantification by the method of external standards. Iridoid structures identified in the extract were derivatives of loganic acid and secoiridoids, cantleyoside, and sylvestrosides III and IV, which are mutual isomers. Anyway, by this method, it was impossible to identify which of these two isomers is present in the analyzed extract. In addition, the extract contained a derivative of sylvestrosides III/IV – possibly sylvestrosides III/IV dimethyl acetal. Other identified secondary metabolites are derivatives of caffeic acid: two hexosides of caffeic acid, three isomers of caffeoylquinic acid, and three isomers of caffeoylquinic acid, and the exact structure of which could not be outrightly confirmed by this method. By quantification, we found that the most represented substances in the prepared extract were the bis-iridoids cantleyoside and sylvestrosides III/IV. Of the polyphenols, the most represented molecules were dicaffeoylquinic acid isomers. Identified iridoids and dicaffeoylquinic acid derivatives are suggested as the antioxidant and antimicrobial active compounds in *D. fullonum* root.

Keywords *Dipsacus fullonum*, LC–MS, sylvestroside, cantleyoside

Acknowledgements This work was supported by grants VEGA 1/0284/20, APVV-19-0056, and APVV-15-0123.

CHARACTERIZATION OF GENES RELATED TO PROBIOTIC PROPERTIES OF *LIMOSILACTOBACILLUS REUTERI* E BY IN SILICO ANALYSIS

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Abstract Nowadays, the whole-genome sequence is more incorporated in routine analysis, identification, and characterization of bacteria. Next-generation sequencing methods based on massive parallel sequencing of amplified DNA fragments have become conventional method. On this basis, the whole-genome sequence can be used in the selection of potential probiotic strains to predict the health beneficial properties. Lactobacilli belong to the most studied probiotic bacteria. The promising potentially probiotic strain *Limosilactobacillus reuteri* E was subjected to whole-genome sequencing by the Illumina Next seq 2 x 150 bp method (genome length 1,902.828 bp) and selected for in silico analysis. We focused on the *pdu-cob-cbi-hem* and exopolysaccharide (Eps) gene clusters. For in silico analysis, the web tools Rapid Annotation using Subsystem Technology (RAST), Bacterial and Viral Bioinformatic Resource Center (BV-BRC), and Basic Local Alignment Search Tool (BLAST) were used.

Using RAST 31% of *L. reuteri* E, genes were subjected to function and divided to subsystems. The most represented gene function covers metabolism of amino acids (106), carbohydrates (104), and proteins (103). Gene products of the *pdu-cob-cbi-hem* cluster are involved in the synthesis of the potent antimicrobial agent reuterin. This cluster was located on contig 5 (under accession JAHQZV010000005) and assumed *L. reuteri* E to produce reuterin.

Genes responsible for exopolysaccharide synthesis, attaching of monosaccharide units and their linking together by glycosylic bounds, were identified in *L. reuteri* E. They were located on contigs 47 (exopolysaccharide biosynthesis glycosyltransferase EpsF (EC 2.4.1.-) and 57 (tyrosine-protein kinase EpsD (EC 2.7.10.2); tyrosine-protein kinase transmembrane modulator EpsC; and cell envelope-associated transcriptional attenuator LytR-CpsA-Psr, subfamily F2). Usually, they are closely attached to the bacterial surface or are released into surrounding environment. They are important for immunomodulatory, antitumor, and antioxidative activities.

Keywords whole-genome sequence, probiotics, lactobacilli, reuterin, exopolysaccharides

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Cu(II) COMPLEXES OF QUINAZOLINONE DONOR LIGANDS AS POTENTIAL ANTICANCER AGENTS

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Abstract Transition metals play a fundamental role in the chemistry of life. In trace amounts, they enable a number of selective catalytic conversions necessary to sustain biological processes. Excessive intake of these elements can cause various toxicological effects including carcinogenesis. However, it is this toxicological potential that forms the basis of transition metal-based anticancer therapy. Metal-based drugs are gaining more and more importance in modern medicine, especially in the field of oncology. The well-known platinum-based compounds are widely used in the cancer treatment. However, high toxicity and significant side effects are often observed. Current efforts are, therefore, directed toward metal-based compounds with lower toxicity and novel mechanisms of action.
In the search for new anticancer agents, three Cu(II) complexes based on quinazolinone Schiff bases were synthesized. The antiproliferative activity of *O,N,O*-quinazolinone donor ligands and their Cu(II) complexes toward human cancer cell lines (Caki-1, HepG2, HT-29) was examined. Experimental data revealed that the studied compounds possess substantial biological activity. Free quinazolinone ligands showed higher antioxidant effect and DNA-protective ability in comparison with their Cu(II) complexes. However, Cu(II) complexes exhibited significant anticancer activity in all tested cell lines. These findings confirm the considerable impact of complexation on bioactivity and suggest that Cu(II) complexes represent model structures for the development of promising anticancer metallodrugs.

Keywords Cu(II) complexes, quinazolinone ligands, cytotoxicity, cancer cell lines

Acknowledgements This work was financially supported by Slovak scientific grant agency, VEGA 2/0071/22 and 1/0429/21.

