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ABSTRACTS

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PHARMACEUTICAL ANALYSIS SECTION

DETERMINATION OF ARTIFICIAL COLORANTS BY SEQUENTIAL INJECTION CHROMATOGRAPHY USING MODERN MONOLITHIC COLUMNS

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This work is dedicated to development and optimization of separation and determination of three water soluble food colorants (Sunset Yellow FCF, Carmoisine, Green S) by sequential injection chromatography (SIC) with spectrophotometric detection with wavelenghts 480, 516 and 630 nm respectively. In the research monolithic columns Chromolith® CN 50-4.6 and Chromolith® Diol 50-4.6 were tested. Mobile phases were based on mixture of water and ammonium acetate. Gradient elution was involved and achieved by automated reproducible mixing of water and ammonium acetate buffer solution in the holding coil of the SIC system. Gradient profile, flow rate and volume of mobile phase were optimized using each column for rapid separation with good resolution of analytes. Analytical characteristics will be presented.

The technique was successfully applied to the separation of colorants in Coldrex Max Grip Lesní Ovoce as a sample. The quantification was performed by standard addition method.

The study was supported by International Visegrad Fund.

QUALITY CONTROL OF FOOD SUPPLEMENTS AVAILABLE ON THE CZECH MARKET USING CORE-SHELL COLUMN CHROMATOGRAPHY

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Many biologically active substances started to be used in commercial preparations, as food supplements. However no analytical methods have been proposed for quality control of nutraceuticals with these substances, such as resveratrol and indol-3-carbinol yet.

In our first study[1] a new HPLC method using fused-core column for fast separation of resveratrol and its glycosylated form polydatin has been developed. Both forms show cardioprotective properties, which are associated with their anti-inflammatory abilities and scavenging of reactive oxygen species. The optimal separation conditions for resveratrol, polydatin and internal standard *p*-nitrophenol were found on the fused-core column Ascentis Express ES-Cyano (100 × 3.0 mm), particle size 2.7 µm, with mobile phase acetonitrile/0.5% acetic acid pH 3 (20:80, v/v) at a flow rate of 1.0 mL min^{-1} and at 60 °C.

In the second study[2] a new HPLC method using core-shell column for separation of indole-3-carbinol and its condensation/degradation products was developed and used for quantitative determination of indole-3-carbinol in nutraceuticals. Indole-3-carbinol is natural glucosinolate identified for prevention of human breast, prostate and other types of cancer. Separation of indole-3-carbinol and internal standard ethylparaben was performed on the core-shell column Kinetex 5µ XB-C18 100A (100 × 4.6 mm), particle size 5.0 µm, with mobile phase acetonitrile/water according to the gradient program at a flow rate of 1.25 mL min^{-1} and at temperature 50 °C.

The both developed methods provided rapid and accurate tool for quality control of nutraceuticals based on extracts with mentioned compounds content.

The study was supported by the project of specific research no. SVV 260 184.

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DEVELOPMENT OF HPLC-FD METHOD FOR DETERMINATION OF ARGININE AND ITS METABOLITES IN CHRONIC WOUND FLUIDS

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Patients with chronic wounds present a serious problem in health care. Prolonged hospitalization creates high costs and impairs the well-being of the patient. Wound healing is a complex and dynamic process affected by many factors. It was discovered that important role in a wound healing process plays nitric oxide (NO) pathway.[1] Amino acid arginine is the sole precursor of nitric oxide and its level is variable during wound healing.[1] Monitoring of arginine metabolism directly in the wound liquid, could be another indicator of chronic wound healing process and significantly improve therapy of non healing wounds.

The aim of this project was to developed HPLC method with fluorescence detection for the determination of arginine, ornithine and citrulline in a fluid from non healing wounds. Separation of these analytes was performed using C18 monolithic column. Sodium acetate buffer (solution A) and mixture of ACN and MeOH (solution B) were used as the mobile phase. Gradient elution mode was applied. Total time of analysis (including a column-wash step) was 14 min. The method was validated by testing its linearity, precision, accuracy, recovery, robustness and detection/quantitation limit values. The method was linear over the range of 2.87–43.05 μmol/L for arginine, 2.97–44.45 μmol/L for ornithine, 2.85–42.81 μmol/L for citrulline and exhibited good correlation coefficient higher than 0.999. This method will be used for clinical practice in the Research laboratory of 3rd Internal Gerontometabolic Clinic in University Hospital Hradec Králové.

The study was supported by the SVV 260 184, Project MH CZ-DRO (UHHK, 00179906), PRVOUK P37/12.

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DEVELOPMENT OF EXTRACTION PROCEDURE FOR THE DETERMINATION OF THIAMINE AND ITS DERIVATIVES IN BIOLOGICAL FLUIDS

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Thiamine, also called vitamin B_1, belongs to a broad group of water-soluble vitamins. All forms of vitamin B_1 are important precursors involved in cellular energy metabolism and proper neuromuscular function.[1] In patients with long term hospitalization various causes, like diabetes, inflammation, cancer, etc., can reduce the amount of vitamin B_1 and thiamine deficiency may develop. Thiamine deficiency results in impaired metabolism and oxidative stress that decreases survival rate of patients.[2] Therefore it is advisable to monitor the level of thiamine and its derivatives.

Whole scale of direct and indirect methods for the analysis of thiamine and its phosphorylated forms were developed as well as reviewed in the past.[3] Some methods are used in clinical practice, but do not meet the requirements needed for simple, fast, precise and accurate determination. Accordingly, the need of novel method that could overcome these problems is necessary.

The new method for the determination of thiamine and its derivatives suitable for clinical applications was developed. The main goal of this project was to optimize extraction procedure that would allow quantitative analysis of thiamine and its phosphorylated forms in human plasma. Several different extraction techniques have been tested with the effort to remove interfering compounds and to increase the sensitivity of the analysis. All reagents have been tested on native and spiked samples to obtain the best results. Up to now the most suitable technique with sufficient purity of sample seems to be the protein precipitation followed by centrifugation, filtration and derivatization. Several precipitation reagents have been tested. So far the best results were obtained using methanol: zinc sulfate mixture. Ultra-filtration and SPE was also tested but so far without satisfactory results. To find the most appropriate extraction procedure several other techniques will be tested in the future. After the optimization and miniaturization of extraction procedure the method will be prepared for further validation.

The study was supported by project SVV 260 184, PRVOUK P37/12 and University Hospital in Hradec Králové, IČO: 00179906.

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DEVELOPMENT OF UHPLC-UV METHOD FOR THE DETERMINATION OF OMEPRAZOLE IN ORAL SUSPENSIONS

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Omeprazole is one of the most widely used drugs from the group of proton-pump inhibitors (PPIs). PPIs block the secretion of gastric acid both selectively and irreversibly by inhibiting H^+/K^+ATPase in parietal cells. Omeprazole plays role in the treatment of the acid-related disorders, such as gastroesophageal reflux disease (GERD), Barrett's esophagus, peptic ulcers disease and Zollinger-Ellison syndrome.

Due to the instability of omeprazole in acidic conditions, enteric-coated tablets or extended-release capsules are commonly used dosage forms. This kind of drug administration is not convenient in pediatric, elderly as well as critically ill patients having problems to swallow solid dosage form. Thus, individually prepared oral suspensions of omeprazole facilitate the administration and the dosage exactly in this group of patients.[1,2]

The aim of this project was development and validation of modern HPLC method for the omeprazole stability monitoring in these suspensions. The separation of omeprazole standard solution, omeprazole related compound (as impurity) and methylparaben (as internal standard) was performed by Kinetex™ C18 column (50 × 2.1 mm, 1.7 µm) using mobile phase 25 mmol/L phosphate buffer (pH 7.6) and acetonitrile (74:26, v/v). All analytes were determinated by UV detection at 300 nm. Method was partially validated.

The newly developed, simple and rapid method will be applied to the analysis of concentration of omeprazole and his stability in six different suspension formulations prepared in the Hospital pharmacy in Motol University Hospital and will serve for Department of Pediatrics, 2nd Faculty of Medicine, Charles University and Motol University Hospital.

The study was supported by project SVV 260 184.

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DEVELOPMENT OF HPLC METHOD FOR DETERMINATION OF EIGHT SYNTHETIC FOOD DYES: COMPARISON OF MONOLITHIC AND FUSED-CORE COLUMN

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Using of synthetic dyes in food industry is quite controversial. There is a positive list of permitted food colourings settled by EU legislation. Although these colours should be harmless, there are numerous studies warning of excessive consumption of synthetic colours in food.[1,2] In addition, they can cause some allergies and rashes in sensitive people.

Two HPLC methods for determination of these dyes were developed and validated, their separation efficiency and validation parameters were compared. The monolithic column Chromolith Performance CN 100 × 4.6 mm with guard column 5 × 4.6 mm (Merck) and fused-core particle column Ascentis Expres ES-CN, 100 × 4.6 mm, 5 μm (Sigma Aldrich) were chosen for separation. Different mobile phases, methanol or acetonitrile, and different buffers and their concentrations were tested for separation on both columns. The dyes were detected at three wavelengths according to their absorption maximum (420, 482 and 625 nm).

Final conditions of dyes separation, advantages and disadvantages of both approaches are going to be presented. Fused-core column showed higher separation efficiency including the shortening of analysis time nearly twice. On the other hand monolithic column showed lower back pressure and blank chromatogram with less interferences in case of some beverage matrices.

Finally, yellow, blue and green synthetic dyes were determined in fruit drinks, and in green beers in Easter seasons 2014 and 2015 to investigate their abuse.

The study was supported by the project of specific research, no. SVV 260 184.

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THE TESTING OF NANOFIBERS AS POTENTIAL SORBENTS IN SOLID PHASE EXTRACTION ON-LINE COUPLED TO HPLC

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Nowadays, SPE (solid phase extraction) is the most popular sample pre-treatment technique. Sorbents for SPE are still innovated and one of these innovations is using of

nanofibers as an extraction phase. Nanofibers can be defined as fibers with diameters less than 1000 nm. They have a large surface area and a great sorption capacity thanks to the extremely small fiber size. Electrospinning is one of the most conventional methods to produce nanofibers. Nanofibers are formed in electrostatic field from solution or melt of polymer. Electrospinning technology can be divided into two groups: needle electrospinning (known also as conventional electrospinning) and needleless electrospinning.[1] Polystyrene, polyamide 6, polyamide 66, and polypyrrole are the most often used polymers in the SPE nanofiber extraction.

Recent trends in sample pre-treatment are also focused on increasing the speed of analysis, the possibility of automation of processes and on-line connection of the preparation step with suitable analytical technique. Sample pre-treatment is realized in the HPLC system, in on-line connection and after extraction, analyte is directly injected to the analytical column.

This work is focused on the application of nanofiber polymers as sorbents in on-line SPE-HPLC. Tested analytes were chosen from the groups of pyrethroids and carbamates. These two groups of analytes have different physico-chemical properties and therefore different behavior in on-line SPE-HPLC system was observed. The conditions for SPE-HPLC (valve switching time, sample washing step, HPLC mobile phase composition) were optimized.

The study was supported by specific research, no. SVV 260 184.

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PARALLEL ARTIFICIAL LIQUID MEMBRANE EXTRACTION – A NEW APPROACH FOR SELECTIVE SAMPLE PREPARATION

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Sample preparation is the key and the most time-consuming step of analytical method. It is crutial for removing interfering matrix components which can affect the results of analysis. In recent years, many different microextraction approaches based on solid phase extraction (SPE) and liquid liquid extraction (LLE) were developed as alternative strategies for sample pretreatment. The advantages of these microextraction techniques consist not only in use of small volumes of organic solvents and sample but also in shortening of sample preparation time. They can accelerate sample processing about tens of minutes. Parallel artificial membrane extraction (PALME) is one of these modern microextraction approaches. PALME can be viewed as a miniaturized version of LLE, where the analytes

in biological matrix pass through a porous membrane wetted by an organic solvent into a water acceptor solution. The goal of this study was to develop a liquid chromatography method using ion trap mass spectrometer for analysis and PALME for the isolation of polar basic drugs from human plasma. The separation was performed on HSS T3 (2.1 × 100 mm, 1.8 μm), using gradient elution with methanol and 20 mM formic acid with run time 11 minutes. Sample extraction was consisted of plasma dilution with phosphate buffer pH 7.0 to total volume 250 μL in the ratio 1:2 and PALME extraction. Polypropylene membrane with 2.5 μL 2-nonanone + 15% of diethylhexyl phthalate (DEHP) was used as an artificial membrane, 50 μL 150 mM trifluoroacetic acid was used as an acceptor solution. The extraction took 45 minutes. The method was validated in terms of precision, accuracy, range, linearity, limit of detection, limit of quantification and matrix effects at 4 concentration levels.

