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BOOK OF ABSTRACTS

51ST CONFERENCE SYNTHESIS AND ANALYSIS OF DRUGS (SAD 2023)

ORAL PRESENTATIONS

I. PLENARY LECTURES

CRYSTALLIZATION-INDUCED DIASTEREOMER TRANSFORMATION: A COST-EFFECTIVE ROUTE TO DIASTEREOSELECTIVELY PURE DRUGS – THE CASE OF APREPITANT

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Abstract

The pharmaceutical industry produces a large amount of waste per 1 kg of active compound, which is much higher compared to other industries, resulting in more than 3 million tons of waste per year. The reason for it is that the preparation of active pharmaceutical ingredients (API) in most cases requires multistep synthetic and purification processes. Very often, these processes contain resolution techniques for the separation of homochiral organic molecules or the isolation of desired diastereomers. Most pharmaceutically active ingredients are known to be chiral, and one enantiomer or diastereomer is generally preferred over the racemic mixture (Stinson, 2001). Therefore, the implementation of novel cost-effective methods for the isolation of a single stereoisomer is highly desired in pharmaceutical production.

One of the efficient methods for the isolation of single stereoisomers from the reaction mixture is called crystallization-induced stereoisomer transformations (CISTs). CIST can, in principle, be divided into two main categories: crystallization-induced enantiomer transformations (CIETs) and the much more common crystallization-induced diastereomer transformations (CIDTs) (Brands & Davies, 2006; Kolarovic & Jakubec, 2021). The use of crystallization-induced diastereomer transformations is presented on the industrial production of the drug aprepitant **1**.

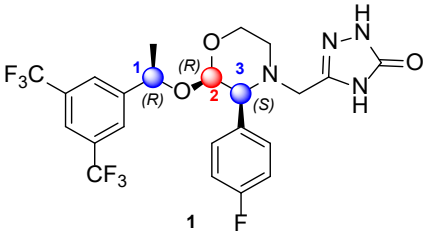
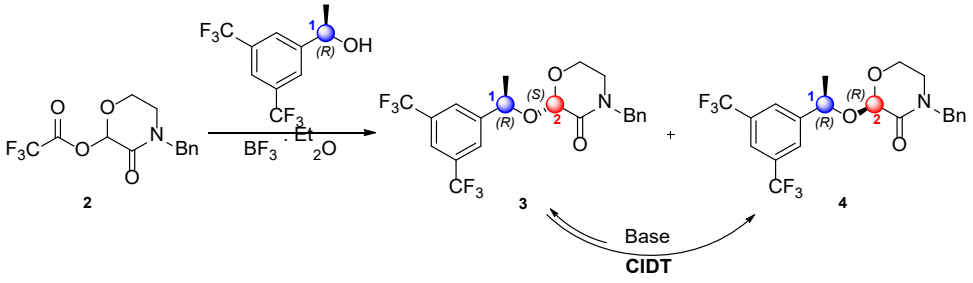


Figure. The structure of aprepitant **1**.