EFFECT OF AIR OXIDATION ON THE PHASE TRANSITION OF PULMONARY SURFACTANT

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Abstract The pulmonary surfactant is a mixture of lipids and proteins that spreads into a film at the air–lung interface. Its main function is to reduce surface tension at the air–liquid interface in the alveoli. This reduces the work of breathing and prevents alveolar collapse at expiration. Oxidative processes in the lungs, which are highly increased during inflammatory processes or by exposure to some air pollutants, can cause inactivation of pulmonary surfactant function. The effect of oxidation on the function of pulmonary surfactant can be attributed to oxidative alterations of proteins and to the peroxidation and hydrolysis of phospholipids.
In this work, we used differential scanning calorimetry (DSC) to study the effect of air oxidation of commercially available porcine pulmonary surfactant Curosuf (PSur) on its phase transition temperature (T_m) based on the length of exposure of the PSur to the air. During the oxidation process, we kept PSur samples incubated at 37°C and continuously stirred for time periods of 1–14 days. The unoxidized PSur measured right after the opening of the original package had phase transition temperature T_m = 27.44 °C. The first significant changes appeared after 3 days from the start of oxidation, when we observed, in addition to the unoxidized fraction, a separated fraction presumably consisted of oxidized lipids with a slightly higher phase transition temperature (T_m=29.36 °C). As the oxidation time increased, the proportion of oxidized lipids increased, as did the phase transition temperature of the oxidized fraction. After 14 days of oxidation, the PSur sample contained only 1 fraction of lipids with the large shift of T_m to 44.5 °C, and we assume that all unsaturated lipids have been already oxidized. The phase transition after 14 days also increased significantly to 23.4 J/g, while for the first 7 days of oxidation, the enthalpy remained relatively unchanged at 18 J/g. Our experiment proved that the oxidation of pulmonary surfactant significantly affects its phase transition. In our experiments, the oxidation of lipids took a relatively long time, but this process can be accelerated by the presence of free radicals that are present in vivo during inflammation.

Keywords pulmonary surfactant, oxidation, differential scanning calorimetry

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TOWARD SUSTAINABLE CHEMISTRY: RECOVERABLE CATALYSTS FOR DEUTERIUM-LABELED ORGANIC COMPOUNDS

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Abstract

Deuterium (D)-labeled organic compounds find widespread applications in many areas including drugs and their analysis.[1,2] To synthesize the D-labeled compounds, catalysts are commonly used due to their regio- and stereo-selectivity, mild reaction conditions, and broad substrate compatibility.[3] However, the reliance on costly catalysts containing precious metals such as Ir, Pd, and Pt poses sustainability challenges, while the recovery of homogeneous small-molecule catalysts from reaction mixtures is costly and difficult.

Our ongoing research focuses on developing immobilized recoverable and recyclable catalysts to synthesize the D-labeled organic compounds. These basically consist of existing catalysts covalently attached to macromolecules such as polymers which can be easily recovered from the reaction mixtures. Utilizing immobilized catalysts can offer several advantages, such as minimizing metal contamination in products/waste streams and facilitating the efficient recovery and reusability of catalysts.[4] These benefits ultimately contribute to cost reduction in synthesis. The transition to recyclable catalysts represents a significant step toward achieving Circular Chemistry, an environmentally sustainable approach that emphasizes the cyclic reuse of catalysts.[5]

The preliminary results from the research done in our labs will be presented, highlighting the significant results and potential implications of the work.

Keywords

H/D exchange, recyclable, circular chemistry, catalysis, isotopic labeling

References

[1] Jansen-van Vuuren, R. D.; Jedlovčnik, L.; Košmrlj, J.; Massey, T. E.; Derdau, V. Deuterated Drugs and Biomarkers in the COVID-19 Pandemic. *ACS Omega* 2022, *7* (46), 41840–41858.

[2] Pirali, T.; Serafini, M.; Cargnin, S.; Genazzani, A. A. Applications of Deuterium in Medicinal Chemistry. *J. Med. Chem.* 2019, *62* (11), 5276–5297.

[3] Atzrodt, J.; Derdau, V.; Fey, T.; Zimmermann, J. The Renaissance of H/D Exchange. *Angew. Chem., Int. Ed.* 2007, *46* (41), 7744–7765.

[4] Toczko, J. F. 9.9 Catalyst Recovery and Recycle: Metal Removal Techniques. In *Comprehensive Chirality*; Elsevier, 2012; pp 209–227.

[5] Keijer, T.; Bakker, V.; Slootweg, J.C. Circular chemistry to enable a circular economy. *Nat. Chem.* 2019, *11* (3), 190–195.

