ABSTRACTS

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SECTION OF BIOORGANIC AND PHARMACEUTICAL CHEMISTRY

INFLUENCE OF ELECTRONIC EFFECTS OF PERIPHERAL SUBSTITUENTS ON INTRAMOLECULAR CHARGE TRANSFER IN AZAPHTHALOCYANINES

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Azaphthalocyanines (AzaPcs) are planar macrocyclic molecules structurally close to porphyrins with unique photochemical and photophysical properties. This project is a follow-up of our recent discovery that intramolecular charge transfer (ICT) can occur at alkylamino substituted AzaPcs. Peripheral amine serves as a donor and AzaPc core as an acceptor of electron in ICT[1]. This phenomenon can be used for sensoric applications[2,3]. The aim of this study is to investigate influence of electronic effects of peripheral substituents of the acceptor moiety of AzaPc (electron withdrawing or electron donating groups) on ICT efficiency.

Synthesis of pyrazine-2,3-dicarbonitriles disubstituted in positions 5 and 6 by various functional group (butoxycarbonyl-, *tert*-butylsulfanyl-, *neo*-pentyl-, butoxy-), precursors "A", was the first step of this work. These precursors were prepared by nucleophilic substitution of 5,6-dichloropyrazine-2,3-dicarbonitrile by appropriate nucleophilic agent (butoxide, *terc*-butylthiolate). 5,6-dineopentyl- and 5,6-dibutoxycarbonyl substituted pyrazine-2,3-dicarbonitriles arose from condensation of diaminomaleonitrile and corresponding diketone. Donor moiety of AzaPc is represented by precursors "B" bearing 4-(2-hydroxyethyl)piperidin-1-yl group (AzaPc 2-4, see Figure 1) or diethylamino group (AzaPc 1, see Figure 1). Both of them were prepared by nucleophilic substitution. Cyclo-

tetramerization of precursors A and B was performed using magnesium butoxide as an initiator of the reaction. Magnesium AzaPc complexes (AzaPc 2-4) are unstable in acidic solution and were converted to metal-free AzaPcs by using *p*-toluensulfonic acid in THF. Required congener of AAAB type was subsequently isolated from mixture of six congeners. Isolated metal-free AzaPc congeners reacted with anhydrous zinc acetate in pyridine to form zinc complexes. Due to unsuccessful attempts with butoxide method, synthesis of AzaPc with butoxycarbonyl substitution (AzaPc 1) was accomplished by metal ion template effect using anhydrous zinc acetate in DMF.

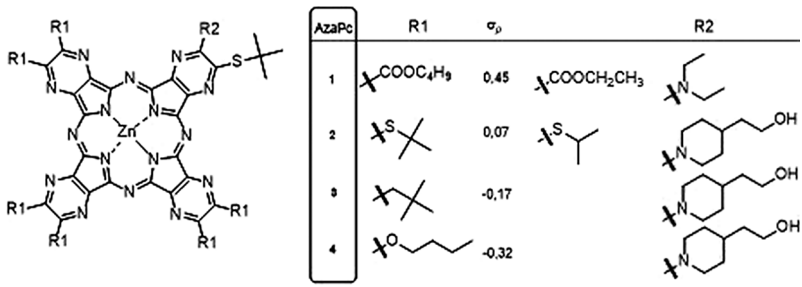

Fig. 1. AzaPc 1-4 substituted by substituents (R1) with diverse electronic effects (σ_p).

The financial support from the GA UK 1182313/2013 is gratefully acknowledged.

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PYRAZINAMIDE DERIVATES: PROPOSED MECHANISM OF ACTION

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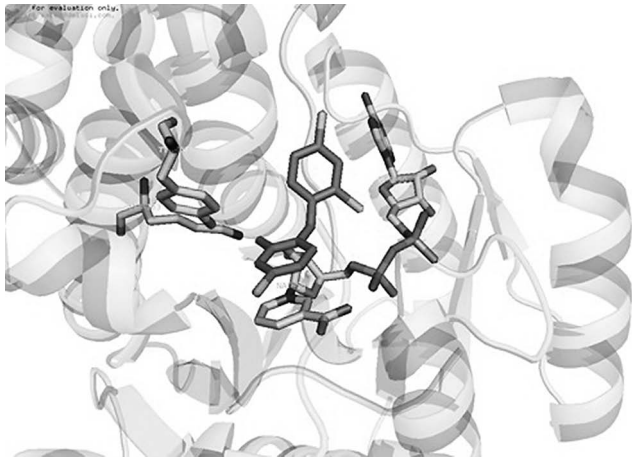
Pyrazinamide is an important sterilizing prodrug that shortens tuberculosis (TB) therapy. However, the mechanism of action of pyrazinamide is poorly understood because of its unusual properties. Spectrum of activity of various pyrazinamide derivates suggests different mechanism of action to pyrazinamide.

Mycobacterium Tuberculosis Enoyl-ACP-reductase (InhA; E.C. 1.3.1.9) has been repeatedly evaluated as effective antimycobacterial target. It is a key enzyme involved in the

long chain fatty acids biosynthesis. It is responsible for the reduction of the double bond between C2 and C3 of the intermediate fatty acid linked to the acyl carrier protein (ACP). InhA proper function disruption leads to insufficient synthesis of mycolic acids, which are crucial for mycobacterium cell integrity. InhA is the target of the current first line drug isoniazid (adduct with NAD) for the treatment of tuberculosis infections. Other inhibitors such as triclosan bind preferentially to the InhA – NAD^+ complex.

Docking studies were carried out in order to verify InhA inhibition as a potential mechanism of action. Used software MOE (version 2012.10) and Autodock Vina (version 1.1.2). The crystal structure of enzyme enoyl reductase was prepared using PDB structure 2H7M as starting geometry.

The results show formation of hydrogen bond with Tyr 158 as well as stacking with NAD^+ pyridine. These interaction are considered to be crucial for effective InhA inhibition and can be observed with most of the currently known inhibitors.



The study was supported by the European Social Fund and the state budget of the Czech Republic. Project no. CZ.1.07/2.3.00/20.0235, the title of the project: TEAB.

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NEW DERIVATIVES OF COMBRETASTATINE

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The aim of this project is to synthesize a small library of α,β-diarylbutenolides, analogous to compounds with interesting antineoplastic and antimicrobial activity previously prepared by our group. Due to their resemblance to naturally occurring cytostatic combretastatin[2], we intended to incorporate methoxy and/or hydroxy groups to the phenyl ring in the β-position. Another modification is hydroxymethylation of γ-position of the furanone in order to improve the hydrophilicity of the compounds.

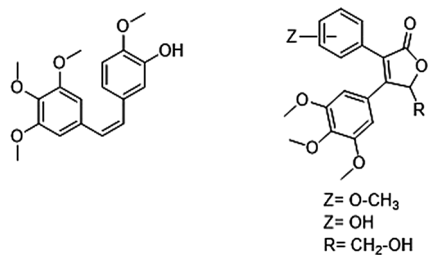

Fig. 1. Combretastatine and furanone analogs.

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MODULATORS OF AB-ABAD INTERACTION FOR TREATMENT OF ALZHEIMER'S DISEASE

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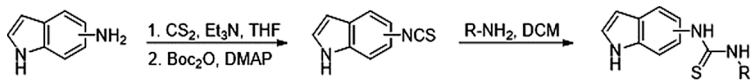
Alzheimer's disease is one of the most frequent neurodegenerative disorders in elderly. Most of the former research was generally focused on extracellular amyloid-beta (Aβ).

However, during last few years intracellular Aβ has grown on its importance. In cells, Aβ binds to variety of proteins, hence it might interfere with their proper function. One of the affected proteins is amyloid-binding alcohol dehydrogenase (ABAD), enzyme directly interacting with Aβ. Among other, the diminishing function of ABAD leads to disruption of energy metabolism and cell homeostasis, consequently resulting in cell death. Therefore, inhibition of ABAD-Aβ interaction represents potential target in AD treatment.

The aim of the project is to prepare series of compounds, disubstituted thioureas. Basic structure was derived from already described inhibitor of ABAD-Aβ interaction, frentizole. Benzothiazole heterocyclic ring was modified by indole-containing moiety with various further functional groups.

For the identification of possible fragment hits against ABAD-Aβ interaction, thermal shift analysis and NMR analyses (STD, WaterLOGSY) were performed providing 8 promising hits. Synthesis of the compound series was performed in two steps (Scheme 1). The final products were purified by column chromatography. Identity and purity of prepared compounds were confirmed by ^{1}H and ^{13}C NMR, ESI-MS and elemental analysis.

In cooperation with University of St. Andrews, the ability of prepared compounds to modulate ABAD activity was evaluated. None compounds showed significant change in ABAD activity. Further *in vitro* experiments, such as capability to inhibit ABAD-Aβ interaction, are currently in progress. Most promising compound will be subjected to molecular modelling studies to reveal potential structural improvements.



Scheme 1. Preparation of substituted indolyl thioureas.

The study was supported by the European Social Fund and the state budget of the Czech Republic (Project no. CZ.1.07/2.3.00/20.0235, the title of the project: TEAB) and project SVV 267 001.

PREPARATION OF NEW PYRAZINAMIDE DERIVATIVES FOCUSED ON MICROWAVE ASSISTED SYNTHESIS AND THEIR BIOLOGICAL EVALUATION

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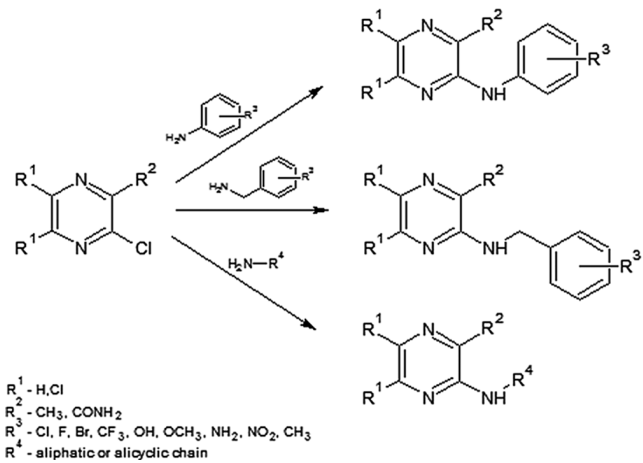
Even though the epidemiological situation of tuberculosis (TB) has been getting better since 2006, this disease is one of the most dangerous and treacherous threat nowadays.

The resistant strains of *Mycobacterium tuberculosis* are appearing more frequently than ever before. The reservoirs of these strains are developing countries situated especially in South Africa and East Asia. Another problem is connected with HIV when this infection leads to higher risk of TB contagion. These reasons resulted in effort to find effective, safer and innovative drugs.

Pyrazinamide is one of the first-line antituberculotic agents with unique properties that mean the sterilising effect of this drug in combination with other one or two drugs from this group. Mechanism of action was studied in many researches and the last known is the inhibition of *trans*-translation in *Mycobacteria* leading to cell's death. This small molecule is very suitable for modifications due to its chemical properties and it is mainstay of this research project in which the synthesis of eight series of pyrazinamide derivatives was completed together with the determination of their biological activities.

Two starting compounds (3-chloropyrazine-2-carboxamide, 5-chloro-6-methylpyrazine-2,3-dicarbonitrile) were prepared according to the published methodology and were treated with various ring-substituted anilines and benzylamines as well as with aliphatic or alicyclic amines. The aminodehalogenation reaction was accomplished using the microwave reactor with focused field. The conditions were proven experimentally to obtain higher yields and shorten reaction times. Prepared compounds were characterized with analytical data such as NMR spectra, IR spectra, elemental analysis or melting point. The lipophilicity was predicted as log *P* using PC software. The experimental measurements of log *k* were completed in order to be compared and correlated with the predicted values.



Antimycobacterial evaluation was carried out against wild strain of *M. tuberculosis* and three other non-tuberculosis stems. Isoniazide and pyrazinamide were used as standards. Another biological evaluation was focused on herbicidal activity measuring the inhibition of photosynthetic electron transport in spinach chloroplasts using DCMU (Diurone®, industrial herbicide) as standard. Last screening included antibacterial and antifungal evaluation of activity against eight bacterial and eight fungal stems. There were used five antibiotic and four antimycotic standards in these assays.

There were found active substances in antimycobacterial and herbicidal screenings and there were predicted structure-activity relationships between the activity and lipophilicity parameters (log *P*, log *k*, σ, π). The most active substances showed better or little less activity than standards.

The publication is co-financed by the European Social Fund and the state budget of the Czech Republic. Project no. CZ.1.07/2.3.00/20.0235, the title of the project: TEAB. This study was also supported by the Grant Agency of Charles University (B-CH/710312), by Ministry of Health of Czech Republic (IGA NZ 13346) and by Ministry of Education, Youth and Sports of Czech Republic (SVV-2013-265-001).

ANTIMYCOBACTERIAL HETEROCYCLIC COMPOUNDS – STRUCTURE-ACTIVITY RELATIONSHIP STUDY

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Enormous spread of tuberculosis together with appearance of multidrug resistant (MDR) and extensively drug-resistant (XDR) strains of *Mycobacterium tuberculosis* (MTB) highlight the necessity for search for new highly potent antituberculotics. In our previous work[1] we found that 2,4- and 3,5-dinitrobenzylsulfanyl fragments are responsible for significant antimycobacterial activities of benzazoles (**1**, Fig. 1).

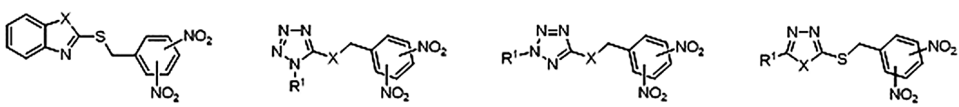

Fig. 1. Structure of prepared compounds.

In continuation of our research series of 1-alkyl/aryl-5-[(dinitrobenzyl)sulfanyl]-1*H*-tetrazoles was prepared (**2**, Figure 1) and structure-activity relationships were studied. Tetrazole derivatives **2** with various alkyl/aryl substituents R^1, with 2,4- or 3,5-dinitrobenzylsulfanyl, selanyl and oxy moieties were synthesized and evaluated for antimycobacterial efficiency. All prepared compounds achieved similar activity as isoniazid (INH) against *M. tuberculosis*. We observed that 3,5-dinitro derivatives displayed higher activity than 2,4-analogs, while benzylselanyl and benzyloxy derivatives showed efficiency comparable with sulfanyl analogs. Substituent R^1 of **2** strongly influenced the activity through the determination of the solubility and lipophilicity of target compounds.

As tetrazole can have 1*H*- and 2*H*-regioisomers, we also explored influence of position of alkyl substituents R^1 on tetrazole ring. It was revealed that 2-alkyl-5-[(3,5-dinitrobenzyl) sulfanyl]-2*H*-tetrazoles and their selenium analogs (**3**, Figure 1) exhibit slightly higher antimycobacterial activity than 1-regioisomers **2**. Furthermore, tetrazole ring was replaced by 1,3,4-oxa- and thiadiazole scaffolds (**4**, Figure 1). Evaluation of antimycobacterial activity of such compounds showed that 2-alkyl/aryl-5-[(3,5-dinitrobenzyl)sulfanyl]-1,3,4-oxadiazoles and 2-aryl-5-[(3,5-dinitrobenzyl)sulfanyl]-1,3,4-thiadiazoles **4** have considerably higher efficiency than tetrazole derivatives **2** and **3** and even than INH. Substituent R^1 had only low influence on efficiency of target substances, while position of two nitro groups on benzylsulfanyl moiety was crucial – 2,4-analogs of **4** were much less active.

The study was supported by the Charles University in Prague (SVV 267001).

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ESTERS OF 6-(DIMETHYLAMINO)HEXANOIC ACID AS SKIN PERMEATION ENHANCERS

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Transdermal drug delivery, compared to conventional routes, has many advantages. But skin acts as a very effective barrier protecting our body from entering of most environmental substances. And drugs are no exception, so if we want to get them into human body in sufficient amounts via transdermal route, we have to overcome the skin barrier. There are many ways how to get drugs through a skin into systemic circulation. One of them is to use permeation enhancers, which are chemical substances with ability of increasing skin permeability for a short time. There are many groups of such substances, for example some terpenes or synthetic derivatives of amino acids such as dodecylester of 6-(dimethylamino) hexanoic acid (DDAK).

Aim of this study was to combine the structure of DDAK and terpenes or other similar structures and to study effect of prepared substances on permeation of model drugs through a human skin *in vitro*.

We prepared esters of 6-(dimethylamino)hexanoic acid with ten selected terpenes or their analogues (menthol, citronellol, linalool, farnesol and borneol, geraniol, nerol, carveol, perillyl alcohol and cinnamyl alcohol) by a two-step reaction.

Permeation-enhancing activity of prepared esters was studied *in vitro*, using Franz cells and human skin. Two model drugs (theophylline and hydrocortisone) were used as markers of permeability. After permeation studies, concentration of model drugs in skin samples

was studied. Activity of each ester was compared to DDAK as a standard and to original terpene.

DDAK enhanced permeation of theophylline and hydrocortisone 23 and 57times, respectively, compared to control. None of prepared substances enhanced permeation of model drug theophylline through a skin more than DDAK. But when more lipophilic hydrocortisone was used, ester of cinnamyl alcohol enhanced its permeation 92times, bornyl ester 67times and citronellyl ester 63times. None of prepared esters increased concentration of both model drugs in skin.

To conclude, by combination of structures of two permeation enhancers groups, we found three potential permeation enhancers, which will be further studied.

The study was supported by the Czech Science Foundation (13-23891S) and Charles University in Prague (GAUK 1404213 and SVV 267 001).

SYNTHESIS AND EVALUATION OF POTENTIAL INHIBITORS OF SOME MYCOBACTERIAL ENZYMES

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The global burden of tuberculosis (TB), the progression of drug-resistance or a threat of latent tuberculosis should serve as a strong impetus for the searching of novel anti-tuberculosis agents. Especially new drugs which should be able to shorten the treatment of TB and overcome emerging resistance are required. The identification of unique mycobacterial pathways and their targeting belongs to the contemporary approaches[1].

Mycobacterium tuberculosis exhibits a tendency to remain latent or persistent for decades before activation. Bacterium has developed ingenious mechanisms to adapt to the hostile environment. These metabolic processes may provide such potential targets[2], which are not affected by conventional therapy.

We have been studied salicylanilide-based inhibitors of mycobacterial isocitrate lyase (ICL) and methionine aminopeptidase firstly[1]. Later, we have investigated some other compounds as potential ICL inhibitors – newer salicylanilide esters with various carboxylic, sulphur- and phosphorus-based acids, phenolic carbamates and thiocarbamates[3,4,5]. Derivatives of itaconic acid, 4-nitrophenol and 4-nitroaniline represent other investigated chemical groups. Some of them are comparable or superior to 3-nitropropionic acid, a standard inhibitor.

Among salicylanilides, esters of 4-(trifluoromethyl)benzoic acid showed the most favorable inhibition properties (inhibition rates of 12–27% at the concentration of 10 μmol/L) with 2-[(3-bromophenyl)carbamoyl]-4-chlorophenyl 4-(trifluoromethyl)benzoate superiority[3], closely followed by salicylanilide *N*,*N*-diphenylcarbamates (20–22%)[5]. In general, 2-methylene-4-[(4-nitrophenyl)amino]-4-oxobutanoic acid showed the highest ICL inhibition.

To identify novel TB drug targets, we evaluated some our derivatives against additional four enzymes related to dormancy of mycobacteria: L-alanine dehydrogenase (L-*Mt*AlaDH), lysine ε-aminotransferase, chorismate mutase and pantothenate synthetase. The most promising results were obtained for L-*Mt*AlaDH with 5-chloro-2-[(3-chlorophenyl) carbamoyl]phenyl diethyl phosphate superiority.

All molecules were evaluated against *Mycobacterium tuberculosis* H37Rv and three nontuberculous mycobacterial strains; the most active compounds were assayed additionally against multidrug-resistant TB. All of these derivatives exhibited activity against all strains within the range from 0.125 to 1,000 μmol/L[1,3,4,5].

Presented enzyme activities cannot be assumed being one and only mechanism of action. As expected and observed previously, there is not a clear relationship of *in vitro* MICs and enzymatic inhibitions.

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MAGIC METALLIC SOLUTION

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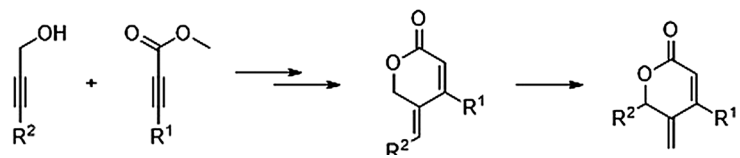
In our research, originally aimed at the synthesis of gelastatin, we have developed a quick and reliable synthetic route towards unsaturated pyranones containing exocyclic double bond at C5. It is based on Stille cross-coupling of precursors that are obtained in one step each from commercially or synthetically easily available precursors. Furthermore, the reaction conditions are mild and therefore tolerate variety of different functional groups. As yet unreported catalytic method has been used based on a species that is easily recyclable and much cheaper than ordinarily used homogeneous transition metal-based catalysts. An unexpected allylic rearrangement of pyranones is also being investigated.



This study was generously supported by the Grant Agency of Charles University (No. 11762/2013) and Charles University in Prague (SVV-265-001).

THE EFFECT OF SIZE OF AZA-CROWN RECOGNITION MOIETY OF SENSORIC AZAPHTHALOCYANINES ON THE SELECTIVITY TO DIFFERENT ANALYTES

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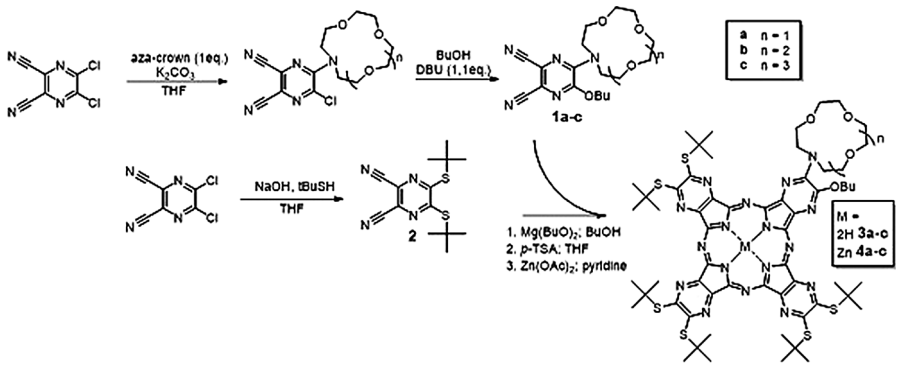
Azaphthalocyanines (AzaPcs) are macrocyclic planar compounds with unique photophysical and photochemical properties. They usually absorb light over 650 nm and possess high quantum yields of fluorescence and singlet oxygen production. Nowadays, AzaPcs are intensively studied as fluorescence sensors. Sensoric properties sensitive to metal cations are based on intramolecular charge transfer (ICT). ICT occurs between donor (peripheral alkylamine) and acceptor (macrocyclic core) moiety of AzaPc and is responsible for

quenching of fluorescence. However, ICT is blocked after binding metal cation into the recognition moiety of AzaPc, which leads to significant increase of fluorescence.[1] The great advantages of AzaPc sensors are emission at longer wavelengths (over 650 nm) and the insensitivity to pH of medium.[2]

This project is focused on the study of the size of aza-crown recognition moiety of AzaPc sensors. A series of unsymmetrical AzaPcs with different size of aza-crowns was prepared according to the reaction scheme below. 5,6-disubstituted pyrazine-2,3-dicarbonitriles (precursors **1a–c** and **2**) were obtained by nucleophilic substitution. Their statistical condensation (in ratio 1:3 for precursors **1**:**2**) led to a mixture of magnesium AzaPc congeners. Magnesium cation was removed from the center by *p*-toluensulfonic acid. Metal-free AzaPcs were easily separated by chromatographic methods and required congener AAAB was obtained (AzaPc **3a–c**). Finally zinc was coordinated into the center of macrocycle by heating the metal free derivates **3a–c** with anhydrous zinc acetate leading to unsymmetrical zinc AzaPc **4a–c**.