Aprepitant **1** is an orally active human antagonist of neurokinin NK_1 receptors, chemically known as 3-{[(2*R*,3*S*)-2-[(1*R*)-1-[3,5-bis(trifluoromethyl)phenyl]ethoxy]-3-(4-fluorophenyl)-morpholin-4-yl]methyl}-4,5-dihydro-1H-1,2,4-triazol-5-one (Figure), developed by Merck and marketed with a trading name of Emend, used for the prevention and treatment of acute and delayed nausea and vomiting in adults related to anticancer chemotherapy. The aprepitant molecule contains three stereogenic centers, of which two are part of the morpholine skeleton. Several synthetic strategies have been developed for the preparation of aprepitant **1** (Zhao et al., 2002; Brands et al., 2003; Dorn et al., 1998; Brands et al., 2006), among which the synthesis starting from enantiopure (1*R*)-1-[3,5-bis(trifluoromethyl)-phenyl]ethan-1-ol was found to be efficient and scalable.
A few methods of the synthesis (1*R*)-1-[3,5-bis(trifluoromethyl)phenyl]ethan-1-ol have been reported mainly from corresponding acetophenone. From them, we have selected the catalytic asymmetric transfer hydrogenation developed by Noyori (Noyori & Hashiguchi, 1997; Fujii et al., 1996; Murata et al., 1999; Palmer & Wills, 1999) using (1*S*,2*R*)-*cis*-1-aminoindan-2-ol and dichloro(*p*-cymene)Ru(II)dimer as chiral ligand and metal source for reduction. As a safe and benign hydride source, propane-2-ol has been used as a stoichiometric reductant. The enantiopure alcohol was prepared with an average yield of 94% and about 90% e.e. The enrichment via recrystallization through DABCO inclusion complexes from *n*-heptane and subsequent DABCO removal led to a purity of alcohol of approximately 99% e.e. (Hansen et al., 2003). The overall yields from starting 1-[3,5-bis(trifluoromethyl)phenyl]ethan-1-one to (*R*)-alcohol were in the range of 67–76 %. The second building block racemic 4-benzyl-3-oxomorpholin-2-yl 2,2,2-trifluoroacetate **2** was prepared from *N*-benzylaminoethanol and aqueous glyoxylic acid followed by acylation with TFAA.



Scheme. Trans-acetalization and crystallization-induced diastereomer transformation (CIDT) processes toward (R,R)-diastereomer **4**.

The crucial step of the synthesis of aprepitant **1** involves a Lewis acid-mediated trans-acetalization coupling of 4-benzyl-3-oxomorpholin-2-yl 2,2,2-trifluoroacetate **2** with enantiopure (*R*)-1-(3,5-bis(trifluoromethyl)phenyl) ethanol that provided a 45:55 mixture of acetal diastereomers **3** and **4** which was converted to a desired *R,R*-isomer **4** in 72% yield via a crystallization-induced diastereomer transformation (CIDT), involving base-catalyzed equilibration in solution. The (*R,R*)-diastereomer **4** is less soluble in the reaction medium and crystallizes out. However, (*R,S*) diastereomer **3** is racemized in the solution by base-mediated deprotonation with lipophilic potassium salt of tetrahydrolinalool. The addition of 4-fluorophenylmagnesium bromide to intermediate **4** was almost quantitative. The adduct was after quenching hydrogenated at ambient temperature and pressure of 1.5 atm in the presence of palladium on charcoal. The enantiomerically and diastereomerically pure aprepitant **1** was finally prepared by reaction with triazolinonyl chloride.
Although there are certain drawbacks and limitations of this method, crystallization-induced diastereomer transformation (CIDT) is widely used in the synthesis and industrial production of active pharmaceutical ingredients (API) due to its efficiency and cost-effectiveness, in which crystallization is combined with racemization into one pot deracemization process leading to enantiomerically and/or diastereomerically pure products in almost theoretical yield.

Keywords CIDT, crystallization-induced diastereomer transformation, aprepitant, active pharmaceutical ingredients

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AMINOPEPTIDASE N AS A POTENTIAL DRUG TARGET

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Abstract Aminopeptidase N (APN) is a broad specificity zinc metallopeptidase with many functions that do not always depend on its enzymatic activity. Among others, it is involved in tumor angiogenesis and metastazing and also serves as a cellular receptor of some coronaviruses. Some APN inhibitors, such as bestatin or tosedostat, were used or tested as anticancer drugs in the past. Within the past two decades, we have prepared several series of potential APN inhibitors. Some of them reached interesting values of inhibitory activity and were also successfully tested for antiproliferation activity in cancer cell lines. We also performed QSAR studies with APN inhibitors prepared by us and other s.

Keywords aminopeptidase N, inhibitors, antitumor activity, anti-infectious activity

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PROGRAMED AND IMMUNOGENIC CELL DEATH MECHANISMS INDUCED BY METAL-BASED DRUGS IN TUMORS

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Abstract After the first studies on cisplatin in 1965, the development of metallodrug gave rise to metal-based treatment regimens in cancer care. There are several clinical trials ongoing, and nowadays, the preclinical testing of active metal compounds benefits from unprecedented abundance of investigational methods. Still, a few new molecules reached the biopharmaceutical pipelines and the antitumor drug market.