ASSESSMENT OF LIPOPHILICITY OF NEWLY SYNTHESIZED CARBONIC ANHYDRASE INHIBITORS USING REVERSED-PHASE HPLC AND SCHRÖDINGER COMPUTATIONAL PLATFORM

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Abstract

A series of 24 potential carbonic anhydrase inhibitors useful for glaucoma therapy have been synthesized. The structure of the studied molecules contains a sulfonamide skeleton typical for carbonic anhydrase inhibitors (brinzolamide, dorzolamide) and also an aryloxyaminopropanol chain present in antiglaucoma active beta blockers (timolol, betaxolol). The lipophilicity of newly synthesized substances was assessed using the RP-HPLC method and QikProp module of Schrödinger computational platform. Chromatographic measurements were performed on a Dionex UltiMate 3000 Series UHPLC System using a Symmetry® C18 5 µm, 4,6 x 250 mm column. A 10 µl of methanolic solution of the substance with an approximate concentration of 0.1 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 (224 nm) was chosen for detection. The analysis was performed in five or six methanol/water mobile phases with volume ratios of 90:10, 85:15, 80:20, 75:25, 70:30, 65:35 (*V/V*). The measurement of each substance in each mobile phase was performed three times. Methanolic solution of potassium iodide was a dead time marker. Based on the retention times of the studied substances (t_R) and the 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. The $\log k_w$ value was, thus, obtained, which is used as a lipophilicity parameter corresponding to the aqueous environment. Subsequently, all experimentally determined $\log k_w$ values were compared with partition coefficients (log*P*) obtained by computational method, and the relationship between lipophilicity and the structure of the studied substances was evaluated.

Keywords lipophilicity, RP-HPLC, Schrödinger platform, carbonic anhydrase inhibitors

DNA CLEAVAGE AND CYTOTOXIC ACTIVITY OF COPPER(II) COMPLEXES BASED ON REDUCED SCHIFF BASES DERIVED FROM SALICYLALDEHYDE AND AMINO ACIDS

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Abstract Metal complexes, which under physiological conditions have antioxidant activity and have the ability to bind and cleave the DNA chain, are important for their use as antineoplastic drugs. We synthesized ligands derived from short-chain amino acids and from salicylaldehyde. The prepared ligands of the type of reduced Schiff bases were subsequently used for the preparation of copper(II) complexes. The aim of the study was to test the copper(II) complexes in vitro, for their capability of cleaving DNA structure. Their cytotoxic activity was also confirmed on *S. cerevisiae* by the resazurin redox method which is based on the preserved healthy mitochondrial function.

Keywords copper(II) complexes, anticancer drugs, DNA cleavage activity, cytotoxicity

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PHOTOCHEMICAL TRANSFORMATIONS OF PYRAZOLONE-TYPE SUBSTRATES

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Abstract In recent years, photochemical transformations have gained popularity in organic synthesis. Since most organic molecules cannot absorb visible light, most photochemical reactions require the use of an external photocatalyst, and the metal-based photocatalysts is the most prevalent. Because of their expensive nature and occasionally complex preparation, it is difficult to achieve large-scale syntheses in the industrial setting. Additionally, metal-based photocatalyst represent a big environmental and economic issue, making the development of novel visible-light transformations in the absence of an external photocatalyst an important challenge.
This study presents a method for the visible-light transformation of pyrazolone-type substrates, without the need for an external photocatalyst. These systems are of important biological value because of their common use as peptidomimetics. The irradiation of the selected compounds under different reaction conditions affords a wide array of final products. Among them, products containing 4- and 7-membered rings are achieved. Such scaffolds are challenging to synthesize using the traditional chemistry but are exceptionally desirable in medicinal chemistry due to their unique structural and biological properties. Our method is compatible with various functional groups and does not require an external photocatalyst, which makes it a cost-effective and environment-friendly solution for obtaining these potentially valuable products.

Keywords photochemical transformations, visible-light, bond cleavage, pyrazolone-type compounds

GREEN TEA AND PEPPERMINT IN MIXTURE SYNERGISTICALLY ENHANCE THEIR ANTIOXIDANT CAPACITY

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Abstract Especially in the case of medicinal plants, analysis of their individual constituents does not provide a satisfactory explanation of their effectiveness. In mixtures, such as medicinal plants or their extracts, the substances finally interact among themselves, which can lead to an increase in their efficacy through synergy, where the final response is higher than the expected one, based on summation of the partial effects. Green tea and peppermint tea are favorite beverages commonly prepared as water infusion from Camellia sinensis Kuntze and Mentha × piperita L. leaves. In addition, they are a rich source of polyphenols, natural compounds with great antioxidant capacity, which can be used in food stabilization or in processes, where quenching of free radicals are desired. In this work, we have studied an antioxidant activity of green tea and peppermint lyophilizates in combinations. Diverse mixtures were prepared before and after lyophilization in various extract ratios. In addition, we arranged the equimolar mixtures of polyphenols present in the peppermint tea: rosmarinic acid and in green tea: epigallocatechin gallate and quercetin, respectively. Antioxidant activity of single lyophilizates and compounds and their mixtures as well were measured using in vitro DPPH radical quenching assay and in the DCF cell-based antioxidant assay. The quantification of interaction as a synergism or antagonism was done by the general Median effect equation according to Chou. Interaction analysis has shown mainly synergy in lyophilizates and compound mixtures in both DPPH assay and DCF cell-based antioxidant assay. Synergy among polyphenols from lyophilizates can partly explain the interactions of the lyophilizates on chemical basement. In conclusion, our study confirmed the old practice of combining the medicinal plants into herbal tea mixtures, where they reach higher effects than assumed.