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NEW EXTRACTION SPECTROPHOTOMETRIC METHODS FOR THE DETERMINATION OF NITROGEN MUSTARD (HN-3) IN ACIDIC ENVIRONMENT

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Nitrogen mustards are compounds that have been used as active ingredients of pharmaceutical preparations. However their effects on human body take also negative consequences such as secondary leukemia genesis because of side DNA alkylation.[1] Tris(2-chloroethyl)amine (HN-3) is military interesting blistering nitrogen mustard that was produced in significant quantities during World War 2. In alkaline environment the alkylating properties are predominant but in acid its tertiary amine structure can be useful for detection and determination of the analyte. The aim of this study is to create and evaluate methods for extraction spectrophotometric determination of HN-3 in acidic environment in field conditions.

Nine dyes form the group of sulfophthaleins, antraquinone dyes and sulfonated azo dyes were suitable for ion pair formation with the analyte. Complexes were extracted from aqueous phase into non-polar solvent (chloroform) and measured spectrophotometrically against blank solutions. Optimization of the determination process covered measuring of absorption curves, optimal pH, ion-pair stoichiometry, optimal dye concentration excess, extraction time, calibration curves, extraction recoveries and distribution ratios.

Because of high polarity the HN-3 is rarely disposed for ion pair formation as tertiary amine. Best molar absorptivities were reached with Bromothymol Blue and Bromocresol Green (301 and 148 $l\,mol^{-1}\,cm^{-1}$ respectively). Highest extraction recoveries were recorded by Bromothymol Blue (0.97), Acid Blue 129 (0.64) and Bromocresol Green (0.61). Best

limits of detection of proposed methods were reached with Acid Blue 25 (36.4 μg ml^{-1}) and Bromothymol Blue (45 μg ml^{-1}).

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USING SOLID-PHASE MICROEXTRACTION FOR PLASMA PROTEIN BINDING STUDY OF DPC

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Solid-phase microextraction (SPME) is a relatively novel technique suitable for extraction of analytes from complex matrices using a small amount of phase attached to a fiber. This method is nowadays thoroughly studied for its use in protein binding (PPB) studies of drugs for its low recovery and reduced matrix interference thanks to higher selectivity of the fiber coating.[1]

Di-2-pyridylketone-4-cyclohexyl-4-methyl-3-thiosemicarbazone (DpC) is novel and highly potent anticancer drug[2] currently under advanced preclinical development. Measurement of PPB is an indispensable part of discovery as well as clinical development of this novel drug. Nonspecific binding of DpC to various materials and its chelation ability complicates the use of standard approaches such as equilibrium dialysis and ultrafiltration as well as employment of metal-based commercially available SPME fibers. Hence the aim of this project was to prepare novel SPME fibers from material resistant to chelation and utilize them for the determination of plasma protein binding of DpC *in vitro*.

In this study silicone fibers were coated with Discovery® DSC-18 sorbent using polydimethylsiloxane (PDMS) as a glue. Development of the SPME method included mainly optimization of extraction and desorption conditions. Potential carry over, matrix effect (PBS and plasma), reproducibility, linearity, accuracy and precision of the method were determined. Optimized procedure was then utilized for determination of PPB[3] of DpC at different concentrations in rat plasma *in vitro*. All samples were analyzed using UHLC-MS/MS to reach sufficient sensitivity for analysis of DpC *in vitro*.

This study was supported by Charles University (project SVV 260 183).

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DEVELOPMENT OF HPLC-DAD METHOD FOR ANTHOCYANIN DETERMINATION IN DIFFERENT HIGHBUSH BLUEBERRY CULTIVARS

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Anthocyanins belong to the most important pigments of the vascular plants which are responsible for many colors in some flowers and fruits. Significant property of anthocyanins is their antioxidant activity which plays important role in prevention of many diseases.[1] High amounts of anthocyanins were found in highbush blueberries (*Vaccinium corymbosum* L.).

The aim of this research was to develop and optimize HPLC method for relatively fast separation and quantification of anthocyanins in highbush blueberry samples. 22 cultivars of highbush blueberry were tested. Anthocyanins were extracted into methanol with the addition of formic acid and then separated using Shimadzu HPLC system equipped with a diode array detector. Compounds were detected at wavelength of 520 nm. Different columns were tested, however column Kinetex PFP (150 × 4.6 mm, 2.6 μm) with guard column Ascentis® Express F5 (5 × 4.6 mm, 5 μm) was finally chosen. Columns were operated at 50 °C. Water solution of formic acid (solution A) and acetonitrile (solution B) were used as the mobile phases. Gradient elution mode was applied. Final time of separation was under 20 minutes. Correlation coefficient in concentration range between 1–100 mg L^{-1} was higher than 0.9991. Repeatability in three concentration levels (5, 20, 100 mg L^{-1}) ranged between RSD 0.21–0.98%. Significant differences in the representation of individual anthocyanins were demonstrated in the observed cultivars.

The study was supported by the NP MZe 20139/2006-13020 and CZ.1.05/2.1.00/03.0116.

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MOLECULARLY IMPRINTED POLYMERS FOR SELECTIVE EXTRACTION OF LOVASTATIN FROM FOOD SAMPLES AND ELIMINATION OF MATRIX EFFECTS

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Lovastatin belongs to the group of cholesterol-lowering drugs and represents the first marketed statin. It is also the only statin that occurs in nature and found application in

clinical practice. This compound is produced by several fungal species of the genus *Trichoderma*, *Monascus*, *Penicillium* and *Aspergillus*. Lovastatin occurs in traditional Chinese food such as Pu-erh tea, Red yeast rice and Oyster mushroom. Liquid chromatography coupled to mass spectrometry (LC-MS) was used for its determination in the food samples.

LC-MS method is well-established and widely used analytical tool in the field of quantitative and qualitative analysis. It is characterized by high selectivity, sensitivity and robustness. However, this method suffers from a major drawback represented by matrix effects. They are caused by co-eluting compounds that affect the process of ionization in the ion source of mass spectrometer. They lead to signal suppression or enhancement and affect important parameters of LC-MS method. Food samples such as tea leaves, mushrooms and fermented rice are complex matrices prone to this undesirable phenomenon.

SPE sorbent based on the technique of molecularly imprinted polymers (MIP) was employed for sample preparation step due to its ability to selectively retain the target analyte and purify the complex samples. The tailor-made cavities for selective recognition of lovastatin were prepared using simvastatin as a template molecule, methacrylic acid as a functional monomer and ethylene glycol dimethacrylate as a cross-linker. Process of polymerization was initiated by azobisisobutyronitrile. Non-imprinted polymer (NIP) was prepared according to the same procedure as MIP, except that no template was used for its synthesis. NIP was employed as a control material for selectivity evaluation due to the absence of cavities. The resulting MIP material was completely characterized in terms of capacity, selectivity, repeatability of extraction procedure and reproducibility of synthesis. The optimized SPE procedure employing MIP sorbent successfully removed matrix effects in all food matrices.

STABILITY OF CARFILZOMIB – UHPLC-PDA-QT OF STUDY OF A PROTEASOME INHIBITOR

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Carfilzomib is an irreversible inhibitor of the protasome – a breakthrough target in cancer treatment.[1] Carfilzomib contains several structural features that might raise stability concerns, such as: 1) peptide bonds, 2) an epoxide, 3) a morfolin ring, 4) four stereogenic centres. Since this drug was granted an accelerated approval by FDA in 2012, only scarce data on analysis and none on stability of carfilzomib are available in the literature. Therefore our goal was to develop and validate a stability-indicating method to provide insight into the inherent stability of this new drug.

We developed and validated (according to ICH guidelines[2]) a novel method for quantitation of carfilzomib using a Nexera® UHPLC system with a PDA detector (Shimadzu, Japan), a C18-bearing silica type C column (Cogent Bidentate C18; 2.1 × 100 mm; 2.2 μm; 120 Å)

and acetonitrile/ammonium formate mixture in gradient mode. This method was used in investigation of carfilzomib's degradation kinetics. Additionally, we employed a Synapt® G2Si QTOF instrument (Waters, United Kingdom) in MS/MS elucidation of the chemical structures of degradation products, that were formed in a forced degradation study.

We found that carfilzomib is: 1) stable at neutral and slightly acidic pH, while it degrades in both low and high pH, 2) acceptably stable in pharmaceutical formulation but is 3) prone to oxidation and photodegration. The decomposition products resulted from peptide bond hydrolysis, epoxide hydrolysis, hydrogen chloride addition, base-catalyzed Robinson-Gabriel reaction, tertiary amine oxidation and isomerization.

Our results document the stability of carfilzomib and provide first information about identity of its degradation products. These results highlight the stability issues that need to be kept in mind for handling/storing.

The study was supported by Charles University project SVV 260183.

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A NOVEL APPROACH TO THE LAB-IN-SYRINGE TECHNIQUE. DETERMINATION OF AMMONIA IN RIVER WATERS

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A novel approach to the Lab-In-Syringe technique, also known as In-Syringe Analysis[1] is presented. It is based on using a secondary entrance to the syringe void through the modified syringe piston. This innovation allows straightforward automation of single-drop microextraction by simplifying the control of drop handling as well as in-drop analyte quantification.

The syringe pump was used in upside-down orientation. Sample homogenization, head-space enrichment by analyte, and syringe cleaning was facilitated by a magnetic micro-stirring bar placed inside the syringe and driven by an external rotating magnetic field.[2]

The system was characterized by the development of a sensitive method for ammonium determination in river and harbour waters. The method is based on head-space extraction of ammonia into a single drop of bromothymol blue indicator formed inside the syringe and on-drop sensing of the colour change using fibre optics. A repeatability of < 5% RSD, a linear range up to 25 μmol L^{-1}, and a limit of detection of 1.5 μmol L^{-1} were achieved. Study of interferences proved excellent robustness of the method towards humic acid,

salts, and detergents, thus being superior to state-of-the-art gas-diffusion approaches. A mean analyte recovery of 106.14% was found analysing spiked water samples.

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SEQUENTIAL INJECTION MANIFOLD AS A TOOL FOR AUTOMATED PERFORMANCE OF DRUG PERMEATION STUDIES

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Sequential injection analysis (SIA) is a second generation of analytical flow techniques introduced by prof. Růžička in 1990.[1] Its universal set-up allows to build an easy manifold to perform sample treatment such as dilution, derivatization, extraction or separation and its detection in one system. SIA manifold is also used for long-term monitoring applications, such as dissolution tests of tablets, liberation tests of ointments or permeation tests of drugs.

Drug permeation studies[2] are tests used for evaluation of drug transport through cellular monolayer, drug interaction with membrane transporters or drug-drug interactions.

This experimental work describes analytical system composed of SIA manifold connected with a Franz diffusion cell as a liberation unit used for drug transport monitoring. P-glycoprotein (P-gp, MDR1) transporter function was evaluated using Rhodamine 123 as a marker of transport and verapamil hydrochloride as a P-gp inhibitor.

Description of flow-based apparatus, its comparison with batch-wise apparatus and experimental data obtained during permeation tests with Rhodamine 123 and verapamil on cell line MDCKII-MDR1 will be presented.

The study was supported by the Grant Agency of the Charles University, project GAUK No. 159415, and by the project of specific research, SVV No. 260184.

