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SECTION OF BIOLOGICAL SCIENCES

CHARACTERISATION OF MULTIDRUG RESISTANT *KLEBSIELLA PNEUMONIAE* ISOLATES BY SPECTROSCOPIC AND GENOTYPIC METHODS

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Ever increasing antimicrobial resistance is currently a worldwide problem, traditionally addressed by DNA-based approaches. This study was aimed at the evaluation of the potential of Fourier transform infrared spectroscopy with attenuated total reflectance (FTIR-ATR) for the characterization of multidrug resistant carbapenemase-producing *Klebsiella pneumoniae* isolates. We analysed 20 *K. pneumoniae* clinical isolates obtained from different community laboratories from Portugal between March 2014 and September 2015. Isolates were primarily characterized by genotypic methods including antimicrobial susceptibility testing, detection of carbapenemases and extended-spectrum beta-lactamases (ESBL), identification of antibiotic resistant plasmids and transposons and genetic relatedness of isolates (multi-locus sequence typing, MLST) and subsequently by FTIR and comparison of spectra by multivariate data analysis. *K. pneumoniae* isolates produced KPC-3 and variably ESBL (SHV or CTX-M types) and were resistant to aminoglycosides (73%), carbapenems (70%) or nitrofurantoin (55%). $bla_{\text{KPC-3}}$ was identified within transposon (Tn) *4401* variant "d" and different plasmids transferring antibiotic resistance (IncFIA, IncN). Using FTIR analysis, we were able in less than 48 hours to distinguish five clones that perfectly matched results obtained by MLST (ST147, ST15, ST231, ST348, and ST109). FTIRATR coupled with multivariate data analysis revealed to be a promising tool to assist quick assessments of clonal relationships among clinically relevant *K. pneumoniae* isolates. Regular monitoring of mechanisms inducing bacterial resistance may significantly help when selecting safe and effective antibacterial therapy especially in hospital settings.

EFFECT OF NUTRITIONAL SUPPORT ON ENERGY EXPENDITURE AND NUTRITIONAL SUBSTRATE OXIDATION IN POLYTRAUMA PATIENTS IN THE ICU

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In recent years, nutritional support became a part of complex therapy, which can decrease morbidity and mortality not only in critically ill patients, but in other pathological conditions associated with energy, vitamin and ion imbalance, too.

The optimal amount of supplied energy and nutritional substrates in polytrauma patients is currently the subject of many professional debates for different metabolism during critical state in comparison with healthy subjects.

The aim of this study was to determine and demonstrate the influence of nutritional support on energy expenditure (EE) and nutritional substrate oxidation (SO) in polytrauma patients in the ICU, because is not well known.

The study was performed on 14 polytrauma patients (11 men and 3 women). Their mean age was 45.29 ± 18.34 years. Examinations were obtained at least 4 hours after nutritional support administration. EE and SO were measured by indirect calorimetry under standard condition.

It was demonstrated that the intake of energy in kcal kg^{-1} d^{-1} ($p = 0.0125$; $r = -0.6461$), carbohydrates in g kg^{-1} d^{-1} ($p = 0.0108$; $r = -0.6563$), proteins in g kg^{-1} d^{-1} ($p = 0.0017$; $r = -0.7576$) reduces protein oxidation in g kg^{-1} d^{-1} which the body did not use as an energy source, but most likely for regenerative and reparative processes of damaged tissues.

This knowledge will be applied in clinical practice for setting of protein doses and optimization of the nutritional support composition thereby it can significantly contributeto increase the survival probability in critically ill patients.

The study was supported by Charles University (Project GA UK No. 772216), Faculty of Pharmacy (SVV 260 295), MH CZ-DRO (UHHK, 00179906) and PRVOUK P40.

GNOTOBIOTIC MICE MODEL AND EXPERIMENTAL INFECTION WITH *FRANCISELLA TULARENSIS*

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Francisella tularensis, the causative agent of a disease called tularemia, is a facultative intracellular Gram-negative bacterium. Because of its very high virulency and mortality rate (if untreated) it is included in the Category A of bioterrorism agents by the Centers for Disease Control and Prevention, USA. However, there is no vaccination for the general public available yet.

The aim of this study is to examine the virulence mechanisms of *Francisella tularensis* using the gnotobiotic mice model. The term "gnotobiotic" comes from the greek words "gnotos" and "bios", meaning "known life", indicating the limited presence/absence of microorganisms in such animal. The model of gnotobiotic animal, as a strictly defined system, minimizes the influence of the organism's microbiota on the results of the study. It is, therefore, widely used in immunology and other biomedical sciences, providing the great options for vaccine development and studies of the immune system, especially the host-pathogen relationship.

We compared the immune response of germ-free and specific pathogen free mice after intraperitoneal infection with two different strains of *Francisella tularensis*. This work also focused on the infectivity of *F. tularensis* and its dissemination into the lungs, spleen and liver of infected mice.

The study was supported by Long-term Organization Development Plan 1011 from the Ministry of Defense, Czech Republic.

GENISTA TINCTORIA IN VITRO – ABIOTIC ELICITATION

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The study is aimed to enhance *in vitro* production of secondary metabolites in *Genista tinctoria via* elicitor treatment. The different concentration of elicitor- selenium dioxide was utilized to affect the level of isoflavonoids occurred in cultures.

Experiment was perfomed in callus and suspension cultures on MS nutrient media supplemented with 10 g l^{-1} of α-naphtylacetic acid as growth regulator.[1] The elicitor was added in the form of solution in concentrations of 9.012×10^{-3} mol l^{-1}; 9.012×10^{-4} mol l^{-1} and 9.012×10^{-5} mol l^{-1}. It was exposed for 6, 12, 24, 48, 72 and 168 hours. The content of

isoflavonoids was determined by HPLC. The flavonoid levels released into media were also measured.

The most effective production of genistin (6.20 mg g^{-1} DW, 8.30 mg g^{-1} DW) in callus culture was measured. It was reached in concentrations of 9.012×10^{-4} mol l^{-1} and 9.012×10^{-5} mol l^{-1} 168 hours after elicitor treatment. The second most satisfactory genistin level 5.20 mg g^{-1} DW was detected after elicitor application in concentration of 9.012×10^{-4} mol l^{-1} after 6 hours.

The most efficient daidzein production (37.10 mg g^{-1} DW) in suspension culture was detected after elicitor treatment in concentrations of 9.012×10^{-3} mol l^{-1} and 9.012×10^{-5} mol l^{-1} after 24 hours. The second most abundant content 11.30 mg g^{-1} DW of daidzein was reached after selenium dioxide treatment in concentrations of 9.012×10^{-3} mol l^{-1} and 9.012×10^{-5} mol l^{-1} after 12 hours.

Isoflavonoids were not released into nutrient media. Selenium dioxide can be recommended to increase efficiently isoflavonoids production in *Genista tinctoria* cultures *in vitro*.

The study was supported by SVV 260 294.

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EFFECT OF SELECTED SESQUITERPENES ON ENZYMES OF HEPATIC PHASE II BIOTRANSFORMATION OF XENOBIOTICS

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Sesquiterpenes are class of terpenoids that consist of three isoprenoid units. These compounds are produced as secondary metabolites in higher plants, but also in fungi and invertebrates. Besides ecological functions, sesquiterpenes play an important role as components of many folk medicines and dietary supplements. In recent years, they have been studied in view of their antioxidant, anti-inflammatory, anti-parasitic and anti-carcinogenic activities.[1] On the other hand, sesquiterpenes could change the activity of biotransformation enzymes and by this way influence pharmacokinetic and pharmacodynamic profiles of co-administered drugs. This fact led us to research of the interaction of these bioactive compounds with the biotransformation system of living organisms. Study of potential modulators of biotransformation enzymes is important, because these compounds may impair the safety and efficiency of pharmacotherapy.

The aim of our project was to study the potential modulatory effect of three acyclic sesquiterpenes farnesol, cis-nerolidol and trans-nerolidol on phase II biotransformation enzymes in rat liver. Enzymes of phase II biotransformation serve as detoxifying step

in drug metabolism. These enzymes are transferases that catalyze conjugating reactions. UDP-glucuronosyltransferases, sulfotransferases and glutathione-S-transferases are the main representatives. In our study, the potential inhibitory effect of sesquiterpenes were tested in hepatic subcellular fractions, while primary cultures of rat hepatocytes served for testing of their potential induction effect. The assessments of enzymes activities were based on the monitoring of speed of specific substrate conversion to product in the selected time interval. The results were analyzed using spectrophotometer.

The activities of hepatic sulfotransferases, UDP-glucuronosyltransferases and glutathione-S-transferases were not affected by any sesquiterpenes tested. The results show that these sesquiterpenes do not influence phase II of drug biotransformation.

The study was supported by SVV 260 294.

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AMARYLLIDACEAE ALKALOIDS OF *NARCISSUS* CV. PROFESSOR EINSTEIN AND THEIR BIOLOGICAL ACTIVITY

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Plants of the Amaryllidaceae family are recognizable due to their aesthetically attractive flowers and are widely used in traditional medicine. More than 500 isoquinoline alkaloids have been already isolated and identified in this family. They are divided into nine main structural types. These alkaloids exhibit diverse biological activities including antitumor, antibacterial, inhibition of acetylcholinesterase (AChE) and others.

Narcissus cv. PROFESSOR EINSTEIN was chosen as a suitable representative for the isolation of alkaloids based on previous screening at our department. In this screening some alkaloids were detected by GC/MS, e.g. lycoramine; pluviine; pancracine; lycorine; 11,12-didehydroanhydrolycorine; haemanthamine; homolycorine; hippeastrine, and inhibition activities of alkaloidal extract against HuAChE and HuBuChE were measured. This *Narcissus* cultivar is characterized by large colored corona and white petals. There is one flower per stem.

Summary ethanolic extract was prepared from about 34.3 kg bulbs. This extract was further divided by column chromatography into about 500 fractions, and based on TLC analysis pooled into 27 subfractions. Subfraction Nr. 17 was used for the isolation of alkaloids in pure form. Whole subfaction was coarsely divided by preparative TLC chromatography into 4 zones. Currently one pure compound in crystalic form has been

obteined. This compound is under structural analysis (NMR, MS, IR). Testing of biological activities (inhibition of AChE, BuChE, PoP etc.) will follow. Also the isolation of other alkaloids still continues.

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CO-IMMUNOPRECIPITATION AS A TOOL FOR THE STUDY OF PROTEIN-PROTEIN INTERACTIONS OF DHRS7 ENZYME

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DHRS7 enzyme is a member of short-chain dehydrogenase/reductase superfamily. The enzyme, at least *in vitro*, is NADPH-dependent reductase of some substance bearing carbonyl group, including androstenedione or all-trans retinal. DHRS7 is close homolog of well-known 11β-hydroxysteroid dehydrogenase 1. Physiological function of DHRS7 is unknown, the level of the knowledge is quite low.[1] Recently, information about some role of the enzyme DHRS7 in diseases as prostate cancer[2] or insulin resistance were published. A role of poorly characterized proteins as DHRS7 can be predicted based on evidence of their interaction with a protein with revealed function,[2] because proteins inside the cell do not usually work alone, they are part of a huge protein complex known as interactome. Knowledge of such protein-protein interactions help us to understand the function and regulation of the protein inside cell or organism.

The aim of the study is initial investigation of protein interacting with DHRS7 by co-immunoprecipitation, a basic method for study of protein-protein interactions. Co-immunoprecipitation procedure with available anti-DHRS7 antibody and pure DHRS7 was introduced to our workplace and optimised. Precleared HeLa cell lysate, where DHRS7 is naturally expressed, was used for study of protein-protein interactions of the enzyme. Imunoprecipitated DHRS7 with potential interaction partners was eluted form protein G particles by 8 M urea and proteins were analyzed by LC-MS. Several proteins were identified as potential interacting partners of DHRS7, the results are necessary to confirm by diverse method.

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THE ROLE OF B_2-RECEPTOR IN EMBRYONIC HAEMATOPOIESIS IN ZEBRAFISH

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Haematopoiesis is a process of blood cells production through individual life. This highly regulated process produces cells up to individual's needs. Embryonal haematopoiesis is divided into two haematopoietic waves – primitive and definitive. The primitive wave precedes definitive wave and produces macrophages, neutrophils and primitive erythrocytes. The definitive wave is the exclusive source of the haematopoietic stem cells (HSCs) and also erythroid precursors. Recently, it has been suggested that β_2-receptors affect both embryonic and adult haematopoiesis. The aim of our study was to describe whether β_2-receptor play role in embryonic haematopoiesis. For this purpose, we have used well established *in vivo* model of zebrafish (*Danio rerio*) for real-time imaging studies either knocked-down for production of β_2-receptors by morpholino antisense oligomers (morpholino) or exposed to model selective β_2-inhibitor (ICI 118551). At time corresponding to onset of the definitive wave, we observed using fluorescence confocal microscopy that morpholino decreased HSCs emergence in the aorta-gonad-mesonephros and colonisation of haematopoietic organs such as the caudal haematopoietic tissue and the thymus. Subsequently, application of ICI 118551 resulted in comparable findings thus confirming β_2-receptor blockade leads to inhibition of definitive wave of haematopoiesis. Later in order to distinguish effect on primitive wave, we have analysed myeloid cell development in embryos with β_2-receptor impaired function (both chemical and knock-down impairment) quantifing colonisation of body by neutrophils and macrophages. The results have shown only delayed but not impaired colonisation of embryo by myeloid cells. We conclude that β_2-receptors play crucial role in emergence of HSCs and their later colonisation of definitive organs while the effect on primitive wave of haematopoiesis remains rather negligible. Thus our study provides new information about zebrafish development and extends knowledge about regulation of vertebrate haematopoiesis. The elucidation of haematopoietic process might contribute to facilitate *in vitro* preparation and proliferation of HSCs in order to employ them in transplantology.