Keywords synergy, green tea, peppermint, antioxidant, polyphenol

Acknowledgements This work was supported by grants VEGA 1/0284/20, VEGA 1/0226/22, and APVV-19-0056.

SYNTHESIS, STRUCTURE, AND BIOLOGICAL ACTIVITIES OF COPPER(II) COMPLEXES OF LIGANDS DERIVED FROM 4-METHOXYSALICYLALDEHYDE AND β-ALANINE AND γ-AMINOBUTANOIC ACID

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Abstract In this study, a series of Cu(II) complexes of Schiff base and reduced Schiff base ligands were prepared. The Schiff bases were prepared from 4-methoxysalicylaldehyde and two short linear amino acids – β-alanine and γ-aminobutanoic acid. The Schiff bases were reduced to prepare the secondary amine compounds. Both Schiff bases and their reduced analogues were used in complexation reactions using copper(II) acetate, chloride, or nitrate. The prepared 4 ligands and 8 complexes were characterized by elemental analysis and spectral methods (^{1}H and ^{13}C NMR, IR). Two of the prepared complexes were suitable for X-ray crystallographic structure determination.

DNA cleavage assay based on partial or full cleavage of plasmid DNA was used to determine antineoplastic activities of the prepared complexes. Agarose gel electrophoresis was used to separate the cleavage products. The complexes were able to cleave pDNA at 1–5 mM concentrations.

Cytotoxicity of the prepared complexes was studied using the Resazurin (7-hydroxy-3*H*-phenoxazin-3-one 10-oxide) model. This test is used as an oxidation–reduction indicator in cell viability assays for yeasts and mammalian cells and cytotoxicity prediction assay for cancer cells. Prepared complexes were able to affect cell survivability in 1–5 mM concentration within 3 hours.

The prepared complexes were used in antiradical (SOD-mimetic) activity INT assay, where their superoxide anion-radical scavenging abilities were determined in 20–55% range, compared to the agent INT (2-(4-Iodophenyl)-3-(4-nitrophenyl)-5-phenyl-2*H*-tetrazolium chloride) at sub-millimolar concentrations.

Keywords copper(II) complexes, Schiff bases, SOD-mimetic activity, cytotoxic activity, DNA cleavage

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SYNTHESIS AND ANTIMICROBIAL PROPERTIES OF QUATERNARY AMMONIUM HOMOCHIRAL SULFONAMIDES

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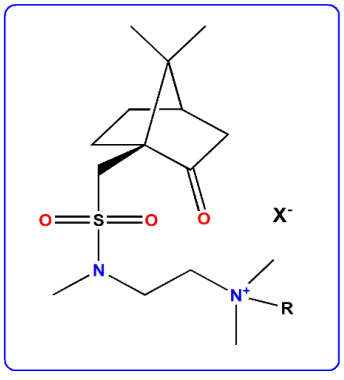
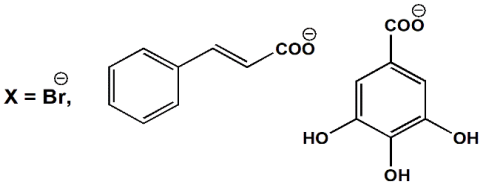
Abstract

The strong bactericidal activity of quaternary ammonium salts (QUATs) with long alkyl chains has been known from 1915 and studied further on a broad range of microorganisms such as bacteria (both G+ and G–) and fungi, certain viruses, and even anticancer agents. The development of resistance in microorganisms toward disinfectants or antibiotics brings the necessity to supply recently applied antimicrobial agents by new, potent, and safe ones, and thus, search for new and effective molecules goes on. The well-known antibacterial effect of essential oils containing bicyclical camphor or borneol brought us to the idea to design and synthesize QUATs bearing hydrophobic camphor-derived sulfonamides, with biologically active contra anions hoping that incorporation of more important antimicrobial active structures in one compound will improve their bioactivity. The introduction of an ester group into the molecule contributes to better biodegradability of such compounds. A group of homochiral quaternary ammonium sulfonamides bearing hydrophobic camphor-derived moieties were synthesized and characterized. The described synthetic procedure is quick and efficient. The novel quaternary ammonium bromides, cinnamates, and gallates were tested as antimicrobial and antifungal agents.