Recent studies proved that the metal compounds are able to induce programed cell death in tumor cells and will more likely become prodrugs. Some of the metal drugs display complementary biologic effect to the monoclonal antibody-based targeted therapy, such as VEGF or EGFR inhibitors, PARP inhibitors, and immune checkpoint inhibitors. Moreover, if they can simultaneously modulate the antitumor immune response in favor of the host immune system, they could serve as adjuvants of immune therapy.

In this respect, we identified the active Pt (II) complexes with curcuminoid ligands, proapoptotic Re(I) and Rh(II) dendrimers, selective Ga(III) compounds, and ferrocene derivates, which damage the tumor cell DNA and immunogenic Pd(II) complexes having the potential to modulate the cytotoxic CD8+ and helper CD4+ T lymphocyte expressions and immune activations. The metal-based compounds, free or encapsulated in targeted nanostructures, if didn't prove to be apoptosis inducers, were re-evaluated for their capacity to trigger two "metallomic" programed cell death pathways: ferroptosis and cuproptosis, seeking evidence that beyond the intracellular accumulation and DNA binding, metal-based drugs exert a fine-tuning on the cells physiological trace metal elements distribution, leading eventually on growth inhibition and exposing tumors to immune cell attack.

Despite the increasing use of biological drugs in cancer care, in the era of the precision medicine, the metal-based drugs still have a central role in the treatment of malignant diseases.

Keywords metallodrugs, cancer chemotherapy, apoptosis, cellular immunity

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NATURAL METABOLITES IN CURRENT PHARMACOTHERAPY

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Abstract The WHO estimates that 80% of the world's human population primarily uses traditional medicine in healthcare. Selection of plant species is based on empirically obtained knowledge and their use in folk medicine (ethnomedicine/ethnopharmacology), botanical relatedness of plants, availability of the analyzed material, or the (non)existence of data on bioactivity or on content substances, knowing that the process of developing a new drug is lengthy, expensive, and uncertain.

The main advantages of natural substances include structural diversity and the fact that less than 0.1% of microorganisms in soil have been studied so far; only about 70,000 fungi out of an estimated 2–5 million species have been identified, or only about 800,000 out of a total of about 20 million insect species have been identified. The limiting factors of using natural substances are their toxicity, bioavailability, solubility, and isolation in the necessary quantity. Cell cultures and biotechnology are, therefore, increasingly used in plants.

Even in the era of biological drugs, natural substances do not lose their relevance. There are currently more than 100 ADCs (antibody-drug conjugates) in various stages of clinical trials (from preclinical to phase III studies), containing warheads from natural sources derived from terrestrial and marine eukaryotic and prokaryotic organisms.

Botanical drugs – extracts approved by the FDA (Veregen, crofelemer) – are also entering the market. One of the reasons for using mixtures is that many disorders have a multifactorial etiology, and additive or synergistic effects can occur between the mixture's components, making the extract more effective than isolated pure compounds.

Keywords secondary metabolites, phytotherapy, botanical drugs

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DRUGS IN THE ENVIRONMENT

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Abstract

Drugs of different chemical structures become increasingly important potential environmental contaminants as pharmacotherapeutic options develop. Their effects on microorganisms, plants, and animals are still being discovered and described. Drugs have been present in nature since they were first prepared synthetically.
One of the first synthetic drugs is acetylsalicylic acid (patent, Bayern, 1899). Its high consumption for treatment may have caused that as early as 1977, acetylsalicylic acid was detected on the effluent of a sewage treatment plant (Missouri River) in an amount of 8.64 (0.55–28.69) kg over a 10-month period (Hignite & Azarnoff, 1977).
Paracetamol has also been detected in the environment and is readily accumulated worldwide (concentrations in the units or tens of ng/l). Bacteria have already been described in the literature that are able to use paracetamol as a carbon and energy source due to the specialized systems and metabolic pathways (Wu et al., 2012). In contrast, studies (Johnston et al., 2002) have found that snakes die after consuming paracetamol.
World consumption of Diclofenac exceeds 2,400 tonnes per year. A study (Santos et al., 2010) described renal and gastrointestinal disturbances in vertebrates. The s (Oaks et al., 2004) described the extinction of up to 95% of the African vulture population after the consumption of diclofenac-treated livestock.
Thanks to state-of-the-art instrumental methods, we can detect and quantify even ultra-low concentrations of pharmaceutical substances in the environment and study the influence of different chemical structures on environmental constituents. By setting up the right pharmaceutical management, we can then ensure a reduction in contamination and potential changes in a wide range of pharmaceuticals that we use in the context of our lifestyle.