Synthesis of desired AzaPc sensors was followed by description of absorption and photophysical properties. Selectivity towards different analytes, determination of appropriate dissociation constants and discussion on the structure-activity relationship was done on the basis of fluorescence titration experiments.



The financial support from SVV 267 001 is gratefully acknowledged.

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FROM NITROGENOUS CATIONIC SURFACTANTS TO *O*-SUBSTITUTED PYRAZINES – SYNTHESIS AND EVALUATION

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The first part of the work deals with the preparation and testing of compounds classified as cationic surfactants, such as disinfection and decontamination agents. Since quaternary cationic surfactants are substances widely used in many applications (pharmaceuticals, chemical industry, food industry etc.), they are still of great interest. There were designed and prepared more than 70 surfactants based on quaternary nitrogen. Substances were derived from structures commonly used (benzalkonium, cetylpyridinium or cetyltrimethylammonium). Synthesis continuously builds on my diploma thesis, where I dealt with the preparation of similar compounds. However the dissertation describes the synthesis of compounds that may include various nucleophilic groups (hydroxyl or oxime) into the structure. Prepared structures were confirmed by NMR, MS, EA analysis. Furthermore, a HPLC method was developed to distinguish the individual homologues in the mixture.

The critical micelle concentration was measured for most compounds as a fundamental characteristic of surfactants. The structure relationship between the value of CMC and lipophilic chain length in the molecule was confirmed.

Selected compounds were then evaluated for expected antimicrobial activity. Some compounds significantly influenced the growth of several strains of bacteria or fungi. The minimum inhibitory and minimum microbicidal concentrations were determined afterwards.

Since structurally similar compounds are also known as micellar catalysts (acceleration or inhibition of some reactions using micellar microenvironment), several prepared compounds were tested for this property. The effect of cationic surfactants as accelerators of some ester cleavage was published in collaboration several times. These esters serve as model compounds of chemical warfare agents and pesticides.

The second part focuses on the preparation of O-substituted pyrazine derivatives as potential antituberculosis compounds. Several of the derivatives were already prepared and indentified by NMR and EA. The property to inhibit the growth of mycobacteria strains and some other effects (fungicidal, herbicidal) will be tested.

The study was supported by the European Social Fund and the state budget of the Czech Republic. Project no. CZ.1.07/2.3.00/30.0061.

SYNTHESIS OF SUBSTITUTED PYRIDINES USING TRIS(2-FURYL)PHOSPHINE GOLD(I) CATALYST

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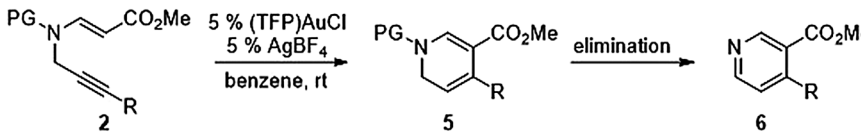
Synthesis of various types of heterocycles is possible from enyne precursors using cationic gold(I) species as a catalyst. In order to expand our research on cyclisation of propargyl vinyl ethers to dihydropyrans using tris(2-furyl)phosphine gold(I) chloride and silver tetrafluoroborate we employed the same catalytic system on protected propargyl vinyl amines. The synthetic protocol has was optimized and a series of substituted pyridines was synthesized.



R = phenyl, 4-bromophenyl, 4-methoxyphenyl, 4-methylphenyl, 4-aminophenyl, thiophene-2-yl, chloromethyl
PG = mesyl, tosyl, 4-fluorbenzenesulfonyl, 4-methoxybenzenesulfonyl, BOC

The study was supported by Charles University in Prague (SVV 267 001 and GAUK 5671/2012) and Czech Science Foundation (P207-10-2048).

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SYNTHESIS OF NEW HIGHLY ACTIVE ANTIMYCOBACTERIAL COMPOUNDS

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It was observed that 5-benzylsulfanyl-1-alkyl(aryl)-1*H*-tetrazole (**I**) and 5-phenyl-2-alkyl(aryl)sulfanyloxadiazole (**II**) substituted with two electron withdrawing groups

(EWG) have the same or higher *in vitro* antimycobacterial activity than first line antituberculosis drug isoniazid.

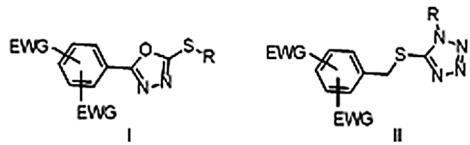

Fig. 1. General formulas of highly active antimycobacterial compounds.

In this work we studied the influence of the specific substitution on phenyl moiety on the antimycobacterial activity (**I**, Fig. 1). We converted one EWG to the electron donating amino group (Fig. 2). This group was further substituted.

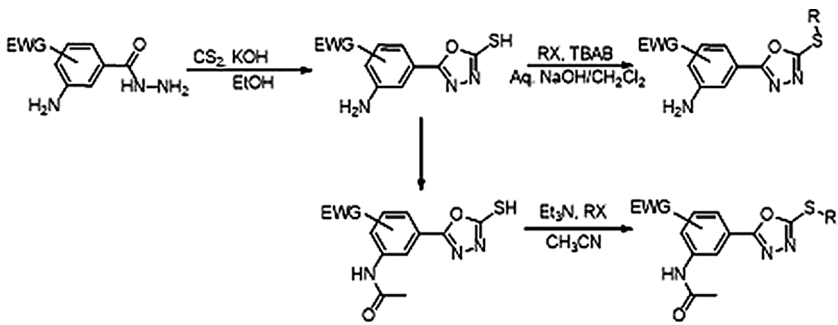

Fig. 2. Conversion of one EWG to amino group and its substitution.

Further structure-activity relationship studies were focused on the connection linker between the specific substituted benzyl group and tetrazole moiety (structure **II,** Fig. 1).

The study was supported by Charles University in Prague (SVV 267 001).

STRUCTURAL ANALYSIS OF ALKALOIDS ISOLATED FROM *BERBERIS VULGARIS* L. (BERBERIDACEAE)

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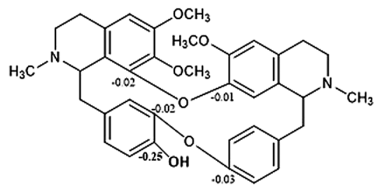
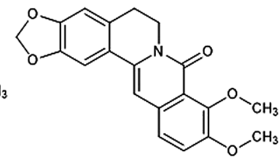
Numbers of alkaloids were isolated from the root bark of *Berberis vulgaris* L. (Berberidaceae). The botanical family Berberidaceae is known for the production of isoquinoline

alkaloids, including, in particular, protoberberines (8-oxoberberine)[1] and bisbenzylisoquinolines (berbamine, etc.).[2]

Typically, these alkaloids consists free and protected phenols. The position of the ether bridge can be usually confirmed by gHMBC or NOESY cross peaks. Because of rapid exchange of phenolic protons, there are no couplings in gHMBC, and NOESY. Isotopic inductive effect of deuterium can be used to determine position of phenol. 5 μL D_2O was added to the sample and the chemical shift change was observed as $\Delta\delta = \delta_D - \delta_H$.



berbamine



8-oxoberberine

The study was supported by grants SVV UK 267 001 and 267 002, GA ČR P207/10/2048, TEAB Nr. CZ.1.07/2.3.00/20.0235.

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SYNTHESIS OF HUMAN SKIN CERAMIDES OF THE EO CLASS

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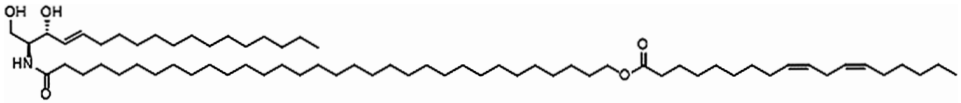
The primary function of the skin is to provide resistance of the body against water loss and penetration of exogenous substances, such as toxins or bacteria. The main skin barrier is situated into stratum corneum, the top layer of the skin. It is composed of corneocytes (flat cells) and the lipidic matrix surrounding them. Lipids, filling the intercellular space of stratum corneum, are composed of equimolar mixture of ceramides, cholesterol and free fatty acids.

Twelve structure types of ceramides occur in the skin. These types are derived from 4 aminoalcohols – sphingosine (S), phytosphingosine (P), 6-hydroxysphingosine (H) and dihydrosphingosine (DS). Primary amino group of these aminoalcohols is acylated by non-substituted acid (N), alpha-hydroxy acid (A) or omega-hydroxy acid esterified with linoleic acid (EO).

The aim of our work is to prepare ceramides of the EO class, because these are not commercially available, and to study their barrier properties.



Synthesis of ceramides of the EO type started from 16-bromohexadecanoic acid, which is commercially available and not very expensive. This acid was converted into protected omega-hydroxy aldehyde. This was the first fragment for the Wittig reaction. The second fragment was obtained also from 16-bromohexadecanoic acid by converting it to phosphonium salt. After the Wittig reaction, the unsaturated product was methylated, hydrogenated and deprotected to obtain 32-hydroxydotriacontanoic acid. Then, the obtained acid reacted with linoleic acid to form an ester. After the reaction with sphingoid base, this ester will provide a ceramide type EO.

This work was supported by the Czech Science Foundation (207/11/0365 and 13-23891S) and by Charles University (SVV 267 001).

SYNTHESIS OF NOVEL NITRO-SUBSTITUTED SALICYLANILIDES AS POTENTIAL ANTIBACTERIAL DRUGS

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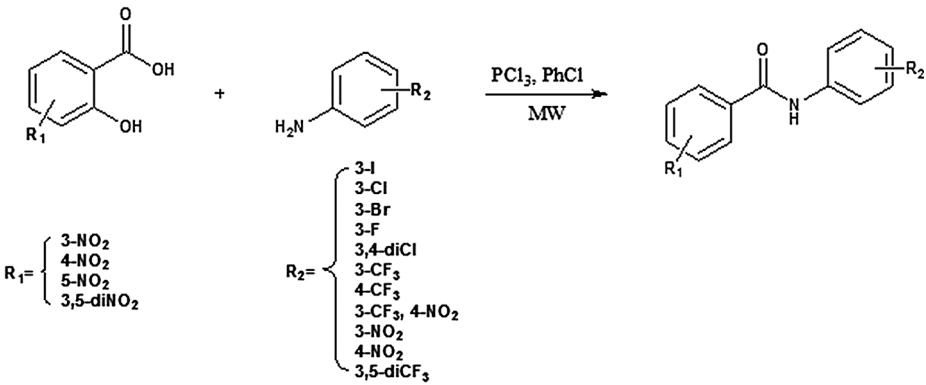
Pathogenic microbes have the ability to mutate in order to survive against chemotherapeutic agents. Thus, antimicrobial drug development has always to be alerted for producing alternative drug candidates. The identification and development of new antibiotics, especially those with new modes of action, is imperative for the treatment of these infections. The continued emergence of multi-drug-resistant bacteria is a major public health concern.

N-substituted 2-hydroxybenzamides, broadly known as salicylanilides, demonstrated a wide range of biological activities, including antiviral potency[1]. The structural motif of the parent salicylanilide allows the synthesis of numerous analogues and thus appears to be a good substrate for drug development. Several derivatives of salicylanilides, developed by our group, have already proved to be active against bacteria and fungi[2]. Despite the already great progress, there is always room for further development. On our ongoing efforts to explore the influence of different substitutions on the parent structure, the introduction of nitro group was examined.

The insertion of nitro group during drug design is considered to be highly risky, as many nitroaromatic compounds have proved to be extremely toxic and mutagenic[3]. On the other hand, the drug portfolio possess a plethora of nitroaromatic compounds[4] hence is acceptable that the general rule has its exceptions. As Computational Chemistry has been established a very useful tool in drug design, the proposed nitro-substituted salicylanilides were screened, in order to predict their toxicity. The software of choice was *ADMET*

Predictor. Surprisingly, the results showed that the suggested structures, even if they all possess nitroaromatic rings, are of minimum to acceptable risk of toxicity.

Herein, the synthesis of 44 nitro-substituted analogues is presented, arising from the conjugation of 4 mono- or di-substituted nitrosalicylic acids with 11 mono- or di- substituted anilines (Scheme 1). All compounds were fully characterized by various spectroscopic techniques (NMR, IR, MS) and their biological activity is currently under evaluation.



Scheme 1. Schematic representation of the synthesis of nitro-substituted salicylanilides.

The study was supported by the European Social Fund and the state budget of the Czech Republic. Project no. CZ.1.07/2.3.00/30.0061.

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EFFICIENT AND SELECTIVE ACCESS TO CYCLOPENTENES FROM MORITA-BAYLIS-HILLMAN CARBONATES *VIA* ASYMMETRIC ALLYLIC SUBSTITUTION – ALKENE METATHESIS SEQUENCE

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One of the most important objectives of organic chemistry is formation of new C-C and C-heteroatom chemical bonds and considerable efforts are devoted to developing synthetic

methodologies allowing full control over stereo- and enantioselective outcome of such reactions. Asymmetric allylic substitution (AAS) offers a powerful tool to address this issue and traditionally involves the use of a transition metal catalyst.[1] With the advent of organocatalysis[2] in the early 2000s and more precisely: studies of Kim et al. on hydrolysis of Morita-Baylis-Hillman (MBH) acetates[3], a complimentary approach to this problem has been proposed and is quickly gaining momentum. Nowadays, various organic Lewis bases are considered to be an equally effective and certainly "greener" alternative to classical organometallic AAS catalytic systems. At the same time researchers focus their attention on post-AAS chemical transformations of adducts, in order to develop concise one-pot cascade synthetic protocols of a wide range of important building blocks for natural product synthesis.

In this contribution we would like to demonstrate that under mild conditions and in presence of a chiral Lewis base, MBH carbonates can be functionalized with C-nucleophiles containing an allyl moiety. Corresponding addition products are obtained in high yields and with moderate enantioselectivities. Further derivatization is achieved *via* an intramolecular ring-closing metathesis step in presence of a commercially available Grubbs-Hoveyda catalyst – a rare example of alkene metathesis in which participate strongly electron-depleted double bonds[4]. As a result a direct access to highly substituted cyclopentene rings, useful and versatile synthons, has been successfully developed.

This work was supported by the Ministry of Education of the Czech Republic (Grant No. MSM0021620857), Czech Science Foundation (Grant No. P207/10/0428), the European Social Fund and the state budget of the Czech Republic. (Project no. CZ.1.07/2.3.00/30.0022). The support of COST Action CM0905 "Organocatalysis" is also acknowledged.

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PERDEUTERATED CERAMIDES – SYNTHESIS AND USE FOR STUDYING THE ARRANGEMENT OF STRATUM CORNEUM LIPIDS

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Ceramides (Cer) are essential constituents of the skin barrier. Together with cholesterol (Chol) and fatty acids (FFA), they fill the intercellular space of the upper skin layer (stratum corneum, SC). Structure of Cer and their role in the skin diseases (atopic dermatitis or psoriasis) have been known for decades, but the importance of the individual structural features in their molecules is poorly understood.

Our long-term aim is to study the structural requirements for Cer function in a competent skin barrier to lay the design criteria for skin barrier repair agents, study the underlying mechanisms, and to identify any diagnostic or predictive parameters for the skin barrier function.

To characterize the arrangement of the SC lipid barrier we prepared natural occurring Cer (NS, NP and NdS) with their acyls labeled by deuterium. These labeled compounds enable us to study the organization of model SC lipid membranes by infrared spectroscopy (IR). Thanks to perdeuteration of carbon chain we can follow phase transitions, conformational or organizational behavior of lipid parts separately and simultaneously. We prepared model lipid membranes containing labeled and unlabeled components – Cer, FFA and Chol and compared the behavior of Cer with different polar head structure. At skin temperature all membranes show well ordered chains and prevailing very tight lateral packing. However we see differences in thermal stability and miscibility of lipids which can be related to different permeability of model membranes.

The study was supported by the Czech Science Foundation (GACR 207/11/0365 and GACR 13-23891S) and the Charles University in Prague (GAUK 652412 and SVV 267 001).

TRANSITION METAL CATALYSIS AND ORGANOCATALYSIS WITH CHIRAL PHOSPHINES

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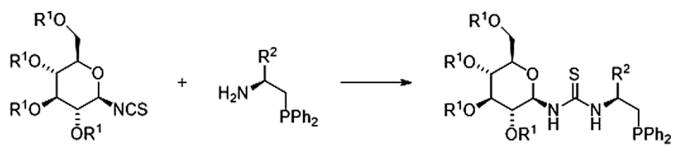
Asymmetric Morita-Baylis-Hillman addition reaction of α,*β*-unsaturated carbonyl compounds to aldehydes usually catalysed by tertiary amine or phosphine represents a powerful synthetic tool allowing stereoselective formation of C–C bonds[1]. This attractive reaction using commercially available starting materials, which can be easily transformed to synthetically interesting products without presence of transition metals, caused very fast development of various more efficient organocatalysts.

In this contribution, we describe bifunctional chiral phosphine-thiocarbamide catalysts **I** bearing glucose derivative. Thiourea is a great donor of hydrogen bonds for strong Lewis bases (e.g. aldehydes) and thus activates electrophiles. On the other hand, second function group of catalysts, phosphine moiety activates double bond of pronucleophile such as acrylates giving very reactive nucleophile[1,2].



R^1 = Me, Ac R^2 = H, Me, CH_2Ph, *i*Pr, CH*i*Pr **I**

This work was supported by the Charles University Grant Agency (Project no. 704213), the European Social Fund and the state budget of the Czech Republic (Project no. CZ.1.07/2.3.00/30.0022).

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DOCKING OF ALKYLAMINO DERIVATIVES OF PYRAZINAMIDE INTO MYCOBACTERIAL PYRAZINAMIDASE PNCA

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Our research group previously reported several 5- and 6-alkylamino derivatives of pyrazinamide (PZA) with antitubercular activity (Fig. 1).[1] PZA acts mainly as a prodrug, which is enzymatically hydrolysed *via* mycobacterial pyrazinamidase (PncA, EC 3.5.1.19) to pyrazinoic acid (POA). On the other hand, some PZA derivatives proved to be active in non-hydrolysed carboxamide form; for example, 5-Cl-PZA acts as a Fatty Acid Synthase I inhibitor and possesses *in vitro* activity against mycobacterial strains with low pyrazinamidase activity as well. To elucidate, whether the discussed alkylamino derivatives of PZA could underwent hydrolysis by PncA or rather function in non-hydrolysed form, a docking study was performed.

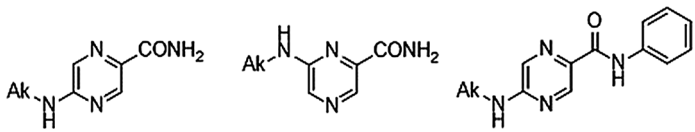

Fig. 1.

The binding poses of the studied compounds in the active site of PncA (pdb: 3PL1) were calculated using the non-commercial software AutoDock Vina 1.1.2.[2] The docking results were evaluated with the respect to the catalytic mechanism proposed by Petrella et al.[3] The long alkyl chains prevented the discussed derivatives from reaching the orientation favourable for the hydrolysis. The carboxamido groups of alkylamino derivatives were significantly directed away from the catalytic triad of the active site of PncA. Based on the results, we conclude that neither 5-alkylamino nor 6-alkylamino derivatives of PZA are likely to be converted to respective carboxylic acids by the PncA of *Mycobacterium tuberculosis* H37Rv.

This publication is a result of the project implementation: "Support of establishment, development, and mobility of quality research teams at the Charles University", project number CZ.1.07/2.3.00/30.0022, supported by The Education for Competitiveness Operational Programme (ECOP) and co-financed by the European Social Fund and the state budget of the Czech Republic. Additional support was provided by the Ministry of Education, Youth and Sports of the Czech Republic (SVV-2013-267-001) and Grant Agency of Charles University (GAUK B-CH/710312).

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SECTION OF PHARMACEUTICAL ANALYSIS

DEVELOPMENT OF NEW HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF RETINOL, ALPHA- AND GAMMA-TOCOPHEROL USING HIGH RESOLUTION MONOLITHIC COLUMN FOR BIOANALYSIS

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Retinol (vitamin A) and tocopherol (vitamin E) belongs to the group of liposoluble vitamins. Retinol has essential biological functions, including its roles in embryonic development, maturation of the immune system, maintenance of epithelial integrity, and in the adult brain for learning and memory. Retinol also prevents the differentiation and promotes the feeder-independent culture of embryonic stem cells. Vitamin E is an antioxidant which intercepts free radicals and therefore protects lipid parts of cell membrane against oxidation and maintains its integrity. Tocopherol is essential for the upkeep of the heart function, for functioning of sex organs and for cell protection. The term vitamin E represents eight structurally related compounds, each differing in their potency and mechanisms of chemoprevention. The most active forms are alpha- and gamma-tocopherol.

In this work retinol, alpha- and gamma-tocopherol were determined using High Performance Liquid Chromatography system (HPLC) and monolithic column as stationary

phase. Monolithic structure of the column has an advantageous character for analysis of biological material. New generation of monolith (Chromolith® High Resolution RP 18e, 100 × 4.6 mm, Merck) was used as stationary phase for simultaneous determination of retinol, alpha- and gamma-tocopherol and tocol as internal standard. As the mobile phase mixture of 85% of methanol and 15% of acetonitrile at flow rate 1.5 mL/min and temperature 25 °C was used. Excitation and emission wavelength for fluorescent detection was carried out at 325 nm and 480 nm for retinol, 295 nm and 330 nm for tocopherol and tocol. Limit of detection (LOD) and limit of quantification (LOQ) for retinol were 24 nmol/L and 80 nmol/L, respectively. LOD and LOQ were for gamma-tocopherol 14 nmol/L and 48 nmol/L, for alpha-tocopherol 35 nmol and LOQ 118 nmol/L, respectively.

This method is suitable for determination of retinol and tocopherol in serum and other types of biological materials.