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UDCA-LPE ALLEVIATES LPS-INDUCED INFLAMMATORY RESPONSE IN THP-1-DERIVED HUMAN MACROPHAGES *VIA* DOWN-REGULATION OF NF-KB AND MAPK SIGNALING PATHWAYS

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Nonalcoholic fatty liver disease (NAFLD) became the most common liver disease in developed countries and is linked with steatohepatitis as a hallmark. It is well-known that the level of protectant phosphatidylcholine (PC) is decreased in NAFLD. The bile acid-phospholipid conjugate ursodeoxycholyl lysophosphatidylethanolamide (UDCA-LPE) was designed in order to specifically deliver PC to hepatocytes. However, previous studies have proved that UDCA-LPE possesses its proper hepatoprotectant capacity[1] and exhibits anti-apoptotic, anti-inflammatory, anti-fibrotic properties and also improved steatosis and hyperlipidaemia in various models *in vivo*.[2,3] These effects may be mediated secondary through modulation of immune system. Therefore, in order to dissect if UDCA-LPE directly influences immune cells *in vitro*, release of pro-inflammatory cytokines TNFα, IL-6 and IL-1β in LPS-induced THP-1-derived human macrophages was measured by ELISA. Moreover, effects of UDCA-LPE on MAPK signalling pathways and nuclear translocation of NFκB were determined by Western blot analysis and immunofluorescence. For deeper investigation, lipid rafts were isolated using Optiprep gradient and recruitment of adaptor proteins TRAF6 and MyD88 into the lipid rafts was assessed by Western blot analysis. UDCA-LPE was able to significantly inhibit release of all measured pro-inflammatory cytokines, nuclear translocation of NFκB and activation of MAPK members JNK1/2 and p38. We therefore may anticipate that UDCA-LPE can exhibit its hepatoprotective properties *via* modulation of immune system in LPS-induced inflammatory response. Due to its versatility, UDCA-LPE has a potential to become a novel therapeutic approach for treatment of NAFLD.

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STUDIES ON GLYCOSPHINGOLIPIDS AS IMMUNE TARGETS IN BIOPROSTHETIC HEART VALVES

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The treatment of valvular heart disease represents approximately 20% of all cardiac surgery. One alternative of this treatment is replacement of diseased valve with bioprosthetic heart valve (BHV). These BHV are manufactured from divergent human or animal tissues *e.g.* porcine pericardium.

This treatment is beneficial for certain groups of patients. However, it suffers from some complications, such as rejection of the xenograft or BHV deterioration. In both of them the immune system is involved and both might result in BHV failure.

This study aims to isolate and characterize important targets of this immune response – glycosphingolipids (GSL). Therefore, 8 non-acidic and 7 acidic GSL from porcine pericardium have been isolated and characterized by mass spectrometry and carbohydrate binding assay.

The acidic GSL from goat erythrocytes have been isolated and characterized. Notable is characterization of new NeuGc-containing GSL-NeuGc-GT1b ganglioside.

And finally, 60 binding assays testing presence of antibodies against various GSLs, in the patient serum collected before, one and six months after BHV treatment surgery. The radioactive iodine-125 and autoradiographic visualisation have been used for detection. Results were mostly negative. However, in a few samples increased reactivity with α-Gal and NeuGc antigens revealed.

This knowledge might contribute to explain immune response against xenografts. Also it might help to further research of strategies preventing early BHV deterioration and therefore increase final outcome of BHV treatment of valvular heart disease.

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ANTIPROLIFERATIVE EFFECT OF NOVEL ANALOGUES OF DEXRAZOXANE

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Anthracyclines (e.g. doxorubicin, daunorubicin etc.) are highly effective and widely used antineoplastic drugs. However, their application is associated with a risk of cardio-

toxicity which still remains unclarified regarding the exact pathophysiological mechanism. The only clinically approved pharmacological prevention of anthracycline-induced cardiotoxicity is cotreatment with dexrazoxane.[1] Although mechanistically unexplained, the remarkable cardio-protective potential of dexrazoxane provides an important tool for investigation of exact mechanism of anthracycline cardiotoxicity.[2] In the Charles University Research Centre for the Study of Toxic and Protective Effects of Drugs on Cardiovascular System, we systematically use the approach of the modification of dexrazoxane structure to study the pharmacological mechanism of dexrazoxane cardioprotection. Moreover, in the view of dexrazoxane therapy controversies such as higher risk of secondary malignancies and interference with anthracyclines efficacy,[2,3] search for dexrazoxane analogues may represent a way to safer cardioprotective agent. The aim of this investigation is to assess antiproliferative effect of four novel analogues of dexrazoxane (JR 415, JAS 1.5a, GK 569 and GK 557) and their influence on anti-proliferative activity of anthracyclines. For proliferation assessments the HL-60 acute promyeolocytic leukaemia cell line was used. Cells were incubated with analogues or their combination with daunorubicin for 72 hours. ŮŮThe proliferation was determined using XTT assay.

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THE EFFECT OF THE PROTEASOME INHIBITION ON THE ANTIPROLIFERATIVE EFFECT OF ANTHRACYCLINE ANTIBIOTICS

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Anthracycline antibiotics (daunorubicin, doxorubicin) belong to the most effective antitumor drugs. In recent clinic practice they have been used most often in the combination with either classic or new targeted antitumor drugs. The new targeted antitumor drugs are also the proteasome inhibitors (bortezomib and carfilzomib). The proteasome is a multienzyme complex in eukaryotic cells which is responsible for intracellular degradation of proteins. The proteasome inhibitors have been largely used in the therapy of multiple

myeloma, but their potential has been also studied in the case of other malignancies. Their use in the combination with anthracyclines could be a possible alternative in the therapy of some tumor illnesses, but the effect of combination of anthracyclines and proteasome inhibitors on tumor cells have not been sufficiently explained. The anthracycline therapy is also accompanied by serious adverse side effect – the cardiotoxicity, which potential could be influenced by the combination with proteasome inhibitors.

The main aim of this study was the evaluation of the antiproliferative activity of proteasome inhibitors (bortezomib and carfilzomib) on human promyelocytic leukemia cells (HL-60) and the influence of these drugs on the antiproliferative effect of daunorubicin on these tumor cells. The next goal was also to study the influence of these drugs on the toxicity of daunorubicin in *in vitro* anthracycline cardiotoxicity model – isolated rat's neonatal ventricular cardiomyocytes.

The antiproliferative activity of proteasome inhibitors was tested on suspension cell culture HL-60. The cell viability was evaluated by MTT test after 72 hours of the incubation of cells with daunorubicin, doxorubicin, bortezomib and carfilzomib in a wide range of concentrations. IC_{50} of all studied drugs was calculated from these data. After that the combination effect of drugs was analyzed using Chou-Talalay method. Bortezomib and carfilzomib showed a quiet profound antiproliferative effects on leukemia cell culture with IC_{50} values in nmol/dm^3 unit order. The combination with anthracyclines did not lead to the significant increase of antiproliferative effect (the combination index was in wide range of concentration scale higher than 1).

The influence of proteasome inhibition by bortezomib and carfilzomib on the viability of isolated neonatal cardiomyocytes was evaluated after 48 hours of the incubation of cells with tested compounds. It was measured the activity of lactate dehydrogenase released from cells to the cultivating medium during the incubation. The values of IC_{50} for bortezomib and carfilzomib were determined. The influence of proteasome inhibition on daunorubicin cardiotoxicity was evaluated on that model. It was found that the proteasome inhibition in our scheme does not significantly influence the toxicity of daunorubicin towards primary neonatal cardiomyocytes. This observation must be supplemented also by other evaluations in the next time schemes and concentrations of bortezomib and carfilzomib.

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FLAVONOIDS IN SOME CULTIVARS OF *SAMBUCUS NIGRA* L. FLOWERS

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The aim of this work was to find out the content of flavonoids in the flowers of eleven cultivars of black elder (*Sambucus nigra* L.) and determine if their contents differ according to the statistical significance. Preparing the list of effects and substances that are contained in the flowers was the goal as well.

The drug *Sambuci nigrae flos* is mostly used for its content of flavonoids and hydroxycinnamic acids in the therapy of colds and diseases of the urinary and respiratory tract. Its benefits were confirmed also by studies testing cardiovascular, antibacterial and antiviral activity, diabetes and obesity, effects on the immune system and also protection against UV radiance.

The content of flavonoids was established by the spectrophotometric method that is listed in the Czech Pharmacopoeia 2009 in the article *Sambuci nigrae flos* as the method for the determination of content. The statistic evaluation of the differences in the flavonoids content between the cultivars was made by ANOVA and the Bonferroni test. The demands of Czech Pharmacopoeia on the minimal content of flavonoids is 0.80%. This requirement was fulfilled in the flowers of these cultivars: Albida, Heidegg 13, Riese auß Voßloch, Sambu, Samdal, Sampo and Samyl. On the opposite side, flowers of the cultivars Allesö, Aurea, Dana and Juicy did not meet the requierements of pharmacopoeia. The highest content of flavonoids was found in the flowers of the cultivar Riese auß Voßloch with the value of 1.360% and the lowest in the flowers of cultivar Dana with the value of 0.3598%. The highest avarage content of flavonoids had the cultivar Sambu, followed by the cultivars in this order: Riese auß Voßloch, Sampo, Albida, Heidegg 13, Samyl, Samdal, Juicy, Allesö, Aurea and Dana. The statistic evaluation proves that there are significant differences in the flavonoids content between cultivars.

All cultivars are potentially appropriate for the harvesting of the flowers excluding Allesö, Aurea, Dana and Juicy, which did not reach the demanded content of flavonoids listed in pharmacopoeia.

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IMPORTANT ROLE OF GLUTATHIONE, S-ALLYLDITHIOGLUTATHIONE, S-ALLYLTRITHIOGLUTATHIONE AND EPIGALLOCATECHIN GALLATE IN ASSOCIATION WITH OXIDATIVE DAMAGE TO DNA

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Cells in organisms affected by various negative influences, both exogenous and endogenous, respond by developing adaptive processes with multiple reparation mechanisms. These mechanisms can be more or less specific. Their main role is to protect the cells against oxidative stress. However, lack of these mechanisms or their disruption is associated with imbalance in the cells. This research deals with oxidative damage at DNA level. The reparation extent in the damaged DNA was assessed on cells A549 *in vitro* using the Comet Assay. The effects of length of reparation period (15 min., 30 min., and 60 min.) and of the type of antioxidant (glutathione (GSH), S-allyldithioglutathione (GSH-A), S-allyltri-

thioglutathione (GSH-B), and epigallocatechin gallate (EGCG)) were evaluated related to the damage initiation and repair in DNA induced by hydrogen peroxide (H_2O_2). The results suggest that EGCG can considerably inhibit the induction and promote the reparation of oxidative DNA damage. This is because of EGCG has protective effect on the induction of single strand breaks and reparation of oxidised pyrimidines detected by means of the enzyme Endonuclease III and oxidised purines detected by the enzyme formamidopyrimidine-DNA-glycosylase. In higher concentrations, GSH and its modifications (GSH-A, GSH-B) were toxic. However, lower concentrations (15–250 μM) of these compounds were effective in diminishing the induction of oxidative damage. These findings suggest that the tested antioxidants may have a very important role in protection of DNA against oxidative stress. In this way, they are promising tools in protection and treatment of many disorders initiated by oxidative stress or disorders inducing oxidative stress by themselves.

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SOLUBLE ENDOGLIN: FOCUS ON FUNCTIONAL ASPECTS IN LIVERS

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Endoglin (CD 105), a type III TGF-β co-receptor, is expressed in the plasma membrane of a number of cell types, including endothelial cells, mesangial cells, cardiac and scleroderma fibroblasts, and hepatic stellate cells (HSC).[1,2] Soluble form of endoglin (sEng; extracellular domain) is considered to be a biomarker of various cardiovascular-related pathologies such as atherosclerosis, preeclampsia, HELLP syndrome, hypertension or diabetes mellitus type II. Only few studies investigated the influence of sEng on other organs.[3] Increased serum levels of sEng have been found in patients with hypercholesterolemia, cystic fibrosis associated liver disease and hepatocellular carcinoma combined with cirrhosis.[1,4,5] As there are no available data focusing on functional aspects of sEng in livers, the aim of this study was to describe the effect of sEng on the mechanisms of bile formation, and cholesterol metabolism in hepatocytes *in vivo.* The expression of transporters was determined by Western blot and qRT-PCR in livers from six-month-old male transgenic mice overexpressing human sEng on CBAxC57BL/6J background and in control mice (wild type) on chow diet. Transgenic sEng animals demonstrated reduced plasma concentrations of cholesterol in comparison to control mice. These changes accompanied increased liver expression of Sr-b1 and Ldlr, the major transporters for uptake of cholesterol into hepatocytes, and reduced HMG-CoA reductase, the rate-limiting enzyme in cholesterol synthesis. Decreased disposition of cholesterol might contribute to reduced plasma levels

of its metabolites, bile acids, in addition to their reduced transport from hepatocytes to blood through down-regulated Mrp4 protein. In conclusion, the results of this study suggest significant role of sEng in the liver homeostasis of cholesterol and bile acids.

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DETERMINATION OF PERMEABILITY AND ACTIVE TRANSPORT OF SELECTED BUTYRYLCHOLINESTERASE INHIBITORS *IN VITRO*

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European Medicine Agency (EMA) and Food and Drug Administration agency (FDA) emphasise drug membrane permeability and drug-drug interactions on ABC transporters expressed in physiological barriers should be investigated for compounds in preclinical studies or for those already clinically used but evidence free. In this work we aimed to assess the capability of several experimental butyrylcholinesterase inhibitors that had been designed to treat dementia to permeate blood-brain barrier and to elucidate role of ATP-binding (ABC) cassette transporters in this transport. For this purpose, we employed *in vitro* bidirectional transport study across monolayers formed by polarized and highly differentiated Caco-2 cells. The permeability values gained from measurements were similar to values of several commonly used drugs for treatment of CNS disorders, e.g. antidepressants, antiepileptics. In addition, the compounds showed values of efflux ratio (basolateral-to-apical/apical-to-basolateral) approximately one which suggest none or negligible involvement of active transport.

All research was carried out in bacteriological laboratory of University of Porto, Portugal with the support of ERASMUS+ exchange program. The study was supported by SVV 260 293.

ANTIOXIDANT AND ANTIPROLIFERATIVE EFFECTS OF SALICYLALDEHYDE ISONICOTINOYL HYDRAZONE DERIVATIVES

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The biogenic chemical element, iron (Fe), is essential for living organisms; however, free cellular Fe may create reactive oxygen species (ROS) *via* Fenton reaction. That could lead to development of oxidative stress, which contributes to cardiovascular pathologies. Therefore, shielding of free or loosely-bound Fe may be an effective therapeutic approach. Salicylaldehyde isonicotinoyl hydrazone (SIH) is experimental tridental and lipophilic Fe chelator, which is able to quickly penetrate through membranes and chelate intracellular Fe. In previous studies, SIH has shown promising properties to protect cardiac cells against oxidative stress[1–4] and has displayed anticancer action.[5] Nevertheless, SIH is poorly stable due to its labile hydrazone bond that makes it prone to plasma hydrolysis.

The aim of this study was therefore to determine the properties of four novel analogues of SIH. Their cardioprotective effect to H9c2 cardiomyoblasts and antiproliferative action against MCF-7 (breast adenocarcinoma) and HeLa (cervical carcinoma) cells were assessed. Three of them displayed either no cardioprotective effect or the effect was reached in high concentrations that were strongly toxic in long-term experiments (72 hours). Only one derivative of SIH (containing 2,2′-dihydroxybenzophenone group instead of salicylaldehyde) showed better properties than SIH. This improvement was observed in all tested parameters. These results make this compound very attractive for further study.