Keywords

drugs, detection, effect, environment

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PYRAZINAMIDE DERIVATIVES: ANTIMICROBIAL ACTIVITY AND BEYOND

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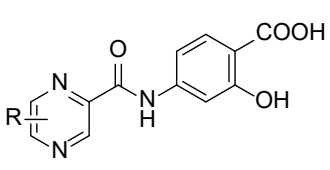
Abstract In the opening parts of the lecture, we will recapitulate the latest theories of the mechanism of action of the first-line antitubercular pyrazinamide (pyrazine-2-carboxamide, PZA). We will present our new antimicrobial derivatives of PZA, including hybrid compounds consisting of PZA and 4-aminosalicylic acid fragment (**I**) (Bouz et al., 2023) and simple derivatives of 3-aminopyrazine-2-carboxamide (**II**). Compounds of general structure **II** were designed as inhibitors of mycobacterial prolyl-tRNA synthetase (Pallabothula et al., 2022). We will also present the results of our hit-expansion study on antistaphylococcal compound **III**, which might be considered a derivative with inversed carboxamide linker.



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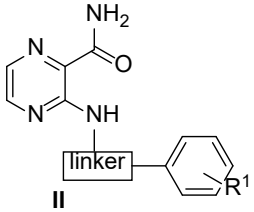

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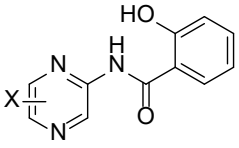

III

Keywords antimicrobial, drug design, prolyl-tRNA synthetase, pyrazinamide

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II. SHORT TALKS

STRUCTURAL ANALYSIS OF DITERPENOIDS ISOLATED FROM THREE *PLECTRANTHUS S.L.* SPECIES

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Abstract The genus *Plectranthus s.l.* (Lamiaceae) consists of three distinct genera: *Coleus*, *Plectranthus s.s.*, and *Equilabium*, which can be genetically and morphologically distinguished from one another (Paton et al., 2019). Due to its significant contribution to traditional medicine, particularly in treating digestive, respiratory, genitourinary, and dermatological disorders, there has been extensive research on the phytochemical composition of *Plectranthus s.l.* All these researches have revealed that diterpenoids are the most prominent group of secondary metabolites found in these plants (Lukhoba et al., 2006).
Our current study focused on exploring the methanolic extracts derived from the aerial parts of *C. comosus*, *C. forsteri* 'Marginatus', and *P. ciliatus*. Through our investigation, we successfully isolated 14 diterpenoids from these extracts, which belong to the abietane, *ent*-clerodane, and *ent*-kaurane classes. Notably, three of these diterpenoids were identified as new natural products, and we also re-evaluated the structure of a known diterpenoid.

Keywords diterpenoid, NMR, 2D NOESY, *Plectranthus*, spirocoleon

Acknowledgements The study was supported by Grant Agency of Masaryk University (MUNI/A/1688/2020) and the NKFIH, Hungary (K-134704).