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DEVELOPMENT OF UHPLC-FD METHOD FOR DETERMINATION OF ARGININE AND ITS METABOLITES IN CLINICAL RESEARCH

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Wound healing process can be divided in three different phases (inflammatory, proliferative and maturation) and each is characterized by certain events that required specific components. Nevertheless, wound healing can progress forwards and backwards depending on some intrinsic and extrinsic factors. If wound-healing process is affected negatively, it can result in chronic wounds. Despite the development of chronic wounds is multifactorial, the nutritional factors have an essential role in their development[1–4]. The amino acid L-arginine is synthesized in the early phase of wound mending by inflammatory cells, mainly macrophages, but its levels becomes critically low after trauma[2].

Several sample preparation methods for arginine, and its metabolites ornithine and citrulline were tested and ultrafiltration using microcon centrifugal filter unit YM-10 membrane (Merck, Darmstadt, Germany) was chosen as an optimal sample preparation. The time of ultrafiltration was investigated in order to optimised and speed up the process. The difference was observed in 20 min, but 30 and 40 min provide the same results. However, the linearity of extraction decreases with decreasing time of extraction. Due this reason, 40 minutes was selected as optimal condition.

Based on previous results, mixture of 3-mercaptopropionic acid and ortho-pthalaldehyd in borate buffer was used for derivatization of target analytes. Different time of derivatization (0, 20 and 40 minutes) was investigated and 2 minutes provided sufficient sensitivity.

The final analyses were performed using UHPLC system Nexera (Shimadzu (Kyoto, Japan) with fluorescence detector (RF-20AXS).

The novel UHPLC-FD method for determination of arginine, citrulline and ornithine was developed. Sample preparation is very simple, only ultrafiltration and automatic derivatization, and 96-well microtitrate plates are used. Due this reason is possible employed method in routine analyses. Sample preparation is suitable for serum and wound exudates. Presented method will be further validated and used in clinical practice and research.

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SELECTIVE ENRICHMENT OF BASIC DRUGS ONTO VARIOUS SOLID PHASE SORBENTS FOR URINE SAMPLE PRE-TREATMENT USING SPE TECHNIQUE

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β-Blockers are an important class of basic drugs that has been used worldwide for treating various cardiac diseases. Athletes may use β-blockers to reduce their heart rates, hand tremors, and to reduce anxiety during athletic activities; therefore, β-blockers are considered doping and prohibited agents for use by athletes during competition. The maximum allowed urinary concentration of β-blockers has been set at 0.5 μg mL^{-1} by the World Anti-Doping Agency (WADA). Thus, the performance of several solid materials based on analyte-interaction such as reversed-phase, mixed-mode interactions (ion-exchange and reversed-phase), and molecularly imprinted polymers (MIP) for selective enrichment of basic drugs namely β-blockers will be presented. Off-line and on-line-SPE sample preparations for β-blockers in complex matrix as urine were investigated for enrichment and cleanup sample. Identification and quantitation were achieved by high performance liquid chromatography-ultraviolet spectrophotometry (HPLC-UV).

The analyte recoveries approaching the range from 94 to 105%, with an RSD ranging from 2 to 4% were obtained. The regression equations for all of the targeted compounds exhibited excellent linearity ($r^2 > 0.9991$) over the concentration range from 10 to 1000 ng mL^{-1}. The limits of detection and quantification for the selected β-blocker compounds in urine were in the ranges of 0.6 to 2.0 ng mL^{-1} and 2.0 to 6.7 ng mL^{-1}, respectively.

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DEVELOPMENT OF ANALYTICAL METHODS AND *IN VITRO* EVALUATION OF AROYLHYDRAZONE PRO-CHELATORS

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Biocompatible iron chelators were originally used for the treatment of iron overload diseases. However, their therapeutic potential has been significantly widened thanks to their strong antioxidative, antiproliferative and cytoprotective properties. One of the lead compounds of aroylhydrazone chelators is SIH (salicylaldehyde isonicotinoyl hydrazone), which has a low toxicity and significant antioxidative effects. However, its main disadvantage is a short biological half-life connected with a rapid degradation in plasma[1]. BSIH (isonicotinic acid [2-(4,4,5,5-tetramethyl-[1,3,2]dioxaborolan-2-yl)-benzylidene]-hydrazide) was synthesized as a pro-drug, selectively converted to SIH only in the presence of oxidative stress, which provides a possibility to target the therapeutic effect exclusively to affected areas[2]. HAPI (*N'*-[1-(2-hydroxyphenyl)-ethyliden]-isonicotinoylhydrazide) is a novel SIH derivative with higher stability in biological matrixes. Its pro-drug was synthesized as well – BHAPI (*N'*-(1-(2-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-benzyloxy)-phenyl)-ethylidene)-isonicotinohydrazide)[3].

The aims of this study were: 1) to develop suitable chromatographic conditions for the analysis of SIH and HAPI along with their pro-drugs, BSIH and BHAPI, respectively, and 2) to utilize developed methods in the *in vitro* activation experiments.

Separation of SIH and its pro-chelator was achieved on Zorbax Bonus-RP column (150 × 3 mm, particle 3.5 μm), using mobile phase composed of 2 mM EDTA in 7 mM NaH_2PO_4, (pH 6.0) and a mixture of methanol and acetonitrile (40:60, v/v), in a ratio of 60:40 ($v_{water}/v_{organic}$). Analyses of HAPI and BHAPI were performed on Ascentis C18 column (100 × 3 mm, particle 3 μm) with mobile phase composed of 15 mM NaH_2PO_4 (pH not adjusted) and acetonitrile in a gradient mode.

Both pro-drugs were incubated (100 μM, 37 °C) in different biological materials (ADS buffer, DMEM medium, DMEM containing H9c2 cells) with 100 μM of epinephrine (or

its oxidized form). Incubations were terminated by addition of organic solvent in defined times and samples were immediately analyzed.

BHAPI proved dramatically faster conversion in all tested media as compared to BSIH. Also higher concentrations of HAPI were detected in the samples in the end of all experiments. The slowest bioactivation was observed in ADS buffer, where 82% and 70% of initial amount of BSIH and BHAPI, respectively, were detected after 24 hours of incubation. Complete conversion of BHAPI in DMEM cell media was revealed in 6.5 hours, while 24 hours incubation was needed in the case of BSIH. However, this process was significantly slower in the presence of H9c2 cells.

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UHPLC-MS/MS METHOD DEVELOPMENT FOR DETERMINATION OF 8-HYDROXY-2′-DEOXYGUANOSINE IN CLINICAL RESEARCH

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Nucleic acids, lipids and membranes are constantly attacked by reactive oxygen radicals (ROS), which leads to damage of these biological structures. After attacking DNA by ROS, 8-hydroxy-2′-deoxyguanosine (8-OH2dG), biomarker of DNA oxidative damage, is released from cells to the circulation. 8-OH2dG could be detected in different kind of human liquids (plasma, urine, saliva etc.). It was reported that increased levels of 8-OH2dG are connected with different diseases. Higher levels of 8-OH2dG were observed in neurodegenerative diseases such as Parkinson's disease, in numerous types of malignancies such as acute leukemia, colorectal carcinoma, hepatocellular carcinoma, and breast or lung cancer etc.

In this study, the new Ultra High Performance Liquid Chromatography (UHPLC) method coupled with UV and Mass Spectrometry detection (MS/MS) for determination of 8-OH2dG and creatinine (correction of urinary excretion and dilution effects) in human urine was developed.

During method development chromatographic parameters with detection conditions and sample preparation procedure were optimized. Different approaches for sample preparation, such as simple protein precipitation (PP) and solid phase extraction (SPE) were used. SPE was tested using various types of cartridges – Spe-ed™ Flow C18, Oasis MCX, HLB, LiChrolut® EN. Based on the results of the sample preparation, SPE method using Oasis HLB Vac Cartridge C18 200 mg / 3 mL (Watters, USA) was selected. Chromatographic separation was performed using UHPLC system Nexera (Shimadzu, Japan). As the stationary phase Aquity UPLC BEH Amide column (Waters, USA) at the dimension 3.0 × 150 mm with 1.7 μm particles protected by security guard was chosen. The column was working in HILIC mode. Mixture of acetonitrile and 10 mM/L ammonium formate buffer at pH 4 in the ratio 87:13 (v/v) was chosen as mobile phase. Analysis was carried out using triple-quadrupole mass spectrometer LCMS 80-30 (Shimadzu, Japan) equipped by electrospray ionization source and working in positive ion mode. Multiple Reaction Monitoring transitions used in MS detection were 284/168, 284/140, 284/112 for 8-OH2dG. Because of extremely high creatinine concentration in urine, on-line coupled UV detection (235 nm) for creatinine was used.

New chromatographic method for the determination of 8-OH2dG in human urine could lead to improvement of diagnostic possibilities, efficiency of the treatment, prognosis predictability and individual treatment approach to cancer patients.

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ONE YEAR OF IN-SYRINGE MAGNETIC STIRRING AS A TOOL FOR AUTOMATION OF DISPERSIVE LIQUID-LIQUID MICRO-EXTRACTION – AN OVERVIEW.

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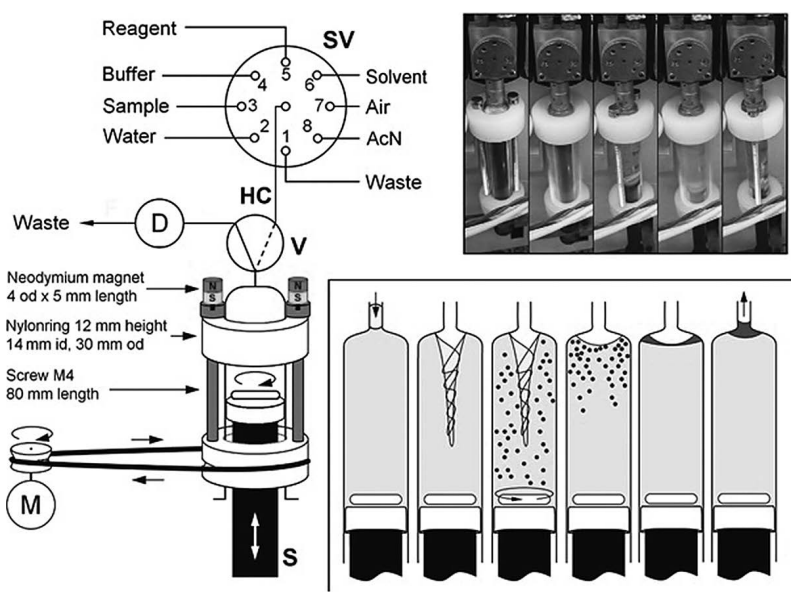
In-syringe stirring as a novel automation approach is overviewed one year after its first report.[1] The technique is based on the use of a magnetic stirring bar inside a syringe pump and forcing its movement by a rotating magnetic field from outside.

Using the syringe pump as part of a Sequential Injection Analyzer (SIA), a simple but highly versatile system is obtained. In contrast to other flow techniques, reproducible and total /or full) homogenization even of several milliliters of solutions is achieved and this within seconds.

The technique is ideally suited for downscaling standard laboratory procedures such as dilution, mixing with reagents for chromogenic reactions, or titration, as well as to automate dispersive liquid-liquid micro-extraction (DLLME) and related extraction procedures.

For DLLME, a water immiscible organic solvent and air are aspirated in addition to the aqueous sample solution into the syringe. Initiating the stirring, the solvent is dispersed into small droplets by the introduced kinetic energy, which enables efficient extraction in less than 1 minute. Stopping the stirring, the droplets coalesce to one drop of organic phase, in which the analyte is then quantified by a directly coupled detection system.



Reported applications and related method development and optimization are shortly overviewed on the examples of the extraction of aluminum from seawater and dichromate, anionic detergents, and cationic detergents from waste waters. Pre-concentration factors in the range of 30, reproducibility of < 5% RSD, and LOD of few nanomol per liter are achievable requiring generally not more than 150 μL of solvent and 4 mL of samples.

The possibility of automation of extract washing, analyte back-extraction, or salting-out assisted extraction are discussed as well as the potential of in-syringe extraction to be used with separation techniques.

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EFFECT OF PASTEURIZATION AND STORAGE ON A-TOCOPHEROL AND RETINOL IN HUMAN BREAST MILK

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Breast milk is an ideal nutrient for term and pre-term infants up to 6 months of age. It is important for host defence, digestion and absorption of nutrient and neurodevelopment.

When a newborn cannot be fed with the milk from their own mother, milk from donor mothers is the best substitution. Human milk banks collect, process and store milk from healthy lactating woman. Breast milk from bank is an alternative for the care and treatment of premature and low-birth-weight neonates and sick newborns. Donor milk must be pasteurized before it is given to infant to avoid the transmission of infectious microorganisms. Milk is frozen before pasteurization then it is pasteurized at 62.5 °C for 30 minutes in pasteurization bath. This temperature safely inactivates Cytomegalovirus (CMV), Human immunodeficiency virus (HIV) and other thermo labile viruses. After pasteurization is milk quickly cooled at 10 °C in 10 minutes and then frozen at −20 °C.

Retinol and tocopherols are sensitive to light and temperature, therefore their concentrations could be affected by pasteurization. The goal of this study was to determine the influence of pasteurization and storage on the concentration of retinol and α-tocopherol in donated human breast milk. Breast milk was collected from eight lactating women aged 29–39 years. Samples of milk were divided into 6 aliquots and 4 aliquots were storaged

in freezer in −27 °C. The samples of breast milk were analyzed before and immediately after pasteurization, others determination of vitamins followed after 1, 2, 4 and 8 weeks.

Extraction procedure was based on liquid-liquid extraction with hot saponification. The first step of sample preparation was deproteination with 2 ml of cold ethanol into the 0.5 ml sample of human breast milk. Then saponification was cary out using potassium hydroxide (80 °C, 30 min). After extraction of retinol and alpha-tocopherol into 2 ml of n-hexane and 1 ml water, the organic layers were separated by centrifugation (3220 ×g, 10 min, 4 °C), evaporated and the residue was dissolved in 375 µl of methanol.

α-Tocopherol and retinol were separated and quantified with reverse phase HPLC performed on system Prominence from Shimadzu (Kyoto, Japan) using methanol as mobile phase. The monolithic column Chromolith® Performance RP-18e, Merck 100 mm × 4.6 mm (Darmstadt, Germany) was used. Analysis of both vitamins took 2 minutes and the DAD detection of retinol and α-tocopherol was carried out at 325 and 295 nm.

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DETERMINATION OF ILLEGAL DYES IN CHILI-CONTAINING SPICES BY ON-LINE SPE-UHPLC-UV/VIS METHOD

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The presented work describes the development of simple, fast and effective on-line SPE-UHPLC-UV/VIS using sub-3 µm particle size column for separation and quantitative analysis of the nine illegal dyes, most frequently found in chili-containing spices. The red dyes Sudan I–IV, Sudan Red 7B, Sudan Red G, Sudan Orange G, Para Red, Methyl Red were separated and analyzed in less than 9 min without labour-consuming pretreatment procedure. The chromatographic separation was performed on Ascentis Express RP-Amide column with gradient elution using mixture of acetonitrile and water, as a mobile phase at a flow rate of 1.0 mL/min and 55 °C of temperature. As SPE column for cleanup was used Guard Cartridge Ascentis Express F5. The applicability of proposed method was proven

for three different chili-containing commercial samples. Recoveries for all compounds were between 90% and 108% and relative standard deviation ranging from 1.1% to 3.8% for within- and from 2.1% to 6.4% for between-day. Limits of detection showed less value than required by European Union regulations and were in the range of 3.3–10.3 μg/L for standard solutions, 5.6–235.6 μg/L for chili-containing spices.

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THE COMPARISON OF POLAR ANALYTES RETENTION ON SILICA AND ZIRCONIA STATIONARY PHASES IN HILIC

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Chromatographic analysis of polar compounds remains a significant challenge due to insufficient retention of these analytes under reversed phase (RP) conditions. Besides normal phase (NPC) and ion-exchange chromatography, hydrophilic interaction liquid chromatography (HILIC) is an up to date approach to overcome this issue. HILIC is usually performed on silica stationary phases, although silica applications can be limited by low pH and thermal stability. These drawbacks can be overcome by replacing silica with metal oxides such as zirconia, titania and alumina.

Our work deals with the retention behaviour of model hydrophilic analytes (4-aminobenzene sulfonic acid, 4-aminobenzoic acid, 4-hydroxybenzoic acid, 3,4-diaminobenzoic acid, 3-aminophenol and 3-nitrophenol) on the polybutadine modified zirconia in HILIC considering various pH, temperature and ACN content in mobile phase. The results were simultaneously compared with a bare zirconia and a silica-based HILIC (s-HILIC) phases.

It was found that the retention of carboxylic acids decreased with increasing ACN content until it reached 60–80% (reversed-phase behavior) and after that the trend reversed and the retention increased. Contrary to this, retention of sulfanilic acid was observed only in high content of ACN. s-HILIC was able to retain all compounds only using mobile phases containing more than 60% of ACN.

The increase in temperature led to increase of retention of carboxylic acids on zirconia phases, while the opposite trend was observed for sulfanilic acid as well as for all analytes on s-HILIC.

Although the retention of sulfanilic acid was influenced by pH alteration only marginally, a steep increase in retention of the carboxylic compounds were observed around pH 6 followed by its marked fall. This behavior suggests that ligand-exchange interactions are involved in retention of the compounds on the zirconia columns under HILIC conditions.

The retention in HILIC mode seems to be a multimodal process with adsorption as the main mechanism. Specifically that a carboxylic acid enters the water rich layer and then the carboxylic moiety interacts *via* ligand-exchange with the Lewis sites on zirconia.

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STABILITY INDICATING HPLC METHOD FOR DETERMINATION OF SODIUM PICOSULFATE, ITS IMPURITIES AND PRESERVATIVES IN PHARMACEUTICAL PREPARATIONS

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The aim of this study was to develop a new sensitive HPLC method with UV detection for simultaneous determination of sodium picosulfate, its two degradation products and preservatives methyl parahydroxybenzoate or sodium benzoate present in pharmaceutical oral solution form. The active substance belongs to the group of stimulating laxatives. A novel money-saving way of preparation of system suitability test solution using controlled partial hydrolysis of sodium picosulfate yielding the main degradation products was developed. The first chromatographic system was optimized for evaluation of preparations with sodium benzoate as preservative using RP-18 chromatographic column (250 × 4.0 mm, 5 μm), acetonitrile – propan-2-ol – phosphate buffer pH 7.0 with addition of cetyltrimethylammonium bromide (43:2:55, v/v/v) as mobile phase. The second method was developed for preparations with methyl parahydroxybenzoate as preservative and was based on separations with HSF5 chromatographic column (150 × 4.6 mm, 5 μm) with acetonitrile – phosphate buffer pH 3.5 (20:80, v/v) as mobile phase. Wavelength of UV detector was set to 263 nm. Both new methods were validated in accordance with ICH requirements which include linearity, precision, accuracy, specificity, range, limits of detection and quantitation and stability of samples. More comfortable normalization method was preferably recommended for purity determination. Both stability indicating methods can be used for routine analysis in pharmaceutical quality control.

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CAPILLARY ELECTROPHORETIC METHOD FOR THE SEPARATION OF INDOMETHACIN, 5-METHOXY-2-METHYL-3-INDOLEACETIC ACID AND 4-CHLOROBENZOIC ACID

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Indomethacin, [1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indol-3-yl]acetic acid, belongs to a group of non-steroidal anti-inflammatory drugs (NSAIDs). Two degradation products, 5-methoxy-2-methyl-3-indoleacetic acid (**I**) and 4-chlorobenzoic acid (**II**), are formed upon decomposition of indomethacin (hydrolysis of its amide group)[1]. The **II** is also mentioned in the Indomethacin monograph of the Czech Pharmacopoeia 2009 as the Impurity A. Capillary electrophoresis (CE) is an analytical separation technique requiring only small amounts of sample and background electrolyte (BGE) while offering excellent separation efficiency. On the other hand, when conducting CE analysis with UV/VIS spectrophotometric detection, the disadvantage of this method is relatively low sensitivity (because of short path length of the detection cell). To overcome this drawback, several approaches of on-line preconcentration techniques have been developed; they are summarized, e.g., in the literature[2]. The aim of our work was to develop capillary electrophoretic method for the separation of indomethacin and its two impurities (**I** and **II**) with use of on-line preconcentration procedure, namely large volume sample stacking with polarity switching. This technique is based on hydrodynamic injection of a large amount of the sample into the separation capillary followed by the application of reversed polarity voltage to remove excessive sample matrix electroosmotically, while anionic analytes are concentrated at the sample-BGE boundary. After this step the voltage polarity is changed and classical CE separation is employed. The method development included testing of the effect of several types of BGE, BGE composition (pH value, BGE and organic modifier concentration), applied voltage and operating temperature on the separation. After conventional CE method optimization, optimal conditions for the large volume sample stacking with polarity switching step were optimized.

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GRAPHENE-BASED SPE SORBENTS – PREPARATION AND PILOT EVALUATION

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Since its direct characterization by Novoselov in 2004, graphene has attracted large attention of scientific community. This perspective material, composed of two-dimensional single-atom-thick sheets of carbon organized in a hexagonal arrays, possess spectacular electrical, electrochemical, optical and mechanical properties[1]. Due to a large specific surface area (theoretical value of 2630 m^2/g) and a broad range of possible interactions, graphene is considered as a promising material in analytical chemistry[2].

The chemical synthesis from graphite is currently the most efficient method for low-cost and large-scale production of graphene. It is based on oxidation of natural graphite with subsequent exfoliation into graphite oxide (GO) sheets and reduction into graphene (reduced GO, rGO). The sheets of rGO and particularly GO are covered with oxygen containing functional groups (e. g. hydroxyl, epoxy and carboxyl), that modify the surface chemistry and are advantageous for further chemical modification[1].

The aim of this work was to investigate the ability of different SPE sorbents for modification with graphene and to evaluate the utility of these newly prepared materials for the extraction of analytes with distinct physicochemical properties.

GO was prepared from graphite powder using modified Hummers method[3]. Following sorbents were chosen for modification – aminosilica (AS), ZirChrom-SHAX (polyethyleneimine-coated zirconia, SHAX), ZirChrom-PEZ (EDTPA-coated zirconia, PEZ). The sorbents were mixed with GO solution and thermal reduction was applied. The model mixture containing a hydrophilic, an acidic, a basic, a neutral and a hydrophobic compound (guaifenesine, ibuprofen, lidocaine, propylparaben and 4'-isobutylacetophenone) was utilized for evaluation of extraction efficiency.

The unmodified sorbents were not able to sufficiently extract most of the analytes. Ibuprofen was sufficiently extracted only on AS and SHAX. On the other hand, lidocaine was quantitatively extracted only on PEZ.

The extraction recovery for all analytes increased significantly after modification and was above 79% on SHAX and PEZ. In the case of modified AS, the extraction efficiency was above 63% for all analytes, except for 4'-isobutylacetophenone, where only 28% of the initial amount was recovered.

The pH modification impacted the extraction efficiency particularly for ibuprofen, while the recovery of the other analytes remained nearly unchanged. In addition, the potential of the SHAX-modified sorbent for sample clean-up of plasma and urine was evaluated. The SHAX-modified sorbent showed insufficient extraction for ibuprofen from plasma sample.