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EFFECT OF IVERMECTIN ON ADULTS OF *HAEMONCHUS CONTORTUS*

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Helminthiasis is a disease caused by helminth parasites including barber´s pole worm (*Haemonchus contortus – H. contortus*) that causes haemonchosis. Haemonchosis is a serious disease harming small ruminant breeding and causing loss of productivity of animals, breeding weakening and ultimately death of the animals. Anthelmintics are widely used in struggle against the disease but frequent use in the past and present has led to developing drug resistance of the parasites. Increasing resistance against compounds with anthelmintic activity is becoming a worldwide problem. The effort is to know the resistance mechanisms and contributing factors which would prevent formation and expansion of the resistance. One of the most widely used substances of veterinary therapy of helminthiasis remains ivermectin (IVE). It is a macrocyclic lactone whose mechanism of action is likely damaging the parasite's chloride channels of glutaminergic synapses.

This project studies the effect of IVE on adults of two strains of *H. contortus* – the sensitive strain, called ISE (Inbred Susceptible Edinburgh) and the multidrug-resistant strain, called WR (White River), both isolated from the abomasum of infected sheep. The expression of selected genes that encode biotransformation enzymes of cytochrome P450 (CYP)[1] family and UDP-glucosyl transferase (UGT) family[2] after exposure to 1 μM IVE for 12 or 24 hours was examined by quantitative PCR. Expression of these genes in affected worms was compared with a control group of untreated adults. Several significant changes were detected in the gene expression. More significant changes were detected in the sensitive strains after the action of IVE in comparison to the resistant strains, especially in males.

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MOLECULAR CLONING OF *YinP* GENE FROM *LEISHMANIA MAJOR* USING TWO RED FLUORESCENT PXG-mCHERRY PLASMIDS. VALUABLE TOOLS FOR GENE EXPRESSION LOCATION

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In the 21st century, leishmaniasis remains a major health problem in numerous developing countries. Around 2 million cases of leishmaniasis are reported every year and estimated mortality is over 20,000 deaths annually.[1] Antileishmanial drugs are often unaffordable for affected people and display severe toxic side effects. Potent human vaccines are not available. This, together with increasing resistance, is a reason why new effective, safe, and affordable medicines are greatly needed.[2]

Leishmaniasis is caused by *Leishmania* species. These parasites are transmitted by phlebotomine sand flies, which also provide to the leishmania an environment necessary for their development into infective forms. The process of transformation into a stage infective for vertebrate hosts is called metacyclogenesis.[3] Nowadays, genes, enzymes, and proteins possibly exhibiting a function in the metacyclogenesis are extensively examined. One of the genes suggested to play a role during the development of the *Leishmania* spp. infective stage is *YinP*.[4]

The main objective of this study was to reveal where *YinP* gene is expressed in the leishmanial cell. Two plasmids, pXG-mCherry12-*YinP* and pXG-mCherry34-*YinP,* were constructed to contribute to finding. In these vectors, *YinP* gen was inserted directly next to the gen for fluorescent protein (mCherry) to generate fluorescent fusion proteins expressed in the parasites. Electroporation was used as a transfection method.

Fluorescent microscopy disclosed that red fluorescence of mCherry fused with *YinP* was localized only in a part of nucleus. Therefore, our results showed that YinP protein is expressed in the nucleus.

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BIOTRANSFORMATION STUDY OF SELECTED SESQUITERPENES

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Sesquiterpenes are 15-carbon compounds that consist of 3 isoprenoid units in their molecule. Sesquiterpenes together with monoterpenes are the main components of plain essential oil and they play important role in plant development, physiology and ecology. In human organism, sesquiterpenes show various biological activities, mainly anti-inflammatory, anti-parasitic and anti-cancer effects. The aim of the present study was to test the possible inhibitory effect of sesquiterpenes caryophyllene, caryophyllene oxide, α-humulene, farnesol and nerolidol on the cytochromes P450 (CYPs) in rat hepatic microsomal fractions. CYPs are the main enzymes catalyzing phase I of biotransformation of drugs and other xenobiotics and their inhibition could have important pharmacological and/or toxicological consequences.[1] In our study, the activities of CYP1A1/2 were assayed using ethoxyresorufin as a specific substrate. Benzyloxyresorufin and midazolam were used as substrates for the measurement of CYP3A4 activity. Our results showed that all tested sesquiterpenes are significant inhibitors of CYP1A1/2 as well as CYP3A4. As CYP3A4 enzyme metabolizes about 50% of all drugs, its inhibition by sesquiterpenes might result in drug-essential oils interactions. On the other hand, inhibition of CYP1A1/2 could represent protective effect of sesquiterpenes, as CYP1A often catalyzes formation of toxic metabolites from environmental pollutants.

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THE EFFECT OF NUTRITIONAL SUPPORT AND NUTRITIONAL SUBSTRATE OXIDATION ON THE COMPOSITION OF BODY FLUIDS IN POLYTRAUMA PATIENTS IN ICU

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Redistribution of body fluids is one of the risk factors in polytrauma patients. Overhydration (OH) and changes in the amount of intracelular water (ICW) and extracelular

water (ECW) are typical characteristics of this state. The aim of this study was to evaluate the influence of administered nutritional support and nutritional substrate oxidation on the composition of body fluids in polytrauma patients, because it has not been well known. The study included 14 patients in which was used indirect calorimetry and bioelectrical impedance spectroscopy in average day 5.3 ± 3.2 of hospitalization in ICU. The average age of the patients was 45.29 ± 18.34 years. Patients received parenteral nutrition, according to the ESPEN guidline. Using correlation analysis there has been demonstrated that intake of energy in kcal d^{-1} ($p = 0.0064$; $r = -0.6892$), ($p = 0.0050$; $r = -0.7031$), carbohydrate in g d^{-1} ($p = 0.0020$; $r = -0.7501$) ($p = 0.0007$; $r = -0.7916$) and proteins in g d^{-1} ($p = 0.0039$; $r = -0.7176$) ($p = 0.0012$; $r = -0.7722$) significantly reduced the amount of TBW, ECW. Daily intake of proteins per kg^{-1} ideal body weight ($p = 0.0487$; $r = -0.5350$) and carbohydrate per kg^{-1} ideal body weight ($p = 0.0285$; $r = -0.5834$) reduced OH. The total energy balance between intake and expenditure and proteins oxidation were associated with TBW. Clinical aplication of result can improve the parenteral nutritional support and contribute to restore the correct distribution of body fluids and thus reduce mortality in polytrauma patients.

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EFFECT OF SELECTED SESQUITERPENES ON THE ACTIVITY OF REDUCING BIOTRANSFORMATION ENZYMES IN RAT

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Sesquiterpenes are secondary metabolites produced mainly in higher plants but also in fungi and invertebrates. Sesquiterpenes are defined as 15-carbon compounds formed from three isoprenoid units. Sesquiterpenes create diverse and extensive group of natural compounds and can be found in human food as well as in folk medicines or dietary supplements. If the sesquiterpenes inhibit or induce drug biotransformation enzymes, they could interact with drugs in concurrent or consecutive therapy.[1] The aim of this project was to evaluate the effect of α-humulene, β-caryophyllene-oxide and β-caryophyllene on the activity of selected reducing biotransformation enzymes in rat liver. In this study, the activities of aldo-keto reductase (AKRs) 1A1, NAD(P)H:quinone oxidoreductase 1 (NQO1) and carbonyl reductase (CBR1) were measured using spectrophotometric assays. These reducing enzymes that catalyze phase I biotransformation of various drugs and other xenobiotics are located in cytosol. The effect of the selected sesquiterpenes was studied in cytosolic fraction prepared from rat liver homogenates and in primary cultures of rat hepatocytes. In the first study, the activities of reducing enzymes were assayed in cytosol with or without individual sesquiterpene. In the second study, hepatocytes were incubated with each sesquiterpene for 24 hours and then the subcellular fractions were isolated. Consequently, the activities of

reducing enzymes were tested in cytosol from hepatocytes treated with sesquiterpenes and in cytosol from untreated hepatocytes. The results did not show any significant induction or inhibition of AKR1A1 and CBR1 based on the effect of sesquiterpenes. On the other hand, incubation of hepatocytes with sesquiterpenes caused mild increase of NQO1 activity.

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CYTOMEGALOVIRUS INFECTION WITH HCMV STRAIN AND ITS RELATIONSHIP TO THE IMMUNOSUPPRESSION

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The main goal of our study is the contribution to the study of *in vitro* interaction of human cytomegalovirus, belonging to the family *Herpesviridae* with selected immunosuppressed host cells.

During the academic year 2015/2016, we were focused on the infection of human lung fibroblasts MRC-5 with human cytomegalovirus strain VR-1590. During the study, basic laboratory techniques have been used. Among these methods, we were employed to work with cell cultures and viral isolates where the concentration of cytomegalovirus has been quantified using plaque-based assay. Moreover, ionizing radiation was applied to ensure the condition of immunosuppressed host. Subsequently, selected signalling pathways of host cells have been examined in relation to the radiation and/or infection using PathScan antibody technology and one-dimensional gel electrophoresis.

In this study, the cytomegalovirus infection model using immunosuppressed host cells has been introduced and quite well optimized. We have also investigated the target signalling pathways of host cells using specific antibody-determined technology. The relationship between immunosuppressed host cells and viral infection has been studied on the basis of changes of selected transduction pathways signals of these cells. PathScan technology will be further optimized in the context of the study of other selected signalling pathway signals in the future.

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EVALUATION OF MITOCHONDRIA-TARGETED DRUGS IN ZEBRAFISH: IMPLICATIONS FOR PARKINSON'S DISEASE

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Parkinson's disease (PD) is the second most common neurodegenerative disorder after Alzheimer's disease. The typical motor symptoms result from dopaminergic neuronal loss in the substantia nigra pars compacta. The aetiology of PD is not clear, although mitochondrial dysfunction has been implicated in the process of neurodegeneration. Zebrafish is a model organism increasingly used in pharmacology and neuroscience research. In this study we tested the effects of a mitochondria-targeted antioxidant (MitoQ) in a zebrafish PD model, and also tested the effects of mitochondria-targeted paraquat (MitoPQ). For induction of the Parkinsonian phenotype we used the dopaminergic toxin 1-methyl-4-phenylpyridinium (MPP^+). MPP^+ induced locomotor impairments in zebrafish, but these were not rescued by co-treatment with MitoQ. MitoPQ induced a concentration- and time-dependent toxicity in zebrafish larvae and embryos, reducing hatching, heart rate and sensory-motor reflexes. Further studies are required to characterize the effects of these drugs in zebrafish. These preliminary data suggest that co-treatment with MitoQ fails to rescue MPP^+ toxicity and, thus, testing a MitoQ pre-treatment before MPP^+ exposure is warranted. Future studies with MitoPQ will include direct comparison with paraquat, and a characterization of the impact on zebrafish mitochondrial function.

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INTERACTION OF XANTHENE-3-ONE DERIVATIVES WITH IRON AND COPPER

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Iron and copper are the essential dietary metals, important for the proper function of cells. Their imbalance can lead to serious diseases. The aim of the study was to assess the interaction of 2,6,7-trihydroxyxanthene-3-one derivatives (synthesized by the group of

Kemal Durić, University of Sarajevo) with transition metals iron and copper in 4 (patho) physiologically relevant pH conditions.

Interaction with iron was determined by use of Ferrozine method. In case of copper Hematoxylin and BCS methods were employed. The rate of chelation and reduction was detected in both cases by measuring absorbance and compared by control samples.

All compounds chelated iron and the potency was not very different among tested congeners and dropped with decreasing pH. According the screening Hematoxylin method, all compounds chelated also Cu^{2+} ions, but more competitive assay with BCS has shown that they are weak copper chelators. The ferric ions reducting ability was seen also in all tested compounds but only in low ratios (compound to Fe^{3+}) at pH 4.5 and partly at pH 5.5 with maximal reduction reaching 50–70%. In a similar way all tested compounds reduced cupric ions, but all of them reached 100% reduction at all pH. The 4′-trifluoromethyl derivative proved to be the most effective iron chelator but it was also the most active in cupric reduction.

These newly synthesized compounds may be of interest for further research in chemotherapy due to their ability to reduce transition metals.

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UDP-GLUCOSYLTRANSFERASES FROM SENSITIVE AND RESISTANT *HAEMONCHUS CONTORTUS* ISOLATES

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Parasite anthelmintic resistance is a great problem of these days. Mechanisms of drug resistance are still not fully understood. Molecular biology methods, *e.g.* gene expression studies, could contribute to understanding of these mechanisms and thus help in resistance management.

Haemonchus contortus is a parasitic nematode of small ruminants, whose multi-resistance to anthelmintics means global problem. The genome and transcriptome have been published recently, allowing extensive gene expression research to be conducted.

This work is a part of complex research, studying changes in expression of UDP-glucosyltransferases (UGTs) in susceptible and resistant strains of *H. contortus*. By now more than 40 UGTs were identified in genome of *H. contortus*. Previous work on faculty showed, that resistant strain (WR) forms significantly more glucose conjugates of albendazol,[1] anthelmintic commonly used for treatment of nematode infections. So far UGT7 was found to be one of possible causes of higher glucose conjugates levels in resistant strain,[2] but further research needs to be carry out.

The aim of this work was to quantify and compare constitutive expression of 8 UGTs in two genetically divergent *H. contortus* strains: drug-susceptible (ISE) and multi-drug-resistant (WR).

Total RNA was extracted from ten adult *H. contortus* males or females from both strains and reverse transcribed to cDNA. QuantStudio 6 Flex Real-Time PCR system (Life Technologies, Applied Biosystems) thermocycler was used for qPCR analyses with SYBR green I detection.

For relative quantification comparative ΔΔCt method was used with gapdh, ama and ncbp as reference genes.[3]

The study was supported by the Charles University (Research project SVV 260 294).

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ENTRY OF *MYCOBACTERIUM BOVIS* INTO MURINE B CELLS: THE ROLE OF B CELLS RECEPTORS AND COMPLEMENT RECEPTORS

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Mycobacterium bovis, a Gram positive, acid-fast, aerobic bacterium, is the etiological agent of bovine tuberculosis and because it is related to *Mycobacterium tuberculosis* it can also cause tuberculosis in humans. In this research we were studying B cells and their role in the entrance of *M. bovis* into them.