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THE INFLUENCE OF MAGNESIUM STEARATE ON THE FLOW AND SHEAR PROPERTIES OF SORBITOL

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The flow properties of powders are very important for handling, transport, storage and the correct dosage of active ingredients and excipients in production of solid dosage forms. The flow, shear and avalanching behaviour of powders depend on the internal friction between grains of the material.[1,2] For evaluation of flow properties, the standard pharmacopoeial methods are used out of them the measurement of the mass flow rate is the most frequent. To measure the angle of internal friction, the shear tester is useful.[3] To increase the flow of powders, glidant such as Magnesium stearate is usually used; but it also can be used as a lubricant which prevents adhesion of tablets on the die or punch wall.[4]

This work studies the influence of the concentration of magnesium stearate MgSt (0.5 wt% or 1.0 wt%, respectively) on the mass flow rate Q (g/s) of sorbitol for direct compression through a circular hopper orifice having a diameter of 1.0 cm. The shear properties of sorbitol and mixtures with MgSt were studied using Jenike shear tester.

The very low values of the cohesion for sorbitol were detected infering the non-cohesive, free-flowing material properties. MgSt increased the mass flow rate and decreased the cohesion properties. The better results were obtained with 0.5 wt% addition of MgSt.

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EVALUATION OF TRANSDERMAL PERMEATION ENHANCERS BASED ON MONOSACHARIDES

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Transdermal route of drug administration is a non-invasive technique with many advantages, compared to other drug delivery routes. However, skin acts as formidable barrier preventing majority of environmental substances from entering into organism. One of the methods how to overcome this barrier is to use permeation enhancers, substances which by various mechanisms reversibly decrease barrier properties of skin. In this study we studied effect of monosaccharide derivatives and their analogues on permeation of two model drugs theophylline (TH) and cidofovir (CDV) through human skin *in vitro*. Afterwards, toxicity, reversibility and mode of action of selected enhancers were studied. The *in vitro* permeation experiments on human skin with model drug theophylline (TH) revealed, that the best activity possess substances 91.1 and 94.1 which significantly enhanced flux of TH 5.9 and 8.3 times, respectively, compared to control. Although none of 91.1 and 94.1 enhanced flux of CDV through human skin, both of them significantly increased CDV concentration in epidermis, with values 7.4 and 6.9 times higher, respectively, compared to control. To determine possible mechanism of action of these enhancers were used infrared studies on isolated human stratum corneum. Results suggested that the mechanism of action of both 91.1 and 94.1 probably involves interaction with barrier lipids. Reversibility of action of selected substances (91.1 and 94.1) after 24h application on human skin was proved using transepidermal water loss measurements. Finally, we studied toxicity of 91.1 and 94.1 on two cell lines; Swiss albino mouse embryonic fibroblasts (3T3) and spontaneously immortalized human keratinocytes (HaCaT). IC_{50} values for substance 91.1 on 3T3 and HaCaT were 25.18 ± 2.79μM and 24.35 ± 0.96μM, respectively. Due to low solubility of 94.1 we were only able to determine that its IC_{50} concentration value is higher than 60μM on both 3T3 and HaCaT cell lines.

The study was supported by Charles University (GAUK 1404213 and SVV 260 183) and the Czech Science Foundation (13-23891S).

BIORELEVANT *IN-VITRO* RELEASE TESTING METHODS FOR CONTROLLED RELEASE PARENTERALS: AN INTRODUCTION

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Controlled release parenteral preparations (microparticles, implants) provide several advantages in comparison with other dosage forms. Despite the rapid development in this area, there is no compendial *in-vitro* release testing apparatus specifically designed for these types of devices. *In-vitro* release tests are irreplaceable tools for characterisation of dosage forms. They provide valuable information about the formulation variables and can predict performance of the device *in-vivo*. The purpose of "biorelevant" *in-vitro* methods is to include (and to recognize) factors which have influence on drug release *in-vivo*. Development in the field of biorelevant dissolution has been predominantly focused on oral dosage forms. To simulate environment at the site of administration in case of implantable devices, use of hydrogels have been suggested. In our proposed novel setup, the controlled release device is incorporated in thin agarose hydrogel and placed in phosphate buffer (pH 7.4). Therefore the possibility of sampling from buffer and use of conventional analysis methods is retained. Initial results are also presented, although more experiments will be needed to make any conclusions.

The study was supported by SVV 260 183.

OPTIMIZATION OF FABRICATION OF NANOPARTICLES WITH HYDROPHILIC BIOLOGICALLY ACTIVE SUBSTANCES

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Nanoparticles have been developed as an important strategy to deliver conventional drugs, recombinant proteins, vaccines and more recently, nucleotides.[1] Nanoencapsulation of drugs through various methods increases drug efficacy, specificity, tolerability and therapeutic index of corresponding drugs.[2]

Although the definition identifies nanoparticles as having dimensions below 0.1 μm or 100 nm, especially in the area of drug delivery relatively large (size above 100 nm) nanoparticles may be used for loading a sufficient amount of drug onto the particles.[3]

The objective of this project is to study and optimize methods of preparation of medicated polymeric nanoparticles from aliphatic hydroxyacids as carriers. Nanoparticles will

contain hydrophilic lowmolecular weight, oligomeric, and polymeric active substances. The aim is to minimize mean size and size polydispersity and to maximize yield and encapsulation efficiency. Efforts will be directed to development of robust methods of preparation of standardised products.

Select samples will be tested in cooperation with The Department of Pharmacology and Toxicology for relevant interactions with biological systems.

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A BIOPHYSICAL INSIGHT INTO A DISTURBED SKIN LIPID BARRIER IN B-GLUCOCEREBROSIDASE DEFICIENCY

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Glucosylceramides (GCer) are precursors for all ceramide (Cer) types in the stratum corneum (SC) and their hydrolysis by β-glucocerebrosidase (GCerase) is important in the formation of the epidermal permeability barrier.[1] Decrease in Cer content in patients with Gaucher disease is related to decreased GCerase activity.[2] We studied how the presence of GCer and/or lack of Cer influences permeability barrier properties of model SC lipid membranes.

The SC model membranes were prepared as an equimolar mixture of Cer or GCer in different ratios, Chol, FFA and 5% of CholS. Also membranes with decreased Cer fraction were prepared. Four permeability markers – water loss through the membrane (TEWL), opposition to electrical current and steady state fluxes of theophylline and indomethacin, were evaluated.

The replacement of 5–25% of hCer by GCer led to impairment of the permeability of the prepared membranes to all 4 permeability markers. At these concentrations, the presence of GCer is a stronger contributor to this disturbance than a lack of hCer. The reduction of hCer to 50 or 0% showed that the lack of hCer disturbs the barrier, while the larger GCer/hCer ratio or complete replacement of hCer by GCer has no negative effects on permeability.

In conlusion, we confirmed that the accumulation of free GCer associated with their incomplete processing contributes to altered permeability barrier properties in skin disorders. However, this barrier perturbation by free GCer seems to be concentration-dependent.

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INFLUENCE OF SUPERDISINTEGRANTS ON THE PROPERTIES OF TABLETS COMPOSED FROM DIFFERENT STARCHES

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Orodispersible tablets are uncoated tablets intended to be placed in the mouth, where they disperse rapidly.[1] Quick disintegration and sufficient radial strenght are two out of the most important properties of them.[2] In this study, the properties of four tablet mixtures prepared from granules made by fluid bed granulation technique were studied.

To prepare the granules, potato starch (PS) and corn starch (CS) as fillers, respectively, and sodium starch glycolate (SSG) or cross-carmellose (CCMC) as superdisintegrants, respectively, were used; povidone (PVP) 10% served as a binder. Granules were evaluated for their particle size distribution, flow properties, compressibility properties and particle density. Then, magnesium stearate (0.5%) was added as a glidant and tablets of 7 mm in diameter and approximately 0.1 g in mass were compressed using the different compression forces to achieve the starting radial strenght of 1 MPa. The diameter and the height of tablets was measured as well as the crushing strenght and the disintegration time.

Results of this study proved, that out of the tested combinations, PS CCMC granules served the best flow and tablet properties. The granules had satisfactory particle size distribution and the mean particle size x_{50} (180 μm), the appropriate angle of repose (39°) as well as the compresibility index (18.14%). Tablets made from PS CCMC had sufficient radial strenght (0.61 MPa) and disintegrated within two minutes.

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DIFFERENT SUBSTITUTIONS OF ISOFLAVONOIDS CORE INFLUENCE MARKEDLY THEIR ANTIPLATELET POTENTIAL

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Beneficial effects of isoflavonoids on the human health, in particular the lower risk of the coronary artery disease was reported.[1] The aim of this study was to compare antiplatelet activities of 17 isoflavonoids and to find the mechanisms of their action.

The screening in the whole human blood has shown that 12 isoflavonoids blocked platelet aggregation induced by arachidonic acid. The structure-activity relationship revealed that 7,4′-dihydroxyl group represented the most efficient functional group. Substitution of these positions by a methoxyl group led to a reduction of the effect while glucose in position C-7 was associated with almost complete loss of the activity. Presence of a 5-hydroxyl group seemed to be beneficial, as well as, the co-presence of a 6-methoxy group. The latter was associated with higher effect than the standard antiplatelet drug, acetylsalicylic acid (ASA). Active isoflavonoids acted as antagonists on thromboxane A_2 receptors and inhibitors of cyclooxygenase-1.

Isoflavonoids with appropriate chemical structure possess even higher anti-platelet activity in comparison with ASA[2] and their advantage over this standard drug might be associated with simultaneous influence on two steps on the platelet aggregation.

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^{61,64}Cu-(SCN)-PHOSPHINATE-IMMUNOGLOBULIN-G M75 AND THEIR BIOLOGICAL TESTING

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The work was focused on a new approach to the labelling of antibody IgG M75 for epitope human carbonic anhydrase IX with copper radioisotopes. Human carbonic anhydrase IX is a membrane enzyme that is significantly expressed in some types of hypoxic cancer cells and copper radioisotopes offer wide range of diagnostic, therapeutic and theragnostic properties.[1] The antibody IgG M75 was successfully conjugated with "phosphinate", recently developed, non-commercial copper-specific chelator. The conjugation method was optimized and provides well-reproducible results. The conjugate was then labelled with two copper radioisotopes: ^{61}Cu (3.339 h) and ^{64}Cu (12.701 h). The resulting labelled conjugates were tested *in vivo* in mice with inoculated colorectal cancer.[2] Obtained data suggest that the prepared labeled antibody is a good candidate for diagnostics of some hypoxic solid tumors by imaging via positron emission tomography (PET).

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THE INCIDENCE OF DYSRHYTHMIAS AFTER ADMINISTRATION OF ANTIPSYCHOTIC OLANZAPINE

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Psychosis is a mental disease with increased risk of developing cardiovascular diseases as well as increased risk of mortality after administration of antipsychotics. Aim of the work was to analyze the effect of antipsychotic olanzapine (OLA 10 mg/kg s.c.) on isolated spontaneously beating rat heart. We used perfusion according to the Langendorff to investigate the effect of OLA. We perfused rat heart for 20 minutes by Krebs-Henseleit solution

followed by 30 min stop-flow ischemia and 45 min. reperfusion (IR injury). Analysis of ECG during reperfusion showed longer QT and QTc intervals durations in the group pre-medicated with OLA. As well as increased incidence of reperfusion-induced dysrhythmias in order ventricular premature beats > bigeminies > trigeminies > salvos. Administration of OLA caused spontaneously terminating episodes of ventricular tachycardia in a time between 10th and 25th minute in reperfusion. Average incidence of ventricular tachycardia during whole reperfusion was 1.6 episodes and duration was 31.5 second per one heart. This represents an increase compared to the IR injury group by 60 percent in the number of episodes and the doubling of their average duration. OLA modulated the activity of the isolated spontaneously beating rat heart and displayed proarrhythmogenic effect during reperfusion.