R = $-C_{10}H_{21}$, $-C_{12}H_{25}$, $-C_{14}H_{29}$, $-C_{16}H_{33}$

$-CH_2COOC_{10}H_{21}$, $-CH_2COOC_{12}H_{25}$
$-CH_2COOC_{14}H_{29}$, $-CH_2COOC_{16}H_{33}$

Keywords quaternary ammonium salts, antimicrobial, camphor sulfonamide

ANTIPROLIFERATIVE METAL COMPLEXES OF THE FLUORINATED CURCUMIN DERIVATIVE: 1,7-BIS(4-FLUOROPHENYL)HEPTA-1,6-DIENE-3,5-DIONE

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Abstract Curcumin and its derivatives have found in the last decades multiple applications in various fields of our daily life. This natural antioxidant compound can be found not only in the food industry and cosmetics but also in the pharmaceutical industry as a potential remedy for multiple diseases. However, curcumin and its derivatives have an inconvenient, poor bioavailability and fast metabolism, which induces the formation of several metabolites and so forth the poor bioactivity in the human body. Therefore, the derivatization of curcuminoids or the coordination to metal centers constitutes a solution for keeping the molecules of aromatic β-dicarbonyl compounds not decomposed. In this way, the curcuminoids are capable to reach biological targets and develop their antioxidant, anti-inflammatory, immunomodulatory, anticancer activities, etc.
Our work was focused on the preparation and structural characterization of transition metal complexes of a mono-fluorinated curcumin derivative. Palladium and ruthenium complexes have been synthesized and biologically tested in vitro on human cancer cell lines and normal healthy cells, using the MTT assay. The palladium complexes were tested on human colorectal adenocarcinomas DLD-1 and RKO and normal colorectal cell CCD-18. The ruthenium complexes were tested on adenocarcinoma human alveolar basal epithelial cell A549, human liver cancer cell HepG2, and lung fibroblast normal cell line HEL299. The biological activity was expressed by the determination of cytotoxicity (IC_{50} values) of synthesized complexes toward the mentioned cancer cell lines. Both categories of complexes show a significant cytotoxicity, over the values shown by classical chemotherapeutic drug CisPt. Moreover, the palladium complexes were proved a fold higher activity as the free ligand displayed in the same biological assay. These preliminary results open a perspective for us to search further for optimization of the structure and activity of curcumin derivatives.

Keywords fluorinated curcuminoid, palladium complexes, ruthenium complexes, cytotoxicity, antiproliferative activity

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The effect of 5-Fluorouracil and LactobaciLli treatment on the model of the intestinal barrier in vitro

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Abstract Caco-2 cell line, a type 1 human intestinal epithelial cell, exhibits spontaneous differentiation and polarization, forming distinct surfaces and structures. We used this cell line as a model to study intestinal mucositis induced by the anticancer and antimetabolic drug 5-fluorouracil (5-FU). We monitored changes in the relative gene expression of enterocyte differentiation markers aminopeptidase (ANPEP), tight junction proteins claudin-1 and occludin (CLDN1, OCLN), and citrate synthase as a marker of intermediary metabolism. We also tested the potential of modulating mucositis through co-cultivation with *Limosilactobacillus reuteri* E.

Caco-2 cells were cultured at 37 °C, 5% CO_2 atmosphere in RPMI medium. Subsequently, cells ($3.85x10^5$ cells) were transferred onto 12-well hanging inserts. The experiment lasted for 15 days, and samples were divided into five groups:

The "time 0" group was cultured for 24 hours. Subsequently, total RNA was isolated from the samples and transcribed into cDNA.

On the 14th day, 5-FU (100 µmol/l) was added to induce mucositis in two groups (5-FU and LRE+5-FU). Lactobacilli were added to the LRE and LRE+5-FU groups on the 15th day for 6 hours. The control samples were used as cultures without treatment. Total RNAs were isolated, transcribed into cDNA, and used for gene expression analysis.

The differentiation of Caco-2 cells into enterocytes was confirmed by increasing trans-epithelial electrical resistance (more than 10-fold between days 0 and 15) and increasing expressions of occludin (12-fold), claudin-1 (64-fold), and aminopeptidase (23-fold).

The addition of 5-FU resulted in decreased gene expression of tight junction proteins compared to the control group (CLDN1 by 96%, OCLN by 60%). Similarly, ANPEP and citrate synthase showed reductions of 90% and 70%, respectively. Administration of lactobacilli improved three parameters, with the expression levels of CLDN1 reaching 40% of the control, ANPEP at 25%, and citrate synthase at 60%.

Keywords Caco-2, 5-fluorouracil, *Limosilactobacillus reuteri* E, intestinal mucositis, tight junction proteins

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SYNTHESIS AND EVALUATION OF BIOLOGICAL ACTIVITY OF NEWLY DESIGNED HYDROXAMATES AS POTENTIAL HDAC INHIBITORS

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Abstract

Histone deacetylases (HDACs) are metalloenzymes involved in the regulation of fundamental cellular processes, such as cell cycle progression, differentiation, and tumorigenesis. The abnormal function of HDACs can induce severe human diseases, including cancer, pulmonary disease, and neurodegenerative disorders. Histone deacetylase inhibitors (HDACi) represent a relatively new generation of drugs with the ability to affect the expression of specific genes, which can repair controlled proliferation or damage apoptotic processes in tumor cells. HDAC inhibitors have considerable anticancer activity forming a complex with the Zn^{2+} ion in the catalytic pocket of enzymes.