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UREASE INHIBITION AS A TOOL AGAINST PATHOGENIC MICROBES: THE ROLE OF METAL COMPLEXES

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Abstract Urease (EC 3.5.1.5) is an enzyme containing and dependent on nickel ions in its active center. It is widespread in nature and found in bacteria, fungi, algae, plants, and other species. The enzyme is also exprimated in several types of pathogenic bacteria and is an important virulence factor in these microorganisms. Prominent among them is *Helicobacter pylori*, an important human pathogen responsible for a variety of adverse medical conditions, the most severe among them being peptic ulcer and gastric cancer. Thus, the development of novel inhibitors of urease represents an important challenge for medicinal chemistry.
Metal complexes are among the most efficacious inhibitors of urease (Habala et al., 2018). Their inhibitory activity depends on the central metal atom as well as on the type of ligands and their arrangement. Bismuth compounds have long been used in the treatment of peptic ulcers and *Helicobacter pylori* infections, with urease inhibition playing an important role in their activity. Many other metal ions exert marked antiurease activity. As part of our work on the inhibition of enzymes by metal complexes, we have been studying the inhibition of urease by complexes with various central metal atoms. The highest inhibition was achieved by copper complexes. The studies are also of theoretical importance as they provide interesting insights into the mechanism of enzyme inhibition by metal complexes.

Keywords urease, enzyme inhibition, metal complexes, bioinorganic chemistry

Acknowledgements This study was supported by the grant VEGA 1/0145/20.

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OPTIMIZATION OF CAPILLARY ELECTROPHORESIS – MASS SPECTROMETRY METHOD FOR ANALYSIS OF MONOCLONAL ANTIBODIES

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Abstract Monoclonal antibodies (mAbs) are a growing group of biopharmaceuticals that are used in therapy for various types of diseases. Structurally, mAbs are large proteins that may potentially cause an immune reaction. Therefore, quality control is required to ensure mAb safety when introduced into organism during treatment. Multiple analytical techniques are used for mAb quality control including electromigration techniques such as capillary electrophoresis (CE). CE has been shown in recent years as suitable for analysis of proteins due to high efficiency separation, low operating costs, low sample, and solvent consumption. CE coupled to mass spectrometry (MS) detection represents a powerful tool for analysis of proteins such as mAbs. This work is focused on the optimization of CE–MS separation and detection conditions for quantitative analyses of various types of mAbs, involving infliximab and bevacizumab. It also demonstrates potential of the developed analytical method for future pharmaceutical applications.

Keywords monoclonal antibodies, capillary electrophoresis, mass spectrometry, method optimization

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NOVEL 1,3,5-TRIAZINYL AMINOBENZENESULFONAMIDES AS POTENT CARBONIC ANHYDRASE INHIBITORS

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Abstract Carbonic anhydrases (CA, EC 4.2.1.1) are metalloenzymes catalyzing the reversible hydration of CO_2, thereby affecting the pH and related physiological processes in various organisms.
In pathogenic bacteria, CAs play an essential role in survival and growth. Inhibition of bacterial CAs leads to growth retardation and growth defects and makes bacteria vulnerable to host defense mechanisms. Bacterial CAs are, therefore, very promising targets in the search for new antibiotics. In humans, 15 different isoforms of CAs can be found, including two tumor-associated (hCA IX, hCA XII). Given the above, it is clear that carbonic anhydrase inhibitors can be drugs for a whole range of diseases. However, a fundamental problem is their selectivity toward a specific isoenzyme.
A series of 1,3,5-triazinyl aminobenzenesulfonamides substituted by aminoalcohol, aminostilbene, and aminochalcone structural motifs were synthesized as potential CA inhibitors. The compounds were tested against vancomycin-resistant *Enterococcus faecalis* (VRE) isolates. To evaluate the selectivity of the compounds against bacterial CAs toward human CAs, the inhibitory activity of compounds against tumor-associated hCA IX and hCA XII, hCA VII isoenzyme present in the brain, and physiologically important hCA I and hCA II were determined. Tested compounds had only a negligible effect on physiologically important isoenzymes.
In conclusion, newly prepared compounds have great potential as antibacterial agents with high activity and, at the same time, with high selectivity for bacterial CA compared to metabolically important hCA isoenzymes (e.g., hCA I, hCA II) found in the human body.