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LC-MS/MS STUDY ON PHARMACOKINETICS OF A NOVEL THIOSEMICARBAZONE IRON CHELATING ANTINEOPLASTIC AGENT – DPC

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Being one of the top causes of death worldwide, cancer has been in the center of interest of many researchers for many decades. Despite undisputable progress in therapy, some tumors remain rather difficult to treat, often due to their resistance towards the currently established chemotherapeutics. Thiosemicarbazone iron chelators target intracellular iron, which participates in numerous metabolic processes. Tumor cells exhibit markedly increased growth and metabolism, which makes them exceptionally sensitive to the effect of thiosemicarbazones. A current lead compound of these novel and promising antineoplastic agents, di-(2-pyridylketone)-4-cyclohexyl-4methyl-3-thiosemicarbazone (DpC), exhibits a high anti-cancer potency with an acceptable toxicity profile. In order to promote the investigation from the preclinical to a clinical phase, it is essential to obtain basic data on the pharmacokinetic behavior of the drug candidate.

The aim of this work was to develop a bioanalytical method applicable for determination of DpC in different biological materials and to utilize it for the analysis of samples from a pharmacokinetic study in rats and an *in vitro* experiment with cancer cells.

A Shimadzu Nexera® UHPLC system coupled with Shimadzu LCMS-8030 triple-quadrupole mass detector were used in all analyses. Waters Acquity® BEH C18 (1.7 μm, 2.1 × 50 mm) column was used with ammonium formate and acetonitrile in gradient mode as a mobile phase. Samples were treated with LLE into acetonitrile/dichloromethane mixture. This method, suitable for analysis of DpC in plasma and MCF-7 cells, was partially validated according to the FDA guideline.

In this study male Whistar rats (n = 6) were injected intravenously with DpC at a dose of 2 mg/kg. Plasma was collected in defined time intervals. Subsequently, breast carcinoma cells (MCF-7) were incubated with DpC (n = 4) at different concentrations (0.5–50 μM)

and temperatures (3 and 37 °C). Cells were washed with cold PBS several times, harvested and analyzed.

The concentration-time profile of DpC in plasma was determined by analysis of samples from the *in vivo* experiment. Basic pharmacokinetic parameters were calculated using Kinetica 6.0 software.

The penetration of DpC into cells appears to be more pronounced at higher temperature, however the profile of the concentration inside of cells is rather similar between higher and lower temperature.

A new UHPLC-MS/MS method for analysis of DpC in different biological materials was successfully developed and validated. It was subsequently applied for determination of plasmatic profile of DpC *in vivo*. This method was also utilized in an *in vitro* experiment aimed at drug penetration into cancer cells.

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AUTOMATED IN-SYRINGE SINGLE-DROP HEAD-SPACE MICROEXTRACTION APPLIED TO THE DETERMINATION OF ETHANOL

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Single-drop head-space microextraction is a microextraction technique appreciated due to several advantages: it uses only a few microliters of the acceptor solution and this does not come into contact with the sample, so it can be applied to the extraction of analytes from complex sample matrices.

The mass transfer of the analyte from the sample into the head-space and further into the acceptor phase can be accelerated either by temperature elevation, mixing or pressure decrease[1]. In this work, decreased pressure was applied, using for the first time a syringe of an automated syringe pump as an extraction vessel for this purpose. Because the volume of the vessel is adaptable by the movement of the piston, negative pressure can easily be reached.

The method was applied to the determination of ethanol in wine samples. The determination was based on the reduction of a single drop of 6 mmol L^{-1} potassium dichromate dissolved in 8 mol L^{-1} sulfuric acid by the analyte. The drop was positioned in the syringe inlet in the head-space above the sample surface (Fig. 1) with posterior spectrophotometric quantification in a coupled flow detection cell.

The entire procedure was carried out automatically using a simple sequential injection analyser system and required less than 5 min per analysis including the washing step. A limit of detection of 0.025%V/V ethanol, repeatability in the range of 0.71 to 3.94% RSD and recovery of 101.4 ± 6.5% were achieved.

Fig. 1. In-syringe single-drop head-space microextraction with visible drop of reagent placed in the syringe inlet with applied vacuum.

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MEPS-MS/MS METHOD FOR THE DETERMINATION OF AMPHETAMINE AND METHADONE IN HUMAN URINE

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Direct injection into the mass spectrometer in combination with optimal sample preparation method is an interesting option for the analysis of biological samples in routine clinical practice considering time and cost requirements.

The aim of this work was to develop and validate method for the determination of amphetamine and methadone in human urine by direct injection into the mass spectrometer and to optimize the sample preparation method using microextraction by packed sorbent (MEPS).

The optimization of MS conditions was made with the regard to injection of relatively large volume directly from MEPS syringe. Optimization of MEPS procedure was a slightly

more challenging than the optimization of MS conditions due to the compatibility of volume and composition of elution solvent. Several types of MEPS sorbents differing in chemistry, particle size and MEPS needles of various constructions were tested. Further, the composition and volume of sample and elution solvent were optimized. The repeated aspiration and discharge during sampling and eluting step had an important influence on recoveries of analytes as well.

The optimal conditions of MEPS-MS/MS method for the determination of amphetamine and methadone were following: the mixture of 0.1% formic acid and methanol in the ratio of 20/80 (v/v) at a flow rate of 0.1 ml/min was used as a mobile phase. Electrospray ionization in positive ion mode was applied. Quantification of analytes was performed by the selected reaction monitoring and using deuterium labeled internal standards. C8 MEPS sorbent was chosen for the extraction. 50 µl of sample were five times aspirated and drained through the MEPS needle during sampling and eluting steps. The developed method was validated at four concentration levels including LOQ concentration. The validation data indicated good linearity ($r > 0.998$), recovery (92–107%), precision (RSD < 17%), accuracy (88–109%) and matrix effects (85–115%).

The combination of MEPS technique with the direct injection into the mass spectrometer enabled simple and fast analysis of amphetamine and methadone in human urine. The results of validation confirmed that developed method was suitable for the analysis of biological samples and that the chromatographic separation step could be excluded.

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SECTION OF PHARMACOLOGY AND TOXICOLOGY

ROLE OF DRUG TRANSPORTERS IN TRANSPLACENTAL PHARMACOKINETICS AND MULTIDRUG RESISTANCE

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Drug transporters are expressed in membranes of many physiological tissues and cancer cells. They are currently recognized as important determinants governing drug absorption, excretion, and distribution into target cells and organs. Members of the solute carrier (SLC)

and ATP-binding cassette (ABC) superfamilies are currently considered of major importance in drug therapy as they can affect pharmacokinetics and cause multidrug resistance in cancer.

Several drug efflux transporters have been found in placenta helping to prevent fetal exposure to potentially toxic drugs administered to pregnant women. Investigation of drug interaction with placental transporters is therefore inevitable for assessing the extent of drug exposure to the developing fetus. Antiretroviral combination therapy of HIV positive women represents one of the important cases, in which detailed knowledge of drug interaction with the transporters is necessary for optimization of pharmacotherapy in pregnancy. Employing cell based *in vitro* studies and *in situ* approaches we have identified several antiretrovirals as substrates of placental ABC or SLC transporters.

Inhibition of ABC transporters in tumor cells represents a challenging approach believed to improve anticancer pharmacotherapy outcomes. We have recently identified the ability of several cyclin dependent kinase inhibitors (CDKi) to inhibit ABC transporters *in vitro*. Moreover, these CDKi were able to synergistically potentiate the cytotoxic effect of anticancer drugs in ABC transporter overexpressing carcinoma cell lines. We therefore suggest that these drugs might work as dually acting agents not only (i) inhibiting the overexpressed cyclin-dependent kinases in cancer, but also (ii) increasing the accumulation of anticancer drugs in multidrug resistant cancer cells.

In conclusion, our results reveal clinically relevant transporter based interactions, which could have impact in pharmacotherapy of pregnant women as well as in anticancer therapy.

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THE TIME-RESOLVED RADIOIMMUNOASSAY MAY OPEN NEW PERSPECTIVES IN PROTEIN KINETICS ASSESSMENT

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The time resolved radioimmunoassay has reached the positive image of the method for ligand-receptor binding assessment. The application of this method also opens the possibility for pharmaceuticals kinetics study including well-known and under development drug substances. The time resolved radioimmunoassay is not the only method strictly focused on real-time detection of ligand-receptor interaction (association and dissociation), but it enables to see the background of the reaction and thus may unveil so far scientists eye hidden interaction processes. The traditional methods allow us to see only basic principle of ligand-receptor interaction, which renders the general information about the kinetics like dissociation constant (K_D) or 50% inhibitory concentration (IC50). When the basic

information about the reaction between two reaction partners is farther analyzed, we can see the strength of the ligand-receptor binding reaction and contributions to this binding coming from more than the only one population of receptors.

The first perspective aim of the time-resolved radioimmunoassay application was its use for the competitive binding study, which is frequently used for first characterization of newly prepared pharmaceuticals. We chose two strategies. Both of them used epidermal growth factor (EGF) as the natural ligand of epidermal growth factor receptor (EGFR) and two therapeutic radiolabeled antibodies as competitors. The first strategy may be referred to as pre-screening method, which studies the competition between competitor and well characterized ligand on the same receptor to quickly test competitor affinity. The second strategy was to compare the traditional manual competitive binding method with the time-resolved radioimmunoassay to define kinetics constants like IC50 and inhibition constant (K_i). The values of measured constants for competitors provided by the classical and time resolved radioimmunoassay did not differ significantly. However, the competitors' values of IC50 and K_i differed between cell lines.

The second application of the time-resolved radioimmunoassay was to combine the real-time measurements with Interaction Map technology to see ligand-receptor interaction in more detail. The time-resolved radioimmunoassay analysis was performed on two living cell lines to extract detailed interaction characteristics of two therapeutic antibodies binding to the same receptor, the epidermal growth factor receptor (EGFR), expressed on two different human carcinoma cell lines as well as binding of its natural ligand, i.e., the epidermal growth factor (EGF). Measured data rendered the information about the employed therapeutic antibodies effect on ligand receptor binding. This effect included heterogeneous interactions where the dynamics in heterogeneity is affected by the interaction itself.

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TRANSPLACENTAL PHARMACOKINETICS OF ZIDOVUDINE IS DETERMINED BY THE ACTIVITY OF ABCB1/ABCG2 AND ENT2 TRANSPORTERS

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Zidovudine (AZT), a nucleoside reverse transcriptase inhibitor, represents backbone of the current therapy of HIV-positive pregnant women. As AZT is listed in pregnancy category C by FDA, knowledge on its drug transplacental kinetics and interactions with placental drug transporters is inevitable to avoid inadequate and potentially dangerous medication for the fetus throughout the gestation. In this study we aimed to investigate whether transplacental pharmacokinetics of AZT is affected by its interaction with placental ATP-binding cassette (ABC) drug efflux transporters and/or equilibrative nucleoside

transports (ENTs) 1 and 2. ABC transporters such as P-glycoprotein (ABCB1) and Breast Cancer Resistance Protein (ABCG2) are known to limit permeation of their substrates across the placenta in maternal-to-fetal direction. On the other hand ENTs are required for uptake of nucleosides by syncytiotrophoblasts. We employed *in vitro* and *in situ* experimental approaches, i.e. transport of AZT across i) monolayers of MDCKII cells transduced with human transporters and ii) dually perfused rat term placenta in closed perfusion setup. Using *in vitro* method we observed that ABCB1 and ABCG2 interact with AZT. Dual perfusion of rat term placenta confirmed active transport of AZT in fetal-to-maternal direction. Application of a non-selective inhibitor of ABCB1 and ABCG2, GF120908 (2 μM), resulted in significant suppression of AZT active transport mediated by ABCB1/ABCG2 in both experimental approaches. Interestingly, uridin (5 mM) and NBMPR (100 μM), non-selective inhibitors of ENTs significantly reduced fetal-to-maternal transport. In order to specify the type of ENT transporter, we employed NBMPR (100 nM) that selectively inhibits ENT1 observing no effect. In conclusion, our findings suggest that AZT transplacental pharmacokinetics is affected by coordinated activity of both influx (Ent2) and efflux (Abcb1 and/or Abcg2) transporters. This should be taken into account when considering co-medication of pregnant women on AZT based therapy. Concomitantly administrated drugs that are substrate/inhibitor of ABCB1, ABCG2 and/or ENT2 can lead to unpredictable drug concentrations in the fetus.

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INTERACTIONS OF DINACICLIB AND PALBOCICLIB WITH ABC TRANSPORTERS ASSOCIATED WITH MULTIDRUG RESISTANCE

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Multidrug resistance is one of the major causes of failure in cancer chemotherapy. This phenomenon is mainly associated with the overexpression of ABCB1 (P-glycoprotein, MDR1), ABCG2 (breast cancer resistance protein, BCRP) or ABCC1 (multidrug resistance associated protein 1, MRP1) in tumor cells. In this project, we evaluated the possible interaction of dinaciclib and palbociclib, cyclin dependent kinase inhibitors currently undergoing clinical trials (phase III) for the treatment of cancer, with ABCB1, ABCG2 and ABCC1 transporters *in vitro*. ABC transporter function was evaluated in both membrane- and cell-based systems. XTT assays showed that dinaciclib was able to confer resistance to all three studied transporters, whereas palbociclib was not. Furthermore, dinaciclib also stimulated the baseline vanadate sensitive ATPase activity of ABCB1, ABCG2 and ABCC1 in Sf9 membrane vesicles overexpressing the respective transporters, suggesting dinaciclib as a substrate of all these transporters. We also determined that dinaciclib in-

creased mitoxantrone accumulation in MDCKII-ABCG2 and daunorubicin accumulation in MDCKII-ABCC1 cells. Palbociclib increased daunorubicin accumulation in MDCKII-ABCB1 and mitoxantrone accumulation in MDCKII-ABCG2 cell lines. In conclusion, dinaciclib is a likely substrate of ABCB1, ABCG2 and ABCC1, three major transporters associated with multidrug resistance, suggesting that cancer cells may develop resistance to this agent. Palbociclib, an unlikely substrate of any of the tested transporters, can overcome ABCB1- and ABCG2-mediated drug resistance by inhibiting the transporter activity and could therefore be used as a modulator of transporter function in the treatment of multidrug resistant tumors in combination with other chemotherapeutic agents.

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ORGANIC CATION TRANSPORTER 1 IS DOWNREGULATED BY PREGNAN X RECEPTOR

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Organic cation transporter 1 (OCT1, SLC22A1) is expressed mainly in the human liver. It is responsible for uptake of many endogenous substances and cationic drugs – e.g. antidiabetic drug metformin or some antivirotics such asacyclovir, zalcitabin.[1] Pregnan X receptor (PXR) plays an important role in regulation of xenibiotic and endobiotic metabolism. It affects gene expression of a wide variety of transporters and drug metabolizing enzymes. PXR is a ligand-activated transcription factor that has a wide variety of ligands, e.g. rifampicin[2].

The aim of this work is to elucidate whether PXR can regulate expression of OCT1 transporter.

First we used human hepatoma cell lines HepG2 and HUH-7 for gene reporter assay. In this series of experiments we used 1.7 kb OCT1 promoter luciferase gene reporter construct and PXR wild-type and mutated expression constructs. We also analyzed the level of OCT1 mRNA in hepatoma cell lines HepG2 and HUH-7 and also in human hepatocytes after treatment with prototype PXR ligands rifampicin and hyperforine.

We observed significant decrease of luminescence in gene reporter assay after co-transfection with OCT1 reporter construct and constitutively-active PXR expression constructs or after treatment with rifampicin in HepG2 and HUH-7 cells. In agreement the level of OCT1 mRNA in these cell lines was decreased after treatment with rifampicin. Additionally, we observed significantly lower levels of OCT1 mRNA in 9 out of 12 tested human hepatocytes after treatment with rifampicin.

We can conclude that PXR down-regulates OCT1 mRNA in both human hepatocytes and hepatoma cell lines.

The study was supported by PRVOUK project.

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SOLUBLE ENDOGLIN AND MARKERS OF ENDOTHELIAL FUNCTION/DYSFUNCTION IN MOUSE AORTA – A PILOT STUDY

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Endoglin (CD 105, TGF-β receptor III, ENG) is a homodimeric transmembrane glycoprotein that plays a regulatory role in several TGF-β signaling pathways. A soluble form of endoglin is generated by the cleavage of the extracellular domain from the intact membrane by MT1-MMP (membrane-type metalloprotease-1). Soluble endoglin was hypothesized to block TGF-β signaling in tissues. In addition, soluble endoglin was proposed to be a marker or a possible cause of endothelial dysfunction in diabetes, hypertension and atherosclerosis in humans and mice, but there are no data available so far showing possible mechanism of that effect. In this pilot study, we hypothesized whether high levels of soluble endoglin might affect expression of markers of endothelial function/dysfunction in mice aorta.

Transgenic mice overexpressing human soluble endoglin on CBAxC57BL/6J background (Sol.eng+) were generated at the University of Salamanca (Spain). Four months old female mice (n = 17) on chow diet were used. Plasma was extracted from a tail tip and the levels of human soluble endoglin were determined by ELISA. Western blot analysis of endoglin, VCAM-1, ICAM-1 and eNOS expression in aorta was performed.

ELISA analysis showed that only seven females were positive for high levels of soluble endoglin (sENG > 1000 ng/ml; Sol.eng+). Other mice reached low levels of soluble endoglin (sENG < 100 ng/ml) and were used as control mice. Western blot analysis revealed no differences in endoglin, VCAM-1, ICAM-1 and eNOS expression in aorta between Sol. eng+ mice and control mice.

High levels of soluble endoglin did not affect markers of endothelial function/dysfunction in aorta, however further studies are required to elucidate a possible influence of soluble endoglin on the function of aortic endothelium.

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ENDOTOXIN-MEDIATED IMPAIRMENT OF RENAL DRUG EXCRETORY FUNCTIONS IN RATS IS PREVENTED BY TWO ANTI-INFLAMMATORY AGENTS, DEXAMETHASONE AND IL-1 RECEPTOR ANTAGONIST, ANAKINRA

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The renal elimination of drugs is highly impaired in sepsis/endotoxin-induced acute kidney injury (AKI). Both glomerular filtration (GFR) and tubular secretion are reduced due to severe inflammatory response. We therefore investigated if two potent anti-inflammatory agents, dexamethasone and IL-1 receptor antagonist, anakinra, may protect renal drug excretory mechanisms during endotoxemia in rats. Rats were divided into four groups: saline controls, LPS-treated, and dexamethasone or anakinra (both in a dose of 10 mg/kg) pre-treated endotoxemic rats. Untreated endotoxemic rats developed within 10 h typical symptoms of AKI characterized by reduced GFR, microalbuminuria, increased fractional excretion of sodium, and decreased tubular secretion of azithromycin, the prototype substrate for multidrug transporters Mdr1 and Mrp2. Administration of either immunosuppressant prevented all these symptoms and restored the azithromycin tubular secretory clearance to control values. This effect was related to up-regulation of basolateral organic anion transporters, but not to Mdr1 or Mrp2, which were paradoxically down-regulated by these drugs. In addition, dexamethasone also increased the urinary clearance of bile acids by reduction of their transporter for reabsorption, Asbt. Effect on immune response indeed revealed more complex action of dexamethasone. Besides reduction of plasma cytokines seen after both agents, it also reduced plasma levels of nitric oxide as a result of reduced iNOS expression in the kidneys and liver. In summary, our data points toward significant role of IL-1β for the development of AKI during endotoxemia. Moreover, both agents were equally able to mitigate AKI imposed by endotoxin and demonstrated significant alleviation of impairment in the expression of major transporters for renal drug elimination.

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INTERACTIONS OF ACYCLIC NUCLEOSIDE PHOSPHONATES WITH SELECTED RENAL SLC AND ABC TRANSPORTERS

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Members of acyclic nucleoside phosphonates (ANPs) possess antiviral and antiproliferative activities. However, several clinically important ANPs may cause renal injury, most likely due to their active accumulation in the renal tubular cells[1]. The goal of this study was to investigate *in vitro* relationships between the affinity of several structurally related potent ANPs to selected human transporters and their cytotoxicity. SLC (solute carrier family) transporters (hOAT1, hOCT2, hCNT2, hCNT3) and ABC (ATP-binding cassette) transporters (MDR1, BCRP), which are typically expressed in the kidney[2], were included in the study. The transport and toxic parameters of the tested compounds were compared to those of two clinically approved ANPs, adefovir and tenofovir. Transport studies with transiently transfected cells were used as the main method in the experiments. Most of the ANPs studied showed the potency to interact with hOAT1. GS-9191, a double prodrug of PMEG, displayed an affinity for hOAT1 comparable with that of adefovir and tenofovir. No significant interaction of the tested ANPs with hOCT2, hCNT2 and hCNT3 was observed. Only GS-9191 was found to be a strong inhibitor for both MDR1 and BCRP. PMEO-DAPy showed the potency to interact with MDR1. Most of the tested substances caused a significant decrease in cellular viability in the cells transfected with hOAT1. Only with the exclusion of GS-9191, a relatively lipophilic compound, the *in vitro* cytotoxicity of the ANPs closely correspond to their potential to interact with hOAT1. The higher cytotoxicity of the compounds with affinity to hOAT1 proved in the inhibitory experiments evidences that ANPs are not only inhibitors but also substrates of hOAT1. Any clear relationship between the potency of ANPs to inhibit the studied efflux transporters and their cytotoxicity was not demonstrated. In conclusion, the study documented that among the studied transporters hOAT1 seems to be the decisive determinant for renal handling in most of the tested ANPs. This transporter may also play an important role in the mechanism of their potential cytotoxic effects. These facts are in good accordance with previous findings in the clinically used ANPs.

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STUDY OF TRANSPORT OF ABACAVIR ACROSS THE DUALLY PERFUSED RAT TERM PLACENTA

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Current prophylaxis of mother-to-child-transmission of HIV infection is based on combination antiretroviral therapy (cART) administered throughout the whole gestation. Despite the fact, that abacavir, a nucleoside reverse transcriptase inhibitor, is as a part of cART used in pregnant women, its transport across the placenta has not been satisfactorily described yet. In this study we aimed to describe effect of ATP-binding cassette (ABC) transporters P-glycoprotein (ABCB1) and Breast Cancer Resistance Protein (ABCG2) on abacavir transplacental pharmacokinetics using both *in vitro* and *in situ* experimental approaches. We firstly determined abacavir transcelullar transport using *in vitro* transport assays across MDCK II monolayers transduced with human ABC transporters. *In situ* method of dually perfused rat term placenta was subsequently employed to further evaluate possible role of these transporters in abacavir transplacental passage on the organ level. *In vitro* method revealed abacavir interactions with both ABCB1 and ABCG2 transporters; *in situ* experimental setup confirmed active abacavir transport in fetal-to-maternal direction that was completely abolished in the presence of dual ABCB1/ABCG2 inhibitor. Based on these results, we propose abacavir to be a substrate of ABCB1 and ABCG2 transporters and we also assume a significant role of these transporters in the transplacental pharmacokinetics of abacavir. These data might be of importance in assessing combination therapy of HIV-infected pregnant women.