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RADIOLABELING OF ANTI-VEGFR2 MONOCLONAL ANTIBODY RAMUCIRUMAB WITH TECHNETIUM-99M

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Vascular endothelial growth factor (VEGF) is one of the most important regulators of angiogenesis, including tumour angiogenesis. VEGF binds on two types of receptors with tyrosine-kinase activity of which VEGF receptor 2 (VEGFR2) is a key receptor in tumour neoangiogenesis.[1] Ramucirumab (RAM), is a novel therapeutic monoclonal anti-VEGFR2 antibody with much higher affinity than its natural ligand.[2] The aim of this work was to evaluate a possibility of labeling RAM with technetium-99m and verify radiochemical purity and stability of radiolabeled antibody. For labeling of RAM with ^{99m}Tc, we optimized a direct method based on reduction of disulfide bridges in antibody molecule with 2-mercaptoethanol[3] using many modifications of the experimental conditions. The radiochemical purity of labeled antibody was assayed by instant TLC on silica gel (ITLC-SG). The sample was analyzed at various times by SE-HPLC with radiometric detection to evaluate a stability of labeled antibody. The introduced methods enable effective labeling of RAM with ^{99m}Tc and result in sufficiently stable radiopreparation. The developed radiolabeled preparation may be used in the follow-up studies to evaluate biological behavior of ^{99m}Tc-RAM *in vitro* and *in vivo*.

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SOLUBLE ENDOGLIN EFFECTS IN AORTA FROM MICE FED HIGH FAT DIET

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A soluble form of endoglin (sEng) is generated by the cleavage of its extracellular domain during development of some pathological conditions. Several authors have suggested the participation of sEng in the mechanisms of endothelial dysfunction. Thus, we tested the hypothesis that high plasma concentration of sEng in *Sol-Eng*$^+$ mice combined with high fat diet might contribute to the development of endothelial dysfunction.

Six-month-old female transgenic mice overexpressing human sEng with low (*Sol-Eng*$^+$ *low*) or high levels of soluble endoglin (*Sol-Eng*$^+$ *high*) were fed a high fat rodent diet containing 1.25% of cholesterol and 40% of fat for 3 months. The expressions of pro-inflammatory P-selectin, ICAM-1, pNFkB, COX-2, and oxidative stress-related markers HO-1, NOX-1 and NOX-2 in aortas of *Sol-Eng*$^+$ *high* mice were significantly higher than in *Sol-Eng*$^+$ *low* mice. Surprisingly endothelium-dependent response induced by acetylcholine was preserved in *Sol-Eng*$^+$ *high* mice compared to *Sol-Eng*$^+$ *low* mice.

These results suggest that high concentrations of sEng in plasma induce the activation of pro-inflammatory, pro-oxidative as well as vasoprotective mechanisms in the vessel wall.

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DIFFERENTIATION AND EPIGENOME AFFECTING DRUGS CHANGE EXPRESSION OF NUCLEOSIDE TRANSPORTERS IN PLACENTAL Bewo CELL LINE

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Nucleoside transporters (NTs) participate in nucleoside uptake by the placenta and contribute to transplacental permeation of nucleoside derived drugs. Evidence on regulation of placental NTs is sparse, therefore this project was aimed to analyze effect of epigenome (butyrate sodium, valproic acid sodium salt and 5-azacytidine) and differentiation (forskolin) affecting drugs on mRNA expression of genes encoding two subfamilies of NTs, i.e. equilibrative nucleoside transporters (ENT1, ENT2) and concentrative nucleoside transporters (CNT2, CNT3) in human choriocarcinoma derived BeWo cell line.[1,2]

Using qRT-PCR we found that only forskolin, cAMP-dependent protein kinase A (PKA) activator that induces syncytiotrophoblast formation in BeWo cells, had significant effect on the expression of *CNT2*. Upregulation of CNT2 in presence of forskolin was reversed by simultaneous application of KT5720, a model PKA inhibitor. Furthermore, significant increase of adenosine, a model substrate of CNT2, uptake was observed, showing higher mRNA expression *CNT2*, is reflected also in its elevated function. Forskolin-induced placentation of BeWo cell line was confirmed by mRNA analysis of endogenous retroviral envelope gene syncytin-1 (HERVW1), product specifically expressed in placental trophoblasts. Interestingly, mRNA expression analysis of *CNT2* mRNA in the first- and third-trimester human placenta also revealed significantly increasing tendency.[3]

In conclusion we propose that placental NTs are not epigenetically regulated. On the other hand expression/function of CNT2 seems to be PKA dependent and changes during placentation. We presume this phenomenon might also occur in the human placenta. As placenta is highly dependent on exogenous supply of pyrimidine nucleosides, we suggest that upregulation of CNT2 during placentation/gestation may be thus attributed to higher placental demands of nucleosides during gestation. It can also be predicted that transplacental permeation of CNT2 substrates increases during gestation.

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RIBAVIRIN TRANSPORT ACROSS PLACENTA AND THE ROLE OF NUCLEOSIDE TRANSPORTERS

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Ribavirin is highly used hydrophilic nucleosid-derived antiviral drug. It is currently recommended for combination therapy of hepatitis C virus (HCV) or HCV/HIV infection. *In vitro* and *in vivo* evidence on teratogenicity or embryocidal effect of ribavirin have shown potential risks, thus considering gestation as contraindication of ribavirin prescription. Ribavirin is suggested substrate of nucleoside transporters (NTs). NTs are divided in two subfamilies – equillibrative nucleoside transporters (ENTs) and concentrative Na^+ dependent nucleoside transporters (CNTs). In the placenta S-(4-Nitrobenzyl)-6-thioinosinu (NBMPR) sensitive ENT1 and NBMPR insensitive ENT2, and CNT2 are most abundantly expressed.[1]

In our study we aimed to determine whether NTs participate in transplacental passage of ribavirin. For this purpose we employed i) an *in situ* dually perfused rat term placenta model, analyzing materno-fetal (M-F) and feto-maternal (F-M) transplacental clearances of ribavirin on the organ level and ii) an *ex vivo* uptake experiment in fragments of human placental fresh villous tissue.

Ribavirin M-F and F-M clearances showed low level of its transplacental permeation, with negligible placental accumulation after the perfusion (≤3% of the ribavirin dose) NBMPR (0.1 μM and 100 μM) decreased ribavirin clearance in both directions to comparable level. Importantly, exposure to NBMPR (100 μM) and/or depletion of Na^+ in buffer resulted in inhibited ribavirin uptake by human fresh villous fragments.

In conclusion, our data document involvement of ENTs and CNTs in transplacental permeation of ribavirin. ENT1 and CNT2 are supposed to be predominant in placenta but further studies should be carried out to specify type NTs involved in ribavirin transplacental transport.

The study was supported by GAUK 324215/C/2015 and SVV/ 2015/260-185.

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INTERACTIONS OF INTESTINAL INFLUX TRANSPORTER hOATP1A2 WITH SELECTED FLAVONOIDS

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The bioavailability of orally administered compounds could be influenced by intestinal transporters. The major human intestinal transporters for uptake of organic anions are organic anion transporting polypeptides OATPs. In this study we focused on hOATP1A2 which is expressed in the apical membrane of human enterocytes. Some dietary flavonoids have been shown to inhibit human OATP subtypes involved in the drug intestinal absorption.[1] Therefore, the potential interactions of flavonoids with hOATPs should be considered as a factor potentially affecting pharmacokinetics of relevant drugs.

The aim of this study was to assess the potential inhibition of hOATP1A2 by natural compounds from the group of flavonoids (quercetin, myricetin, galangin, pinobanksin, pinocembrin, chrysin, fisetin) *in vitro*.

HEK293 cells transiently transfected with hOATP1A2 were used as the experimental model. The cells transfected with the empty vector served as a control. Inhibitory studies measuring hOATP1A2-mediated uptake of the typical substrate, radiolabelled [^{3}H]-estrone 3-sulfate, were employed to determine the inhibitory potency of flavonoids. Quercetin, the known OATP inhibitor, served as a comparator.

All mentioned flavonoids showed the statistic significant inhibition of the hOATP1A2-mediated uptake. The most potent hOATP1A2 inhibitors seem to be fisetin and pinocembrin with IC_{50} of 0.2 μM and 2.0 μM, respectively.

According to the obtained results, these natural compounds could potentially affect in varying degrees uptake of the drug substrates transported by human OATP1A2 (e.g. statins) into the enterocytes. So there is a possibility of food-drug interactions in humans at the level of absorption.

The study was supported by grant of the Czech Science Foundation (GAČR) no. 303/12/G163 and Charles University (SVV 260185 and PRVOUK P40).

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THE HAEMODYNAMIC EFFECTS OF FLAVONOID METABOLITE 3-(3-HYDROXYPHENYL)PROPIONIC ACID IN RAT

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The flavonoid intake is supposed to be associated with a lower cardiovascular mortality.[1] Paradoxically, the oral bioavailability of flavonoid aglycones is low.[2] The aim of this study was to test whether metabolites formed in the gastrointestinal tract by microflora could contribute to the effect.[3] A series of quercetin metabolites formed by both human enzymes and colon microflora were tested *in vitro* on isolated rat aortic rings precontracted with norepinephrine. A number of them caused vasodilatation with 3-(3-hydroxyphenyl) propionic acid (3HPPA) being clearly the most potent one. The vasodilatory activity of 3HPPA was confirmed by *in vivo* experiments on both normotensive and spontaneously hypertensive rats. Subsequent experiments indicated that arterial blood pressure decrease after 3HPPA was caused by the peripheral effect of the compound on vascular beds and could be NO-based. This is the first study showing that a metabolite of flavonoids formed by human microflora has haemodynamic effect.

The study was supported by the Charles University (grant No. 253115 C, the program "Research and Study of the Drugs" (PRVOUK P40)) and by the The Czech Science Foundation (project No. P303/12/G163).

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INTERACTIONS OF ANTIRETROVIRALS WITH THE MAIN PLACENTAL TRANSPORTERS

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Current prevention of mother-to-child transmission of HIV infection is based on administration of combination antiretroviral therapy (cART) during the whole pregnancy. One

of the prophylactic mechanisms of cART is the presence of antiretrovirals in the fetal circulation that can be, nevertheless, associated with the potentially harmful effects on the developing fetus. To select optimal therapy while minimizing risks it is inevitable to have detailed knowledge of all the factors affecting transplacental transport of drugs.

The aim of this study was to investigate whether the main placental transporters are able to affect distribution of selected antiretrovirals between mother and fetus. Employing variety of *in vitro*, *in vivo*, *in situ* and *ex vivo* methods we determined the role of the drug efflux transporters in the transplacental pharmacokinetics of the tested drugs.

We suggested that antiretrovirals zidovudine, abacavir and tenofovir disoproxil fumarate are the substrates of placental ABCB1 and ABCG2 transporters. However, passive diffusion and/or other transporters enabled the penetration of abacavir and zidovudine into the fetus. On the other hand, the transplacental transport of lamivudine and parent drug tenofovir was not affected by the activity of ABC efflux transporters. Further we detected that long-term administration of tenofovir and emtricitabine to pregnant rats altered expression of the main drug efflux transporters in the selected organs of neither fetus nor mother.

The presented results contribute to the complex knowledge regarding transplacental pharmacokinetics of antiretroviral drugs.

The study was supported by Grant Agency of Charles University (SVV 2015/260-185).

RESPONSE OF SERUM INFLAMMATORY MEDIATORS TO CHEMOTHERAPY OF BREAST CANCER IN MICE AND HUMANS

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Breast cancer (BC) is the most common cancer in women worldwide. Despite advances in early-stage diagnostics and new therapeutic approaches, BC still remains one of the most frequent causes of cancer mortality in women. One of the major factors behind pathophysiological complexity of BC and variability in response to chemotherapy seems to be reaction of immune system but data in this area are still sparse. The aim of study was therefore to evaluate changes in serum concentrations of cytokines accompanying chemotherapy in humans and mice with BC. Mice with Ehrlich BC tumor showed biphasic change in IL-1, IL-5, IL-6, IL-12, MCP1, VEGF, TNF, IP10/CXCL10, MIG/CXCL9, and KC/GRO1 where in comparison to control group, a decrease was seen at 5th day after tumor implantation, which was followed by marked increase at 10th day where tumor present in advanced stage. Chemotherapy with cisplatin led to almost complete disappearance of tumor and attenuation of immune response including IL-2 concentrations.

Doxorubicin showed only partial effect on tumor size and cytokine response. In comparison, neoadjuvant chemotherapy (NCT) in patients with BC produced reduction in IL-1, IL-2, cFGF, EGF, and MIP-1, but also increase in IP-10, INF, and IL-2R when compared with levels measured before initiation of NCT. In conclusion, our data showed marked difference between mice and humans with BC in systemic cytokine/chemokine response to administration of chemotherapy. On the other hand, similarity in both species was found in reaction of IL-1 and IL-2, which may suggest significant role of these two cytokines in BC pathophysiology, and therapeutic response.