Keywords carbonic anhydrase, inhibitors, 1,3,5-triazine, sulfonamide

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ADVANCING DIAGNOSTIC CAPABILITIES OF FLUORESCENT PROBES: UNVEILING THE STRUCTURE-BINDING AFFINITY RELATIONSHIP IN IMAGING AMYLOID B PLAQUES FOR ALZHEIMER'S DISEASE

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Abstract

Alzheimer's disease (AD) is characterized by the accumulation of amyloid β (Aβ) plaques in the brain, which contribute to cognitive decline. Fluorescent dyes have become valuable tools for imaging Aβ plaques, allowing their visualization and examination. These dyes have specific properties, such as high photostability and solvatofluorochromic properties, which allow an accurate detection of Aβ plaques. Advances in the synthesis and design of novel fluorescent probes have improved their specificity and binding affinity to Aβ aggregates and offer potential as diagnostic tools for the early detection and study of AD (Zetterberg et al., 2021; Fua & Finney, 2018; Rejc et al., 2017).

Therefore, we focused on the synthesis of a series of novel fluorescent probes. The probes consisted of a central π-system (phenylethynyl or phenyl) end-capped with electron-donating (EDG) and electron-withdrawing groups (EWG). By incorporating different functionalities, we successfully modulated the optical properties and binding affinities of the probes to Aβ *in vitro, in cellulo,* and *ex vivo* (Figure 1).

Our results show that the synthesized probes exhibited selective binding properties to Aβ fibrils, and their interactions with the fibrils were supported by docking studies and molecular dynamics simulations. These results provide valuable insights into the potential application of these probes as diagnostic tools for AD. This study highlights the crucial relationship between structure-optical properties and structure-binding affinities in the development of highly effective fluorescent probes for optical imaging of Aβ plaques.

Although our results are promising, further investigation and optimization are required to fully exploit the diagnostic capabilities of these probes and to advance their application in AD basic research and clinical diagnostic. Continued research efforts will facilitate the exploration of their full potential and enhance their effectiveness as valuable tools for AD diagnosis.

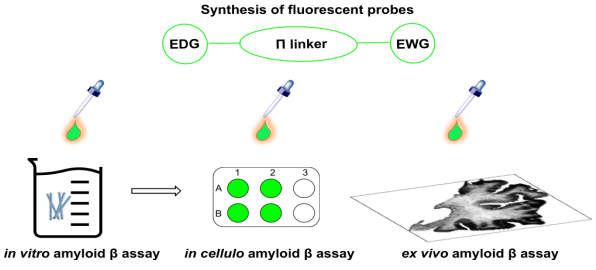

Figure 1: Simplified structure of fluorescent probes together with executed biochemical assays.

Keywords

Alzheimer disease, amyloid β, diagnostic tool, fluorescent probes

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BIOLOGICALLY ACTIVE DECAVANADATES

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Abstract The decavanadate anion, $H_xV_{10}O_{28}^{(6-x)-}$, is the main species in vanadate solutions at concentrations above 1 mM and in the pH range 2–6. This anion itself has many biological effects, which result mainly from its ability to interact with biomacromolecules (proteins or enzymes). The coordination compounds incorporating $H_xV_{10}O_{28}^{(6-x)-}$ are scarce, and the conditions of their formation are unclear. Herein, we deal with a systematic study of the formation and isolation of $H_xV_{10}O_{28}^{(6-x)-}$ complexes under variable conditions. Five new substances were prepared and characterized: $(Hnad)_2\{[Co(H_2O)_3(nad)_2]_2[\mu\text{-}V_{10}O_{28}]\}\cdot 6H_2O$ I, $\{[Co(H_2O)_4(iso\text{-}nad)_2]_3\}V_{10}O_{28}\cdot 4H_2O$ II, $\{[Co(H_2O)_4]_2[Co(H_2O)_2(\mu\text{-}pza)_2][\mu\text{-}V_{10}O_{28}]\}\cdot 4H_2O$ III, $\{[Co(H_2O)_4(\mu\text{-}pza)]_3V_{10}O_{28}\cdot 4H_2O$ IV, $(NH_4)_2\{[Ni(H_2O)_4(2\text{-}hep)]_2\}V_{10}O_{28}\cdot 2H_2O$ V, nad = nicotinamide, iso-nad = isonicotinamide, pza = pyrazinamide, 2-hep = 2-hydroxyethylpyridine.
Compounds I and III are decavanadate complexes, and compounds II, IV, and V are complex salts with $V_{10}O_{28}^{6-}$ anion. ^{51}V NMR spectroscopy confirmed that substances I and III are stable in aqueous solutions. Although no interactions with the model proteins thaumatin, lysozyme and proteinase K were observed, in the reaction of catalytic oxidation of water, substance I achieved up to 9-fold efficiency compared to uncoordinated $H_xV_{10}O_{28}^{(6-x)-}$, producing 143.37 nmol O_2, demonstrating a high cooperative effect of the decavanadate and Co(II) center. In addition, compound I is less toxic to A549 cell lines by 40% (0.05 mM) and HeLa by 26% (0.1 mM).