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IMMUNOHISTOCHEMICAL EVALUATION OF ENDOGLIN, VCAM-1 AND P-SELECTIN EXPRESSION AND CO-EXPRESSION IN AORTAS OF APOE-DEFICIENT MICE DURING ATHEROGENESIS

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Both P-selectin and VCAM-1 represent crucial cell adhesion molecules that are the hallmarks of endothelial dysfunction and atherogenesis. Endoglin, an accessory receptor of TGF-β signaling pathway is a homodimeric transmembrane glycoprotein that has been demonstrated to play a role in vascular physiology and pathology, including atherosclero-

sis. It was also demonstrated that endoglin might be involved in inflammation and plays a role in leukocyte adhesion and transmigration in venules *in vitro* and *in vivo*. Thus, in this study targeting arteries, we wanted to evaluate endoglin expression in two different parts of aorta, namely aortic sinus and ascending aorta. Moreover, we aimed to assess its potential co-expression with cell adhesion molecules in aortas of apoE-deficient mice, or rather its possible contribution to leukocyte accumulation during atherogenesis.

Ten-week–old female apolipoprotein E-deficient mice on a C57BL/6J background (n = 24) were randomly subdivided into three groups and were fed either chow diet (for another two months) or Western type diet (for another two or four months). Immunohistochemical staining of endoglin, VCAM-1 and P-selectin in aortic sinus and ascending aorta was performed.

Endoglin expression was detected only in endothelial cells. Endoglin expression varied during atherogenic process in aorta but not in aortic sinus. Moreover, endoglin expression seemed to be weaker in aorta when compared to aortic sinus. Endoglin expression was detected only in endothelium covering atherosclerotic lesions but not in non-atherosclerotic endothelium regardless of the plaque size. Endoglin was not co-expressed with P-selectin and VCAM-1 in aortic endothelium in any studied group.

In conclusion, endoglin expression seems to be related to the atherogenic process especially in the extracardial part of aorta. Moreover, endoglin does not seem to be co-expressed with cell adhesion molecules that are critical for inflammation and atherogenesis, suggesting it might not participate in leukocyte accumulation in aorta of apoE-deficient mice during atherogenesis.

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STUDY OF DRUG INTERACTION WITH MATE1 TRANSPORTER USING ASP+ ACCUMULATION METHOD

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Multidrug and toxin extrusion proteins (MATEs) are together with human organic cation transporters (OCTs) the major transporters for secretion of cationic drugs. While OCTs act as uptake transporters, MATEs are H^+ exchangers localized in the apical membrane of hepatocytes and renal proximal tubular cells ensuring efflux of drugs into bile and urine.

To study drug interactions with MATE1, OCT1 and OCT2 transporters, we have established a method assessing cellular uptake of fluorescent substrate ASP^+ into Madin-Darby canine kidney (MDCK) cells stably expressing human MATE1, OCT1 and OCT2 transporters. Uptake of 1μM ASP+ has been evaluated under various time conditions in the presence of metformin, MPP+ and cimetidine, the known MATE1 substrates showing

variable affinity to the transporter. Furthermore, two antiretroviral drugs, lamivudine and emtricitabine have been evaluated for their ability to influence ASP+ accumulation in MATE1, OCT1 and OCT2 expressing MDCK cells.

While high affinity MATE1 substrates, MPP+ and cimetidine, were able to decrease ASP+ accumulation independently of the preincubation time (0, 5 or 15 min) at least 5min preincubation was needed to see the inhibition of ASP+ uptake by metformin. Lamivudine revealed the greatest decrease in ASP+ uptake when added 5 minutes before addition of the fluorescence substrate, whereas emtricitabine decreased the ASP+ accumulation most profoundly with the 15 minutes-preincubation period. Our data indicate that emtricitabine and lamivudine are low affinity substrates of MATE1. We also emphasize the importance of testing various time conditions, when assessing drug interaction with MATE1 transporter in the uptake assay.

The study was supported by the Grant Agency of the Charles University in Prague (GAUK 1148213/C/2013) and SVV/2013/267-003.

IN VITRO EVALUATION OF COPPER-CHELATING PROPERTIES OF FLAVONOIDS

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Flavonoids are polyphenolic, naturally occurring compounds which possess a wide variety of biological effects. *In vitro* studies have documented a number of positive cardiovascular effects including direct scavenging of free radicals, inhibition of reactive oxygen species-forming enzymes, influence on inflammatory processes, inhibition of platelet aggregation and direct vasodilatory action[1]. Transient metal (particularly iron and/or copper) chelation probably represents a substantial contribution to the desired activity of flavonoids, because free or loosely bound metal ions are harmful for biological systems.

This *in vitro* study was aimed at the comparison of copper (cupric as well as cuprous) chelation properties of various flavonoid compounds by the simple, rapid but precise spectrophotometric method using bathocuproinedisulfonic acid disodium salt, developed by our research group[2].

25 flavonoids (structurally belonging to flavones, flavonols, flavanones and flavanols) were tested at four (patho)physiologically relevant pH conditions (4.5–7.5) for their copper chelation activity and compared with the clinically used chelator, trientine. Marked differences based on structural features were observed and the chelation was dependent on the acidity of the environment in most cases. We found essential structural elements necessary for significant chelation activity: 2,3-double bond, 4-keto group and free 5-hydroxyl (in the case of flavones) and/or 3-hydroxyl group (in the case of flavonols). On the contrary,

the presence of 3′,4′-dihydroxyl group did not substantially influence the chelation activity in this assay. Baicalein (5,6,7-trihydroxyflavone) was the most potent flavone at all tested conditions and it reached or even surpassed the activity of trientine at more acidic conditions. A similar characteristic was observed in the case of 3-hydroxyflavone and its congener kaempferol (3,5,7,4′-tetrahydroxyflavone) at the ratio 10:1 (flavonoid to copper ions, respectively).

In conclusion, some flavonoids acted as potent copper chelators *in vitro*, even in comparison with the clinically used chelator. Related properties (e.g. the reduction of cupric ions) should be taken into account to further understanding of the complex action of these compounds.

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IN SILICO IDENTIFICATION OF MICRO RNAs PREDICTED TO TARGET NUCLEAR RECEPTORS AND OTHER TRANSCRIPTION FACTORS INVOLVED IN THE REGULATION OF DRUG METABOLISM ENZYMES

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Several nuclear receptors (NRs) were shown to play important regulation role in both constitutive and inducible gene expression of many drug metabolism enzymes (DMEs) such as cytochrome P450s (CYP). Among the most studied nuclear receptors belong constitutive androstane receptor (CAR), pregnane X receptor (PXR), hepatocyte nuclear factor 4α (HNF4α), vitamin D receptor (VDR) and glucocorticoid receptor (GR) with respect to their significance in transcriptional regulation of CYP genes. Moreover, it has been reported that coactivators and corepressors associate with NRs thus subsequently promote or suppress transcription machinery at the promoter of target genes, respectively. While many research papers implied function of NRs and coregulators in regulation of DMEs transcription, so far, their own regulation has not been fully clarified. Recently, microRNAs (miRNAs), short, noncoding RNAs has been suggested to be involved in post-transcriptional regulation of several NRs expression.

In the present study, we attempted to update current list of in silico identified miRNAs predicted to regulate NRs and coregulators involved in CYPs expression with respect to new discoveries in human miRNAs and advances in bioinformatic algorithms.

Taken together, we identified many miRNAs targeting 3′-UTR of evaluated transcription factors using various computational programs. MiRNAs identified were further compared with current experimental results reported in literature. The extent of false positive rates and characteristics of each *in silico* approaches will be discuss during presentation and interesting hits further comment.

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CHARACTERISTICS OF L6E9 MYOBLASTS – *IN VITRO* MODEL FOR STUDY OF DIFFERENT ENDOGLIN ISOFORMS

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Endoglin is an accessory receptor for TGFβ with important role in atherosclerosis. It has been demonstrated that there are two different alternatively spliced isoforms of endoglin called L-endoglin (long) and S-endoglin (short), which may have different effects on TGFβ signaling. Our previous *in vivo* study, we showed that atorvastatin upregulates endoglin related signaling proteins including phosphorylated Smad2. L6E9 myoblasts represent unique *in vitro* model for studying of endoglin related signaling. L6E9 myoblast mock cells are free from both human and rat endoglin expression. L-endoglin transfected L6E9 myoblast mock cells express only human L-endoglin. In this pilot experiment, we aimed to describe basic characteristic of mock and L-endoglin transfected L6E9 myoblast mock cells with respect to TGFβ1 and atorvastatin treatment.

The rat myoblast cell line L6E9 were grown in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum (FBS). 24 hours after plating, cells were serum starved in DMEM with 2% FBS for 24 hours and treated with either TGFβ1 (10ng/ml) or atorvastatin at concentration 5 μM in DMSO 0.1% (v.v) for 30 minutes. Endoglin and phosphorylated Smad2 (pSmad2) expression were determined by Western blot analysis. Cells were homogenized in RIPA lysis buffer and amount of protein onto the gell was 30 μg. Membranes were incubated over night at 4 °C with primary antibodies at the following concentrations: anti-human endoglin at 1:500 (sc-20632, Santa Cruz Biotech.) and pSmad2 at 1:500 (3108P, Cell Signaling).

Flow cytometry showed that L6E9 myoblasts do not express any endogenous rat endoglin in any studied cell lines. TGFβ1 treatment induce Smad2 phosphorylation in both mock and L-endoglin positive cells suggesting that endoglin is not part of this signaling cascade in L6E9 myoblast cells. Atorvastatin treatment did not induce Smad2 phosphorylation in either mock or L-endoglin cells suggesting that Smad2 might not be target for statins effect in these cells.

In conclusion, L6E9 myoblast cells seems to be interesting *in vitro* model for studying of endoglin importance in TGFβ signaling in various experimental conditions. Our pro-

spective studies will be focused on the role of endoglin on different signaling pathways that might play role in atherosclerosis (e.g. eNOS).

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STUDY OF RENAL TRANSMEMBRANE TRANSPORT OF PEPTIDES USING CELLULAR RENAL MODELS

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High expression of peptide receptors on tumours provides an attractive target for peptide-receptor radionuclide therapy and scintigraphy. Radiolabeled somatostatin analogues are effective therapy against selected neuroendocrine tumours. Other radiolabeled peptides such as bombesin or gastrin analogues are under preclinical and clinical investigation. However, retention of radiopeptides in the kidney can cause renal radiotoxic injury. The main transport mechanism responsible for the renal uptake is still not fully identified, but the experimental studies point out the role of endocytic receptor megalin[1]. However, transport by influx transporters for organic anions (OATs), organic cations (OCTs) and fluid phase endocytosis can contribute to the renal accumulation, in addition involvement of efflux transporters MDR1 or BCRP in renal handling of the radiopeptides has not been studied. This study was aimed at evaluation of the role of renally expressed aforementioned transporter mechanisms on the cellular accumulation of radiolabeled somatostatin, gastrin and bombesin analogues using an *in vitro* model.

To evaluate a contribution of active endocytosis by megalin, pig kidney epithelial cell line LLC-PK1 stably expressing this system was affected by RAP as a competitive inhibitor, and by albumin as a proven substrate/inhibitor of megalin. Rottlerin was used as a known inhibitor of fluid phase endocytosis and incubation under low temperature (4 °C) served to reveal general role of active transport process in the cell uptake. We investigated possible inhibitory effect of studied peptides on BCRP and MDR1 transport activity employing stably transduced MDCKII cells. HeLa cells transiently transfected with hOAT1 and MDCKII cells with hOCT2 were used to analyse the contribution of organic transporters on peptides uptake. Six peptides were tested: ^{177}Lu-DOTA-[Tyr^3] octreotate, ^{177}Lu-DOTA-[1-Nal3]octreotide, ^{177}Lu-DOTA-sargastrin, ^{177}Lu-DOTA-[Pro^1,Tyr^4]bombesin, ^{177}Lu-DOTA-[Lys^3]bombesin and ^{177}Lu-PCTA-[Lys^3]bombesin.

Cellular accumulation in the used cellular models was significantly inhibited under lower incubation temperature, so we confirmed that this process is at least partly active. Incubation with the specific megalin ligands, albumin and RAP, resulted in a significant inhibition of the accumulation of all studied radiopeptides. Therefore, this type of transport *via* receptor-mediated endocytosis may be responsible for influx of the radio-

peptides. Fluid-phase endocytosis plays a role simultaneously, since rottlerin decreased intracellular accumulation of the radiopeptides. However, the inhibition was less intensive than that of the aforementioned substances. The transporters for organic anions and cations seem to play no significant role in the renal uptake. Efflux transporters BCRP and MDR1 did not exhibit any detectable effect on accumulation of these radiolabeled peptides.

The study was supported by Charles University in Prague (Project SVV 267003) and grant GAUK No.376411/FaF/C-LEK.

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EFFECTS OF ATORVASTATIN AND TNF-ALPHA TREATMENT ON ENDOGLIN AND eNOS EXPRESSION IN ENDOTHELIAL CELLS

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Endoglin (TGF-β receptor III, CD105) is able to regulate eNOS expression in blood vessels suggesting its role in atherosclerosis. Statins were demonstrated to increase eNOS and endoglin expression *in vivo* in mouse atherosclerotic aorta. In this study, we focused on endoglin and eNOS expression during inflammation and after atorvastatin treatment in HUVECs. We also hypothesized whether statin induced eNOS expression depends on endoglin.

HUVECs were exposed to TNFα (10 ng/ml) for 2, 6 and 16 h to mimic inflammation. Atorvastatin (ATV) was added for 24h at a concentration of 5 μM, DMSO 0.1% (v/v) was used as control. In atorvastatin pretreatment model, cells were treated 24 h by ATV, then rinsed and cultured with TNFα for 16 h. Endoglin and eNOS expression was also studied after endoglin silencing. HUVEC cells with siRNA of endoglin were prepared by using Amaxa HUVEC Nucleofector kit and pre-designed siRNA from Ambion. The protein expression was determined by flow cytometry and Western blot analysis and soluble endoglin by means of ELISA.

ATV treatment significantly increased endoglin and eNOS expression in HUVECs. TNFα treatment for 16h significantly reduced endoglin expression, together with significant increase of soluble endoglin in medium. Pretreatment of ATV, before TNFα exposure, significantly prevented decrease of endoglin and eNOS expression, when compared to cells treated only by TNFα. Endoglin silencing significantly reduced the expression of endoglin. Atorvastatin had no effect on eNOS expression in endoglin siRNA HUVECs.

Inflammation results in reduced expression of endoglin and eNOS in HUVECs, which could be prevented by atorvastatin treatment. Moreover, atorvastatin induced eNOS expression seems to be dependent on endoglin expression. Since endoglin and eNOS play important role in various cardiovascular pathologies including atherosclerosis, hypertension, diabetes, preeclampsia and hereditary hemorrhagic telangiectasia, we propose that statin effects on tissue and soluble endoglin in these diseases should be evaluated in clinical studies.

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SECTION OF CLINICAL AND SOCIAL PHARMACY

OPINIONS AND ATTITUDES OF CZECH CITIZENS ON SELECTED ISSUES OF DRUGS IN SOCIETY (REPRESENTATIVE SOCIOLOGICAL SURVEY)

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The social sciences in health care and pharmacy enable various points of view on the issues often managed not only by health care professionals but also other specialists inside or outside of health care system. This multidisciplinary often leads to divergent views and offers various opinions and solutions. The paper is devoted to the opinions and attitudes of Czech citizens on the issue of healthcare and pharmacy which can be a source for understanding the relation-ships in pluralist health care system as a whole. This article presents the results of a representative sociological survey. The sample of 1797 respondents is a representative sample of the Czech population aged over 15 years in terms of gender, age and region. The research is fo-cused on issues of self-medication, choice of pharmacy, out of pocket expenditures on prescription drugs and over-the-counter medicines, experiences with their side effects, evaluation of leaflets' comprehension. According to the results, the most of the population during illness tries to treat itself before seeing a doctor (54.1% always, 30.9% sometimes). The over-the-counter drugs are bought mostly in classical pharmacies without self-service (96.1%). The choice of pharmacy determines primarily proximity and much less personal experience or price. They spend around 150 CZK for the over-the-counter medicines and around 143 CZK for the prescription drugs per mounth; 77.9% of Czech citizens understand leaflets. This data helps to understand the perception, orientation and behavior of the patient in the healthcare system and can lead to higher effectiveness and satisfaction of all stakeholders.

The study was supported by SVV 265/005.

ANALYSIS OF SELF-REPORTED ADHERENCE AND LDL CHOLESTEROL GOAL ACHIEVEMENT IN PATIENT CHRONICALLY TREATED WITH STATINS

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Introduction: Non-adherence to long-term therapy demonstrates the serious problem leading to therapy failure. Asymptomatic diseases such as dyslipidemia can contribute to this and thus measuring patient related adherence with established assessment tools should be required to achieve optimal health outcomes.

Objective: The aim was to analyze the adherence using subjective (self-reported questionnaires) and objective (health outcome achievement) tools in statin users.

Methods: In cross-sectional prospective study outpatients ≥ 18 years aged treated with statins at least 3 months were addressed. Anonymous structured interview was conducted to determine self-reported medication adherence (by Medication Adherence report Scale, MARS-CZ) and other supplementary patient characteristics. At the day of interview medication records were reviewed and the low density lipoprotein cholesterol (LDL-c) goal achievement as the primary aim of statin therapy was assessed. Statistical analysis was performed using software R (R Foundation for Statistical Computing, Austria) and correlations were analyzed by Fisher´s exact or Goodman-Kruskal gamma tests ($p < 0.05$).

Results: From 157 eligible patients 136 (86.6%) completed the interview (67 men, mean age 66.1 ± 10.5 years and 69 women, 65.9 ± 10.7 years). 92.6% patients reported high adherence according to MARS-CZ. Significant correlation between level of adherence by MARS-CZ and LDL-c goal achievement was observed. All patients who achieved their LDL-c goal had high adherence to statin therapy and 9 patients from 10 having low adherence did not achieve LDL-c goal. Physicians' judgment, i. e. at least 50% cholesterol decrease, in relation to MARS-CZ score was also significant.

Conclusion: Although the adherence distribution was positively skewed, this study showed that MARS-CZ can be used as an effective tool to measure medication non-adherence in statin users in a combination with cholesterol parameters assessment. Therefore if patients do not achieve their LDL-c goal, it can be alerted to a signal of non-adherence.

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PRAKTICKÝ LÉKÁRNÍK – A JOURNAL DEVOTED TO PRACTICAL ISSUES OF PHARMACY PRACTICE

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Praktický lékárník, a professional journal focused on pharmacy practice, started in 1932. In this period, 4 journals concerned with pharmacy were issued: two rather scientific and professional (*Časopis československého lékárnictva*, *Vojenské zdravotnické listy*) and two dealing with internal problems of the profession and policy (*Věstník Svazu československého lékárnictva*, *Lékárnické listy*). The aim of this research was to recognize the focus and content of the journal *Praktický lékárník* and its position among other contemporary pharmaceutical journals. Another objective was to recognize its importance for issues of work rationalization (scientific management) in pharmacy practice and possible connection with *Section of Pharmacy of Commission for Rationalization and Standardization in Medicine, Veterinary Medicine and Pharmacy* (working between 1929 and 1932). The work is based on the study of the issues of *Praktický lékárník*, other contemporary pharmaceutical journals, archival records and pertinent secondary sources.

In accordance with the publisher's intention, *Praktický lékárník* was concerned with the issues of everyday practice in pharmacies. Usable information on compounding of medicines, pharmaceutical control or pharmacotherapy was published, as well as articles on economic, administrative and legal aspects of pharmacy practice. Although not primarily focused on scientific management and related modernization of pharmacies, it devoted an intensive attention to these topics. However, no evident connection with the work or members of the Section of Pharmacy was found. In 1941, Commerce-Industry company, the publisher of the journal, stopped publishing it. In 1942, the remaining pharmaceutical journals were merged into a new journal, *Lékárnický věstník*, by decision of the Protectorate administration.

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DIAGNOSTIC AND TREATMENT DELAY AMONG PULMONARY TUBERCULOSIS PATIENTS IN THE ARAL SEA REGION OF UZBEKISTAN

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Introduction: Tuberculosis (TB) remains a severe global health problem and ranks as the second leading cause of death from infectious diseases worldwide after the human immunodeficiency virus (HIV). According to the World Health Organization Uzbekistan has a high burden of TB, including multi-drug resistant TB. Early diagnosis and prompt effective therapy plays an important role in TB control. Delay in diagnosis results in clinical deterioration and ongoing transmission in the community.

Objective: The aim of this study was to evaluate the extent of delay in diagnosis and treatment of patients with pulmonary tuberculosis and identify risk factors for patient and health system delay.

Methodology: Patients with newly diagnosed smear-positive pulmonary tuberculosis, 15 years of age and older, diagnosed in three tuberculosis hospitals in the Aral Sea region of Uzbekistan were interviewed and hospital medical and TB DOTS clinic records were collected.

Results: Among 300 patients enrolled, the mean duration of delay from onset of symptoms until treatment with anti-tuberculosis drugs was 59.2 days. Detailed analysis of the factors affecting the health-seeking behavior and timely treatment showed the presence of patient or health system factors. Self-medication was the first choice for more than half of patients. The main predictors for delay were sociodemographic (illiteracy, rural residence), economic and stigma.

Conclusion: Diagnostic and treatment delay of pulmonary tuberculosis patient was identified in a region with high prevalence of drug resistant TB. Effective strategies involving regulatory enforcement prohibiting sales of anti-TB drugs without prescription to be implemented together with public education sessions emphasizing the curability of TB.

The study was supported by Charles University in Prague (Project SVV 265 005).

RESEARCH OF MEDICATION ADHERENCE: WHY IS CRONBACH'S ALPHA SOMETIMES POINTLESS?

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Introduction: Cronbach's alpha was developed to provide a measure of the internal consistency of a test or scale (range from 0 to 1). Internal consistency describes the extent to which all items in a test measure the same concept and hence it is connected to the interrelatedness of the items within the test.

Objectives: The objective is to discuss appropriateness of calculation of Cronbach's alpha as a measure of the internal consistency of tests in research of medication adherence and to point out associations with validity and reliability of questionnaire tools.

Methods: Based on the paper of key importance for medication adherence measurement *Voils et al. J Clin Epidemiol 2011; 64: 250-4,* we reviewed literature from PubMed to clarify importance of Cronbach's alpha as a psychometric parameter of multi-item measures (questionnaire tools) in the research of medication adherence. The following key words were used: medication (non)adherence, Cronbach's alpha, validity, reliability, internal consistency, multi-item tools, self-reports, latent variable, psychometrics, measurement.

Results: Adherence can be measured directly as a measured variable (number or percentage of missed doses over a specified period) or indirectly as a latent variable. Measurement of a latent variable should be properly based on one of the two models. In an effect indicator model the latent variable determines the values of the indicators. By contrast, the causal indicator model involves indicators that are relatively unique, additive components of a construct. Internal consistency is reported because effect indicators should be positively and highly correlated. Causal indicators may be positively or negatively correlated or uncorrelated. The most important issue for validity assessment of causal indicator models is whether the items adequately represent all components of a construct.