The study was supported by IGA NT/13473-3/2012.

EFAVIRENZ DECREASES RENAL EXCRETION OF LAMIVUDINE THROUGH INHIBITION OF OCT1, OCT2 AND MATE1 DRUG TRANSPORTERS

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Efavirenz (EFV) is a non-nucleoside reverse transcriptase inhibitor that constitutes an important part of combination antiretroviral therapy (cART) in HIV positive patients. It is often combined with other antiretrovirals, mainly nucleoside reverse transcriptase inhibitors (NRTI), many of which are substrates of solute uptake carriers OCTs (SLC22A) and efflux transporters MATEs (SLC47A). These membrane proteins are functionally expressed in kidneys and contribute to vectorial transfer of many drugs across tubular cells from blood to urine. Drug–drug interactions (DDI) on these transporters can affect renal drug excretion of the drug substrate and/or its accumulation in the renal tissue.[1] The aim of our study was to evaluate inhibitory potential of efavirenz towards OCT1, OCT2 and MATE1 transporters and possible transporter-mediated DDI between efavirenz and another NRTI used in cART, lamivudine, which has been previously shown to be actively excreted into urine by OCTs and MATE transporters.[2]

The inhibitory effect of efavirenz to OCT1, OCT2 or MATE1 transporters was measured *in vitro* using accumulation and transport assays in MDCK cell lines stably expressing the human SLC transporters. ASP^+ and MPP^+ were used as model substrates of OCT1, OCT2 and MATE1. Effect of efavirenz on pharmacokinetics of lamivudine was further evaluated in male Wistar rats *in vivo*.

Employing *in vitro* approaches, efavirenz was able to inhibit uptake of ASP^+ into relevant MDCK-OCT1, MDCK-OCT2 and MDCK-MATE1 cells with IC_{50} values of 4.2, 7.5 or 53.7 μM respectively. Efavirenz (10 μM) significantly decreased transcellular transport and intracellular accumulation of MPP^+ (2 nM) as well as lamivudine (10 nM) across cellular monolayers of MATE1 expressing cell lines. When applicated intravenously to Wistar rats, 10 μM efavirenz significantly decreased renal excretion of lamivudine by up to 92.13% and increased lamivudine accumulation in kidney 10.39 fold, exceeding thereby the effect of cimetidine as a control inhibitor of OCT and MATE transporters (causing inhibition of renal excretion by 55.73% and renal lamivudine retention enhanced 9.56 fold).

Taken together, our data suggest that efavirenz is an inhibitor of OCT1, OCT2 and MATE1 transporters able to cause pharmacokinetic DDI able to influence renal excretion of lamivudine *in vivo*. Further study will be needed to justify these findings in clinical pharmacotherapy.

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SOLUBLE ENDOGLIN EFFECTS ON ENDOTHELIAL CELLS *IN VITRO*

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Endoglin (Eng) is a transmembrane accessory type III receptor for the transforming growth factor-β (TGF-β) and its expression is up-regulated in proliferating endothelial cells. Soluble form of endoglin (sEng) has been identified in plasma of patients with preeclampsia, atherosclerosis and other cardiovascular diseases. Hence, endothelial dysfunction plays important role in many cardiovascular pathologies, we decided to test, whether high soluble endoglin induces changes in the expression of markers of endothelial dysfunction, inflammation, and oxidative stress in human umbilical vein endothelial cells (HUVEC) *in vitro*.

HUVEC were obtained from Lonza from Clonetics™ Laboratories. Cells were cultured in gelatin-coated flasks in EGM-2 medium. HUVEC were exposed to recombinant human sEng (50 ng/ml or 500 ng/ml) for 3 and 16 hours. qRT-PCR was used for the evaluation of changes in the expression of mRNA of selected markers.

The results of this study showed that treatment of HUVECs with 500 ng/ml recombinant human sEng for 16 hours induced significant increase in the expression of pro-inflammatory markers. Surprisingly, markers of endothelial function/dysfunction and oxidative stress were not significantly affected. Significantly increased expression of membrane endoglin mRNA might represent potentially compensative and possibly protective mechanism how endothelial cells react on soluble endoglin treatment. In conclusion, this study shows that high concentration of soluble endoglin affects endothelial cells with respect to inflammatory markers. However, these results deserve further study, particularly on the protein level.

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CAN ANTIDEPRESSANT PREMEDICATION REDUCE ISCHAEMIC-REPERFUSION INDUCED INJURY?

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In 2012 was ischaemic heart disease the leading cause of death in the world according to WHO. Antidepressants can affect heart function. Adverse effects of antidepressant drug therapy occure mostly at the beginning of therapy. More than 10% of drug intoxications in Slovakia during year 2013 were caused by antidepressants. Aim of work was to clarify if one application of antidepressants to rats (30 mg/kg s.c. of amitriptyline, citalopram or venlafaxine) 24 hours before experiment with isolated rat heart is sufficient to modify ventricular function during the reperfusion phase. Isolated hearts were perfused according to the Langendorff (20 min stabilization + 35min ischaemia and 45 min reperfusion). In I-R (ischaemic-reperfusion – premedicated by vehicle) group was lowest incidence of dysrhythmias during reperfusion phase. Premedication with amitriptyline induced the highest incidence of different VPB forms in the end of reperfusion. Hearts from this group reacted on the beginning of reperfusion with increased heart frequency, ventricular tachycardia and fibrillation and increase in bradycardia epizodes incidence and duration at later stages of reperfusion. Citalopram premedicated hearts reacted predominantly by bradycardia. The incidence of dysrhythmias in this group was the highest at the beginning of reperfusion. Hearts affected by venlafaxine reacted at the beginning of reperfusion by similar incidence of bradycardia and ventricular tachycardia. Incidence and duration of dysrhytmias were increased after antidepressant premedication. Incidence of dysrhytmias was in order: I-R < Citalopram < Venlafaxine < Amitryptyline group. Antidepressant premedication didn't reduce effect of I-R injury on the heart's function. Antidepressant premedication worsened function of hearts after ischaemia.

This work was supported by grants VEGA, SR 1/1342/12 and UK/430/2015.

BIOORGANIC AND PHARMACEUTICAL CHEMISTRY SECTION

INTRAMOLECULAR TSUJI-TROST ALLYLATION – ENANTIOSELECTIVITY OUTLOOK

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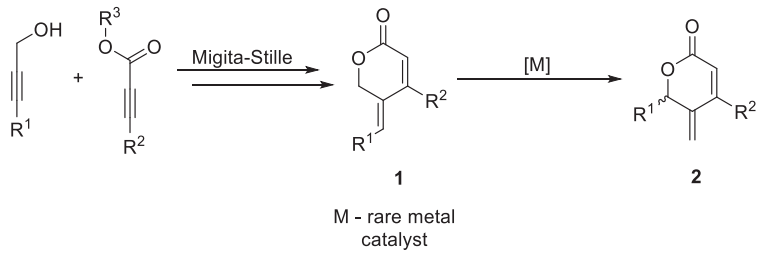
Our group previously reported an interesting by-product of Migita-Stille coupling.[1] The original required products (**1**) underwent a rearrengement to isomeric 5,6-dihydro-

5-methylene-2*H*-pyran-2-ones (**2**). Plausible mechanism for this transformation has been proposed.

The rearrangement introduces a new chiral center to the pyranones. The newly formed compounds (**2**) may be further utilized as precursors to norditerpenoid structures through the reactivity of electrophilic Michael-acceptor sites and ester moiety reduction. Therefore we performed screening of catalysts, chiral ligands, solvents and additives to achieve the highest possible enantiomeric excess.



Scheme 1.

This work was supported by Grant Agency of Charles University (project No. 1176213), Czech Science Foundation (project No. 15-07332S) and Faculty of Pharmacy in Hradec Králové (SVV-260-183).

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DESING AND SYNTHESIS OF NOVEL 3,4-DIARYLSUBSTITUTED FURANONES FOR GROWTH-INHIBITORY AND PRO-APOPTOTIC EFFECT AGAINST LEUKEMIA CELLS

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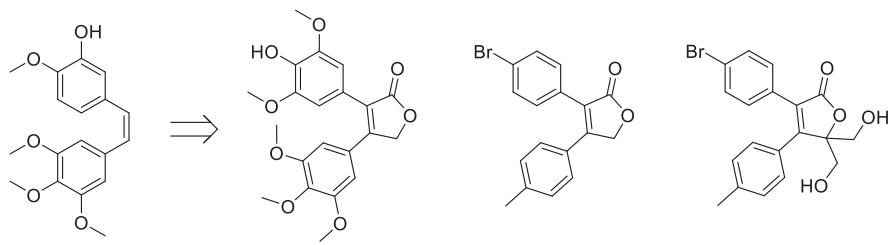
Herein we report the synthesis, derivatization and cytostatic activity evaluation of 3 libraries of α,β-diphenyl furanones.

The first library was derived from natural combretastatin. Since the *cis*-stilbene structural pattern as well as 3,4,5-trimethoxy substitution are essential for antitumor activity, the first library of molecules was characterized with high oxygenation of both phenyl rings.

In the second series of compounds, different substituents were attached. Furanones bearing halogen on C3 aromatic core and alkyl or alkoxy group on C4 found to possess sig-

nificant antineoplastic activity against human leukemia cancer cell lines. More specifically, several compounds proved to possess activity at the submicromolar range. Furthermore, the effect on healthy cell lines was also investigated. No toxicity observed up to a concentration of 40μM in medium.

In order to increase the hydrophilicity of our analogs a third library was developed by the introduction of two hydroxymethyl groups at the structure. Unfortunatelly, the activity of the obtained molecules was decreased.



Combretastatin A4 | Example of 1st library K562 (IC_{50} > 50 μM) | Example of 2nd library K562 (IC_{50} < 1 μM) | Example of 3rd library K562 (IC_{50} > 50 μM)

This work was supported by Charles University (GAUK 1906214, SVV 260-183) and Czech Science Foundation (15-073325).

NOVEL BIOLOGICALLY ACTIVE QUINAZOLINES

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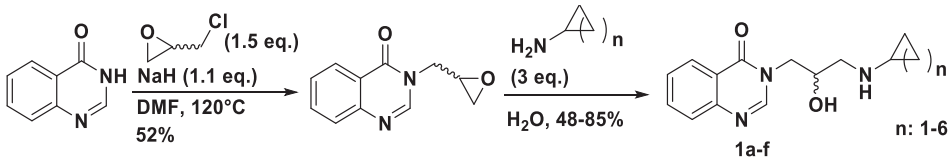
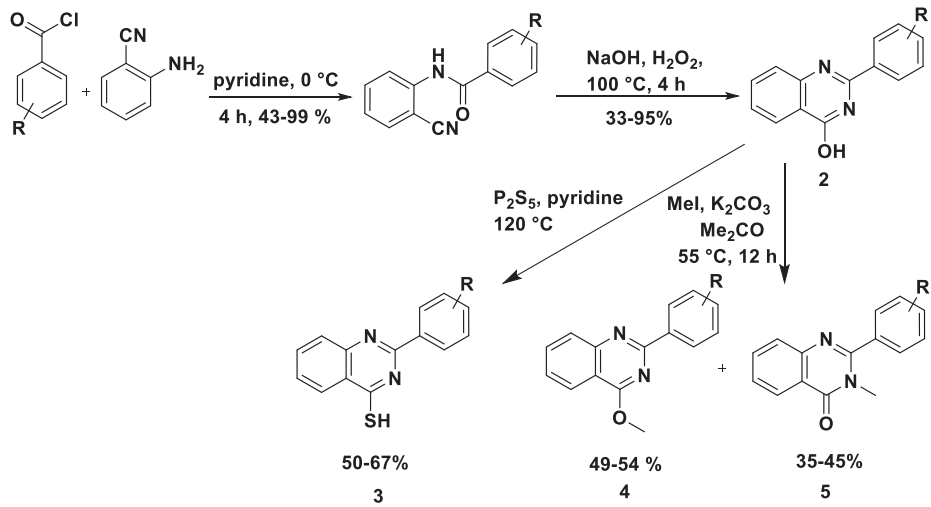
The most active bronchodilatory compounds from previous screening contained (piperidine-1-yl)propyl moiety attached to quinazoline ring.[1,2] Another series of derivatives (**1a–f**) bearing hydroxyl group on the three-membered carbon linker were synthesized (Scheme 1). Bronchodilatory activity was tested and the relationship between the biological effect and the prepared compounds will be discussed.