Keywords decavanadate, cobalt, cytotoxicity, water oxidation catalysis, proteins

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COPPER(II) AND ZINC(II) COMPLEXES OF REDUCED SCHIFF BASES: SYNTHESIS, STRUCTURE DETERMINATION, AND BIOLOGICAL ACTIVITY

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Abstract In recent years, Schiff bases and their metal complexes have been gaining a more attention because of their simple preparation, a wide range of biological activities, unique chemical properties, and structure arrangements. Copper and zinc complexes with Schiff bases are well known for their antimicrobial, anticancer, or antiradical activity. In our study, we present the preparation of four Schiff bases obtained by condensation of cyclohexane-1,2-diamine and fluorinated benzaldehydes, followed by reduction with $NaBH_4$. Reduced form of the prepared bases was used in coordination with metal salts to produce the respective complexes. The structures of the original and reduced Schiff bases as well as their metal complexes were characterized by single-crystal X-ray analysis, ^{1}H and ^{13}C-NMR, IR spectroscopy, and elemental analysis. The antimicrobial activities of reduced Schiff bases and their metal complexes were evaluated in vitro against *E. coli, S. aureus,* and *C. albicans*. Some of the compounds exhibited a very good antimicrobial activity. Metal complexes showed significantly higher activity compared to the corresponding free ligands. This observation confirms the fact that complexation with a metal ion enhances biological activity of ligands. All compounds were evaluated for urease inhibition against jack bean urease. Antiurease activity was observed in all copper complexes.

Keywords antimicrobial activity, metal complexes, Schiff bases, zinc, copper

APPLICATION OF SPECTROSCOPIC TECHNIQUES FOR EVALUATING FUNGAL VIABILITY

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Abstract Microscopic filamentous fungi represent one of the most significant factors causing the contamination of cleanroom surfaces, pharmaceuticals, and healthcare products. Filamentous fungi can produce a wide variety of enzymes capable of inducing different degradation processes, and they are known as producers of many mycotoxins and allergens.
Early detection and characterization of contamination make it possible to apply relatively noninvasive methods for its removal, and it can provide information necessary for preventive contamination control.
With a focus on determining the viability of fungi, a few techniques are currently used, but ongoing and intense research aims to develop new, time-saving, easier, nondestructive, and noninvasive methods. In this regard, combining spectroscopic techniques and statistical data processing seems promising to fulfil these requirements.
The main goal of the research was to study the spectral properties of vital and devitalized filamentous fungi – *Alternaria alternata*, *Aspergillus niger*, *Cladosporium herbarum*, *Penicillium chrysogenum*, and *Trichoderma atroviride* inoculated on the substrate in two phases – conidia and mycelium. UV-Vis-NIR and NIR Fibre Optics Reflection Spectroscopy (FORS), FTIR spectroscopy, and Raman spectroscopy, each combined with the principal component analysis (PCA), were set to determine whether the spectra of vital and devitalized forms of studied samples differ.
Based on the obtained results, it is possible to state that UV-Vis-NIR and NIR spectra of vital and devitalized forms of filamentous fungi generally differ, at least in one studied spectral region. FTIR spectroscopy seems to be a less sensitive method, and, in most cases, PCA could not clearly distinguish the spectra of vital and devitalized fungi. Portable Raman spectrometers were unable to detect the signal of fungi conidia. On the other hand, the spectra of viable conidia were obtained using the Raman spectrometer with microscope DXR™3.