Based on the common three-motif pharmacophore model of HDACi, we have designed a series of hydroxamate derivates. Compounds with variously substituted anilids as a capping group and hydroxamic acids as a zinc-binding group were synthesized. The antiproliferative activity of the series was investigated in the monocytic leukemia cell line THP-1 and evaluated by WTS-1 analysis. The first registered HDACi, Vorinostat®, was used as a positive control for the detection. The most important part of the study was to verify the ability of the hydroxamates to inhibit the enzymatic activity of HDAC classes I and II (HDAC1, HDAC10). Based on the previous data demonstrating the antiproliferative activity of new hydroxamic acids in THP-1 cells, for the assessment of HDAC inhibition, we selected compounds whose antiproliferative effect after 72 h of incubation was quantitatively comparable to the effect of Vorinostat®. A series of tests confirmed that the synthesized hydroxamic acids have antiproliferative activity, an effect on the cell cycle progression, and induction of apoptosis.

The most potent inhibitors are compounds that contain methyl or bromine substituent at the para position at the aromatic ring with IC_{50} less than 1.6 μM.

Keywords hydroxamic acid, histone deacetylase inhibitors, anticancer agents

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A STUDY OF THE MICELLIZATION OF CARBISOCAINIUM CHLORIDE IN AQUEOUS SOLUTION USING THE OPTICAL DENSITY METHOD

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Abstract The critical micellar concentration (CMC) of carbisocainium chloride in an aqueous solution was determined by measuring the optical density on the 96-well microtiter plates. The measurement was carried out using the Epoch 2 Microplate Reader in distilled water at temperatures T = 294.15–313.15 K. The CMC values were determined by the dependence of the optical density (*OD*) on the concentration (*c*) of carbisocainium chloride. The thermodynamic parameters of micellization, molar Gibbs energy ($\Delta G°$), enthalpy ($\Delta H°$), and entropy ($\Delta S°$) were calculated according to the pseudophase separation model and subsequently analyzed. In the measured temperature range, the contribution of enthalpy increased with increasing temperature, and the contribution of entropy, on the contrary, decreased. It was also found that temperature does not affect $\Delta G°$, and at the same time, the effect of temperature on $\Delta H°$ was more significant. This means that as the temperature increases, the micellization process becomes more exothermic. Finally, the enthalpy-entropy compensation was also determined, which showed a linear course. The value of the compensation temperature (*Tc*) was 304.16 ± 1.51 K.

Keywords critical micelle concentration, thermodynamics, optical density

SIMPLE AND SENSITIVE ANALYSIS OF CLENBUTEROL IN URINE MATRICES BY UHPLC–MS/MS METHOD WITH ONLINE-SPE SAMPLE PREPARATION

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Abstract

Clenbuterol is one of the most misused anabolic agents in professional sports. Therefore, the monitoring of clenbuterol in body fluids such as human urine is related to the development of rapid, selective, sensitive analytical methods that produce the reliable results. In this work, these requirements were met by a two-dimensional separation method based on online solid-phase extraction coupled with ultra-high-performance liquid chromatography–tandem mass spectrometry (SPE–UHPLC–MS/MS). The developed method provides favorable performance parameters, and it is characterized by minimum manual steps (only dilution and the addition of an internal standard) in the sample preparation. A limit of quantification (LOQ) of 0.1 ng/mL, excellent linearity (0.9999), remarkable precision (1.26% to 8.99%), and high accuracy (93.1% to 98.7%) were achieved. From a practical point of view, the analytical performance of the validated SPE–UHPLC–MS/MS method was demonstrated on blinded spiked urine samples from 10 healthy volunteers. The estimated concentrations of clenbuterol were in accordance with their corresponding nominal values, as supported by the precision and accuracy data (relative standard deviation ≤5.4%, relative error ≤11%). The fulfilment of the World Anti-Doping Agency's screening and confirmation criteria indicates that the proposed method is suitable for implementation in routine use in toxicologic and antidoping laboratories. Due to its high orthogonality and separation efficiency, the SPE–UHPLC–MS/MS method should also be easily adapted to the separation of structurally related compounds (such as clenbuterol metabolites). Thus, future antidoping applications could also include monitoring of clenbuterol metabolites, providing a longer detection widow.

Keywords

clenbuterol, ultra-high-performance liquid chromatography, tandem mass spectrometry, online SPE extraction, antidoping analysis

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SYNTHESIS OF ANTIBACTERIAL CINNAMAMIDES

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Abstract Nature is an inspiring and rich source of compounds with biological activity. Most of the drugs approved for clinical use in the last 30 years have been small molecules either directly of natural origin or structural analogues of natural products. Many plant secondary metabolites show biological activity and serve as templates for research and development of synthetic analogues. One such example is cinnamic acid and its derivatives, such as coumaric, ferulic, caffeic, or sinapic acid, which can be isolated from many plant sources. Cinnamic acid and its derivatives have shown interesting antimicrobial, antiproliferative, antiparasitic, neurological, and anti-inflammatory activities.
Substituted *N*-phenyl amides of 3,4-dichlorocinnamic acid have been the subject of earlier studies. Some derivatives have shown excellent antimicrobial and antimalarial activities. The newly synthesized compounds that are the subject of this study are direct structural analogues differing in phenyl substitution. Forty derivatives with multiple substitutions of both electron-donating and electron-accepting groups were synthesized and characterized, and the antimicrobial activity against Gram-positive bacteria of the genus *Staphylococcus* was determined. Some derivatives have shown an excellent activity comparable to the clinically used antibiotics ampicillin and ciprofloxacin.