Scheme 1.

As a result of random screening, 2-aryl-quinazoline-4-oles (**2**) have been found as potential ligands towards CAR receptor. We have synthesized a library of 65 sulfur (**3**), *O*-alkylated (**4**) and *N*-alkylated (**5**) analogues (Scheme 2).[3] The evaluation of affinity to CAR receptor displayed promising effects.



Scheme 2.

The study was supported by Charles University (GAUK 398315), SVV-260-183 and Czech Science Foundation (15-073322S).

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SYNTHESIS OF AZAPHTHALOCYANINE CONTAINING ANIONIC GROUPS

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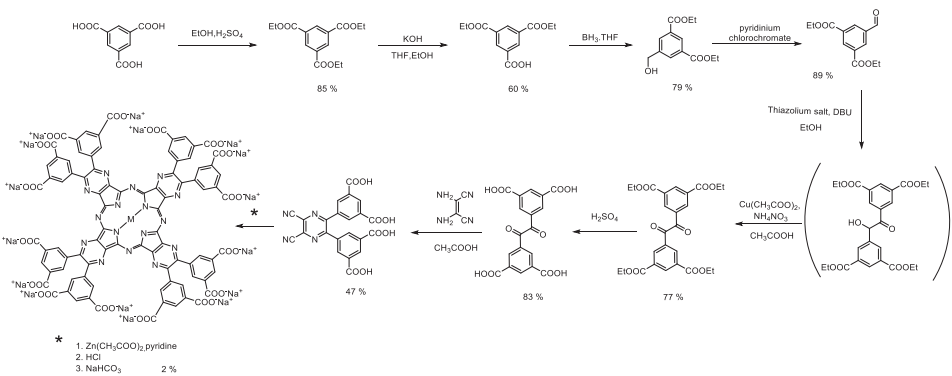
Phthalocyanines and their aza-analogues (e.g. tetrapyrazinoporphyrazines, TPyzPz) represent an interesting group of organic dyes with interesting photophysical properties (strong absorption in area over 650 nm and strong singlet oxygen production) highly suitable for the use in photodynamic therapy of cancer. The aim of this work was a synthesis

of a water-soluble sodium salt of zinc TPyzPz with eight 3,5-dicarboxylatophenyl substituents. Firstly, pyrazinedicarbonitrile precursor was prepared by multistep reaction pathway according to Scheme below. TPyzPz substituted with sixteen free carboxylic groups was synthesized in a template reaction with zinc(II)acetate in pyridine. This zinc(II) TPyzPz was converted into the sodium salt and the product was then purified by gel chromatography. Sixteen negative charges in rigid arrangement on periphery of the macrocycle inhibited aggregation in water or buffers of pH > 5.8. Strong aggregation was observed in buffers of pH < 4.8 due to the protonation of carboxylate functions and loss of repulsive forces. Final TPyzPz was tested on photodynamic activity *in vitro* on HeLa cells (IC_{50} = 5.7 ± 1.1 μM). Strong interactions with serum proteins and dependence of photodynamic activity on pH inside the cellular compartments was observed.[1]



The work was supported by SVV 260 183 and by Czech Science Foundation (13-27761S).

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SALICYLANILIDE-OLIGOTUFTSIN CONJUGATES: SYNTHESIS AND BIOLOGICAL ACTIVITY

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The global tuberculosis epidemic and increasing emergence of drug-resistant tuberculous as well as atypical strains call for intensive research on new antimycobacterial agents, especially on new structures with innovative mechanisms of action and without cross-resistance.[1]

Salicylanilides (2-hydroxy-*N*-phenylbenzamides) have exhibited a significant *in vitro* activity against *Mycobacterium tuberculosis* including drug-resistant strains and atypical mycobacteria at low micromolar concentrations. However, they share pronounced cytotoxicity and poor solubility.[2] These obstacles can be overcome, *i.a.*, by employment of drug delivery systems. We selected peptide carriers based on repeated oligotuftsin sequence $[TKPKG]_n$, which are nontoxic, stimulate the immune system and target macrophages specifically.[3]

Oligotuftsin-based peptide carriers were obtained using solid-phase synthesis and Fmoc/tBu strategy. N-terminus and/or lysine side chain amino group(s) were modified by varied substituents (carboxylic acids, fluorescent labels, peptides etc.). Then, the carriers were coupled with convenient salicylanilide derivatives *via* oxime bond, purified and characterized.

The conjugates were evaluated for their *in vitro* extracellular antimycobacterial activity (two strains of *M. tuberculosis*, *M. abscessus*), intracellular activity in infected macrophages, cytotoxic and cytostatic properties for various cell lines, and cellular uptake. Salicylanilide-oligotuftsin conjugates showed improved activity and cellular uptake together with decreased toxicity.

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PERMEABILITY AND MICROSTRUCTURE OF MODEL LIPID MEMBRANES CONTAINING 6-HYDROXYCERAMIDES

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Ceramides (Cer) based on 6-hydroxysphingosine (H) have been found only in human epidermis; however, their role in the skin barrier homeostasis is not fully understood. In this work we focused on the total synthesis of *N*-lignoceroyl-6-hydroxysphingosine (CerNH). Moreover, we aimed to study the permeability and microstructure of model lipid membranes based on CerNH in comparison with CerNS, CerNdS and CerNP. CerNH was prepared by using alkynylation of (*S*)-Garner aldehyde[1,2] with protected (*R*)-pentadec-1-yn-3-ol as a key step. Model membranes were composed of Cer/free fatty acids (C_{16}–C_{24})/cholesterol/cholesteryl sulfate. Their permeability was assessed in Franz-type diffusion cells using the following permeability markers: flux of two model compounds (theophylline and indomethacin), electrical impedance and water loss through the membrane. To elucidate the mechanisms of Cer effects on skin permeability, their biophysical properties were investigated by X-ray powder diffraction (XRPD) and infrared spectroscopy (ATR-FTIR). Using XRPD we found the short periodicity phase and crystalline

cholesterol in all membranes. In addition, in CerNH-based membrane we observed also a long periodicity phase with a repeat distance d = 10.6 nm. Next, using ATR-FTIR we showed differences in lipid mixing, packing and thermotropic phase behaviour. In membranes containing hydroxylated Cer, *i.e.* CerNP and CerNH, free fatty acids did not mix with the Cer chains.

The work was supported by the Czech Science Foundation (13-23891S) and Charles University (GAUK 1868214 and SVV 260183).

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INHIBITION OF ALDOSE REDUCTASE BY CHALCONES: SYNTHESIS, ENZYME ASSAY AND MOLECULAR DOCKING

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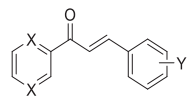
Aldose reductase (AKR1B1) is an important enzyme in polyol pathway, that is distinctly activated in metabolism of glucose under hyperglycemic conditions. Products of this pathway are implicated in long-term complications of diabetes.[1] In the aim of the search for new inhibitors, chalcone derivatives and its pyrazine analogues have been tested on isolated rat lens ALR2. Chalcones were prepared by Claisen-Schmidt condensation of acetofenone or pyrazin-2-ylethan-1-one with position isomers of hydroxybenzaldehyde or vanilline. In the enzyme assay, IC_{50} of the most active compounds ranged between 25–50 μM. In comparison with polyhydroxylated chalcones,[2] they exhibited lower activity. Interactions of the most potent chalcones in the active site of AKR1B1 were discussed in accordance with results of *in-silico* molecular docking.



X = CH, N
Y = 2-OH; 3-OH; 4-OH; 3-OCH_3,4-OH

The study was supported by PRVOUK P40 (Charles University) and by the project SVV 260 183.

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CO_2 SENSORS USING PHENOL SUBSTITUTED AZAPHTHALOCYANINES AS THE FLUORESCENT INDICATOR

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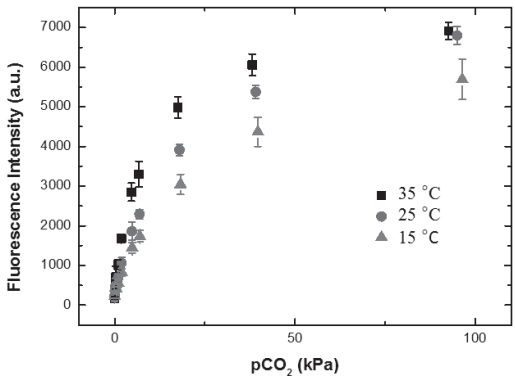
Azaphthalocyanines (AzaPcs) are planar macrocyclic compounds with extensive system of double bonds. AzaPcs may be used as indicators for metal cations[1,2] or pH sensitive indicators.[3] The principle of their fluorescence sensing properties is based on blocking of intramolecular charge transfer upon analyte binding that leads to increase of fluorescence.

This project studies the possibility to use the pH sensitive AzaPcs as the fluorescence indicators in optical sensor devices for CO_2 detection. First, conditions for immobilization of AzaPc indicator into several matrices were optimized (e.g. type of hydrogel, solvent). Polyethylene terephthalate support foil was then coated by "cocktail" containing AzaPc indicator, matrice, solvent and base in thickness of the 75 μm of the wet film. Finally, prepared foils were characterized by spectral methods (absorption, emission) and sensitivity towards CO_2 was examined.



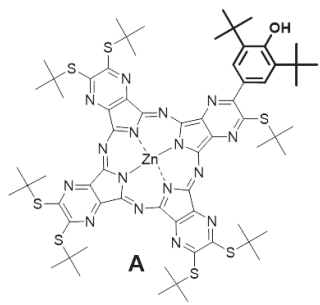


Fig. 1. Temperature dependency for compound **A** in Hydrothane 5 matrice

The study was supported by scholarship Aktion Austria-Czech Republic and SVV 260 183.

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SYNTHESIS OF SUBSTITUTED PYRIDINES BY GOLD(I) CATALYSIS

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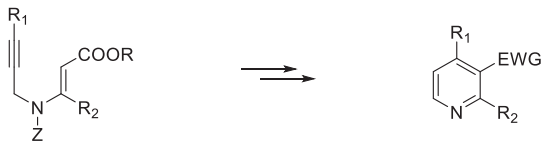
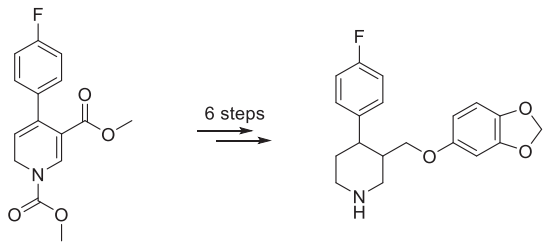
Synthesis of various types of heterocycles is possible from enyne precursors using cationic gold(I) species as a catalyst. In order to expand our research[1] on cyclisation of propargyl vinyl ethers to dihydropyrans using tris(2-furyl)phosphine gold(I) chloride (TFPAuCl) and silver tetrafluoroborate, we employed the same catalytic system on enynes with other heteroatoms. The synthetic protocol was optimized and a series of substituted nitrogen heterocycles synthesized. Crucial step of the synthesis is gold(I) catalysed endo-cyclization of 1,5-enynes. Compared with synthesis of pyrrols,[2] forming of dihydropyridnes was observed. The resultant dihydropyridine derivatives are potential precursors for paroxetine synthesis.[3]



R_1, R_2 = H, aryl, heteroaryl, alkyl
Z = Ts, MBS, Boc, -COOMe



This work was supported by Charles University (SVV 260 183 and GAUK 5671/2012) and Czech Science Foundation (Project No. 15-07332S).