Keywords filamentous fungi, viability, spectroscopy, principal component analysis

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LEVOMENTHOL IN LIQUID MEDICINES PREPARED IN A PHARMACY AND ITS DETERMINATION

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Abstract Levomenthol, (1*R*,2*S*,5*R*)-5-Methyl-2-(1-methylethyl)-cyclohexanol, is many years used as an active pharmaceutical substance in pharmaceutical formulations. They are prismatic or acicular, colorless, shiny crystals practically insoluble in water, very soluble in ethanol (96%). Quality control procedures have been developed and validated for levomenthol 1% and 10% alcohol solutions (ethanol 60%) prepared in pharmacies.
As control methods for the identity tests of levomenthol and ethanol 96% in Solutio levomentholi ethanolica 1% and 10%, the official methods of the current European Pharmacopoeia, relative density (2.2.5), specific optical rotation (2.2.7), and refractive index (2.2.6), were used. For the determination of the levomenthol content of a medicinal product, procedures have been developed using optical rotation with Kruss digital polarimeter and absorption spectrophotometry in the visible region after the chemical reaction of levomenthol with salicylaldehyde (L-SAL) in H_2SO_4 on calibration with the external standard method and reaction of levomenthol with 4-dimethylaminobenzaldehyde (L-DMAB) in H_2SO_4 at 100°C on a one-point calibration. The stability of the color products formed was monitored by measuring the absorbance at 562 nm (L-SAL) and 547 nm (L-DMAB) as a function of time and the presence of water. The absorption spectra were recorded by a qualified spectrophotometer Shimadzu UV 1800.
The developed determination methodologies were validated in the range of 80–120% of the concentration level of levomenthol in the formulation using the parameters: precision (SD or RSD) as repeatability, intermediate precision, reproducibility, accuracy as recovery, linearity, and range. All validation parameters met the criteria that were defined by the regulatory ity. Identity and assay procedures were developed and approved by the Pharmacopoeia Committee SUKL for the monograph of the drug in the Slovak Pharmaceutical Codex, 3rd edition.

Keywords levomenthol, absorption spectrophotometry UV–VIS, optical rotation, relative density, analysis of drugs

DETERMINATION OF STEROIDAL SAPONINES IN *TRIBULUS TERRESTRIS* FOOD SUPPLEMENTS BY LC–MS/MS METHOD

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Abstract

Tribulus terrestris L. (TT) is a plant used in the traditional folk medicine and to sport nutrition to improve health and performance. Its positive effects on the body are mainly due to its steroidal saponin content. The content and composition of saponins vary depending on the area from which they originate. The aim of the work was to develop an LC–MS/MS method for the determination of the main steroid saponins in dietary supplements containing TT. The saponins were determined in seven dietary supplements intended for athletes. The main steroidal saponins: protodioscin, protogracillin, diosgenin, gitogenin, hecogenin, ruscogenin, and tigogenin have been specified. Protodioscin, a typical steroid saponin of the Bulgarian chemotype, was found only in one sample, although its contents were labeled on several food supplements.

The other samples of tested food supplements declared a standardized extract of TT, but the determined content of saponins did not correspond to the stated data on the product. The results showed that the composition of tested food supplements did not correspond to the label, even the yohimbine (used to be prescribed as a treatment for erectile dysfunction) was detected in one product instead of steroid saponins from TT. Based on the results of the analysis, it is appropriate to alert the consumer's attention to the importance of ensuring the correct choice of a nutritional supplement and its quality.

Keywords

LC–MS/MS analysis, *Tribulus terrestris L.*, food supplements, steroid saponins