The fact that B cells are responsible for production of specific antibodies against pathogenic bacteria is known for a long time but they have a role in antibody-dependent also as in antibody-independent immune response. It means, that not only T cells are important in the early protective immune response against intracellular pathogens. B cells are also important. They can communicate with bacteria through their receptors. We used flow cytometry to show the role of B cells receptors and complement receptors allowing the entry of *M. bovis* into B cells after infection. We focused on the effect of blocking B cells receptors BCR, FcR and complement receptors CR½, CR3, CR4 on peritoneal CD19+ cells during the infection. We have also observed the effect of opsonisation by complement and antibody on the infection.

The study was supported by Grant "Long-term organization plan 1011" received from the Czech Ministry of Education, Youth and Sports.

INFLUENCE OF PRECURSOR FEEDING ON PRODUCTION OF NAPHTODIANTHRONES AND FLAVONOIDS IN *HYPERICUM PERFORATUM* EXPLANTATE CELL CULTURES

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The goal of this work was to influence the production of hypericin (naphtodianthrone), hyperoside and quercitrin (flavonoids) in the suspensional *Hypericum perforatum* explantate cell cultures. The precursors of naphtodianthrones and flavonoids were added into the medium in final concentrations 10 mg/l, 50 mg/l and 100 mg/l. Taking of samples were in time intervals 72 and 168 hours. As the precursors of naphtodianthrones and flavonoids were used kalium acetate, cinnamic acid, sodium cinnamate, tyrosine and shikimic acid. Cultures were cultivated on the Murashige and Skoog medium with the addition of the growth stimulator α-NAA. Concentration of hypericin, hyperosid and quercitrin was detected by HPLC analysis.

Positive influence on the production of hypericin in cultures was registered by adding of tyrosine. The concetration of hypericin in cells raised in this experiment from 0.003 % (control culture) to 0.03% (culture with tyrosine concentration 100 mg/l).

Highest influence on the production of hyperoside in cultures was registered by adding of cinnamate and shikimic acid. Quercitrin was not detected in the cultures.

The study was supported by SVV 260 294.

ASSESSMENT OF DRUG-INDUCED DNA-PROTEIN COVALENT COMPLEXES

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A number of proteins form covalent bonds with DNA as obligatory transient intermediates in normal nuclear transactions. Currently, more than 20 proteins are known to form covalent complexes with DNA including DNA repair involved proteins, DNA glycosylases, DNA methyltransferases, DNA polymerases and topoisomerases. Drugs that trap these complexes have proven to be potent therapeutics in both cancer and infectious disease.[1] Anthracyclines are amongst the most potent groups of antineoplastic drugs. The mechanism of anthracycline antineoplastic effect had been formerly attributed to their ability to intercalate to DNA. Lately, this effect was identified to be mediated by topoisomerase II poisoning with the subsequent cell-cycle break, formation of double-strand breaks and apoptosis.[2] Besides the highly favourable antineoplastic effect, anthracyclines have also highly unfavourable and life-threatening side effect – cardiotoxicity. The mechanism of

cardiotoxicity is elusive, although it was traditionally attributed to the iron-mediated oxidative stress. Nevertheless, current literature now points to the topoisomerase II-mediated DNA damage in cardiomyocytes as one of the possible explanation of anthracycline cardiotoxicity.[3] Hence, the aim of this study is to optimize the isolation and immunodetection of DNA-topoisomerase II covalent complexes induced by daunorubicin in various cell lines and relationship of the induction of these complexes and DNA damage assessed by other methods (comet assay).

The study was supported by SVV 260 294.

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EVALUATION OF SYNERGISTIC EFFECT OF DAUNORUBICIN AND RIBOCICLIB BY THE COMBINATION INDEX METHOD

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ABCB1 (P-glycoprotein, MDR1) is the well-known member of ATP-binding cassette (ABC) transporter family. This transport protein is able to efflux a wide variety of structuraly unrelated substrates including drugs out of the cells and thereby represents one of the cellular deffence mechanisms against potentially harmful substances. Activity of ABCB1 overexpressed in cancer cells causes decrease of intracellular concentrations of administered chemotherapeutics under the cytotoxic level and leads to the development of multidrug resistance (MDR) and treatment failure.

Cyclin-dependent kinases (CDK) play an important role in cell cycle progression. Their deregulation is observed in cancer cells and therefore the CDK have recently become a useful target of anticancer treatment. Our previous studies revealed that cyclin-dependent kinase inhibitors (CDKI) can also inhibit ABC transporters, including ABCB1.[1,2] This feature might be exploited in battling MDR by administering CDKI simultaneously with other chemotherapeutics that are substrates of these transporters.

The aim of this project was to study ribociclib, inhibitor of CDK 4 and 6 that is now undergoing phase III clinical trials for treatment of breast cancer and has been revealed as ABCB1 inhibitor in our previous studies. Using XTT test we studied the antiproliferative effect of simultaneous administration of ribociclib and antracycline antibiotic daunorubicin (DNR), the well confirmed ABCB1 substrate in MDCKII-ABCB1 cell line overexpressing human ABCB1 transporter. Obtained data were evaluated by Chou-Talalay method and the

combination index values were calculated to determine the resulting antiproliferative effect of this drug combination.

Combination index values indicate that concomitant treatment of MDCKII-ABCB1 cells by ribociclib and DNR leads to significant synergistic activity of both antineoplastic drugs. Fact that this effect was not observed in ABCB1 non-expressing parental cells suggests that this synergism is a consequence of ABCB1-mediated efflux inhibition, which indicate that ribociclib might be beneficial in the anticancer therapy not only as CDK inhibiting drug, but also as MDR overcoming agent.

The study was supported by SVV 260 293.

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HYPERICUM PERFORATUM L. IN VITRO – ABIOTIC ELICITATION

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The subject of this study is the evaluation of secondary metabolites production in *Hypericum perforatum* L. cultures *in vitro* after elicitor treatment. The aim was to find if orthosilicic acid as abiotic elicitor increases the flavonoid and hypericin production in *Hypericum perforatum* L. cultures *in vitro*. Experiment was carried out in callus and suspension cultures of *H. perforatum* using Murashige – Skoog nutrient medium[1] supplemented with 10 mg ml^{-1} αnaphtylacetic acid as growth regulator. The elicitor was added in the form of solution in 3 different concentrations ($C_1 = 10.4047 \times 10^{-3}$ mol l^{-1}, $C_2 = 10.4047 \times 10^{-4}$ mol l^{-1}, $C_3 = 10.4047 \times 10^{-5}$ mol l^{-1}), it was affecting 6, 12, 24, 48, 72 and 168 hours. The content of flavonoids and hypericin was determined by HPLC. Secondary metabolites release into nutrient medium was also a part of this study.

The increasing flavonoid and hypericin production in callus cultures after elicitor application at any concentrations was not observed. The maximum flavonoid content (0.04 mg g^{-1} DW) in suspension culture was detected after 72 h of elicitor treatment in concentration of C_1 where the maximum hyperoside production was observed. The maximum hypericin production (0.21 mg g^{-1} DW) in suspension culture was detected after 12 h of elicitor application in concentration of C_1 (10.4047×10^{-3} mol l^{-1}). The second significant increase in hypericin production (0.17 mg g^{-1} DW) in suspension culture after 24 h of elicitor treatment in the same concentration was reached. Flavonoid and hypericin release into nutrient medium was not detected. The elicitor orthosilicic acid is able to increase the flavonoids and hypericin production in *Hypericum perforatum* cultures *in vitro*.

The study was supported by SVV 260 294.

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EFFLUX TRANSPORTERS OF TAPEWORMS

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Multidrug resistance in parasitic helminths can be considered as worldwide problem with high socioeconomic impact. Several mechanisms are known to be responsible for the resistance development in parasitic helminths. Efflux of transporters belongs to one of them as activity of these transporters can cause decrease of drug levels in cells leading up to loss of therapeutic efficacy. The knowledge of P-glykoprotein-like (Pgp-like) efflux transporters expression, function and distribution may help to better understand the multidrug resistance development in tapeworms. For our purposes rat tapeworm (*Hymenolepis diminuta*), a typical representative of rodent parasite often used as a laboratory model for Cestoda class, was used. Aim of our study was to investigate: I) distribution of Pgp-like transporters over the adult tapeworm body (scolex, immature proglotides, mature proglotides and gravid proglotides), II) influence of rifampicin (RIF, 1μM, Pgp inducer in mammals) and albendazol (ABZ, 1 μM, anthelmintic drug) on Pgp-like transporters expression. Based on the search and comparison of *H. diminuta* draft genome with *H. microstoma* and *Echinococcus granulosus* annotated genomes five Pgp-like homologs (*Hd-Pgp 1-5*) were tentatively identified. Quantitative PCR was used to determine expression of observed genes. *Drosha* (*drosha ribonuclease III*) and TBP (*TATA box binding protein*) were selected and used as reference genes due to their highest stability. For *Hd-Pgp 1*, *3* and *5* significant differences in basal distribution were observed among the parts of body in *H. diminuta*. Significant increase in expression for *Hd-Pgp 1* and *3* after RIF treatment was observed. Expression of *Hd-Pgp 5* was decreased by both RIF and ABZ treatment. Our experiment confirmed presence and different distribution of Pgp-like genes in *H. diminuta* and ability of two of them to be induced by Pgp inducer RIF. Based on the findings Hd-Pgp 1 and 3 seems to be good candidates for future investigation of efflux transporters role in drug resistance development.

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ESTABLISHMENT OF EPITHELIAL-MESENCHYMAL TRANSITION MARKERS IN CELLS *IN VITRO*

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Epithelial-mesenchymal transition (EMT) is a process during which motile mesenchymal-like cells develop from non-motile parent epithelial cells. Physiologically, EMT plays important roles during embryonic development and wound healing. Loss of control over this mechanism can lead to fibrosis and cancer progression.[1] Motile mesenchymal-like cells can pass through the basal lamina, get into the blood vessels and spread to distant tissues. Transition is regulated by EMT biomarkers. The biomarkers comprise wide spectrum of proteins, including cell surface proteins (E-cadherin, N-cadherin), cytoskeletal proteins (vimentin), extracellular matrix proteins (fibronectin) and transcription factors (SNAIL,TWIST).[2] In this study, expression of EMT biomarkers was evaluated using RT-PCR and Western blotting. The ability to migrate was assessed using real-time analysis with the x-CELLigence system. Two known triggers of EMT, the StemXVivo™ EMT Inducing Media Supplement (IS) and TGF-beta, were compared in human oral cancer cell lines DOK and H376.[3] TGF-beta has been shown as more effective, especially in 5 ng/ml concentration, in comparison with IS. More sensitive to the TGF-beta treatment was the cancer cell line DOK.

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ELICITATION OF BIOACTIVE COMPOUNDS PRODUCTION IN *IN VITRO* CULTURES OF *PANAX GINSENG*

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Panax ginseng C. A. Meyer belongs to the family of Araliaceae. It is one of the most important traditional medicinal plant. The main biological acitive compounds are the

secondary metabolites: ginsenosides (triterpenoid saponins) and polyacetylenes.[1] The pharmacological efficacy of *Panax ginseng* includes the improving of brain function, anti-tumor activity, anti-diabetic, analgetic and anti-stress effects, adjusted blood pressure.

The aim of this *in vitro* study was to test the effects of jasmonic acid and coronatin on the growth of biomass and the biosynthesis of secondary metabolites in *Panax ginseng* cultures. The adventitious roots were cultivated in Erlenmeyer flasks with the liquid SH medium[2] for 6 weeks using rotary shaker which was placed in the dark at 24 ± 1 °C. Various concentrations of coronatin and jasmonic acid were added in the medium. After cultivation, the amount of biomass was measured and ginsenosides were extracted with methanol. The extractes were analyzed using UPLC.

The obtained results showed that jasmonic acid and coronatin increased the production of secondary metabolites, although it did not increase the total root biomass.

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DEVELOPEMENT OF A RELIABLE TEST SYSTEM FOR HUMAN PURINERGIC P2X3 RECEPTORS

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Purinergic P2X3 receptor is a ligand-gated ionotropic channel that occurs in all mammalian tissues. The highest occurrence has been observed in central and peripheral nervous system and smooth muscles, where P2X3 receptors participate in pathological disorders such as visceral and neurophatic pain, inflammatory reactions and psychiatric disorders.[1] Compounds capable of blocking P2X3 receptor activity could be therefore used as potential drugs for treatment of these states.[2] P2X3 receptor belongs to fast-desensitizing ionotropic channels, which makes the measurement of its activity very difficult. It was described that one point S15V mutation, in which the amino acid serine in a position 15 is replaced by amino acid valine, slows down the desensitization rate and the signal becomes easily measurable. This simple mutation may be used as an effective tool for characterization of insufficiently explored P2X3 receptor.[3]

The P2X3 S15V receptor DNA was inserted into retrovirus and, subsequently, human 1321N1 astrocytoma cells were infected. Retroviruses carried not only receptor DNA but also a resistance to antibiotic G418, which allowed the selection of successfully transfected

cells. Human cells expressing P2X3 S15V receptor were tested *via* fluorescence-based calcium assay, in which the intracellular calcium levels are indicated fluorescently. When the method was completely optimized the testing of known P2X3 agonist, antagonist and allosteric modulators plus yet untested potential P2X3 antagonists was performed. The measured data was analyzed in GraphPad Prism.

P2X3 S15V receptor mutant is a reliable surrogate for characterization of P2X3 ionotropic channel. The response to P2X3 agonists, antagonists and allosteric modulators was comparable to literature with one exception, compound called A-317491. Moreover, blocking activity of one widely used drug and its metabolite was observed at P2X3 S15V receptor. Very interestingly, this could mean that the mechanism of action of this worldwide used compound was finally discovered.

The study was supported by SVV 260 291.

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EFFECT OF CADMIUM CHLORIDE ON P-GLYCOPROTEIN EXPRESSION AND FUNCTION AT THE BLOOD-BRAIN BARRIER

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The blood-brain barrier (BBB) separates the central nervous system (CNS) and the peripheral blood circulation and regulates the material and signal transport between these compartments due to its specialised structure and cellular constitution.[1] The endothelial cells forming the BBB are characterized by the expression of different multidrug resistance proteins which belong to the ATP-binding cassette (ABC) transporter family. These transmembranous ABC export proteins actively transport molecules out of the BBB endothelia into the bloodstream and protect the brain against harmful xenobiotics, toxins and metabolites. On the other hand, ABC export proteins constitute obstacles to the delivery of many therapeutic drugs across the BBB into the CNS, thus the efficacy of CNS pharmacotherapy is limited.[2] One of the most important efflux transporters is P-glycoprotein (P-gp).[3] Cadmium is a heavy metal that is dangerous to human health. It gets to the environment as an industry contamination and then to the human body through smoking tobacco, drinking water or inhalation of polluted air.[4]

In this work, the impact of cadmium chloride ($CdCl_2$) on the functional activity and expression of P-gp at the BBB was studied. Monolayers of the hCMEC/D3 cell line and freshly

isolated rat brain capillaries were used as model systems for the BBB. In hCMEC/D3 cells, short-term treatment with 0.5–50 μM $CdCl_2$ in pyruvate supplemented Hanks' balanced salt solution (HBSS-P, up to 3 hours) lead to a concentration-dependent decrease in accumulation of the fluorescent P-gp substrate, Rhodamine 123, compared to untreated control cells indicating a $CdCl_2$ induced increase of P-gp-mediated transport. These findings were confirmed in studies with isolated rat brain capillaries by measuring the luminal fluorescence of the specific P-gp substrate, NBD-CsA, *via* confocal laser scanning microscopy: capillaries exposed to 0.5–20 μM $CdCl_2$ showed a higher luminal NBD-CsA fluorescence compared to untreated control capillaries.