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SYNTHESIS OF HAEMANTHAMINE DERIVATIVES

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Isochinolines alkaloids are one of the brodest class of the secondary metabolites and with more than 2500 known structures constitute very large class of alkaloids with various biological properties.[1] Among these class of isochinolines alkaloides, the amarylidaceae alkaloids represent large group of natural compounds with antitumor, antibacterial, antifungal, antimalarial, antiviral, analgetic, acetylcholiesterase and butyrylcholinesterase inhibitory activity.[2]

Haemanthamine is one of the isochinolines alkaloid of the Amarylidaceae family. It is 5,10-β-ethanophenantridine derivative, which displayed pronounced cytotoxic activity againts various cancer cell line such as MOLT-4, HepG2, Hela, MCF7, CEM, K562, G-36, Bj human fibroblast, A 549, OE21, Hs683, U373, SKMEL and B16F10.[3]

In this work several derivatives of Haemanthamine were prepared and their inhibitory activities on acetylcholinesterase and butyrylcholineesterase as well as cytotoxic activity will be studied.

The study was supported by Charles University (SVV 260 184).

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SIGNALING ROLE OF CERAMIDES AND THEIR METABOLITES IN MAMMALIAN EPIDERMIS

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Ceramides (Cer) are essential lipids, participating in the formation of the mammalian epidermal barrier in the uppermost layer of the skin, the stratum corneum. As a minor component, they can be found in the cellular membranes. Cer and their metabolites (Cer-1-phosphate, sphingosine-1-phosphate, glycosphingolipids) are also well known to have a signaling role. They regulate proliferation, differentiation and apoptosis in epider-

mal keratinocytes and modulates innate immune function. The modulation in the signaling role of these molecules can be applied to cutaneous disease prevention and therapy.[1]

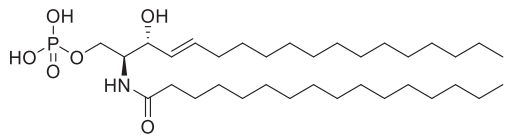

Fig. 1. Structure of Cer NS-1-phosphate.

This work focuses on the synthesis and evaluation of two Cer metabolites, Cer NS-1-phosphate and Cer NdS-1-phosphate. Few synthetic approaches have been published until now, but it's still a challenge to synthesize these compounds because of their low reactivity and high insolubility. For our synthesis, we decided to utilize a recently developed 3 steps synthesis, using a biphasic system for the introduction of the dimethyl phosphate group, followed by a hydrolysis to Cer-1-phosphate. The overall yield of this synthetic pathway is around 40% (even in the higher quantities, up to 100 mg).

The study was supported by the Czech Science Foundation (13-23891S) and by Charles University (SVV 260 183).

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BENZYLDERIVATIVES OF 3-AMINOPYRAZINE-2-CARBOXAMIDE: SYNTHESIS AND ANTI-INFECTIVE EVALUATION

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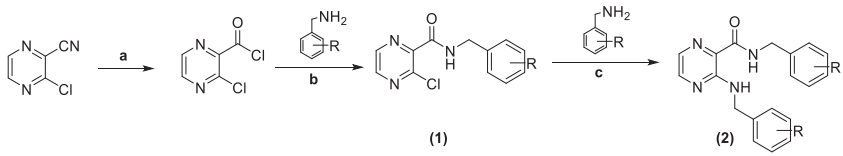
Pyrazinamide (PZA) is a first-line antitubercular drug and has been used for over sixty years. PZA has a significant role in shortening of tuberculosis treatment. As a small molecule PZA and metabolically derived pyrazinoic acid (POA) offer many ways how to affect mycobacteria: acidification of cytoplasm,[1] inhibition of trans-translation (liberation of ribosomes trapped in faulty protein synthesis),[2] inhibition of Fatty Acid Synthase I (synthesis of mycolic acids)[3] and aspartate dexarboxylase (involved in energetic metabolism).[4]

Series of substituted *N*-benzyl-3-chloropyrazine-2-carboxamides **(1)** and *N*-benzyl-3-(benzylamino)pyrazine-2-carboxamides **(2)** were prepared from the starting 3-chloropyrazine-2-carbonitrile *via* well known procedures.



Reagents and conditions:
a) 1. NaOH (aq); 2. acidification; 3. $SOCl_2$, DMF, PhMe, reflux
b) TEA, acetone, RT
c) pyridine, MeOH, microwave irradiation

R = 2-CH_3; 4-CH_3; 4-OCH_3; 2,4-OCH_3; 2-Cl; 3-Cl; 4-Cl; 2,4-Cl; 3,4-Cl; 2-F;4-F; 3-CF_3; 4-CF_3

Prepared compounds were characterized with analytical data and tested against four mycobacterial strains – *M. tuberculosis* H37Rv*, M. kansasii, M. avium* and *M. smegmatis*. Additionally, antibacterial and antifungal activity was determined. The most active compounds against *M. tuberculosis* (MIC = 12.5 μg mL^{-1}) were structures with double 3,4-dichlorobenzyl or 2-methylbenzyl substitution both in amide and amine moiety. Compund with 2-chlorobenzyl substitution in amide moiety showed the highest activity against *Streptococcus aureus* (MIC = 7.81 μmol L^{-1}). Structure-activity relationships within presented series and in comparison with previously published compounds will be presented.

The study was supported by the Grant Agency of Charles University, project B-CH/1594214, and SVV 260 183.

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NITRO GROUP-CONTAINING HETEROAROMATIC COMPOUNDS AS ANTITUBERCULAR AGENTS: STRUCTURE-ACTIVITY RELATIONSHIPS STUDY

VALÁŠKOVÁ, L.,[1] ROH, J.,[1] KARABANOVICH, G.,[1] NĚMEČEK, J.,[1] STOLAŘÍKOVÁ, J.,[2] VÁVROVÁ, K.,[1] KLIMEŠOVÁ, V.,[1] HRABÁLEK, A.[1]

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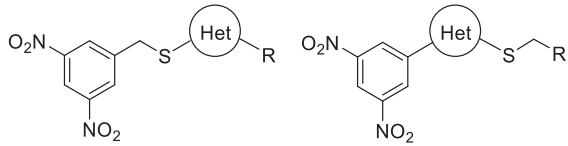
Tuberculosis (TB) is worldwide health and economic problem. Strains causing this infectious disease are becoming more and more resistant against therapy which has remained the same for more than 40 years. TB brings complication mostly in the underdeveloped world where higher incidence, poorer diagnosis and healthcare cause increased mortality and transmission of the infection as well as formation of resistant strains. A few totally drug resistant strains have appeared last years.

Our group has developed heterocyclic compounds containing 3,5-dinitrobenzyl/phenyl fragment (Figure 1). These compounds showed high activity against various mycobacterial strains, including resistant and dormant strains. Nitro groups are often carriers of increased toxicity, but not in case of our lead compounds. As a follow up to previous SAR study we examined the effects of various changes in 3,5-dinitrobenzyl/phenyl fragment to antimycobacterial activity.



Het = tetrazole, 1,3,4-oxadiazol
R = aryl, alkyl

Fig. 1.

This work was supported by the Charles University Grant Agency (project 361215/2015), Czech Science Foundation (project GAČR 14-08423S) and Charles University (project SVV 260 183).

SYNTHESIS OF TRICLOSAN DERIVATES AND THEIR ANTIMYCOBACTERIAL ACTIVITY

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Development of novel tuberculosis chemotherapeutics against existing drug resistant strains is based on several strategies.[1] One of them involves the identification and inhibition of enzyme drug targets. *Mycobacterium tuberculosis* enoyl-acyl carrier protein reductase (InhA) important in fatty acid biosynthesis pathway, is a target of the frontline chemotherapeutic, isoniazid (INH). The majority of INH-resistant clinical isolates arise from mutations in KatG, the enzyme responsible for activation of INH, into its active form. Thus compounds that inhibit InhA without first requiring KatG activation could be active against the majority of INH-resistant strains of *M. tuberculosis*. Triclosan is well known inhibitor of InhA and a widely used broad-spectrum biocide.[2] Recent progress in the development of novel diphenyl ether-based InhA inhibitors[3] inspired us to prepare a new series of triclosan esters.

We have synthesized 28 triclosan esters based on various aliphatic, alicyclic, aromatic and heteroaromatic acids by the Steglich esterification or direct acylation with various acyl chlorides in presence of trimethylamine. Prepared derivatives were evaluated for their *in vitro* antimycobacterial activity against *M. tuberculosis* $H_{37}Rv$, *M. avium* and two strains

of *M. kansasii*. The best *in vitro* activity was found for 5-chloro-2-(2,4-dichlorophenoxy) phenyl 4-bromobenzoate with minimum inhibitory concentrations (MIC) of 16 μmol/L against *M. tuberculosis* $H_{37}Rv$. Triclosan ester of isonicotinic acid showed the best activity against atypical strains. Its MIC values was comparable with INH for *M. kansasii* 6509/96 and better for *M. avium* and *M. kansasii* 235/80 strains.

The study was supported by SVV 260 183.

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SECTION OF PHARMACOGNOSY AND TOXICOLOGY OF NATURAL PRODUCTS

ALKALOIDS FROM *NARCISSUS* CV. PROFESSOR EINSTEIN (*AMARYLLIDACEAE*) – ISOLATION AND BIOLOGICAL ACTIVITY

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More than 500 *Amaryllidaceae* alkaloids (AmA) have been detected in plants of many different species belonging to the *Amaryllidaceae* family. These alkaloids showed wide range of biological activities. They are isolated from plant material and tested for their possible use in treatment of various illnesses. The most important AmA is galanthamin which is already used in the treatment of Alzheimer's disease as an inhibitor of human erythrocytic acetylcholinesterase (HuAChE; $IC_{50,HuAChE} = 1.5 \pm 0.2$ μM).[1] Some alkaloids display more biologic activities together. Active AmA serve as a template for a synthesis of series of semisynthetic analogues. Among the most widely used template AmA belong lycorine and haemanthamine.

From our previous screening on plants of *Amaryllidaceae* family, *Narcissus* cv. PROFESSOR EINSTEIN was chosen for detailed phytochemical work. Summary alkaloidal extract has been prepared from fresh bulbs (34.336 kg) and separated by column chromatography (Al_2O_3). Almost five hundred fractions were collected and, based on analytical TLC, pooled into 27 subfractions. Three substances were already obtained in crystallic form – isomer of hippeastrine, lycorine and haemanthamine, so far. Lycorine and haemanthamine have been already previously isolated at our department. They are currently used for the preparation of their semisynthetic analogues. Hippeastrine and other AmA which

are supposed, according GC/MS analysis, to be isolated in future will be screened for their biologic activity e. g. inhibition of HuAChE and HuBuChE (human butyrylcholinesterase), POP (prolyl oligopeptidase), GSK 3β (glycogen synthase kinase-3β), AKR1C3 (aldo-keto reductase 1C3) and others.

The study has been supported by SVV 260 184.

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ISOLATION OF ALKALOIDS FROM *NARCISSUS DUTCH MASTER* AND THEIR BIOLOGICAL ACTIVITIES

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Alzheimer's disease (AD) is one of the most frequent causes of dementia in the world. Deficit of the neurotransmitter acetylcholine (ACh) in the cortex participates on the development of the AD, which results in the damage of cholinergic functions, and this is responsible for the memory loss. Acetylcholinesterase (AChE) is enzyme which hydrolyzes ACh and terminates process on nerve impulse transmission. Second important enzyme is butyrylcholinesterase (BuChE) which hydrolyzes ACh and another esters. The level of AChE decreases during AD but level of BuChE increases. This fact is the reason for looking at new substances with inhibition of activity against both cholinesterases. Today, inhibition of AChE is the most important goal in the treatment of AD.

Amaryllidaceae plant family is source for structurally unique compounds, Amaryllidaceae alkaloids. Some of these alkaloids show biological activities as anticancer, anticholinesterase and antiviral. For this cause the Amaryllidaceae family is interesting goal in searching for active substances with various biological activities. Galanthamine, an Amaryllidaceae alkaloid is already used as inhibitor of AChE in therapy of AD.