Conclusion: The distinction between causal and effect indicator model is important for proper understanding of validity of multi-item instruments. Internal consistency expressed as Cronbach's alpha to reflect validity and reliability of a causal indicator model is misleading.

The study was supported by project SVV 267 005.

ADHERENCE TO CALCIUM AND VITAMIN D SUPPLEMENTATION IN POSTMENOPAUSAL WOMEN AT RISK OF OSTEOPOROSIS-RELATED FRACTURE (PILOT STUDY)

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Introduction: Osteoporosis is an important health, social and economic problem. Supplementation with calcium and vitamin D significantly increases the effect of antiresorptive medication and the overall treatment effect is not simply the sum of the individual effects. While adherence to bisphosphonates is at least partially monitored in clinical practice, adherence to supplementation therapy escapes attention.

Objectives: The objective of the study was to evaluate treatment adherence to calcium and vitamin D (Caltrate tbl.) in a sample of postmenopausal women (over 55 years) treated for osteoporosis or osteopenia in clinical practice.

Methods: This was a pharmacoepidemiological observational study. Data were obtained in osteocenter in University hospital in Hradec Králové (Department of Clinical Biochemistry). Adherence was measured by a unique combination of methods: electronic bottles type Medication Events Monitoring System (MEMS) and self-reported questionnaire. The measuring of the adherence covered a period of 90 days in each patient.

Results: The mean age of the sample (n = 36) was 72 years (range 56–82). Based on MEMS, the mean proportion of days covered (PDC) was 64% (range 2.2–106.7%), the mean proportion of days covered concerning weekend days was 58%. As much as 50% of the women used Caltrate in the evening (6 pm – 12 am). Based on the questionnaire, 10% of women experienced gastrointestinal disorders related to calcium. When assessing last 30 days, 98% of tablets were used on average on the basis of the questionnaire and only 61% of tablets based on MEMS.

Conclusion: The mean PDC during the observed period of 3 months was 64%. The mean PDC decreased from 64% to 61% in the third month. By comparing the results based on MEMS and the questionnaire, women markedly overestimate their adherence with the use of calcium and vitamin D in the questionnaire.

The study was supported by project SVV 267 005.

COMPARISON OF DRUG DOSING RECOMMENDATIONS FOR ANTIMICROBIAL DRUGS BASED ON KIDNEY FUNCTION ESTIMATING EQUATIONS IN OBESE PATIENTS

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Introduction: The glomerular filtration rate (GFR) is the best overall index of kidney function. However, it cannot be directly measured. In clinical practice, GFR is routinely estimated by using a number of methods[1]. Mathematical estimations of GFR, based on serum creatinine, are a clinically useful method to follow renal function. Convention supports the use of Cockcroft-Gault (CG) for the purposes of drug dosing. Drug manufacturers rely on this equation to stratify patients by severity of kidney function when studying the pharmacokinetics of new drug. New equations were developed to estimate GFR more accurately than CG. One example is the Modification of Diet in Renal Disease (MDRD) equation. The impact of using MDRD has not been formally evaluated with respect to drug dosing[2]. This equations (CG, MDRD) were developed in non-obese populations. Obesity includes various different pathophysiological factors like glomerular hyperfiltration, intraglomerular hypertension, insulin resistance, increased sympathetic activity and hyperactivity of the renin-angiotensin system[3].

Objective: The aim of the project was to determine whether a difference exists when determining antimicrobial dosage adjustments in obese patients with chronic kidney disease, based on estimation of GFR using the MDRD and CG equations.

Methods: We conducted an observational analysis of 60 obese patients with chronic kidney disease. Patients with body mass index (BMI) >30 kg/m^2 were included. Patients were > 18 years and had stable renal function, and they were > 3 months after renal transplantation. GFR was calculated using the 4 – variable MDRD equation and the CG equation. Dosage discordance rates of the selected antimicrobials were determined on the basis of manufacturer renal dose recommendations.

Results: Patients were screened between Januar 1 and October 30, 2013. A total of 60 patients were screened. Demographic data of the patients: BMI 33 (30.4-36.9), age (yrs) 53 (37–52), S_{Cr} (μmol/l) 185+/− 0.55. They resulted in discordant dosages of the antimicrobial drugs (meropenem, cefepime) 11.5–20% ($p < 0.001$) of the time. When doses of antimicrobials were discordant, the MDRD equation would have resulted in the prescription of higher doses.

Conclusion: This analysis demonstrated statistically significant differences between the CG and MDRD equations in obese patients. The clinical significance of these differences is uncertain in the absence of data regarding clinical outcomes that would result from the use of discordant doses.

The study was supported by 2014 SVV 265 005.

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NURSING HOME RESIDENTS AND DEPRESSION

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Introduction: Depression is very common psychiatric disease in elderly. It increases morbidity and can lead to malnutrition, poor hydration, decreases quality of life and physical activity. Nursing home population is more sensitive to depressive symptoms than older people living in community.

Objective: The purpose of this part of our study was to find out associations between poly-pharmacotherapy, several measures of functional status and depression in nursing homes residents.

Methods: This cross-sectional study was conducted in three nursing homes from the Czech Republic. All respondents including in study were ask to fill GDS 15, ADL, IADL and MMSE. We collected clinical and demographic data from patient medical records. Charles Comorbitity Index was calculated for each respondent from medical diagnoses. For statistical analyses was used PASW 18.0.

Results: 103 (76.3%) of the 135 contacted residents were interviewed. The number of women was 82 (79.6%) and the mean age of all respondents was 83.2 (SD = 7.6). A score within a normal range on GDS 15 had 51.5% of respondents, 34.0% showing moderate and 14.5% severe depressive symptoms. Multiple regressions showed that only ADL, IADL and MMSE were significantly correlated with depressive symptoms.

Conclusion: Our findings indicate a strong relationship between depressive symptoms or depression and functional states of nursing home residents but poly-pharmacotherapy probably doesn't have an impact to depression in this group people. Depression is commonly under-diagnosing therefore nursing staff should be more focus on its monitoring and appropriate treatment.

The study was supported by the Grant of Charles University in Prague SVV 267 005.

THE IMPACT OF ATORVASTATIN AND SIMVASTATIN USE IN THE PREVENTION OF CARDIOVASCULAR DISEASES IN THE CZECH REPUBLIC: COST-EFFECTIVENESS ANALYSIS BASED ON MODELING APPROACH

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Objectives: To assess the impact of atorvastatin compared to simvastatin use in the Czech Republic into cardiovascular diseases (CVD), Life-Years Gained (LYG) and Quality-Adjusted Life Years (QALY), based on the real proportional consumption of both statins in particular strengths (10 mg, 20 mg, 40 mg).

Methods: Life-time cost-effectiveness Markov cohort model was developed with 1 year cycle length and 5 health sates, i.e. Alive without CVD, Alive with experience of CVD, Non-fatal CVD, Fatal CVD and Death. The probability of transition among health states were derived from Framingham equations[1] or from SCORE equations[2], i.e. probability of the first non/fatal CVD), Czech life-tables (i.e. for background mortality)[3] and international cohort studies (i.e. probability of subsequent CVD)[4].

Patients enter the model with base-line risk characteristics: age, proportions of males, diabetics, smokers, level of systolic blood pressure and cholesterol (total and HDL) level. The efficacy data for particular statin and its strength were derived from latest meta-analyses[5]. Drug acquisition costs of atorvastatin 10 mg and 20 mg were 10% higher compared to simvastatin 20 mg and 40 mg. The costs of fatal, non-fatal CVD and one-year follow-up after CVD were 1,410 EUR, 1,460 EUR and 580 EUR.

Results: Over a life-time horizon, the highest costs are attributed to atorvastatin cohort (3,025 EUR), however this cohort also revealed the highest LYG and QALY gained, simultaneously with the highest proportion of non/fatal CVD averted. Atorvastatin compared to simvastatin provides 7.77 QALYs vs. 7.69 QALYs, 10.85 LYG vs. 10.75 LYG, 56.02% vs. 58.44% of non-fatal CVD (RRR is 4.14%) and 34.9% vs. 36.27% of fatal CVD (RRR is 3.78%), see Fig. 1.

Atorvastatin compared to simvastatin provides increment total costs of 468 EUR, which provides ICER for atorvastatin vs. simvastatin 5,693 EUR/ QALY and 4,475 EUR/ LYG.

Conclusions: Over a life-time horizon (from the statin treatment initiation), in a cohort of 100 patients atorvastatin compared to simvastatin provides 776.8 QALYs vs. 768.6 QALYs (incremental gain of 8.22 QALYs), 1085.0 LYGs vs. 1074.6 LYGs (incremental gain of 10.40 LYGs). On a population level, if all patients currently on simvastatin (165 thousand patients in the Czech Republic) were treated with atorvastatin, there would be a gain of 17,160 life-years, or 13,563 QALYs from the statin treatment initiation to the end of life. Hence, these values represent the loss of health in the population of the Czech Republic.

The study was supported by Pfizer, s.r.o. Czech Republic.

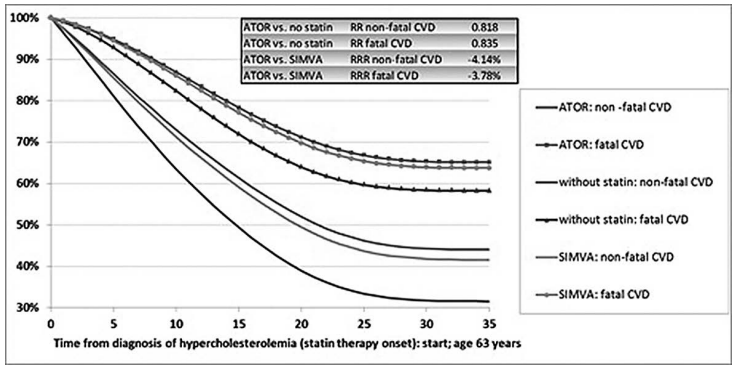

Fig. 1. Cumulative number of fatal and non-fatal CVD of each intervention. The percentage is proportion of patients free from particular complication, i.e. fatal, non-fatal CVD.
RR – relative risk, RRR – relative risk reduction

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PREVENTION OF INTRAVENOUS DRUG INCOMPATIBILITIES IN AN INTENSIVE CARE UNIT

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Introduction: Drug incompatibilities are relatively common in patients and may result in morbidity or mortality of patients and add to the cost of the therapy. They make up 25% of medication errors occurring in intensive care units (ICU). The risk of incompatibilities cannot be completely eliminated; however, we can significantly help to reduce this risk.

Materials & Methods: The data were collected by the direct observation of nurses administering intravenous medications at Department of Gerontology and Metabolism in University Hospital Hradec Králové. Data were collected in 2010–2013 in patients with two or more intravenous drugs. Interventions in intravenous drug incompatibilities include

direct education of medical and nursing staff, processing a wall board of incompatibilities and a brochure of proper administration of intravenous drugs.

Data to assess the effectiveness of the proposed rules were obtained at least 2 months after all educational interventions. To assess the incompatibilities the Handbook on Injectable Drugs (Trissel LA, 2009) was used. The results were processed using descriptive statistics.

Results: In the analysis of 50 patients before the intervention 9.43% incompatible medicines out of 318 submitted were found. Frequently observed incompatibilities include insulin, ranitidine, furosemide and ciprofloxacin. The analysis after intervention followed 25 patients and of the 121 medicines administered intravenously was 6.61% incompatible. Among incompatible medicines belonged furosemide, fluconazole, cotrimoxazole, amiodarone and pantoprazole. After the interventions made was meant incompatibilities decline of 29.9%.

Discussions, Conclusion: The proposed method to reduce the risk can be used to prevent incompatibilities and to apply them in a real environment in intensive care. Comparison with similar studies in this area shows a comparable incidence of incompatibilities before the intervention and similar improvements in problem after education and intervention (eg Bertsche, 2008). To confirm our proposed method and the results obtained, it is necessary to carry out further studies on similar sites in the Czech Republic.

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THE TRENDS IN ANTIBIOTIC PRESCRIBING BY PRACTICAL DENTISTS IN THE CZECH REPUBLIC SINCE 2006.

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Objectives: The aim of the study was the evaluation of the antibiotic prescribing in practical dentistry, as some warning signals of antibiotic overuse can be observed in Czech Republic.

Methods: The individual antibiotic prescriptions were extracted from the database of the General Health Insurance Company. The proportion of dentist's prescription of antibiotics and the rate of prescriptions of particular antibiotics were both in Defined daily doses

per 1000 insurees and day (DID) and in number of prescriptions calculated. The studied period were the years 2006–2012.

Results: The proportion of antibiotic use in dentistry incresed from 0.63 DID in 2006 to 0.75 DID in 2012. We found the decline in use of narrow-spectrum penicillins by 4.8%, tetracyclines by 3.5% and macrolides by 3.6%, accompanied by increasing rate in prescriptions of aminopenicillins combined with beta-lactamase inhibitor by 8.9% and lincosamides even by 8.5%. The consumption of clindamycin and amoxicillin combined with clavulanate in DID increased by approimately 60% since 2006 thanks to the exclusive prescribing two commercial oral products only.

Conclusion: We noted the trends in antibiotic prescriptions in dentistry, including an growing share of the total consumption of antibiotics in primary health care area, the rising consumption of broad-spectrum types of antibiotics, and estimate the proportion of patients with poorly chosen dose of antibiotics. All of these outstanding issues have not been described in public, and discussed in the community of Czech dentists yet. The factors contibuting to this unfavourable trend are especially the commercial influence or the defensive medicine practice.

ADVERSE DRUG REACTIONS: ANALYSIS OF SPONTANEOUS REPORTING SYSTEM IN EUROPE IN 2007–2009

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Purpose: Spontaneous reporting systems in European countries are crucial for collecting adverse drug reaction (ADR) reports. The aim of this study was to evaluate reporting activity among countries and their strategy to increase the number of reports. We also established the best measure for assessment quantity of reports.

Methods: This was a retrospective observational study based on questionnaires and annual reports. The most reliable measure of reporting was determined by Spearman correlation coefficients.

Results: Data collected in spontaneous reporting systems in 26 European countries were analysed. In 2007, 2008 and 2009, the average value of reports per year per million inhabitants based on the safety databases of countries was 208, 236, 286, respectively; in comparison, that of EudraVigilance was 311, 453 and 435, respectively. Twelve countries reached a significant level for signal detection of ADRs in 2009. The population-based reporting ratio (PBRR) was correlated to the total expenditure on health ($\rho = 0.499$, $p = 0.023$, $n = 21$), public expenditure on health ($\rho = 0.477$, $p = 0.035$, $n = 20$), density of physicians ($\rho = 0.336$, $p = 0.136$, $n = 21$) and expenditure on pharmaceuticals ($\rho = 0.365$, $p = 0.114$, $n = 20$). Strategies of regulatory authorities to increase reporting were determined.

Conclusions: The results of this study make several noteworthy contributions regarding national spontaneous reporting systems. The relevance of the PBRR for the measurement

reporting activity is clearly supported by the current findings. This study also shows that there is a general trend towards increased reporting activity. This is maintained by regional centres and encouragement of reporting. A further study would be helpful to assess the effectiveness of reporting systems at both the national and European level.

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PHARMACY STUDENTS OF JEWISH ORIGIN OR RELIGION AT THE CHARLES-FERDINAND UNIVERSITY FROM YEAR 1882 TO YEAR 1918 AND CHARLES UNIVERSITY AND GERMAN UNIVERSITY FROM YEAR 1919 TO YEAR 1938

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In my contribution I will deal with the pharmacy students of Jewish origin or religion, at the Charles-Ferdinand University in Prague in the years of the Austro-Hungarian Empire, especially from the division of the university to the Czech and German parts in 1882 and at the Charles University and German University from year 1919 to year 1938 in the years of Czechoslovak Republic.

In the years 1654-1804 the faculty organized only examinations. In 1804 pharmaceutical higher education in Austria officially started in the form of one-year course at the Faculty of Medicine. In 1864 the study was organized by the Faculty of Arts, after 1919 by the Faculty of Nature Sciences. In the winter semester of 1834/1835 the study was extended for two years.

The first student of pharmacy before the division of the university in 1882 was Josef Jeiteles who graduated in chemistry in 1852. Next year he passed the doctoral degree in chemistry. In the years 1834–1890 four Jewish students graduated from the German part of the University. Prominent figure was Max Fanta, member of Prague Pharmacy family who completed the study in 1884.

After the division of the university the majority of students studied at its German part. In 1919 was created Czech Charles University, separated from the German University. In 1939 was Charles University closed by the Nazis, so no students could study there including the Jewish ones.

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FAT-RELATED AND FIBER-RELATED DIET BEHAVIOR AMONG TYPE 2 DIABETES PATIENTS FROM DISTINCT REGIONS

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Objective: Diet is a key part of type 2 diabetes mellitus (T2DM) management. Optimal diet education is impossible without the knowledge of individual's eating habits. As culture seems to be the most important determinant of food intake it is important to conduct specific diet behavior research for each country or cultural group. We aimed to analyse and compare eating habits, with emphasis on fat- and fiber-related behavior (FFB), in patients with T2DM from distinct cultural areas.

Methods: Observational cross-sectional study was conducted in the Czech Republic (CR) (n = 200), the United States (US) (n = 207) and Yemen (n = 200). Patients filled out the Fat- and Fiber-related Diet Behavior Questionnaire (FFBQ)[1]. Clinical and therapy-related data were obtained from their medical records. Chosen parametric and non-parametric tests were used for statistical evaluation in PASW software.

Results: There were significant differences in all aspects of FFB among countries ($P < 0.0001$). The best fat-related behavior reported was from patients from the CR. Patients from the US stated the worst fat-related behavior and the best fiber-related behavior. Patients from Yemen reported the worst fiber-related behavior. Overall men presented a little better scores in the "avoid fat as flavoring" subscale than women ($\beta = 0.088 \pm 0.044$; $P = 0.043$). Patients from Yemen exhibited significantly different diet behavior in the "modify meat" subscale in dependence of gender ($\beta = 0.466 \pm 0.134$; $P = 0.0005$). Patients from all studied countries reported the best results in the "modify meat" and "avoid fat as flavoring" and the worst in the "substitute high fiber" subscales.

Conclusions: Patients across countries tend to modify the dishes they are used to rather than to remove them from their diet or replace them by other types of food. There seem to be some differences in diet preferences between men and women but it is necessary to further investigate this. Professionals involved in the diet education of T2DM patients should be aware of the specificity of diet in their country when advising patients keeping general recommendations. We suggest being as particular as possible and concentrating on fiber-related behavior.

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SECTION OF PHARMACEUTICAL TECHNOLOGY

INVESTIGATION OF INFLUENCE OF METHOD OF FIXING OF PORCINE SUBLINGUAL MEMBRANES ON THE PERMEATION OF NANOPARTICLES *IN VITRO*

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Sublingual route of administration offers numerous properties which could be advantageous for non-invasive administration of macromolecular biological actives (e.g. peptides, proteins, nucleotides) or drugs incorporated into/onto nanoparticle carriers.

In our previous work, we studied permeation of Chromeon 470-marked nanoparticles through fresh cut or frozen porcine sublingual membranes *in vitro* in modified Franz diffusion cells. The membranes were fixed using silicone grease. When examining the acceptor side of the membranes with fluorescence microscopy, we noticed higher occurrence of permeated nanoparticles near the borders of the area available for permeation.

The aim of this work was to check, whether a method of fixing the porcine sublingual membranes into modified Franz diffusion cells can influence the amount of nanoparticles, which permeate through the membrane *in vitro*.

We tried an alternative method of fixing the membranes into the diffusion cells.

Caffeine was used as a membrane integrity marker. Concentrations of both nanoparticles and caffeine were assessed simultaneously using HPLC equipped with fluorescence and UV-VIS detectors.

Distribution of the nanoparticles on donor and acceptor sides of the sublingual membrane was examined with fluorescence microscopy.

The nanoparticles were more frequently located near the outer boundary of sublingual membranes. However, the method of fixing the membranes didn't have statistically significant influence on the amount of permeated nanoparticles. We can conclude our previous and rather surprising results to be verified from the point of view of correct membrane fixation at experiments.

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MODIFIED PLGA AS FLUCONAZOLE CARRIER

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Copolymers of lactic acid and glycolic acid (PLGA) are widely used as drug carries in modern pharmacy. Adding polyhydric alcohols to the reaction mixture leads to a radical modification of otherwise linear PLGA polymer – branched molecules are formed. For example, if dipentaerythritol is used, branched, star-like oligoesters are gained[1]. The rate of hydrolytic degradation can be influenced by shifting the ratio of reactants. To ease workability and final application of developed drug transport system, various plasticizers are often incorporated.

In this work we focused on the development and improvement of modern drug carries based on copolymers of D,L-lactic acid, glycolic acid and dipentaerythritol. Slight modifications in reactant ratios led to terpolymers of different molecular weights and polydispersity index. Furthermore, one of selected plasticizers (triethyl citrate, ethyl pyruvate, ethyl salicylate, methyl salicylate, triacetin and tributyrin) was added to improve the workability, especially the drug incorporation. As the model drug, fluconazole was utilized. Besides thermoanalytical, rheological and mucoadhesion tests, also the dissolution studies were performed to reveal the fluconazole release model of each system. After specified time let for hydrolysis, the dissolution medium was collected. It appeared to be a complicated mixture of various oligoester molecules, plasticizer and fluconazole. Using the spectrophotometric determination of fluconazole was therefore unsuccessful. For that reason HPLC was employed. A new analytical method was developed for this purpose. A fused-core particle column, mixture of phosphate-citrate buffer and acetonitrile (86:14 v/v) at constant flow rate 0.63 mL/min and UV detection at 260 nm were used. The retention time of fluconazole was 3.8 min, the analysis was completed within 6 min[2].

The study was supported by PRVOUK P40.

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EVALUATION OF FLOWABILITY OF POWDERS AND GRANULES IN PHARMACEUTICAL TECHNOLOGY

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Powders represent the dominant material in the pharmaceutical industry. Many pharmaceutical active ingredients or excipients are used in the form of powder. An important property of pharmaceutical powders and granulates is their flowability. Flow behaviour is critical for handling, storage and transport of pharmaceutical powders and granules which is closely associated with the production of solid dosage forms, e.g. the compression of tablets, and is essential to ensure the homogeneity of the product. Standardization of manufacturing processes and the quality of the final product are not possible without understanding and the precise describing the flowability behaviour of the material.

Flowability expresses the ability of powder substance to flow. Flow is not a simple process; it is influenced by the properties of the material itself as well as the equipment used in the flowability testing. This review summarizes the information about the flow behaviour of materials and methods of its evaluation. It presents information on different types of hoppers and the requirements of their geometry.

In order to characterize the flowability, the mass flow rate Q (g/s) is most often used. The complex influence of the flow rate due to an orifice diameter of a hopper D_0, the mean particle size d and powder density ρ_b is described by the flow equations. To study the possibility of optimization of the parameters of flow equation and its exponent n, and the possibility to increase the precission of the flow rate estimation for pharmaceutical materials has a major practical impact in pharmaceutical technology in the manufacturing of the solid dosage forms and opens up the oportunity of cooperation with the pharmaceutical industry in the practical use of the experimental results.