Long-term influence of $CdCl_2$ on P-gp function and expression was studied in hCMEC/D3 cells after 48 hour treatment. Incubation with 0.5–10 μM $CdCl_2$ in differentiation medium for 48 hours caused less Rhodamine 123 accumulation compared to non treated control cells. Relative P-gp mRNA and protein expression levels were both up-regulated after 0.5 μM and 1 μM $CdCl_2$ exposure compared to untreated control indicating a genomic up-regulation of functionally active P-gp. None of the used concentrations and exposure periods of $CdCl_2$ affected the metabolic activity of hCMEC/D3 cells as revealed by measuring cell viability with a Resazurin-based assay. These results show that transport activity as well as gene and protein expression levels of P-gp at the BBB can be altered by low concentrations of $CdCl_2$.

This work was supported by Erasmus+ programme.

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SECTION OF CHEMICAL SCIENCES

SYNTHESIS OF QUINAZOLINES WITH POTENTIAL AFFINITY TO CAR RECEPTOR

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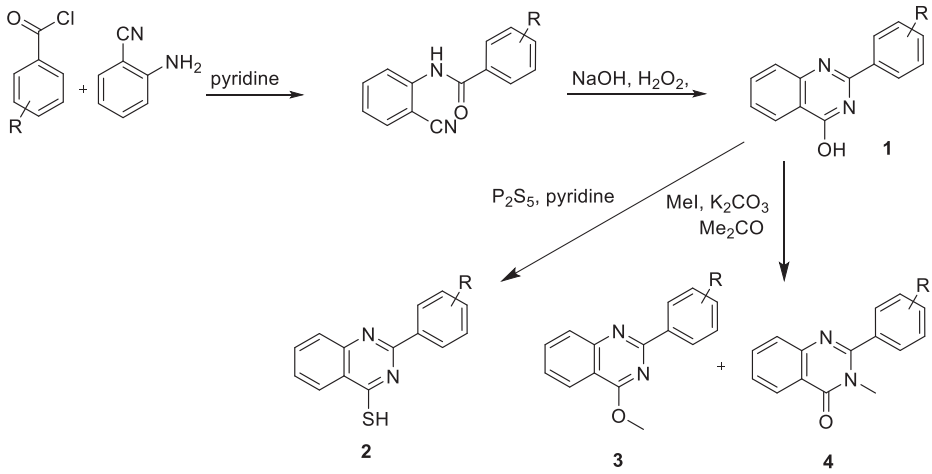
This project deals with a synthesis of 2,4-disubstituted quinazoline derivatives with the affinity to CAR receptor. CAR receptor (constitutive androstane receptor) is a nuclear recep-

tor controlling genes expresison and is one of the key regulators of endobiotic and xenobiotic metabolism. As a result of random screening, 2-aryl-quinazoline-4-oles (**1**) have been found as potential ligands towards CAR receptor.[1] We have synthesized a library of sulfur (**2**), *O*-alkylated (**3**) and *N*-alkylated (**4**) analogues (Scheme 1).[2] The evaluation of affinity to CAR receptor displayed promising effetcs.



Scheme 1. Synthesis of the title compounds.

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DESIGNING A METHOD FOR A HOMOGENOUS LIQUID-PHASE MICROEXTRACTION IN A LAB-IN-SYRINGE SYSTEM

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The sequential injection analysis (SIA) is a technique derived from flow injection analysis. It has been used to automate laboratory procedures. The “Lab-In-Syringe” is a modified SIA used to carry out parts of the experiment inside the used syringe pump. Using a PTFE-coated magnetic-propelled stirring bar inside the syringe[1] allows, for

example, to homogenously mix the syringe contents and perform a liquid-phase microextraction (LPME)[2].

In this work, the approach to perform salt-assisted in-syringe homogenous LPME (HLPME) was explored and evaluated for the first time. Starting with a one-phase system, the analyte was extracted from water into n-propanol. For this, a highly-concentrated solution of magnesium sulfate was used to increase the polarity of the aqueous phase. The high polarity causes the separation of the two normally miscible liquids.

Measuring the absorbance in the organic phase was studied both in-syringe and at the outlet and yields precise analysis of the sample content. Astraphloxine and riboflavin were used as model analytes. The method performance and parameters were studied, evaluated and improved for the highest preconcentration factor and the fastest phase separation.

The highest achieved preconcentration factor was 6.68. The fastest phase separation took < 5 s. The reproducibility of 3 repetitive extractions was generally < 1% RSD.

In conclusion, the in-syringe salt-assisted HLPME presents an interesting approach to perform a fast and precise automated extraction for the analyte preconcentration using an environment-friendly and HPLC compatible solvent.

The study was supported by SVV 260 292.

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OPTIMALIZATION AND VALIDATION OF SPE METHOD FOR UHPLCMS/MS DETERMINATION OF QUERCETIN AND ITS 9 METABOLITES IN BIOLOGICAL MATERIAL

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The aim of this study was to develop and validate a new extraction method for the preparation of biological samples for the determination of quercetin and its 9 metabolites: phloroglucinol, 3,4-dihydroxyphenylacetic acid, homovanilic acid, 3-hydroxyphenylacetic acid, 3-(3-hydroxyphenyl)propionic acid, rutin, quercetin-3-glucuronide, tamarixetin and isorhamnetin. Due to the best retention and subsequent elution of all analytes the polymeric ion exchanging cartridge MAX was chosen. The mixture of 95% methanol and 0.5% trifluoracetic acid in water was chosen as optimal solvent for elution. The combination of 0.01M ammonium formate buffer pH 5.0 and 1% methanol in buffer pH 5.0 were chosen as washing solvent.

The determination of quercetin and its 9 metabolites was performed using UHPLC-MS/MS. The best selectivity between the critical pair of analytes with the same molecular weight (tamarixetin and isorhamnetin) was achieved using BEH Shield RP18 column and gradient

elution with methanol and 0.1% formic acid. The ionization was performed in electrospray polarity switching mode. The quantification was performed by triple quadrupole and selected reaction monitoring (SRM) mode. The method was validated in terms of linearity, sensitivity (LOD, LOQ), accuracy, precision, selectivity and matrix effects. Good linearity demonstrated correlation coefficients value ≥ 0.990. Method precision was expressed as RSD ≤ 20.0%. The results of method accuracy were between 79.6–116.6% except for three metabolites (PG, HVA, PAA) for which the results of accuracy 31.1–61.4% were affected by losses during a washing step. The advantage of newly developed SPE method for the preparation of biological samples prior to UHPLC-MS/MS analysis is simultaneous analysis and extraction of the compounds with different physicochemical properties.

The study was supported by SVV 260 292.

CHROMATOGRAPHIC BEHAVIOUR OF BORON CLUSTER COMPOUNDS

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Boron clusters compounds (BCC) are purely synthetic inorganic species. Their three-dimensional boron skeleton is formed by three-centred two-electron bonds forming an electrondeficient structure with delocalized cloud of electrons. The most perspective and studied compounds are related to three 12 vertex structures – *closo*, *nido* and *arachno* (Fig. 1).

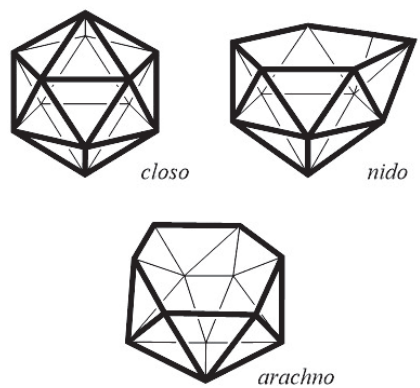

Fig. 1. Structures of boron clusters compounds.

Derivates of BCCs are obtained by both exoskeletal and endoskeletal substitution. If at least three atoms of BCC are replaced by other atoms or groups of atoms they result compound may miss its symmetry and become chiral.[1] BCC are being explored as artificial

pharmacophores owing to their size, geometry, electron structure and metabolic stability. BCC have been also successfully used in boron neutron capture therapy.[2,3]

Properties of boron-cage compounds such as extreme acidity and hydrophobicity considerably influence their chromatographic behaviour in an HPLC system. The BCC anions are problematic to separate chirally using β-cyclodextrin by HPLC. Nevertheless, electrophoretic separations of anionic clusters with dissolved cyclodextrins[1] proved that these selectors can, in principle, discriminate almost any type of substituted BCCs.[1,4]

The aim of our work was to explore the influence of chromatographic conditions (organic modifier, pH of the aqueous phase and temperature) on retention of mainly anionic species with respect to their chiral separation in order to elucidate the above mentioned discrepancy between CE and HPLC.

The study was supported by SVV 260 291.

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SYNTHESIS AND EVALUATION OF 1-*O*-ACYL CERAMIDES

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The extracellular matrix of the uppermost layer of the skin, the *stratum corneum* (SC), consists of ceramides (Cer), cholesterol (Chol), free fatty acids (FFA) and cholesteryl sulfate (CholS). The SC Cer play an important role in the correct barrier function of mammalian epidermis. A new type of sphingolipids, *i.e.*, 1-*O*-acyl-Cer, have been found in human SC very recently; however, their role in the SC is unknown.[1] These Cer species contain sphingosine (S) that is *N*-acylated with non-hydroxylated (N) or α- (A) hydroxylated fatty acid, and moreover, a hydroxyl group at C1 in S is esterified by an additional fatty acid – lignoceric (C24) and palmitic (C16) acid. Because 1-*O*-acyl-Cer are not commercially available, we aimed to synthesize physiological 1-*O*-acyl Cer, *i.e.*, Cer-24NS16, Cer-16NS16 and Cer-24AS16. Moreover, we aimed to study their effects on the barrier properties and microstructure of model SC lipid membranes. The 1-*O*-acyl-Cer were synthesized by an esterification of Cer-NS16 or Cer-AS16 with palmitic or lignoceric acid using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (WSC) and 4-dimethylaminopyridine (DMAP). Cer-AS16 was prepared by α-bromination of palmitic acid, substitution of bromine by hydroxyl and *N*-acylation of sphingosine. Next, model SC lipid membranes were prepared with followed composition: Cer-NS24/FFA $C_{16\text{-}24}$/Chol/CholS as a control

membrane and then 5, 10, 20, 30, or 100% of Cer-NS24 was replaced by Cer-24NS16. The permeability of model SC lipid membranes was assessed in Franz-type diffusion cells using flux of theophylline and indomethacin, electrical impedance and water loss through the membrane. To elucidate the mechanisms of 1-*O*-acyl-Cer on skin permeability, their effects on the membrane biophysics will be investigated by infrared spectroscopy and X-ray powder diffraction. These results should elucidate the behaviour of 1-*O*-acyl-Cer with other skin lipids and the role of this new class of sphingolipids in the skin lipid barrier.

The study was supported by Czech Science Foundation (GAČR 13-23891S) and Charles University (SVV 260 291).

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SYNTHESIS OF AMPHIPHILIC PHTHALOCYANINES WITH QUATERNIZED IMIDAZOLES

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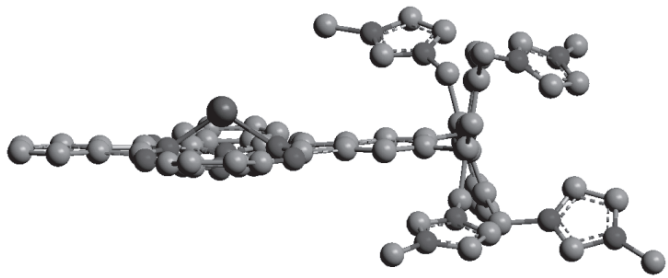
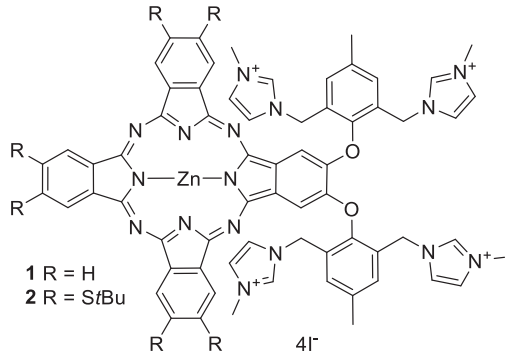
The photodynamic therapy is a curative method of cancerous and non-cancerous diseases. It uses light, oxygen and photosensitizer to destroy the cancer cells. Photosensitizer absorbs energy of light and produces singlet oxygen. Singlet oxygen attacks cellular structures like cellular membrane, lysosomes, mitochondria, etc. causing thus damage leading to cell death.

Previous study revealed that zinc phthalocyanine with methylated 2,6-[bis(imidazol-1-yl)methyl]-4-methylphenoxy substituent had excellent photodynamic activity against HeLa cells and low toxicity.[1] That is why, we decided to synthesize a series of similar compounds and **2** bearing this substituent but with rather amphiphilic character. They bear interesting spatial features to be potentially incorporated into lipid bilayer or a double stranded DNA.





The synthesis started by condensation of bis(hydroxymethyl)-*p*-cresol and imidazole to obtain 2,6-[bis(imidazol-1-yl)methyl]-*p*-cresol substituent. In the following reaction, this phenol was subjected to nucleophilic substitution with 4,5-dichlorophthalonitrile to obtain the corresponding phthalonitrile precursor for cyclotetramerization. Its co-cyclotetramerization with phthalonitrile or 4,5-bis(*tert*-butylsulfanyl)phthalonitrile initiated by magnesium butoxide resulted in the corresponding magnesium complexes of **1** and **2**, respectively. The magnesium complexes were converted into metal-free phthalocyanines by treatment with *p*-toluenesulfonic acid and subsequently zinc cation was incorporated to the molecule centre by zinc acetate. At the end, imidazole nitrogen was alkylated with methyliodide. The quaternized compounds were tested on HeLa cells for their photodynamic activity and inherent toxicity.

The study was supported by SVV 260 291 and Czech Science Foundation No. 13-27761S.