Keywords cinnamamide, synthesis, antibacterial activity

Acknowledgements The work was supported by project MUNI/A/1210/2022.

EFFECT OF SILVER IONS ON ANTIOXIDANT AND ANTIBACTERIAL ACTIVITY OF EXTRACTS OBTAINED FROM *OPHIOCORDYCEPS SINENSIS*

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Abstract

Mushrooms from the genus *Cordyceps* are characterized by a wide range of biological effects due to the diverse amount of substances contained in them and are used as an important source of bioactive compounds. In China, they have been used as a medicinal preparation of traditional Chinese medicine for centuries. They represent an important source of bioactive substances that are used in the treatment of various diseases. The presence of various chemical components makes them interesting also from the point of view of searching for new master structures. There are also a number of professional works dealing with the ability of substances isolated from the extracts of these mushrooms to form complexes with various metals. It is believed that the polysaccharides, which are the main component of the extracts, are involved in the complexation with metals. This improves and deepens their biological effects.

The work deals with antioxidant and antimicrobial activities. The possibility of the formation of complexes of extracts with silver ions was monitored. Extracts alone as well as extracts with added $AgNO_3$ showed antioxidant activity. Among *Ophiocordyceps sinensis* extracts without $AgNO_3$ addition, the highest antioxidant activity was shown by the sample extracted by reflux, which was cultivated on chickpea (2R), with an IC_{50} value of 6.11 $mg.ml^{-1}$. The sample obtained by reflux extraction cultured on corn (1R) had the lowest activity, with an IC_{50} value of 9.34 $mg.ml^{-1}$. The antioxidant activity was reduced by the addition of silver nitrate in some samples; on the contrary, it was increased or even manifested in some, even though the sample without $AgNO_3$ did not show it. After adding $AgNO_3$ to the extracts obtained by maceration, the highest antioxidant activity was recorded in the sample cultivated on chickpea (2M), with an IC_{50} value of 4.99 $mg.ml^{-1}$, and the lowest activity was recorded in the 2R sample, which had an IC_{50} value of 10.08 $mg.ml^{-1}$. *Ophiocordyceps sinensis* extracts showed antimicrobial activity only after adding $AgNO_3$, and pure extracts did not show any effectiveness against the tested pathogenic bacteria (*Escherichia coli* and *Staphylococcus aureus*). Antibacterial activity was proven only against a strain of Gram-negative bacteria.

Keywords *Ophiocordyceps sinensis*, **antioxidant activity, antimicrobial activity**

SYNTHESIS AND STUDY OF BIOLOGICAL PROPERTIES OF NEW CARBAMATES WITH A MODIFIED BASIC FRAGMENT IN THE ARYLOXYAMINOPROPANOL CHAIN

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Abstract

Drugs with a carbamate functional group are an important part of many drugs and prodrugs. Today, this functional group is part of many approved drugs that act as chemotherapeutics (mitomycin C, irinotecan), cholinesterase inhibitors in the treatment of neurodegenerative diseases (rivastigmine, neostigmine, physostigmine, pyridostigmine), human immunodeficiency virus (ritonavir, amprenavir), anticonvulsants (felbamate, retigabine), and muscle relaxants (methocarbamol, metaxalone). Carbamate functional group is also part of prodrugs with different therapeutic applications (irinotecan, bambuterol, gabapentin enacarbil, capecitabine). Propranolol was the first clinically approved β-blocker introduced by James Black in 1964, and his discovery is considered a turning point in the treatment of angina pectoris and is one of the most significant points in pharmacology of the twentieth century. Since then, more than 20 new β-blockers have been patented. β-blockers are a specific type of drugs that are indicated for various diseases: angina pectoris, cardiac arrhythmias, atrial fibrillation, heart failure, hypertension, glaucoma, hyperthyroidism, and other. Currently, the design and synthesis of drugs within the framework of disease therapy are focused on the preparation of new drugs that could influence several biological systems at the same time. Using this design, two or more pharmacophores are combined with each other within a single molecule, while this new molecule, a new drug, should have properties that are characteristic of both starting pharmacophores. These new multipotent compounds are referred to as "multi-target-directed ligands" (MTDLs).

The work deals with the synthesis of new carbamate derivatives with an aryloxyaminopropanol fragment in the molecule. By modifying the basic part of the aryloxyaminopropanol chain to benzylpiperidine and substituted benzylpiperazines, a series of new derivatives were prepared. The ether functional group was also replaced with an ester functional group in the aminopropanol chain. Selected compounds were tested for their antimicrobial and anticholinesterase activities and their effect on β-adrenoreceptors.