PREPARATION OF DISPERSIONS FOR FIBER COATING USING A "DIP-COATING" TECHNOLOGY

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The presented study is concerned with coated biodegradable fibers based on modified biodegradable polymer hyaluronic acid. The special fiber-surface coating is based on a "Dip coating" technology. The coated fibers include pharmaceutically active additives that are supposed to be released at the spot of a fiber application within the human

body. Fibers have been coated with an application of dispersions, consisting of three main components: solvent mixture, binder and API. The purpose of the study was to determine a suitable dispersion composition with optimal rheological and adhesive characteristics for the further fiber coating. Within the dispersion formulations, the attention has been paid especially to the molecular weights of the binding polymers and the solvent mixtures. The quality of the dispersion has been evaluated by rheological methods to assess the spreadability parameters. Furthermore, a thermal stability of the dispersion, surface tension and the degradation kinetics have been taken into account. From the results obtained, several dispersion compositions have been selected that can be used to modify the fiber surface and influence the kinetics of the release of the incorporated active substance.

The study was supported by Contipro Biotech s.r.o.

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EFFECTS OF SPHINGOMYELIN/CERAMIDE RATIO ON PERMEABILITY AND MICROSTRUCTURE OF MODEL STRATUM CORNEUM LIPID MEMBRANES

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Lipids filling the intercellular space of the stratum corneum (SC) are essential for the skin barrier function in terrestrial mammals. The highly ordered skin barrier lipids include three main groups of hydrophobic compounds – ceramides (Cer), free fatty acids (FFA) and cholesterol (Chol) – in approximately equimolar fractions, with a minor amount of cholesteryl sulphate (CholS). Sphingomyelin (SM) conversion to ceramide (Cer) by acid sphingomyelinase (aSMase) is an important event in the skin barrier development. The deficient aSMase in diseases such as Niemann-Pick disease and atopic dermatitis coincides with impaired skin barrier recovery after disruption[1,2]. We studied how an increased SM/Cer ratio influences the barrier function and microstructure of model stratum corneum (SC) lipid membranes. Previously we presented experiments on a simple membrane model with only one CerNS. Increased SM/Cer ratio provided similar or better barrier towards permeation of various markers. In contrast, in the membranes composed of isolated human SC Cer (hCer)/Chol/FFA/CholS, partial or full replacement of hCer by SM increased water

loss. Partial replacement of 25% and 50% of hCer by SM also increased the membrane permeability for theophylline and alternating electric current, respectively, while higher SM content did not alter or decrease the membrane permeability. The X-ray powder diffraction revealed that the replacement of hCer by SM interferes with the formation of long periodicity lamellar phase (LPP) with repeat distance $d = 12.7$ nm. The presence of LPP plays a key role in the water barrier homeostasis. Our results suggest that the SM-to-Cer processing in human epidermis is essential in preventing excessive water loss, while the permeability barrier for exogenous compounds is less sensitive to the presence of sphingomyelin.

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EVALUATION, OPTIMIZATION AND UTILIZATION OF SURFACTANTS IN GRANULATION PROCESS

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The main topic of this work was evaluation and optimization of a granulation process on a laboratory scale fluid bed granulator. Fluid bed granulation is widely used technique of granulate production, as a mid-product for tablet production. During the experimental work, parameters of process and product were evaluated. In repeated granulation runs, granulate was produced, with 90% of share in whole product. (Fig. 1)

As a main parameter of the granulation process, the spraying rate was determined. The most optimal setting for given formulation and process was 9 g/min in combination with active pharmaceutical ingredient, its particles were smaller than 500 μm.

Due to temporal unavailability of fluid bed granulator, it isn't possible to continue in the work on the topic. It was decided to focus on the topic of multifuctionality of surfactants. With help of high-shear granualtion, they could be incorporated in to the granulate. Surfactants are usually mixed with granulate and they are used as lubricants. As a component of granulate, surfactants serve as disintegrants, they can also affect dissolution of active pharmaceutical substance, disintegration of tablet and bioavailability of active pharmaceutical ingredience. In experiments, three surfactants will be used: Sodium lauryl sulfate,

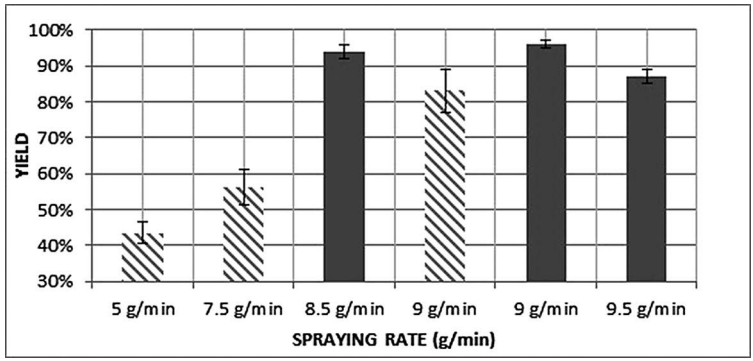

Fig. 1. Yield size in granulation runs, which were produced with different spraying rate (blue shaded – caffeine < 1000 μm, red full – caffeine < 500 μm).

SPAN® 20 and TWEEN® 20. Model active ingredient will be paracetamol. Granulate will be prepared on high-shear device Roto Junior. Granulate will be examined according to the pharmacopoeia criteria, that means particle size distribution, angle of repose, dissolution of active pharmaceutical ingredient and moisture content. From granulate mixed with lubricants, tablets will be pressed. Tablet pressing process parameters will be evaluated, such as kinetic of crushing process, disintegration, active pharmaceutical ingredient dissolution. Newly, methods of solid-phase physic, such as infrared and Raman spectroscopy, will be used.

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SECTION OF PATHOBIOCHEMISTRY AND XENOBIOCHEMISTRY

ABC-LIKE FLIPPASE AND ITS IMPORTANCE FOR BACTERIUM *FRANCISELLA TULARENSIS*

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Francisella tularensis ABC transporter protein encoded by *FTS 1402* belongs to the group of ATP-binding cassette (ABC) transporters as highly efficient translocation systems

of various compounds across the lipid bilayers of cellular membranes. In bacteria, ABC transporters have a major role in a glycoconjugate biosynthesis and some of them are even essential for the virulence of pathogenic bacteria including *Francisella tularensis*. Insertional inactivation of *FTS 1402* resulted in a mutant bacterium with affected virulence in BALB/c mice as a consequence of a reduced bacterial growth and elimination of the microbe from the mice organs, yet still inducing protective immunity, when administrated subcutaneously. *FTS 1402* gene product with a predicted role as a flippase is involved in a *Francisella* protein glycosylation pathway. Additionally, the *FTS 1402* mutant strain undergoes complement-mediated lysis at higher concentrations of human sera, indicating the affected biogenesis of presumably capsule but not LPS, which is still being synthesized and expressed on the surface. Reduced adherence of the mutant strain to pneumocytes indicates another role of surface glycoconjugates as an adhesin molecules. Reduced virulence, adherence, and sensitivity to complement of the *FTS 1402* mutant strain are likely the result of the impaired production of other cell surface glycoconjugates, such as capsule/CLC, and not affected glycosylation of membrane-associated glycoproteins since the simultaneously studied PglA mutant, which is deficient in solely glycosylation, has the wild-type level of virulence and adherence to pneumocytes and is also fully resistant to serum bactericidal activity.

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PC12 CELLS: A MODEL FOR STUDY OF CATECHOLAMINE NEUROTOXICITY IN PARKINSON'S DISEASE ETIOLOGY

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Parkinson's disease (PD) is the second most common form of motor system degeneration and it is characterized by a progressive loss of dopaminergic neurons in the substantia nigra pars compacta in the ventral midbrain. The etiology of the disease is unknown and the factual cause of cellular destruction in PD remains a mystery. It is possibly multifactorial and there are many potential causes, including catecholamines, metal ions or oxidative stress. These three can be linked into one unit called catecholamine toxicity, when catecholamines undergo autoxidation promoted with free iron ions resulting in formation of reactive intermediates and oxidative stress with its toxic effects towards cells. Understanding the pathogenesis of the disease is important for developing therapeutics and requires usage of suitable model systems. One of model systems for study of PD is PC12 cell line. PC12 cells are derived from a pheochromocytoma of the rat adrenal medulla and exhibit phenotypic properties associated with original tissue, *i.e.* they synthetize, store and can release catecholamines (principally dopamine and norepinephrine). They also respond

to nerve growth factor (NGF) and after several days' exposure they undergo a dramatic change in phenotype and acquire a number of properties characteristic of sympathetic neurons, *e.g.* proliferation arrest, long neurites growth, electrical excitability and a number of changes in composition associated with enhanced neuronal differentiation. All this along with other advantages like relative stability of this cell line, its homogeneity, high degree of differentiation, vigorous response to NGF, potential for genetic manipulation and large number of studies regarding their characterization, makes PC12 cells a useful model for the study not only of neuronal differentiation and function but also of neurotoxicity.

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RESISTANCE OF CANCER CELLS TO ANTHRACYCLINES INDUCED BY REDUCTIVE METABOLISM: THE ROLE OF ALDO-KETO REDUCTASE 1C3

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Pharmacokinetic drug resistance is one of the main obstacles that emerge during cancer chemotherapy. In this study, we investigated the possible role of AKR1C3 in the resistance of tumor cells to anthracyclines. First, we tested the reducing activity of AKR1C3 toward anthracyclines using incubations with a purified recombinant enzyme. AKR1C3 was shown to most efficiently catalyze the formation of daunorubicinol, followed by idarubicinol and doxorubicinol. We further examined the intracellular reduction of daunorubicin and idarubicin by employing the transfection of A549, HeLa, MCF7 and HCT 116 cancer cells with an AKR1C3 encoding vector. The production of daunorubicinol and idarubicinol was markedly accelerated in transfected cells; this acceleration was significantly blocked by the recognized AKR1C3 inhibitor 2′-hydroxyflavanone. To demonstrate the participation of AKR1C3 in anthracycline resistance, we conducted MTT proliferation assays, which employed transfected HeLa, MCF7 and HCT 116 cells. The introduction of AKR1C3 into these cells significantly reduced the antiproliferative effect of both daunorubicin and idarubicin in all three tested cell lines. In another experiment, we combined daunorubicin and idarubicin with 2′-hydroxyflavanone in non-transfected A549 cells, which endogenously express considerable amounts of AKR1C3. As a result, we observed that 2′-hydroxyflavanone efficiently sensitized A549 cells to anthracycline treatment. In the final part of our work, we tracked the changes in AKR1C3 expression after anthracycline exposure to determine whether intrinsic cell resistance, which is

caused by the normal expression of AKR1C3, could be strengthened by enzyme induction. Interestingly, we recorded a reciprocal correlation between the extent of induction and endogenous levels of AKR1C3 in particular cell lines. Therefore, we suggest that the induction of AKR1C3 following exposure to daunorubicin and idarubicin, which seems to be dependent on endogenous AKR1C3 expression, eventually might potentiate an intrinsic resistance given by the normal expression of AKR1C3. In conclusion, our data suggest a substantial impact of AKR1C3 on the metabolism of daunorubicin and idarubicin, which affects their pharmacokinetic and pharmacodynamic behavior. In addition, we demonstrate that the reduction of daunorubicin and idarubicin, which is catalyzed by AKR1C3, contributes to the resistance of cancer cells to anthracycline treatment. The concomitant administration of anthracyclines with a specific AKR1C3 inhibitor, such as 2′-hydroxyflavanone, might be a successful strategy for the combination chemotherapy of AKR1C3 overexpressing tumors.

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CYTOPROTECTIVE IRON CHELATOR SIH AND OXIDATIVE STRESS-ACTIVATED PROCHELATOR BSIH: COMPARISON OF THEIR PROPERTIES

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Iron imbalance plays an important role in oxidative stress associated with numerous cardiovascular diseases. Shielding of free or loosely-bound iron *via* its chelation may be an effective therapeutic approach. Salicylaldehyde isonicotinoyl hydrazone (SIH) is able to quickly penetrate through cellular membranes and efficiently chelate labile intracellular iron pool. SIH has been previously demonstrated as extremely effective in protection of cardiac cells against oxidative stress. The main drawback of SIH is related to its rapid hydrolysis due to the labile hydrazone bond resulting in a short half-life in biological environment. Recently, novel oxidative stress-activated prochelator BSIH has shown potential to protect cardiomyocytes against oxidative stress-induced cell damage with exceptionally low own toxicity resulting from lack of interference with physiological cellular iron homeostasis[1].

H9c2 rat embryonal cardiomyoblast-derived cell line was used in our experiments. Neutral Red Uptake Assay and xCELLigence System were used for assessment of cellular viability. Calcein Assay and Calcein-AM Assay were used to determine efficiency of prochelator activation by H_2O_2 to effective chelator in buffered solution and inside the

cells. Epifluorescence microscopy was used for photodocumentation of mitochondrial inner membrane potential by JC-1 probe and lysosomal integrity by LysoTracker Blue. IC_{50} and EC_{50} were calculated using the CalcuSyn software. HPLC-UV method was used for determination of SIH and BSIH stability.

Our *in vitro* experiments have demonstrated conversion of BSIH in the presence of H_2O_2 to effective Fe chelator SIH in solution as well as in cells where its activity reached even 70% of chelating activity of SIH. We also confirmed higher stability of BSIH in medium and in cells as compared to SIH. Whereas SIH showed dose-dependent decrease of cellular viability, BSIH did not display any own toxicity when incubated with the cells for up to 72 hours and concentrations up to its solubility limit of 600 μM. SIH significantly protected cells against injury caused by H_2O_2 (200 μM) at concentrations <10 μM, whereas BSIH significantly protected H9c2 cells at concentration <60 μM and at its higher concentrations its protective effect was even more pronounced than the parent chelator SIH.

In conclusion, our results demonstrate very good properties of prochelator BSIH. It shows higher stability and does not display almost any own toxicity while retaining the useful protective properties of parent chelator SIH. Therefore BSIH could be applicable in prevention and/or in treatment of cardiovascular disorders with a known (or presumed) role of oxidative stress without a risk of toxicity due to the iron deficiency, which is typical adverse effect of Fe chelators.

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THE METABOLISM OF DEXRAZOXANE: PHARMACOKINETICS OF THE PARENT STRUCTURE AND ITS METABOLITE ADR-925

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Dexrazoxane is the only clinically available protective substance against anthracycline cardiotoxicity. Although the mechanism of dexrazoxane cardiotoxicity is not completely understood, it is widely accepted that it represents a pro-drug of an EDTA-like structure named ADR-925, which is may chelate various ions including iron. By chelating iron,

it could prevent iron-catalysed redox cycling of anthracyclines with subsequent oxidative damage to various cellular components. On the other hand, the parent structure of dexrazoxane was shown to interact with topoisomerase II, which is also a direct target of antracyclines. By topoisomerase poisoning, anthracyclines exert their antineoplastic activity, but this may also cause DNA damage in non-proliferating cardiomyocytes. Together with the limited regenerative capacity of the cardiac muscle, the DNA damage with subsequent cell death could also be a potential explanation of the anthracycline cardiotoxicity. Unfortunately, the direct relationship between the metabolism of dexrazoxane and its cardioprotective activity is only poorly characterised. In this study, we isolated neonatal rat cardiomyocytes and treated them in culture with 100 μM dexrazoxane and ADR-925 for 3, 6, 9, 12 and 24 hours to assess the amounts of both agents inside the cardiomyocytes. Moreover, the concentrations of the agents were monitored in the culture medium. We found that dexrazoxane is hydrolysed in the culture medium independently of the cardiomyocytes. The maximal concentration of dexrazoxane within the cells is reached after 6 hours of incubation. After 9 hours, ADR-925 is detected in the cells. We also found that ADR-925 passes through the plasma membrane into the cells. After incubation with ADR-925, it is detectable in the cells after 3 hours of incubation and after 24 hours its concentration inside the cells exceeds the maximal concentration of dexrazoxane by 30%. Together with the *in vivo* data obtained from the rabbit model of anthracycline cardiotoxicity and cardioprotection these findings could provide a valuable insight into the pharmacokinetic/pharmacodnamic relationship of the dexrazoxane cardioprotection of anthracycline cardiotoxicity.

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MONEPANTEL – METABOLISM AND EFFICACY

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The increased drug-resistance, especially characteristic for nematodes, has contributed to the development of new anthelmintic class: amino-acetonitrile derivatives (AADs). In 2009 (after a gap of 25 years) the launch of this new class of anthelmintic to the market was a welcome development. Monepantel (MOP) is the first compound from this class developed as an anthelmintic (Zolvix^{TM}, Novartis Animal Health) against drug resistant nematodes. During the last few years this new anthelmintic drug has been intensively studied. Many authors examined this drug from different perspectives. MOP is an anthelmintic for livestock (currently only sheep and goats), with molecular mode of action which is different to all other anthelmintics. MOP has a broad-spectrum of activity against gastrointestinal nematodes of sheep, including adults and L_4 larvae of the most important species.

The key feature of MOP is its full effectiveness against strains of nematodes resistant to benzimidazoles, levamisole, macrocyclic lactones and closantel.

In our laboratory, MOP metabolism and efficacy against lower developmental stages of *Haemonchus contortus* were studied. After oral administration, MOP is quickly absorbed into the bloodstream and quickly metabolized to MOP sulfone that has a similar efficacy as the parent molecule. Fourteen other MOP metabolites are formed in ovine hepatocytes, urine and faeces. Unlike to sheep, *H. contorus* adults are able to form only 4 MOP metabolites. Based on obtained results, schemes of metabolic pathways of MOP in sheep and nematodes were proposed.

In *in vitro* study, high MOP efficacy against lower larval stages (L_1–L_3) of *H. contortus* was observed. Larval susceptibility was not dependent of the sensitivity status of the nematode isolate. On the other hand, ovicidal effect of MOP was very low.

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BIOCHEMICAL CHARACTERIZATION OF HUMAN DEHYDROGENASE/ REDUCTASE (SDR FAMILY) MEMBER 3

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Dehydrogenase/reductase (SDR family) member 3 (DHRS3), also known as retinal short-chain dehydrogenase/reductase retSDR1 was identified as an all-trans-retinal dehydrogenase in cone photoreceptors. Knowledge about this enzyme is quite poor, only subcellular localization, tissue expression at the mRNA level and one substrate have been described so far (Haeseleer et al. 1998). It is a membrane-bound enzyme belonging to the Short-chain dehydrogenase/reductase (SDR) superfamily. Some members of this superfamily metabolize some endogenous and also xenobiotic compounds. They also play an important role in some serious diseases such as some types of cancer or metabolic syndrome. Despite the importance of SDR members, there are still many uncharacterized or poorly characterized enzymes, including DHRS3.

The aim of this study was to describe selected biochemical properties of DHRS3, especially its membrane topology, selected posttranslational modifications, cofactor preference, substrate specificity and tissue localization at protein level. Recombinant form of human DHRS3 was prepared using Bac-to-Bac Baculovirus expression system and Sf9 insect cells. We have demonstrated that DHRS3 is an integral protein with C-terminus oriented into the cytosol. Activity of the microsomal fraction with overexpressed DHRS3 from Sf9 cells was verified by all-trans-retinal. Further, catalytic activity of this enzyme was tested. It has been found that DHRS3 is NADPH dependent reductase and it participates besides reduction of retinal in metabolism of nitroacetophenone, DL-glyc-

eraldehyde, NNK and androstendion *in vitro*. Expression of DHRS3 protein only in liver has been confirmed so far.

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NOVEL ZINC AZAPHTHALOCYANINE AND PHTHALOCYANINE PHOTOSENSITISERS WITH LOW INHERENT TOXICITY SHOWED HIGH ANTITUMOR ACTIVITY UNDER PHOTODYNAMIC TREATMENT *IN VITRO*

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Photodynamic therapy is relatively novel approach in the treatment of localized tumors using specific compounds – photosensitizers. This technique includes two distinct steps: uptake of the compound into malignant cells with subsequent irradiation by activating light. Photosensitizer produces cytotoxic and highly reactive oxygen species (ROS) damaging and destroying the cancer cells through damage of important macromolecules. Additionally, the generated ROS may trigger the activation of signal pathways leading to induction of apoptosis.

Several phthalocyanine (Pc) and one azaphthalocyanine (AzaPc) photosensitizers were studied in this work. In general, (Aza)Pcs are often hydrophobic compounds with poor solubility in aqueous media. However, all studied compounds bear cationic peripheral or non-peripheral substituents that render them highly hydrophilic. Mouse fibroblasts cell line (3T3) was used to evaluate inherent toxicity of studied (Aza)Pcs in dark. All compounds showed low dark toxicities with TC_{50} values in $\approx 10^{-4}$ M range. Photodynamic treatment experiments were performed mainly on human cervix carcinoma cell line (HeLa), but melanoma (SK MEL – 28) and colorectal carcinoma (HCT 116) cell lines were also used to establish the ability of compounds to damage different tumor cell lines. Irradiation of the cells with red light ($\lambda > 570$ nm, 12.4 mW/cm^2, 11.2 J/cm^2) induced strong photodynamic effect with EC_{50} values in 10^{-8} M range. Epifluorescence microscopy was used for analyses of morphological changes and apoptosis/necrosis during experiment. Cell shrinkage and disintegration that starts even during irradiation itself was observed. Deter-

mination of subcellular localization of studied compounds using fluorescent probes was also performed. Further investigations will focus on particular cell death pathways and intracellular ROS production.

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QUANTITATIVE ANALYSIS OF mRNA EXPRESSION OF CARBONYL REDUCING ENZYMES IN HUMAN TISSUES

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A variety of compounds of endogenous or xenobiotic origin containing carbonyl groups are metabolized into corresponding alcohols by NAD(P)H-dependent reductases. Carbonyl reduction is a significant step in biotransformation of endogenous (aldehydes, steroids, prostaglandins, retinoids and fatty acid derivatives) and xenobiotic (NNK, alfatoxin B1, quinones, doxorubicin and daunorubicin) compounds[5]. Based on structural and functional data, carbonyl reducing enzymes have been generally classified into two distinct protein families: the short-chain dehydrogenase/reductase (SDR) and aldo-keto reductase (AKR) superfamilies. SDR represent one of the largest and oldest groups of enzymes, whose members occur in all life forms including humans. The SDR superfamily currently contains more than 160,000 primary structures available in sequence databases[4]. The SDR enzymes also play an important role in some serious pathological diseases as some types of cancer, obesity, metabolic syndrome and endometriosis[1,3]. Over 75 SDR genes have been identified within the genome of human beings[4].

The most of the known information refers about the role of cytosolic forms of carbonyl reducing enzymes in the metabolism of xenobiotics (e.g. CBR1, AKR1C1-4). The knowledge about microsomal forms is limited and only one well-known microsomal form involved in biotransformation of xenobiotics – 11β-hydroxysteroid dehydrogenase 1 (11β-HSD1) – has been reported so far[2]. But it is probable that other microsomal members of SDR superfamily participate in biotransformation. This study has investigated expression and tissue localization of mRNA of three human poorly characterized microsomal enzymes included to SDR superfamily – DHRS3, DHRS7 and DHRS12.