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NEW ANALOGUES OF IRON CHELATOR SALICYLALDEHYDE ISONICOTINOYL HYDRAZONE

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Iron (Fe) is an element essential to all living cells. However, this transition metal may also catalyze the Fenton reaction which results in the formation of toxic reactive oxygen species (ROS), such as hydroxyl radicals.

Salicylaldehyde isonicotinoyl hydrazone (SIH) is a tridental chelator selectively forming complexes with Fe ions. As a result of its low molecular weight and optimal lipophilicity, SIH can be administered orally. It readily enters the cells, effectively chelates the intracellular Fe ions, and is therefore able to very efficiently inhibit the Fedependent processes, such as production of ROS, but also the synthesis of some proteins and enzymes and the processes they regulate (e.g., cellular growth and proliferation).

In this work we focused on the design and the synthesis of novel SIH analogues with modified ligands, in particular the thio-analogue of SIH – thioSIH, and the analogues derived from (di)hydroxybenzophenone (Fig. 1).

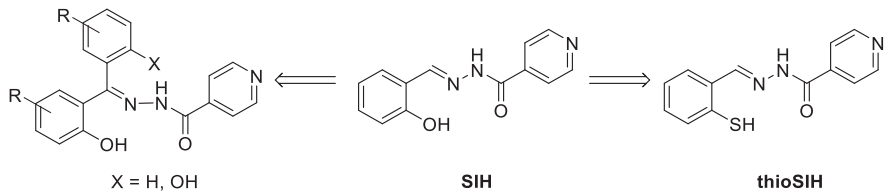

Fig. 1. Structure of SIH and its analogues studied in this work.

We started the synthesis of thioSIH from thiosalicylic acid *via* its reduction to 2-mercaptobenzyl alcohol and subsequent oxidation to 2,2′-dithiodibenzaldehyde. Schiff base condensation with isoniazid provided thioSIH-disulfide, which was then reduced to thioSIH using dithiothreitol. (Di)hydroxybenzophenone analogues were prepared by condensation of hydrazides with appropriately substituted benzophenones.

The study was supported by the Czech Science Foundation (13-15008S) and Charles University (SVV 260 291).

SYNTHESIS OF ULTRA-LONG CERAMIDES

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Ultralong ceramides (UC), minor but essential components of the extracellular lipid matrix in the uppermost skin layer *stratum corneum*, play a critical role in the survival of mammals in dry land. Deeper understanding of the role of UC in human (patho)physiology or the potential use of UC is hampered by their limited availability.[1]

Skin sufrace lipid analysis, performed by Colton and Downing[2] has shown that skin surface lipids of *Equus asinus* (Donkey sebum) contain 56% of ω-lactones, of which 51.2% is mono-unsaturated dotriacontanolide (lactone of dotriacontanoic acid).

The aim of this project was to isolate dotriacontanolide from donkey sebum by column chromatography, then perform saturation of double bond, and finaly open the lactone to N-succinimidyl ester, that can be further used in complete synthesis of UC. UC have already been successfully synthesized with high yields in our laboratory by using N-succinimidyl ester activation group.

First, we have succesfully performed isolation of mono-unsaturated dotriacontanolide from Donkey sebum. Then, we succesfully performed hydrogenation of the double bond using paladium catalyst. Finaly, we have found a way, how to open this ω-lactone to *N*-succinimidyl ester of 32-hydroxydotriacontanoic acid.

The study was supported by Czech Science Foundation (GAČR 13-23891S) and Charles University (SVV 260 291).

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SYNTHESIS AND EVALUATION OF QUATERNARY AMMONIUM SALTS

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Surfactants (surface-active agents) are substances having property of absorbing onto the surfaces or interfaces of the system and ability to decrease surface or interfacial fee energies. Surfactants have an amphipathic molecular structure consisting of hydrophobic and hydrophilic group in the case of a surfactant dissolved in aqueous medium.[1] Quaternary ammonium salts (QAS) have numerous applications in many industry sectors. For example, some of the QAS are used as disinfectants, solubilizers, antiplaque agent etc.[2]

Several homologous series of quaternary ammonium salts were prepared by nucleophile reactions. As a starting material were used 1-(2-hydroxyethyl)pyrrolidine, 1-(2-hydroxyethyl)piperidine and 1-(2-hydroxyethyl)imidazole which reacted with C10-C18 bromoalkanes reagents and C8-C12 dibromoalkanes reagents with only even number of carbons. Products were verified by nuclear magnetic resonance (NMR) and high resolution mass spectrometry (HRMS). Critical micellar concentration (CMC) will be measured by conductometry at Krafft temperature. Minimum inhibitory concentration (MIC) will be tested on several types of microorganisms (bacteria and fungi).

The study was supported by SVV 260 291.

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SYNTHESIS AND CHARACTERISATION OF FLAVONOIDS AS POTENTIAL ANTICANCER AGENTS

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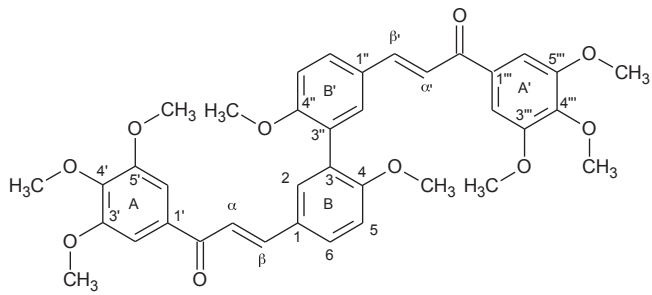
With more than 3 million new cases and 1,7 million deaths each year, cancer represents the second most important cause of death and morbidity in Europe.[1] Search for new anticancer agents is one of the most important ways towards significant advancements in cancer therapy. At present, 50% of anticancer drugs are natural compounds or can be traced to natural products.[2] Flavonoids, 2-phenyl-1,4-benzopyrones derivatives, are known for their antioxidant, anti-inflammatory, vasculoprotective, antimicrobial and anticancer properties.[3] This project is focused on on flavonoids with open C ring, named chalcones. Specifically, synthesis of two original molecules will be discussed: bichalcone (I) and chalcone-polyamine conjugate. Linking chalcone to polyamine is a promising strategy towards increasing water solubility and targeting the molecule to cancer cells. The derivatives were obtained *via* Claisen-Schmidt condensation and Suzuki reaction; various synthetic approaches will also be discussed. The compounds are currently being subjected to biological evaluation, which has provided positive preliminary results.



The study was supported by SVV 260 291.

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SYNTHESIS OF LIPOPHENOLIC DERIVATIVES OF HYDROXYTYROSOL, RESVERATROL AND PHLOROGLUCINOL

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Lipophenols are conjugates of (poly)phenolic derivatives with a lipid moiety (Fig. 1) that are designed here to access lipophilic antioxidants.

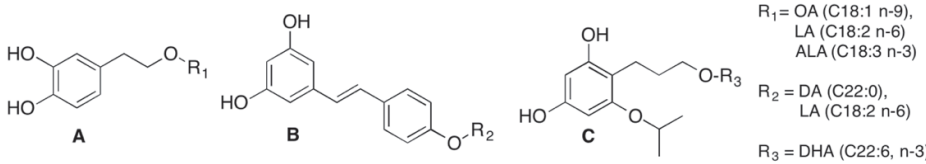

Fig. 1. Target lipophenolic derivatives of hydroxytyrosol (A), resveratrol (B) and phloroglucinol (C).

First part of this work targets olive oil lipophenols. Three new lipophenolic compounds, conjugates of hydroxytyrosol (most abundant olive oil phenol derivative) and three different unsaturated fatty acids were synthesized in two steps, in good yield and high purity. These products will be used as standards for determination of their presence in extra virgin olive oil (EVOO) and in liver samples of rats being fed by EVOO to examine possible metabolization.

Second part of the work targets lipophenols as potential antioxidant and anti-carbonyl-stress agents in retinal diseases, where those factors are involved in the physiopathology. (Poly)phenols linkage to specific lipophilic fatty acids can increase their bioavailability, potentially enable vectorization to the target retina tissue and bring synergic effect of both moieties. Lipophenolic conjugates previously synthesized within the team showed promising results *in vitro* against oxidative and carbonyl stress.[1] Two new conjugates of resveratrol were synthesized in five steps, in gram amounts. Products will be used for *in vitro* experiments to investigate the effect and the importance of the docosahexaenoic acid (DHA; C22:6(n-3)) part in previous obtained results.

Finally, to increase antioxidant properties of phloroglucinol lipophenolic derivatives, a promising six-step pathway was tested to link the DHA and the alkyl-phloroglucinol moities through a new kind of linker.

The study was supported by Erasmus+ study program and Charles University (SVV 260 291).

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SYNTHESIS OF NEW SELENINIC ACID DERIVATIVES AS POTENTIAL ANTILEISHMANIAL AGENTS

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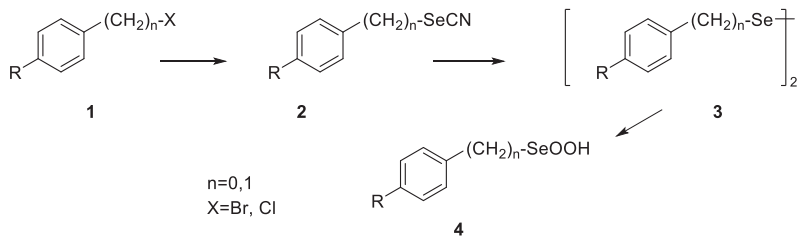
Selenium containing organic compounds have been known to possess promising *in vitro* antiparasitic activity against *Leishmania* infection. Various selenocyanate **2** and diselenide **3** derivatives with different functional groups in the aryl ring and in the aliphatic chain, displaying promising leishmanicidal activity and higher selectivity indexes than those obtained for the reference drugs, miltefosine and edelfosine have been recently synthesized.[1]

Within this presentation, the synthesis of new seleninic acid compounds **4** using the above mentioned selenocyanates **2** and diselenides **3** as reagents is described. The reaction sequence started from commercially available benzyl halides **1** with subsequent nucleophilic attack by potassium selenocyanate. The target seleninic acids **4** were obtained from selenocyanates **2** via reduction with borohydride leading to the diselenide intermediates **3** which were further oxidized by hydrogen peroxide.



Four new seleninic acid compounds **4** have been prepared and submitted to screening for their *in vitro* activity against *Leishmania infantum* intracellular amastigotes.

The study was supported by SVV 260 291.

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THE USE OF NANOFIBERS AS A SORBENT FOR SOLID PHASE EXTRACTION

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Sample preparation is an important and necessary part of every analytical process. This step is required for removing interferences from matrices and for the analyte pre-concentration. Especially in biology and pharmacy, where the matrices are very complex and analytes are presented at trace levels, so this step cannot be avoided. The current methods of sample preparation require a significant part of whole analytical process and could contribute to result error. Because of these disadvantages there is an interest to improve this step of analysis. The emphasis is placed on the possibility to lower sample volume, to reach a higher specifity or selectivity in extraction, to reduce organic solvents consumption and to develop fully automated methods.

Solid phase extraction (SPE) is a very popular sample preparation method especially for liquid samples because of its simplicity and wide range of application. There are many ways how to achieve above written goals. One of them is to find a sorbent material with high analyte recovery, sorptive capacity, selectivity, mechanical and chemical stability. The use of nanofibers in SPE has a high potential to meet all these requirements.

In our study, two kinds of nanofibers – polyamide 6 and polyamide 66 were used as sorbents. They were prepared by electrospinning at the Technical University of Liberec. The nanofibers were packed in SPE cartridge and their efficiency was tested for several groups of substances, namely parabens, steroids and flavonoids, with different polarity and molecular weight. These substances were dissolved in water. The sample concentration and extraction conditions were optimized. HPLC coupled to spectrophotometric detection was utilized for the evaluation of the amount of extracted analytes. Results of this study show analytes which could be extracted using polyamide 6 or polyamide 66.

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SYNTHESIS OF DEXTRAZOXANE ANALOGUES

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Anthracyclines (ANT) such as daunorubicin or doxorubicin belong to the group of widely used anticancer drugs. However, the administration of anthracyclines is connect-

ed with a high risk of the cardiotoxicity leading to the development of the congestive heart failure. The only compound effective against the ANT cardiotoxicity is dexrazoxane (DEX). The mechanism of its unique cardioprotective effect is unknown yet. There are two main hypothesis: 1) DEX can interact with the topoisomerase 2β in the heart and thus protects it from the effects of ANTs; 2) the main metabolite of DEX – ADR-925 – chelates the iron ions, which catalyze the formation of reactive oxygen species, thus provides the protection to the heart from the oxidative stress. The aim of this study was to prepare two close analogues **1** and **2** of DEX (Fig. 1) and to study their cardioprotective activity.

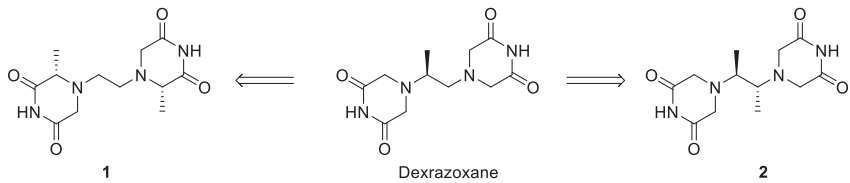

Fig. 1. Structure of DEX and its intended analogues **1** and **2**.

Analogue **1** was prepared *via* three-step procedure involving the synthesis of the corresponding tetracarboxylic acid and its cyclization in formamide. Analogue **2** was synthesized *via* five-step procedure starting from *meso*-2,3-dibromobutane. The cardioprotective effects of both DEX analogues **1** and **2** will be studied *in vitro* and *in vivo*.

The study was supported by the Czech Science Foundation (13-15008S) and Charles University (SVV 260 291).

SYNTHESIS OF LOW-SYMMETRY AZAPHTHALOCYANINE FOR THE LABELING OF DNA PROBES INCREASING THE SUSCEPTIBILITY OF THE MOLECULAR-BIOLOGICAL METHODS

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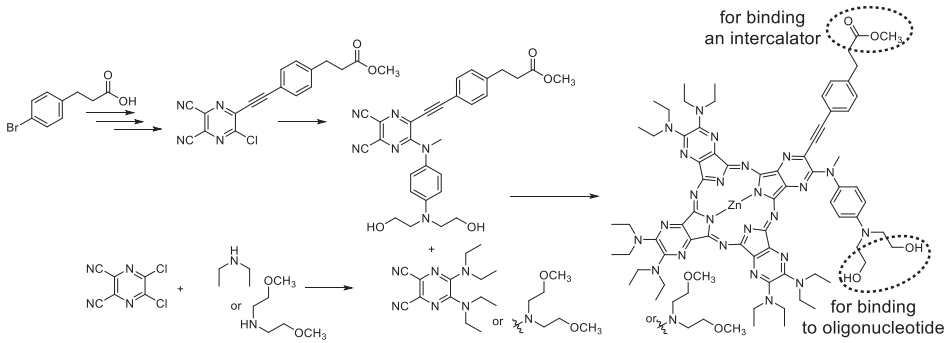
Azaphthalocyanines (AzaPc) are planar macrocyclic compounds. The alkylamino substituted derivatives can be used as dark quenchers in DNA-hybridization assays. The probe usually contains a quencher and a fluorophore. If these two moieties are close enough, the emitted fluorescence is quenched by the quencher. After the hydrolysis of the oligonucleotide probe, the fluorescence appears because of the long distance between the quencher and fluorophore. Thanks to the large system of conjugated double bounds, AzaPc absorb over a wide range of wavelengths from 300 nm to 750 nm. Such absorption covers all fluorophores used in hybridization assays nowadays, thus, AzaPc may serve as a universal dark quencher.