Keywords

synthesis, carbamates, aryloxyaminopropanol, piperidine, piperazine

NEW FLUOROPHORES FOR THE DETECTION OF GLYCANS

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Abstract Changes in the composition of glycans correlate with the progression of many diseases, and therefore they are investigated as disease markers. However, their analysis is complicated because they do not contain a chromophore or charges that would enable their electrophoretic separation. Our goal was to develop a new fluorescent marker covalently bound to glycans providing fast labeling kinetics, high quantum yield, increased detection sensitivity using MS, and at the same time, carrying a charge in the structure, which will enable the studied glycans to be separated electrophoretically.

The basic skeleton of this fluorophore is based on pyrrole and indolizine. The synthesis was based on two building blocks, synthon A and synthon B, which were subsequently connected. The plan was to prepare variants of the fluorophore carrying electron-donating or electron-accepting groups. These substitutions allow for variability in the absorption maximum wavelength and, thus, fluorescence wavelength. The molecule also holds a trimethylammonium functional group responsible for electrophoretic mobility and a propanoate chain, which is necessary to attach the fluorophore to the glycan.

The prepared substances will also be characterized in cooperation with the Institute of Analytical Chemistry of the Academy of Sciences of the Czech Republic. The usability of the newly developed fluorophores will be demonstrated by profiling glycoproteins associated with breast cancer.

Keywords fluorophore, pyrrole, glycan

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Creation and optimization of the In vitro model for studying inflammation-driven changes of lipid metabolism in hepatic cell line

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Abstract Besides direct immunological function, inflammation is associated with complex changes in organism such as quantitative and qualitative alternations in lipid metabolism (decreased HDL, LDL levels, pro-inflammatory remodeling of HDL, disrupted cholesterol efflux, increased LDL oxidation, etc.). These alternations may explain the increased cardiovascular morbidity in patients with chronic inflammatory diseases. The research in this area is important; however, there are only a few reliable models for studying inflammatory changes in lipid metabolism recently. Therefore, we decided to create and analyze an *in vitro* model of inflammation-driven changes in hepatic cell line HepG2 induced by conditioned media (CM) from THP-1 monocytes exposed to different inflammatory stimuli: lipopolysaccharide (LPS) for 8 hours, phorbol-12-myristate-13-acetate (PMA) for 24 hours, or their combination (PMA for 24 hours, LPS 4 hours). After stimulation, the medium in THP-1 cells was replaced with serum-free RPMI, which was collected after 24 hours and added to HepG2 cells. The effect of CM from differently stimulated THP-1, variable exposure times, and CM dilutions on mRNA expression in HepG2 were tested by quantitative real-time PCR. According to our results, the most suitable was the stimulation of HepG2 cells with CM from THP-1 treated with a combination of PMA and LPS diluted in a ratio 1:3. While 4-hour long exposure of HepG2 to CM influenced mainly mRNA expression of inflammatory genes and some transcription factors (increased IL-β, NF-κB, SAA, decreased PPARα expression), the longer exposures (20/24 hours) were associated mainly with changes in lipid metabolism-associated genes (relative decrease of ApoAI, PON1, ABCA1, apoC3 expressions when compared to CM-unexposed controls at the same time points).

Keywords inflammation, lipid metabolism, HepG2

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1,2,3-TRIAZOLIUM SALTS: THE PATH TO MESOIONIC N-HETEROCYCLIC OLEFINS

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Abstract 1,3,4-Trisubstituted-1*H*-1,2,3-triazolium salts, which upon deprotonation give access to mesoionic N-heterocyclic carbenes (MICs), are a widely investigated class of compounds. Because of MICs' unique electronic properties, they quickly found success in the field of (organo)catalysis. Similarly, 1,3,4,5-tetrasubstituted-1*H*-1,2,3-triazolium salts upon deprotonation yield mesoionic N-heterocyclic olefins (mNHOs)[1], which raised our interest as a new class of carbon-based ligands. mNHOs were first described by Hansmann in 2020[1], and so far, limited examples have been reported. They are stronger donors than N-heterocyclic carbene ligands but form weaker metal-ligand bonds.[1] To date, only three rhodium complexes with different mNHOs have been described.[1]
Herein, we present the synthesis of 1,3,4,5-tetrasubstituted-1*H*-1,2,3-triazolium salts and their corresponding mNHOs. A series of differently substituted 1,2,3-triazolium salts were designed to explore the coordinating ability of mNHOs to transition metals and the effects of different substituents on coordination as well as properties of potential mNHO-transition metal complexes.

Keywords triazolium salts, mesoionic *N*-heterocyclic olefins, coordination

Reference

[1] Hansmann, M. M.; Antoni, P. W.; Pesch, H. Stable Mesoionic N-Heterocyclic Olefins (MNHOs). *Angewandte Chemie International Edition* **2020**, *59* (14), 5782–5787.