The total of 16 tissues were collected from humans. The RNA was extracted using Trizol reagent from all frozen tissues and subsequently RNA was reversely transcribed to

cDNA. The expression and tissue localization of mRNA was determined using real-time SYBR Green qPCR with specific primers.

Tissue localization together with knowledge of enzyme activity of these three carbonyl reducing enzymes will open the way for studies of its potential role in the metabolism of important endogenous and xenobiotic compounds in human.

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MICRO RNAs IN MOUSE MODEL OF OBESITY

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Obesity and metabolic syndrome are increasing health problems worldwide. Among other ways, nutritional intervention using phytochemicals is important approach in the treatment and prevention of these diseases. Recent studies have shown that certain phytochemicals could alter the expression of specific genes and microRNAs (miRNAs) that play a fundamental role in the pathogenesis of obesity[1]. For study of the obesity and its treatment, monosodium glutamate (MSG)-injected mice with developed central obesity, insulin resistance and liver lipid accumulation are frequently used animal model. To understand the mechanism of phytochemicals' action in obese animals, the study of selected genes expression together with miRNA quantification is extremely important.

Green tea, one of the most popular beverages consumed in Asian countries, contains a series of polyphenols known as catechins. Molecular mechanisms underlying chemopreventive effects exerted by green tea have been extensively investigated. Besides a wide spectrum of proved biochemical and pharmacological activities showing mainly cancer preventive effect, administration of green tea extract can diminish body fat and reduce the risk of obesity-related diseases in mammals.

This study describes the identification and characterization of appropriate reference RNA target for the normalization of microRNA qPCR data in the liver and small intestine

of MSG mice. A panel of ten potential reference genes was examined and the effects of selected normalizers on the relative quantity of established obesity and diabetes-related microRNAs (mir-221 and mir-29b, respectively) were assessed. Moreover, we tested the effects of green tea catechins on expression of these miRNAs in obese and normal mice.

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IDENTIFICATION OF CMY-2-TYPE CEPHALOSPORINASES IN CLINICAL ISOLATES OF *ENTEROBACTERIACEAE* BY MALDI-TOF MASS SPECTROMETRY

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Objectives: Several research groups have demonstrated the use of MALDI-TOF mass spectrometry (MS) for the identification of antimicrobial resistance mechanisms, as the detection of β-lactamase activity. However, so far the attempts to visualize any native β-lactamases by MALDI-TOF MS in wild-type bacteria have been mostly unsuccessful. The aim of this study was to exploit the possibility to detect CMY-2-like cephalosporinases in *Enterobacteriaceae* clinical isolates using MALDI-TOF MS.

Methods: The described assay was tested against a group of 38 *Enterobacteriaceae* strains, which were previously well characterized. Periplasmic proteins were prepared using a modified sucrose method[1] and analyzed by MALDI-TOF MS. The peaks detected were compared with the observed molecular mass of the purified CMY-2 enzyme. The protein content of periplasmic extracts was characterized by SDS-PAGE, and protein bands observed at approx. 40,000 g/mol were identified by the in-gel tryptic digestion, followed by MALDI-TOF/TOF MS[2].

Results: The MALDI-TOF MS measurement of the molecular mass of the purified CMY-2 β-lactamase detected one major peak with m/z of 39,852. In the mass spectra of the tested isolates, the presence of a peak with m/z of ca. 39,850 was found in most of *Klebsiella pneumoniae* (8/10) and *Escherichia coli* (11/12), and all *Enterobacter aerogenes* (2/2) isolates producing CMY-2-like enzymes. Among *Proteus mirabilis* isolates, the peak was found in only three of the six CMY producers. The lack of the ~39,850-m/z peak was observed for *E. coli* and *K. pneumoniae* reference strains, and all of the non-CMY-pro-

ducing isolates. In the SDS-PAGE analysis, protein bands of around 40,000 g/mol, which co-migrated with the purified CMY-2 β-lactamase, were detected in extracts of all of the CMY-producing strains that were positive in the MALDI-TOF MS assay. The identification of the ~40,000-g/mol bands revealed multiple tryptic peptides, being fragments of CMY-2-like polypeptides, for all of the isolates that were positive in the MALDI-TOF MS assay. Consistently, such peptides were not detected in extracts from corresponding gel fragments for the isolates that were negative in the MALDI-TOF MS assay. In the preliminary analysis of other AmpC-type β-lactamases, peaks of ~39,670-m/z and ~38,900-m/z were observed for ACC-4 and DHA-1 enzymes, respectively.

Conclusion: In this study, we showed for the first time that MALDI-TOF MS has the potential to detect the most clinically important acquired AmpC β-lactamases, such as CMY-2-like, ACC and DHA types, in clinical isolates of *Enterobacteriaceae*.

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QUANTITATIVE ANALYSIS OF THE ANTI-PROLIFERATIVE ACTIVITY OF COMBINATIONS OF IRON-CHELATING AGENTS NHAPI AND DP44MT WITH THE ESTROGEN RECEPTOR ANTAGONIST TAMOXIFEN

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Recent studies have demonstrated that several iron chelators possess marked potential as anti-neoplastic drugs and as agents that can ameliorate some of the adverse effects associated with standard chemotherapy. Anti-cancer treatment employs combinations of several drugs that have different mechanisms of action. However, data regarding the potential interactions between iron chelators and established chemotherapeutics are lacking.

Using two estrogen receptor-positive (MCF-7 and T47D) breast cancer cell lines and one estrogen receptor-negative (MDA-MB-231) breast cancer cell line, we explored the combined anti-proliferative potential of two iron chelators, namely: *(E)-N'*-[1-(2-hydroxy-5-nitrophenyl)ethyliden] isonicotinoyl hydrazone (NHAPI), and di-2-pyridylketone

4,4-dimethyl-3-thiosemicarbazone (Dp44mT), plus estrogen receptor antagonist tamoxifen standardly used for breast cancer treatment. Our quantitative chelator-drug analyses were designed according to the Chou-Talalay method for drug combination assessment.

All combinations of these agents yielded concentration-dependent, anti-proliferative effects and showed synergistic interactions on both estrogen receptor positive cell lines. On the contrary, synergism was not observed on MDA-MB-231 cells without estrogen receptors. The most potent combinations of NHAPI with tamoxifen were deeply studied using MCF-7 cells by electrical impedance data, a mitochondrial inner membrane potential assay and cell cycle analyses. All the above mentioned methods confirmed the synergistic combination of NHAPI and tamoxifen on estrogen receptor positive cell lines and it showed a link between iron metabolism and estrogen signaling.

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METABOLISM OF ANTHELMINTIC DRUGS IN GIANT LIVER FLUKE

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Giant liver fluke (*Fascioloides magna*) belongs to the group of dangerous flukes, which cause severe damage to the liver of its host. The host spectrum is wide including most of the domestic and wild herbivores. Its spread in Europe including Czech Republic is emerging in last years[3]. Drugs from the salicylanilide group like closantel (CLO) and rafoxanide (RFX) are one of the most commonly used. Another choice could be use of benzimidazoles (BZD) like triclabendazole (TCBZ) or albendazole (ABZ)[4,5,6]. Deactivation of the drugs *via* metabolism of the fluke could cause the failure of the treatment. The aim of our study is to assess the metabolism of the selected drugs (CLO, RFX, TCBZ, ABZ, and mebendazole-MBZ) in giant liver fluke. In our study we used the *F. magna* adults collected from naturally infected red deer (*Cervus elaphus)* and fallow deer (*Dama dama*). In *ex vivo* experiments flukes were incubated in RPMI medium with 10 μM BZD drugs or 1 μM salicylanilide drugs for 24 hours and then the metabolites were analyzed by liquid chromatography coupled with mass spectrometry. The mass weights found indicate that *F. magna* is able to reduce mebendazole. Despite other helminths recently studied there were no signs of ability to oxidize ABZ or TCBZ[1,2]. There were no metabolites of CLO found. On the other hand small amount of phosphorylated RFX was found. Obtained results indicate, that *F. magna* has very different metabolism compared to other helminths and deserve further research.

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SECTION OF PHARMACOGNOSY AND NATURAL DRUGS TOXICOLOGY

ALKALOIDS OF *NERINE BOWDENII* WATSON (AMARYLLIDACEAE) AND THEIR BIOLOGICAL ACTIVITY

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Nowadays, AD is the most predominant cause of dementia in the elderly. The etiology of AD is still unknown, but *postmortem* studies have shown two characteristic pathologic hallmarks, senile plaques (SPs) and neurofibrillary tangles (NFTs). In AD patients, deficit of the neurotransmitter acetylcholine (ACh) in the cortex results in a degradation of cholinergic functions' level, and thus the memory impairments. Not only acetylcholinesterase (AChE) participates in the cholinergic regulation of central nervous system (CNS) in humans, but also butyrylcholinesterase (BuChE), which is able to hydrolyze ACh, as well as other esters. BuChE is associated with the NFTs and SPs and its activity increases in the AD brain, where it co-localizes with Aβ fibrils. In severe AD, levels of AChE and choline acetyltransferase are decreased by approximately 90%, while the concentration of BuChE increases[1].

The Amaryllidaceae is a very important plant family, which produce structurally unique alkaloids with a wide range of interesting physiological effects. These alkaloids are restricted to this family and are best known due to galanthamine, alkaloid used for the treatment of Alzheimer's disease (AD). It is a selective and reversible inhibitor of acetylcholinesterase that increases the level of acetylcholine in the brain.

The genus *Nerine* is the second largest within Amaryllidaceae with ca 30 species. *Nerine bowdenii* is an autumn-flowering perennial bulbous plant group, whose species inhabit

areas with summer rainfall and cool, dry winters. Previous investigations led to the isolation of more than 30 Amaryllidaceae alkaloids. Some of these have been tested for their AChE inhibitory activity, but not against BuChE and prolyl oligopeptidase (POP).

After extraction and fractionation of the extract by column chromatography, we were able to isolate more than 10 alkaloids by now which were put through nuclear magnetic resonance (NMR) for structure observation and tests on cholinesterase inhibitory activity and POP.

Cholinesterase inhibitory activity was tested *in vitro* using human erytrocyte AChE and plasmatic BuChE. The activity was measured spectrophotometrically by Ellman's method. The values of IC_{50} were determined and compared with values of IC_{50} of reference substances or substances used in therapy (galanthamin, rivastigmin, huperzin A)[1].

POP is a serine peptidase which digests small peptide-like hormones, neuroactive peptides, and various cellular factors (e.g. vasopressin, substance P, and thyrotropin-releasing hormones) that are involved in the processes of learning and memory[2].

Two of the isolated alkaloids (undulatine and ambelline) were also tested for their cytotoxic activity.

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EVIDENCE-BASED HERBAL MEDICINE: PHYTOCHEMICAL INVESTIGATION OF *AZORELLA COMPACTA*

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In its broader aspect, this project has been meant to support the meaning of traditional use of herbs on scientifically-based evidence. Specifically, the herb *Azorella compacta*, family *Apiaceae* is the matter of our concern. It grows indigenously in the high Andes of Peru, Bolivia, Chile and Argentina. In the traditional folk medicine, the herb has been being used in the form of teas and draughts for the treatment of colds, flu, rheumatism, pulmonary diseases, and relieving of pains[1]. These traditional, simply available preparations haven't been scientifically explored in detail yet considering the quantification and

identification of active compounds. Current scientific research has demonstrated biological activities of compounds identified in this plant and managed to reveal compounds with new type of skeletons[2,4].

The objective of the project is set as the distinguishing groups of bioactive compounds, the determination of their total content and the evaluating their biological activity regarding antioxidant and immunomodulating capacity in the aqueous and ethanolic extracts; and the carrying out their basic separation, detection and preliminary identification by comparing research paper data in the first year. Following the obtained results, fractionation, and isolation, identification and quantification of extracted secondary metabolites from the extracts will be accomplished using advanced instrumental techniques such as HSCCC, HPLCp, ESI- MS, NMR and HPLC-DAD. Fractions, respectively particular compounds, will be tested for their antioxidant and immunomodulating activity too.

First experimental results evaluating immunomodulating activity of the extracts show the capacity to stimulate immune cells.

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BERGENIA GENUS, PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITY

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Bergenia genus belongs to the widely used medicinal herbs in India, China, Nepal or Russia. In Indian system of medicine Ayurveda, *Bergenia* (called *Pashanbheda*) is used for therapy of cough and pulmonary diseases, to increase imunity, to dissolve kidney or bladder stones, to wound healing, disinfection, as a tonic and laxative. Previous investigations showed that *Bergenia* plants contain biologically active chemical compounds, such as polyphenol bergenin (C-glucoside of 4-O-methyl gallic acid), its derivative norbergenin, arbutin (phenolic monoglucoside of hydroquinone), catechins and other polyphenols[1,2]. The main attention is focused on bergenin and arbutin for antiviral, antibacterial and antioxidant potential. Our study was focused on the evaluation of arbutin and total tannin contents and antioxidant activity of extracts prepared from green leaves of *Bergenia crassifolia* (L.) Fritsch.*, Bergenia ciliata* (Haw.) Sternb. and *Bergenia* x *ornata* Stein. collected in different seasons. It was also studied the influence of meteorological data on the presence of phenolic compounds. Garden of Medicinal Plants, Mendel university in Brno, Faculty of Horticulture in Lednice has provided plant material. For the determination of total tan-

nin and arbutin content spectrophotometric methods described in Czech Pharmacopoeia were used. The highest total tannin content was determined in the leaves of *B. crassifolia* (48.7 mg g^{-1} DW) and *B. ornata* (36.9 mg g^{-1} DW). The highest amount of arbutin was in the leaves of *B.* ornata (51.0 mg g^{-1} DW) and *B. crassifolia* (41.7 mg g^{-1} DW)[3]. Free radical scavenging potential, in DPPH, ABTS, NADH and FRAP assays, of the water leaf extracts was revealed. It was found that extracts of *B. crassifolia* and *B. ornata* are the most active radical scavengers. Antioxidant activity correlated well with the content of total tannin. Significant correlation was found also between environmental factors and phenolic concentrations.

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ALKALOIDS FROM *FUMARIA OFFICINALIS* L. (FUMARIACEAE) AND THEIR CHOLINESTERASE INHIBITORY ACTIVITY

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Alzheimer's disease (AD) is the most common form of dementia among the elderly. This progressive, degenerative disorder of the brain is characterized by a loss of memory and cognition, together with a declined ability to perform basic activities of daily living. The current approved therapy includes the anticholinesterase inhibitors and the NMDA blockers. Cholinesterase inhibition is the most used therapeutic treatment for the symptoms of AD, although spectrum of used anticholinesterase compounds is relatively limited. In the screening of natural inhibitors of acetylcholinesterase, alkaloid extracts from species of Fumariaceae have demonstrated AChE inhibitory activity[1]. The main compounds of genus Fumaria are isoquinoline alkaloids belonging to different structural types[2].

Herbs of *Fumaria officinalis* L. were selected on the basis of bio-guided spectrophotometric Ellman´s method[3] as a source of isoquinoline alkaloids for study of their selected biological activities. The herbs were extracted with ethanol and the mixture of summary tertiary alkaloids was fractionated in alumina chromatography column using step gradient

elution with petrol, chloroform and ethanol. Repeated column chromatography, preparative TLC and crystallization led to isolation of other isoquinoline alkaloids (dihydrofumariline, fumarophycine, *O*-methylfumarophycine, parfumidine, corydamine, corlumine/adlumine, fumaritine, cheilanthifoline N-oxide and a spirobenzylisoquinoline alkaloid with formula weight 355) to others isolated from *F. officinalis* in this study previously. Last two mentioned alkaloids haven't been isolated so far. The chemical structures of isolated compounds were determined on the basis of spectroscopic techniques and by comparison with literature data. Isolated alkaloids were tested on ability to inhibit human erythrocyte acetylcholinesterase and serum butyrylcholinesterase. Established IC_{50} values of these compounds were compared with standards galanthamine, huperzine A and eserine. None of isolated compounds have showed any promising inhibitory activities towards human cholinesterases ($IC_{50} > 100$ μM).

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IN VITRO ANTIPLATELET ACTIVITY OF FLAVONOIDS ON ARACHIDONIC ACID BASED AGGREGATION

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Flavonoids are polyphenolic compounds widely occurring in fruits and other plant parts. The consumption of diet rich in these substances may be related to reduction of risk of cardiovascular diseases and tumors. Moreover, a specific group of flavonoids, i.e. isoflavones, possess additional estrogenic and thus anti-osteoporotic activities (e.g. genistein, daidzein and glycitein isolated from *Glycine max*).

In this study, the influence of a set of 15 flavonoids was tested on three steps of the arachidonic acid based aggregation cascade including antagonism at thromboxane A_2 receptors and inhibition of thromboxane synthase and cyclooxygenase-1 (COX-1) by use of human platelets and recombinant ovine COX-1 enzyme.

As we reported before, the mechanism of antiplatelet activity of flavonoids apparently does not include inhibition of thromboxane synthase but seems to be based on antagonism of thromboxane A_2 receptors and inhibition of COX-1. However, the structural charac-

teristics for the both activities are not identical. The ideal structure for antagonism on thromboxane receptors is a planar structure, consisting of the 2,3-double bond and the 4-keto group, with 7-hydroxyl and 4′-hydroxyl groups (e.g. genistein, apigenin), while 3-hydroxyl group generally markedly decreased the inhibition potential. In case of COX-1, only isoflavones containing free 7-hydroxyl and 4′-hydroxyl groups are active, while analogous flavones are not. Moreover, it has to be mentioned that their activity on inhibition of COX-1 seems to be lower than that of acetylsalicylic acid.

Some experiments necessary for the conclusion of the structure-activity relationship are in progress now.

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ISOLATION OF PROTOPLASTS FROM PLANT CULTURES *IN VITRO*

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Plant cultures *in vitro* serve as objects for research of plant secondary metabolites and their metabolism[1]. One step of metabolism is storing of these metabolites into vacuole, which has function as storage organelle and also protect cell against potentially toxic effects of these substances[2]. Secondary metabolites are transported into vacuole through special channels such as ATP-binding cassette transporters[3]. These proteins belong to a large family which is also present in bacteria and animals. A function of these transporters can be observed on isolated vacuoles after mixing with activators/inhibitors of transport and certain amount of secondary metabolite. For these experiments are better older cultures *in vitro*, because their metabolic activity is relatively low. *Genista tinctoria* L., *Fagopyrum esculentum* Moench., *Hypericum perforatum* L. and *Silybum marianum* (L.) Gaertn. were chosen for this study. Every chosen plant contain some flavonoid glycoside (genistin, rutin, hyperoside) or aglycone (taxifolin). The first step of the secondary metabolites transport research is removing cell wall which protect protoplast. The cell wall is composed from cellulose and other polysaccharides as hemicellulose and pectin. The cell wall can also contain lignin, which creates cross-links in the cell wall and increase its hardiness[4].

G. tinctoria, F. aesculentum and H. perforatum were used as cell suspension cultures. Only the samples of *S. marianum* were prepared from callus before observation. Several types of enzymes were used for protoplast isolation – Pectinase P 4625, Macerozyme R-10, Driselase Kyowa Hakko, Rhozyme HP-150, Pectolyase Y-23, Driselase Fluka and Cellulase Onozuka R-10. The 1% water solutions of these enzymes were added to cell cultures and the mixtures were observed for 2 hours. The sufficient amount of protoplast was gained only from *S. marianum* after application of most enzymes, but there were not many free protoplasts remaining in plant cultures. Further experiments are neccesary for isolation of protoplasts from these cultures *in vitro*.

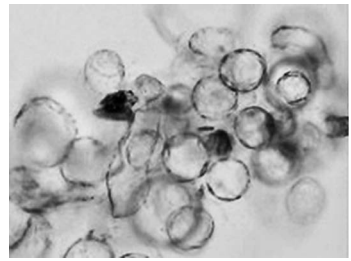

Fig. 1. Silybum marianum (L.) Gaertn. cells with Pectolyasa Y-23 after 80 minutes.

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ALKALOIDS OF SOME *NARCISSUS* SPECIES AND THEIR BIOLOGICAL ACTIVITY

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Alzheimer's disease (AD), one of the most common neurodegenerative diseases, is characterized by progressive and irreversible loss of neurons. AD is characterized by decreased levels of neurotransmitter acetylcholine in the cortex, which is hydrolyzed by acetylcholinesterase. In healthy brain, AChE is the most vital enzyme compared to others. In late AD stages butyrylcholinesterase is the main hydrolysing enzyme as its content will increase by up to 90% in comparison to normal state. BuChE cleaves ACh in a manner similar to that of AChE to terminate its physiological action.

Plants of Amaryllidaceae species are important for producing specific compounds, known as Amaryllidaceae alkaloids. These alkaloids have interesting physiological effects such as antitumor, antiviral, antimalarial and acetylcholinesterase activity. Alkaloidal extracts of eight *Narcissus* species have been tested for their inhibiting effects on acetylcholinesterase

and butyrylcholinesterase and alkaloid pattern. Fourty-two alkaloids were determined by GC/MS, thirty of them were identified from their mass spectra, retention times and indexes. Interesting biological activities were demonstrated by extracts of *N. poeticus* cv. Pink Parasol IC_{50} HuBuChE = 3.3 ± 0.5 μg/ml (IC_{50} HuAChE = 191.3 ± 20.2 μg/ml) and *N. poeticus* cv. Gigantic Star IC_{50} HuAChE = 3.2 ± 0.6 μg/ml (IC_{50} HuBuChE = 16.2 ± 1.0 μg/ml).

For phytochemical studies was chosen *N. poeticus* cv. Pink Parasol, because of its interesting inhibition activity towards BuChE and presence of lycorine and homolycorine type of alkaloids. Many of them are new structures that have not been isolated yet. Its biological activity is also unknown.

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NICOTINAMIDE AND NICOTINIC ACID TREATMENT OF *PISUM SATIVUM* L. SHOOT CULTURE CAUSES AN EPIGENETIC EFFECT

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Nicotinamide (NIC) is a well-characterized constituent of the pyridine dinucleotide coenzymes NADH and NADPH, which are involved in many enzymatic oxidation-reduction reactions in living cells. In plant tissues NIC can be metabolized to nicotinic acid, N-methyl nicotinic acid (trigonelline) and N-glucose nicotinic acid[1]. Epigenetics, reflected in DNA methylation, is closely associated with stress and defence in plants[3]. The purpose of the present study was to investigate the influence of nicotinamide (NIC) and its metabolites nicotinic acid (NiA) on *Pisum sativum* shoot cultures DNA methylation. The analysis of DNA methylation was made by the luminometric methylation assay (LUMA)[2]. It is important to point out that the method used gives a general picture of changes in DNA methylation and not a quantification of the methylation level. The results of both treatments by NIC and NiA decreased the level of DNA methylation in *Pisum sativum* L., and they are reflected in methylation changes in CCGG sequences. When comparing both of the treatments in connection of hypomethylation of DNA the role of nicotinic acid as a stressor seems to be for *Pisum sativum* shoot culture more noticeable, but the role of DNA hypomethylation, as a link between various types of stressors and the induction of plant defensive metabolism, needs other studies. One of the property of NIC, and NiA, which is good to mention, is a low toxicity considering their use in biotechnological systems[1].

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