The aim of this study was to prepare an AzaPc, which will be possible to attach inside an oligonucleotide strand. Furthermore, a planar moiety bounded to AzaPc will intercalate into oligonucleotide probe that will result in an increase of the strength of arrangement of the probe. First, the appropriate precursors were synthesized according to scheme below. Reaction conditions were optimized. The final non-symmetrical AzaPc was then obtained by statistical condensation of two precursors under Linstead condition and desired congener was separated. Target AzaPc will be used for the synthesis of oligonucleotide probes and quenching properties will be studied in hybridization assays.



The study was supported by SVV 260 291.

NITRO GROUP-CONTAINING TETRAZOLE AND OXADIAZOLE DERIVATIVES AS POTENTIAL ANTITUBERCULAR AGENTS

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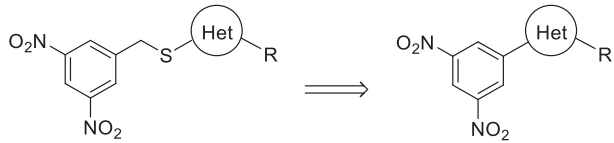
Tuberculosis (TB) is widespread infectious disease predominantly caused by *Mycobacterium tuberculosis* (*M.tb*.). According to World Health Organization, estimated 9.6 million new TB cases and 1.5 million deaths from TB were registered worldwide only in 2014.

In the previous work of our group it was found that 1,5- and 2,5-disubstituted tetrazoles and 2,5-disubstituted oxadiazoles bearing 3,5-dinitrobenzylsulfanyl fragment exhibit outstanding antimycobacterial activities with minimal inhibitory concentration (MIC) values as low as 0.03 μM. In the continuation of this work, we modified these lead structures by removing the methylsulfanyl linker and prepared a series of nitro group-substituted tetrazole and oxadiazole derivatives.



Het = tetrazole, 1,3,4-oxadiazole

Fig. 1. General formula of the compounds studied in this work.

The antimycobacterial activities of the prepared compounds as well as their effects on mammalian cell viability were evaluated. In the case of tetrazole derivatives, both 1,5- and 2,5-disubstituted isomers were prepared to determine the role of the position of substituents in the antimycobacterial activity. Furthermore, several water-soluble analogs of the most promising compounds were prepared.

The study was supported by Czech Science Foundation (14-08423S) and Charles University on Prague (SVV 260 291).

SYNTHESIS OF ANALOGUES OF CARDIOPROTECTIVE DRUG DEXRAZOXANE

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Anthracyclines such as daunorubicin or doxorubicin are widely used anticancer drugs. However, the administration of anthracyclines is connected with cardiotoxicity leading to irreversible damage of heart muscle and congestive heart failure. The reason of anthracyclines toxicity is unknown yet, there are two main theories. It is assumed that the complexation of anthracyclines with intracellular iron ions catalysis the formation of reactive oxygen species (ROS). The second theory involves inhibition of topoisomerase II (TOP2). The only known drug effective against the anthracycline cardiotoxicity is dexrazoxane (DEX). The cardioprotective mechanism of DEX is also unknown yet. In this study we deal with the synthesis of two types of DEX analogues – achiral methyl-DEX analogue **1** and sulfonamide analogues **2** (Fig. 1). New DEX analogues should clear up the structure-cardioprotective activity relationships of DEX and possibly help us in the development of new cardioprotective agents.

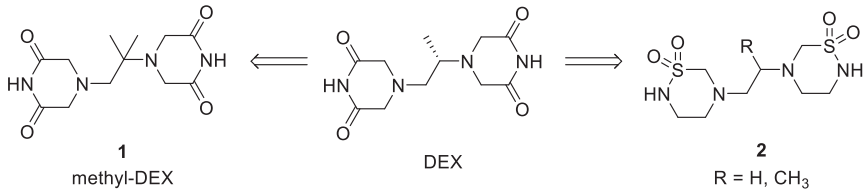

Fig. 1. Structure of dexrazoxane (DEX) and its analogues **1** and **2**.

Sulfonamide groups in analogs **2** should mimic the original imide groups in DEX because of their similar acid-base properties. The methyl-DEX analogue **1** would provide the information about the role of a chiral center and the connection linker in the cardioprotective activity of DEX.

The study was supported by the Czech Science Foundation (13-15008S) and Charles University (Charles University Research Centre UNCE 204019/304019/2012 and project SVV 260 291).

SYNTHESIS OF AZAPHTHALOCYANINE FLUORESCENCE SENSORS WITH THE IMPROVED SELECTIVITY TOWARDS DESIRED METAL CATIONS

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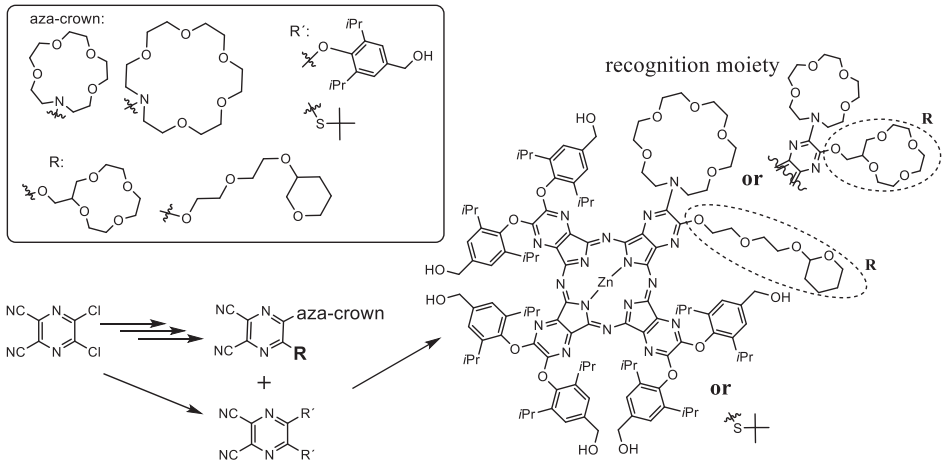
The impact of azaphthalocyanines (AzaPc) bearing aza-crowns on the periphery to be used as fluorescence sensors for metal cations has already been demonstrated in our previous works.[1] The great advantage of these compounds is the emission in the red part of the spectrum because such light is not absorbed by endogenous chromophores. Previous projects served mostly as a proof of concept where sensitivity of AzaPc sensors to a series of alkali and alkaline earth metal cations was shown. The aim of this study was focused on the improvement the selectivity of a recognition moiety for particular cations. This can be achieved by the attachment of various substituents “R” close to aza-crown moiety (see scheme below).

Firstly, appropriate precursors were prepared using mostly nucleophilic substitution and reaction conditions were optimized. Then, cyclotetramerization of two different precursors (A and B) using a template method with zinc acetate in high boiling solvent provided a statistical mixture of congeners. It was followed by an isolation of desired unsymmetrical AzaPc of AAAB type. Finally, sensoric properties of prepared AzaPc were studied by the mean of fluorescence titration experiments.



The study was supported by SVV 260 291.

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SYNTHESIS OF PHENOL-BASED PH SENSORS

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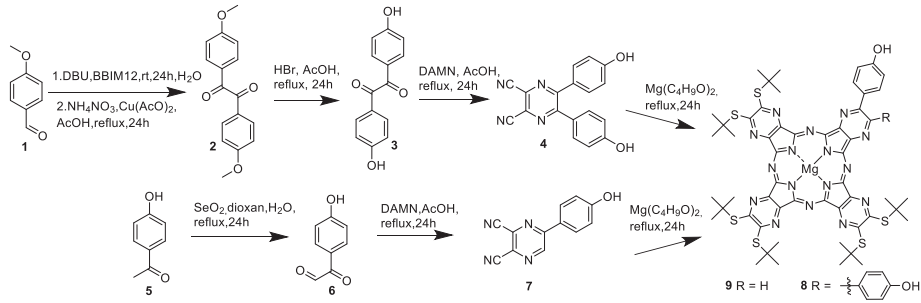
Tetrapyrazinoporphyrazines (TPyzPzs) bearing suitable phenol moieties were found to be potentially useful pH sensors.[1] In this study, we focused on expanding the series of phenol derivates synthesized previously. The main reason of our effort was the fact, that pK_a of previous compounds (12.5–12.7) was physiologically useless.

Two different strategies to synthesis of precursors were developed. Synthesis of the precursor with two phenols started by benzoin condensation of *p*-anisaldehyde **1** to obtain corresponding acyloin, which was oxidized to diketone **2** and dealkylated to **3**. Subsequently, the pyrazine **4** was obtained by condensation of this diketone with diaminomaleonitrile. Synthesis of precursor with one phenol began with oxidation of 4-hydroxyacetophenone **5** to ketoaldehyde **6**, which was used in condensation with diaminomaleonitrile to the pyrazine **7**. The mixed cyclotetramerization of **4** or **7** with 5,6-bis(*tert*-butylsulfanyl)pyrazine-2,3-dicarbonitrile (**6**) provided the mix of congeners and the magnesium TPyzPzs **8** and **9** with the phenol recognition moieties were isolated by repeated column chromatography. The magnesium complexes were used for preparation of metal-free and zinc derivatives. Finally, acid-base sensing properties were evaluated by absorption and fluorescence spectrophotometry.



The study was supported by SVV 260 291.

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SYNTHETIC APPROACH TOWARDS ANTIMICROBIAL NOSTOTREBIN 6

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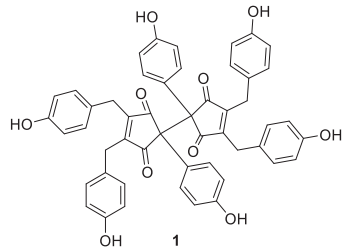
Growing demand for novel highly effective antimicrobial agents is one of the prevailing trends in current medicinal research due to increasing antimicrobial resistance. Particularly promising anti-infectives have been found among naturally occurring products providing us with a broad spectrum of lead structures. Cyclopentenedione-based compounds are examples of such compounds which belong to an emerging class of biologically active agents.

Nostotrebin 6 **(1)** is a polyphenolic secondary metabolite containing the cyclopentenedione moiety. It possesses an antimicrobial activity and is also an efficient inhibitor of both acetylcholinesterase and butyrylcholinesterase.[1] To date, nostotrebin 6 can be obtained solely using specific methods of a long-term cultivation and extraction from natural sources.[2] Synthetic approaches based on the orchestration of established reactions with the emphasis on further development of Pdcatalyzed processes will therefore be discussed.



The study was supported by Charles University (SVV 260 291 and GA UK 262416) and Czech Science Foundation (15-07332S).

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MICROWAVE ASSISTED SYNTHESIS OF AMINOPYRAZINE-2-CARBOXAMIDE DERIVATIVES AND THEIR BIOLOGICAL EVALUATION

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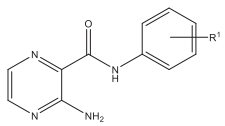
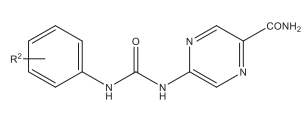
Tuberculosis (TB) is a major global health problem. In 2014, there were 1.5 million TB deaths.[1] Resistant tuberculosis-forms, namely multi-drug resistant (MDR) constitute a serious problem and emphasize the need for novel antitubercular drugs. Pyrazinamide (PZA) is the first-line antituberculotic drug, which has a sterilizing effect on semi-dormant tubercle bacilli.[2] PZA is also very suitable for chemical modifications.

This work is focused on preparation of aminopyrazinamide derivatives. Substituted 3-amino-*N*-phenylpyrazine-2-carboxamides **(1)**, 5-(3-phenylureido)pyrazine-2-carboxamides **(2)** and 5-(3-benzylureido)pyrazine-2-carboxamides **(3)** were prepared using microwave irradiation.



(1)



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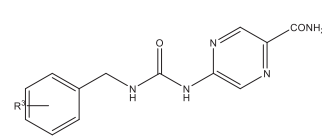

(3)

R^1 = H; 4-CH_2CH_3; 2,5-diCH_3; 2,4-diOCH_3; 3,4-diCl; 2,4-diF; 3-CF_3; 4-OH; 2-Cl-5-CH_3
R^2 = H; 2-Cl; 4-Cl; 3-CF_3
R^3 = H

Final compounds were characterized by NMR and IR spectroscopy, elemental analysis and melting point. Substances will be tested against four mycobacterial strains – *M. tuberculosis* H37Rv*, M. kansasii, M. avium* and *M. smegmatis*, antibacterial and antifungal assays will be performed.

The study was supported by the Grant Agency of Charles University, project B-CH/1594214, and by ERASMUS+.

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SECTION OF SOCIAL AND TECHNOLOGICAL SCIENCES

FLOW EQUATION OF GRANULES PREPARED FROM SORBITOL

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Pharmaceutical tablets offer the easiest and most convenient type of administration, plus, they have many advantages including simple, fast and economical production. The most applied way of tablet manufacturing is through the compression of granules made by wet granulation which confirms the importance of granulation in pharmaceutical industry.[1] To prevent any production problems, the detail description and prediction of the flow behaviour of particulate material is necessary.

The aim of this thesis is to study the flow behaviour of size fractions of the granules prepared from sorbitol by wet granulation method. Particles were characterized by analytical sieving using a vibratory sieve shaker; a bimodal distribution was observed. The size fractions of the granules were classified with the particle size x (mm) as the geometrical mean of the used screens.

The bulk as well as the tapped densities, the angle of repose, and the flow rate were estimated. No difference was observed between the bulk densities measured in a cylinder, in a volumeter and/or for a pile of material, respectively. The polynomial relationship between the bulk density and the particle size, as well as between the angle of repose and the particle size were identified. The excellent to good flow properties were noted for the granules.

The influence of the particle size x in the range of 0.200–1.000 mm and the aperture sizes D of 0.60–1.50 cm on the mass flow rate Q (g/s) through the circular orifice of a conical stainless steel hopper was investigated. The power relationships were detected. A mathematical model of Jones-Pilpel power regression was applied to describe the relationship between the mass flow rate of the sorbitol size fractions and the diameter of the hopper orifice. Using the actual parameters of the J-P equation, the average precision of the predicted mass flow rate of approx. 4% was detected leading to conclusion that J-P equation represents the suitable model for the sorbitol granules.

The study was supported by Grant Nr. 322315/2015 GAUK and by SVV 260 291.

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THE QUALITY OF DIETARY SUPPLEMENT INFORMATION ON THE INTERNET

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Dietary supplements in recent years become part of the daily diet considerable part of the population of all European countries. According to the survey of 2015 internet purchases through OTC drugs and dietary supplements people buys more than two fifths of the internet population aged 15–59. The internet is often used by consumers as a source of information. If people buy a dietary supplement they get all information about a dietary supplement is from the web sites.

To assess quality of information presented on the Internet for the top selling dietary supplements from the clinical and regulatory point of view.

We searched the Internet using the 3 most commonly used search engines in the Czech Republic – Seznam, Google and Centrum starting July of 2015 till November of 2015. One-hundred top selling dietary supplements in the Czech Republic in 2014 were extracted from IMS Health database. Dietary supplement information was evaluated from the clinical (information important for the client and health care professional) and regulatory point of view (compulsory information required by law).

One-hundred and ninety-one websites were evaluated, of which 25 were excluded (18 websites did not refer to a specific dietary supplement (e.g., Wikipedia, Slovakia web sites, etc.) and 7 websites referred to information on a medicinal product). In total, 166 websites were analysed. The average regulatory (6 criteria) and clinical (10 criteria) score was 3.83 and 3.82, respectively. Nine percent of webpages (73 from 775 webpages) contained dietary supplement information referring to the treatment, cure or prevention of disease and symptoms and were considered as a serious misconduct.

The quality of information on dietary supplements sold *via* internet may vary and may not be always reliable. A consumer should consult a pharmacist before using a dietary supplement in order to be fully informed about its proper use.

The study was supported by SVV 260 295.

POLYMERIC STABILIZERS MAINTAINING THE SATURATION SOLUBILITY OF ITRACONAZOLE NANOCRYSTALS AFTER DISSOLUTION PROCESS

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The increase of bioavailability of poorly water soluble drugs is still an issue. One of the techniques improving aqueous drug substance solubility, and consequently enhancing bioavailability, is formation of nanoparticles. However, the bioavailability is determined by the concentration of the dissolved drug achieved at the time of absorption.

The solution to this issue represent sufficiently stabilised nanocrystalline drugs.[1] In this study, the solid nanoparticle formations of an antifungal agent itraconazole (ITZ) are presented. Wet milling[2] was employed to create the nanosuspension stabilised by binary mixture of stabilisers or by a single stabiliser. An aggregation inhibitor Poloxamer 407 (F127) in combination with a polymeric precipitation inhibitor hydroxypropyl methylcellulose (HPMC) or polyvinyl pyrrolidone (PVP) at various ratios, or a single precipitation inhibitor, were utilised.[3]

The solubility tests showed the importance of utilised stabilisers over nanosized particles size. The highest solubility levels and the most successful maintenance of high solubility values were obtained in samples containing single polymeric precipitation inhibitors, followed by binary mixtures with the amount of F127 exceeding the amount of precipitation inhibitor. The order can be concluded: HPMC>PVP>F127+HPMC>F127+PVP. The physical state, pre-dissolved or solid, of the precipitation inhibitor influences the solubility level. Hygroscopic properties of PVP enhance its affinity to water and, hence the addition of solid excipient is more beneficial.

The study was supported by project no. SVV 260 291.

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SIX-MONTH TRAINEESHIP IN THE PHARMACY FROM POINT OF VIEW OF STUDENTS

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The six-month traineeship at a pharmacy is an obligatory course for final year students who have been studying Pharmacy at FAF UK in Hradec Králové.[1] The purpose of this course is to fix the theory and let the students transform their knowledge into practical skills.

The thesis has been divided into two parts. The theoretical part is focused on the issue of the six- month traineeship at a pharmacy mainly in the term of legislation. The practical part deals with the analysis of a questionnaire survey which has been done among the final year students of Pharmacy at FAF UK in Hradec Králové as the main objectives are to evaluate their opinion on benefits and negatives of their traineeship including change proposals. The responds of the students from academic years 2007/2008 and 2014/2015 were used and processed using Excel. The evaluation was done with the help of filter functions and then it was statistically compiled. The feasibility of the suggested changes was compared to the valid Czech legislation[2] and European Union directives.[3]

Different viewpoints have been used to analyze the survey results in the discussion. For example, whether the student did the placement at a hospital or private pharmacy, in a chain store or not. The academic years were also compared. In total there were 173 students of the year 2007/2008 to answer the survey. These people attended their traineeship in 112 hospital pharmacies and in 230 private pharmacies. Compared to this there were 20 less students in 2014/2015 and they completed their practical experience in 109 of hospital and 222 of private pharmacy places. The most frequent changes that the students proposed were shortening of the length of the traineeship, the obligation to attend at least two different pharmacies where one would be at a hospital and finally they also suggested the possibility to spend their placement in another pharmaceutical branch such as science or industry.

The study was supported by SVV 260 295.

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3. Směrnice evropského parlamentu a rady 2005/36/es ze dne 6. července 2005 o uznávání odborných kvalifikací, v platném znění.

STUDY OF SKIN BARRIER DEFECTS BY LIPID MONOLAYERS

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Ceramides (Cer) together with free fatty acids and cholesterol form the intercellular space of the uppermost skin layer, the *stratum corneum* (SC). This lipid matrix presents the skin barrier, which protects human organism from environmental factors (endogenous substances, physical radiation) as well as prevents the body from water loss. Cer are synthetized from polar precursors: glucosylated Cer (GluCer) and sphingomyelins by splitting the polar part by the hydrolytic enzymes β-glucocerebrosidase (β-GluCer-ase) and sphingomyelinase. A lack of these enzymes leads to accumulation of precursors and a non-functional skin barrier is formed. The goal of this work was to study the β-GluCer-ase defects by monolayer lipid models of the SC. The impact of GluCer quantity on lipids organisation was evaluated by several techniques (Langmuir monolayers at the gas-liquid interface, Langmuir-Blodgett, atomic force microscopy and Brewster angle microscopy).

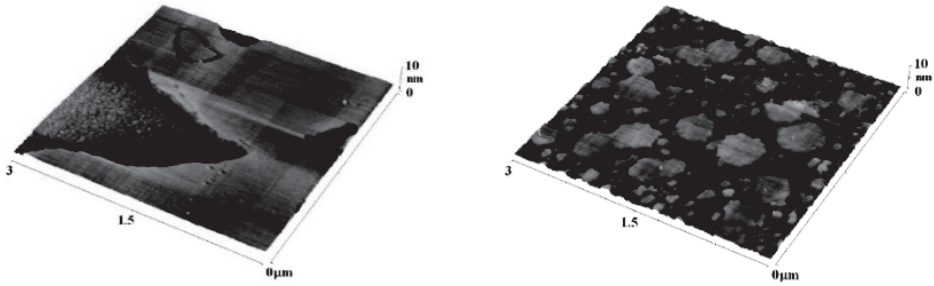

Fig. 1. Model of SC without GluCer (left) and separated domains of the model with 75% GluCer (right).

At the gas-liquid interface with increasing GluCer ratio lipid mixtures are less willing to organise at lower surface pressures. It seems that with increasing surface pressure (20 mN m^{-1}) the mixtures with higher GluCer ratio collapse earlier and the lowest compressibility of lipid monolayer is apparent in the mixture containing 75% GluCer. On the solid support, increasing amount of GluCer causes a decreased ratio of tight-packed (higher) phase lipid monolayers and their separation into a lot of domains with smaller areas.

The study was supported by the Charles University (SVV 260 291) and the Czech Science Foundation (13-23891S).

BRANCHED POLYESTER AS A CARRIER OF POORLY SOLUBLE DRUGS IN SOLID DISPERSIONS

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The purpose of this work was to formulate a mucoadhesive biodegradable polymeric system with salicylic acid (SA) based on a solid molecular dispersion of this drug in poly(lactic-co-glycolic acid) linear (PLGA), or branched on tripenterythritol (PLGA/T). The SA incorporation into the polyesters was carried out either by solvent method using butanone and its evaporation in a vacuum dryer, or by melting at 90 °C followed by thorough homogenization. The drug–polymer miscibility and content of residual solvent were found out employing Differential scanning calorimeter (DSC 200 F3 Maia® Netzsch, Germany) was used for the thermal analysis of the polyester carriers, SA, and their blends. The cooling and heating rates were 10 °C/min. The amount of SA released was carried out in the shaking water baths maintained at temperature of 37.0 °C. It was allowed to shake at a constant shaking frequency 50 min^{-1} and shaking amplitude 22 mm. The dissolution medium used was 15.0 mL of Phosphate buffer solution pH 7.4 (Ph. Eur. 7.0). At predetermined time intervals (1 h, 3 h, 5 h, 1 d, 2 d, 4 d, 7 d) dissolution medium was withdrawn and replaced. The drug content in the withdrawn samples was determined spectrophotometrically at λ 296 nm. The results were the means of three runs.

The DSC scans indicated a transformation of crystalline form of SA into an amorphous dissolved in polyester carriers, and decreased Tg value of polyesters due to the presence of butanone residuals in formulation. The SA release from polymeric matrices was strongly dependent on the conformation and molar weight of the polyester. Branched PLGA/T exhibits low burst effect and prolonged SA release for 168 hours.

A polymeric system based on poly(lactic-co-glycolic acid) branched on tripenthaerythritol seems promising for controlled release of SA for topical application on the skin and mucosal surfaces.

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A COMPARISON OF COMPRESSIBILITY OF MICROCRYSTALLINE CELLULOSE AND PELLETS

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This work compares compressibility of two types of microcrystalline cellulose Comprecel 102 and Avicel PH-200) and two types of microcrystalline cellulose pellets (Cellets

100 and Cellets 200). The flowability, the angle of repose, the moisture content, the particle size distribution, the bulk and tapped density and the Hausner ratio belong among the most important qualities of materials for the compaction. All of them were evaluated in the materials used in this work. Moreover, the force-displacement method was used to describe the compaction process. The compressibility was also characterized by the three-exponential compaction equation.[1] In the end, the properties of the prepared tablets were measured. They were the radial strength[2] and the friability.

The results revealed that both types of Cellets have better flow properties than the powdered microcrystalline celluloses. It is mainly because of the higher bulk and tapped density, the narrow particle size distribution and the remarkably smoother surface of the pellets. However, these better flow properties also influenced the compressibility and consequently the quality of prepared tablets that were very often substandard. The radial strength of tablets made of pellets was very low and also the friability was very high. It is believed that the pellets tend to fragment during the compaction process.[3]

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MEDICATION ADHERENCE IN PATIENTS AFTER KIDNEY TRANSPLANTATION

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Kidney transplantation (KT) is the best treatment option for patients with end-stage renal disease. Despite its numerous benefits, it requires the necessity of lifelong medical regimen of immunosuppressive treatment (IS) with accent on a strict medication adherence. It is common that patients with renal failure suffer from various comorbidities and take frequently many other drugs every day. To minimize risk of therapy failure i.e. graft rejection, we intend to perform an observational prospective study with the main aim to evaluate patient's medication adherence as the leading preventable cause of graft rejection. Furthermore, we analyze other drug-related problems (DRP) and factors potentially related to non-adherence with focus on patients' opinions and attitudes to IS. This study is undertaken in period from 2016 to 2017 at the Haemodialysis Centre in the Teaching

Hospital Hradec Králové. Patients who accomplish the inclusion criteria are asked to participate in the study during their regular visit of nephrologist. In the first part of the study, the anonymous motivational interview is performed to determine the patients´ attitudes and self-reported adherence by Czech validated questionnaires BMQ-CZ (Beliefs about Medicines Questionnaire) and MARS-CZ (Medication Adherence Report Scale). Patients are also asked about their lifestyle, education, or self-medication, and finally, the interview is focused on patient's specific IS by a dedicated person. In the second part of the study, medication records are reviewed with the accent on parameters indicating impaired kidney function as well as the entire pharmacotherapy and potential DRP. Continuously, identified data are discussed with the physicians to suggest appropriate arrangements for the patients. Non-adherence to therapy is the very complex problem which requires combination of several interventions to capture it. This is the first complex study conducted in the Czech Republic in patients after KT and is expected to obtain a unique set of data about the medication taking behaviour in this population.

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POTENTIALLY INAPPROPRIATE MEDICATION USE (PIM) IN OLDER PATIENTS IN EUROPE AND DIFFERENCES IN APPROVED PIM LISTS IN DIFFERENT EUROPEAN COUNTRIES

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Explicite criteria of potentially inappropriate medication use in older patients (PIMs) have been published for the first time in the USA in 1991 and in the last two decades they were used in epidemiological research in several European countries. However, in the Central and Eastern Europe, only a few studies on this topic have been published until now. For this reason, the EU COST Action IS 1402 (2015–2018, Working Group 1b) focused primarily on research regarding this issue in Eastern and Central European countries. One of the first goals was to determine the approval rates of PIMs in different EU countries.

Six EU countries participated in this study, namely Serbia, Hungary, Spain, Turkey, Portugal and the Czech Republic. Data were collected using specific questionnaire containing items assessing approval rates of PIMs, their availability in various doses and drug forms, most frequently prescribed brand names, OTC availability of PIMs, etc. University researchers from different countries filled in the questionnaires in the period Sept–Dec 2015. All explicite criteria of PIMs published by 2015 year were used to determine the approval rates of PIMs in different countries.

From the whole list of PIMs (N = 484 items) known in the scientific literature, the majority of PIMs (81.7%) were approved for clinical use at least in one of the participating country. The total prevalence was similar in the Czech Republic (45.2%), Turkey (48.6%),

Spain (52.1%) and Hungary (54.1%), while in other countries it substantially differed – in Serbia (33.9%) and Portugal (68.5%). The lists of PIMs approved for clinical use in the Southern EU countries (Spain, Portugal) and Turkey were different from the lists of PIMs available on pharmaceutical markets in the Czech Republic, Serbia, and Hungary.

This pilot study before a broader epidemiological survey confirmed substantial differences in the lists of PIMs available for clinical use on pharmaceutical markets of different EU countries. Even if the total prevalence of PIMs approved for the clinical use did not differ substantially across many participating countries, individual lists of PIMs confirmed significant regional differences.

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