The summary ethanolic extract was prepared from the fresh bulbs of *Narcissus Dutch* Master L. More than three hundred fractions were collected by column chromatography (on Al_2O_3). Fractions were pooled into 16 subfractions. So far, seven pure alkaloids have been isolated. The isolated compounds were identified as homolycorine, seco-isopowellaminone, lycorenine, oduline, masonine, haemanthamine and tetrahydromasonine by comparison with the literature data and results of MS and NMR studies. The alkaloids are screened for their biological activities (the inhibition activity against HuAChE and HuBuChE, prolyl oligopeptidase (POP), glycogen synthase kinase-3β (GSK 3β), aldo-keto reductase 1C3 AKR1C3).

ANTIPLATELET EFFECTS OF FLAVONOIDS ON ARACHIDONIC ACID BASED PATHWAY

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Flavonoids, common natural compounds of human diet, have a positive influence on various cardiovascular diseases according to experimental studies.[1] The aim of this study was to analyze anti-platelet activities of 29 flavonoids on three consecutive steps of the arachidonic acid cascade in detail. The isoflavonoids genistein and daidzein were shown to possess a marked cyclooxygenase-1 inhibitory activity, which was higher than that of acetylsalicylic acid using the isolated ovine enzyme, and physiologically relevant, although lower than in human platelets. Flavonoids with isolated 7-hydroxyl group and/or a 4′-hydroxyl group acted as antagonists on thromboxane A_2 (TXA_2) receptors. None of the tested flavonoids possessed an effect on TXA_2 synthase in a clinically achievable concentration.[2] An interesting finding is that the substitution of the free 7-hydroxyl group by a glucose did not block the activity. Several flavonoids inhibited two steps of the arachidonic acid pathway of platelet activation.

In conclusion, the consumption of flavonoids in food, particularly the isoflavonoids genistein and daidzein, may affect platelet aggregation.

The study was supported by grant of The Czech Science Foundation No. P303/12/G163.

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TRANSPORT MECHANISMS IN CELL MEMBRANE OF RED CLOVER

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The red clover (*Trifolium pratense*) from family *Fabaceae* contains several groups of secondary metabolites, which are used in medicine. Isoflavonoids belong to a group of phytoestrogens, because they can bind to corresponding hormonal receptors and substitute or block their activity.[1] These secondary metabolites are produced inside plant cell and they are stored in a vacuole or transported to other parts of the plant. They can also be excreted outside to the soil; some isoflavonoids can attract nitrification bacteria, which are typical

for *Fabaceae* plants. The plant cell has several mechanisms as ABC or MATE transporters, which can be used for transport of these metabolites.[2] Transport studies were performed with suspension cultures of *T. pratense*, var. DO-8, which were derived from a callus. The cell suspension was treated with metavanadate and vanadyl sulphate for increasing of secondary metabolites at first. The highest content of secondary metabolites were found after application of these elicitors in concentration of 10 μmol L^{-1} and cultivation for 24 hours. The various inhibitors of transport were added then and their effect on secondary metabolites concentration was observed in medium and suspension. Inhibitor of proton pumps sodium orthovanadate and calcium channel blocker verapamil inhibited transport of some isoflavonoids as genistin. NH_4Cl and ABC inhibitor probenecid had no effect. The study will continue with more inhibitors as brefeldin, which can inhibit vesicular transport.[3]

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ALKALOIDS OF *VINCA MINOR* L. AND THEIR EFFECT ON ACTIVITY OF SELECTED ENZYMES AS BENEFIT TO PROGRESS OF ALZHEIMER'S DISEASE

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Indole alkaloids are known as the source of cholinesterase inhibitors for the treatment of Alzheimer's disease.[1] 62 kg of dried aerial parts of *V. minor*, an ornamental plant from Apocynaceae family, were extracted in our laboratory. The mixture of the alkaloids (201 g) was chromatographed on alumina (total 531 fractions, combined in 16 fractions). The fractions 73–110, 128–146 and 147–214 were further separated using combination of flash chromatography and preparative TLC on silica-gel.

Two compounds were isolated from the fraction 73–110 and identified by GCMS and NMR as vincaminorine and vincaminorein.[2] Alkaloids (+)-minovincine, (+)-eburnamonine and vincorine were isolated from the fraction 128-146. Their structures were determined by NMR. Potentially new alkaloid named kosimonine was isolated from fraction 147-214. On its spectral characteristics (NMR, MS) and physical features (optical rotatory) is working presently.

All isolated alkaloids were tested for their inhibition activity on HuAChE and HuBuChE by Ellman's method.[3] Alkaloids exerted significant inhibitory of HuBuChE; the most potent inhibitors were vinkaminorein and vincorine with IC_{50} values of 71 ± 0.49 and 9.75 ± 0.45 μM respectively.

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PATHOBIOCHEMISTRY AND XENOBIOCHEMISTRY SECTION

SELECTED SESQUITERPENES FROM *MYRICA RUBRA* ESSENTIAL OIL POTENTIATED ACCUMULATION AND EFFICACY OF DOXORUBICIN IN CANCER CELLS.

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Our previous experiments have shown that *Myrica rubra* essential oil (MEO) inhibited proliferation of Caco 2 cancer cells. Selected sesquiterpenes, α-humulene (HUM), caryophyllene oxid (CAO), *trans*-nerolidol (NER) and valencene (VAL), form significant proportion of MEO.[1] All listed sesquiterpenes alone have ability to decrease cancer cell proliferation in concentration dependent manner with IC_{50} in range 24–57 μg/mL. To investigate possible enhancement of efficacy of doxorubicin (DOX), frequently used cytostatic, we tested combinations of DOX with HUM, CAO, VAL and NER. CalcuSyn software was used for distinction of synergism, antagonism and additive effect as it was described by Chou.[2] Based on the obtained results, CAO, NER and VAL seem to synergistically increase effect of DOX, while HUM has only additive effect. DCF assay showed ability of HUM, CAO, VAL to increase production of ROS. On the contrary, NER decreased ROS production in cancer cells. All of the listed sesquiterpenes increased accumulation of DOX in Caco-2 cells, with CAO being the most effective.

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EXPRESSION PROFILE OF DHRS1 IN HUMAN TISSUES

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Dehydrogenase/Reductase Member 1 (DHRS1) belongs to superfamily of short-chain dehydrogenases/reductases (SDR). Wide variety of endogenous and xenobiotic substrates are metabolised by some members of SDR superfamily. However from group of 75 human genes, only 20% of its members are regarded as well-characterized. DHRS enzymes are group of 17 enzymes from SDR superfamily that are generally poorly characterized, but there is first knowledge of a role of some members (e.g. DHRS4, DHRS7)[1] in metabolism of some xenobiotic or endogenous substrates.

The aim of this study is determination of expression pattern of DHRS1 in normal human tissues collected in short post-mortem interval. RNA was isolated from tissues and its integrity was checked by 3′:5′ assay.[2] DHRS1 on mRNA expression level was measured by real-time reverse transcription polymerase chain reaction with absolute quantification in 15 tissues collected from 4 males. The expression pattern on protein level was measured by western blot in whole tissue homogenates. Highest expression of DHRS1 mRNA was detected in liver and lower expression was also detected in thyroid, testis, kidney and adrenals, but expression on protein level was detected only in liver. These results represents part of effort to characterize previously unknown enzymes and tissue expression levels together with their *in vitro* activity may suggest their potential role in human organism.

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FIRST IDENTIFICATION OF DHRS1 EZYME AS SPECIFIC MOLECULAR TARGET OF ANTICANCER DRUG ORACIN

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For several millennia the human medicine is based on application of small bioactive molecules that are administered in the form of plant extracts or synthetic compounds. However, their use in modern medicine is not possible without a detailed understanding of their biochemical effects and identification of their molecular targets. Chemical proteomics based on the specific recognition between the bioactive molecule and the target molecule is currently the most widely used techniques for identification of molecular targets of small molecules.[1] Carbonyl-reducing enzymes, which play an important role in physiology due to their involvement in metabolism of various endogenous (e.g. prostaglandins, steroid hormones) and xenobiotic (e.g. anthracyclines, oracin) substrates, also represent such target biomolecules. Although the majority of today known carbonyl-reducing enzymes represent soluble proteins, there are many membrane-bound members in short chain dehydrogenases/reductases (SDR) superfamily. However, the knowledge on their role in metabolism of xenobiotics is quite poor. Based on the research on the reduction stereospecificity of anticancer drug oracin, there were predicted microsomal carbonyl-reducing enzymes involved in the metabolism and inactivation. However, previous attempts to purify these enzyme failed.[2]

The aim of this project was to develop suitable affinity carrier with immobilized ligand oracin capable to selectively purify carbonyl-reducing enzymes from human liver tissue and subsequently determine the potential role of theses enzymes in biotransformation.[3] Thus, affinity carrier was implemented into purification protocol of human microsomal carbonyl-reducing enzymes. Obtained fractions exhibited metabolic activity towards oracin with desired stereospecificity of its reduction. Using mass spectrometry proteins DHRS1, RDH16 and 17β-HSD6, with unknown affinity and metabolic activity towards oracin were successfully isolated and identified. Furthermore, enzyme 11β-HSD1 with already described affinity towards oracin was identified too. The selectivity of enzyme DHRS1 towards oracin was subsequently demonstrated by incubation of recombinant protein with affinity carrier and by Drug Affinity Responsive Target Stability (DARTS) method. The confirmation of DHRS1 specific interaction with this drug may indicate its potential role in biotransformation of other xenobiotics.

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CYCLIN DEPENDENT KINASE INHIBITORS AS MODULATORS AND SUBSTRATES OF ABC TRANSPORTERS

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Cyclin-dependent kinases play an important role in cell cycle regulation and their enhanced activity can lead to the development of various malignancies. Therefore, these kinases have become a rational target for inhibition in cancer therapy. ABC efflux transporters influence the pharmacokinetic properties of various drugs and their overexpression in cancer cells lead to multidrug resistance (MDR) against diverse cytotoxic therapeutics. Three members of the ABC transporter family play a prominent role in the pharmacokinetics and MDR: ABCB1 (P-glycoprotein), ABCG2 (breast cancer resistance protein) and ABCC1 (multidrug resistance-associated protein 1).

The aim of this work was to elucidate the interactions of novel CDKIs, dinaciclib and palbociclib, with ABC transporters using *in vitro* methods. Moreover, we aimed to determine whether these interactions affect the efficiency of conventionally administered anticancer drugs in human cancer cells.

Applying the MDCKII cell model and monolayer transport assays, we identified dinaciclib as an inhibitor of ABCC1, but at the same time a substrate of ABCB1 and ABCG2 transporters. Using the accumulation method in MDCKII cell lines overexpressing ABC efflux transporters we revealed inhibitory properties of palbociclib towards ABCB1 and ABCG2 and synergistic antiproliferative effect of both CDKIs when applied in combination with conventional cytotoxic drugs that are substrates of ABC transporters. Our data thereby show the ability of studied CDKIs to interact with ABC transporters as inhibitors and/or substrates and suggest that transporter-mediated drug-drug interactions (DDIs) should be taken into account, when introducing the CDKIs into anticancer pharmacotherapy. The DDI of CDKIS with conventional cytotoxic drugs caused by the inhibitory activity of CDKIs towards the ABC transporters can be exploited to battle the problem of MDR.

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NEWLY SYNTHESIZED ANALOGUES OF DEXRAZOXANE – INITIAL *IN VITRO* CHARACTERIZATION

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Anthracyclines (ANT) belong to the most frequently used antineoplastic agents. However, their cardiotoxic effect is still within the dose-limiting factors in clinical practice. Although known for decades, the mechanisms of ANT cardiotoxicity are still elusive. The only clinically available protective compound against anthracycline cardiotoxicity is dexrazoxane (DEX). Nevertheless, DEX use in the clinical practise is limited, mainly due to the anticipated risk of antagonism in the antineoplastic effects of DEX and ANT or possible adverse effects of DEX. These hurdles could be minimized by rational design of DEX analogues. Unfortunately, the precise mechanism of DEX cardioprotection or structure-activity relationship of DEX or other bis-dioxopiperazines have not been fully resolved. Although the traditional hypothesis presumes DEX metabolism to iron-chelating EDTA analogue ADR-925 with subsequent protection from the reactive oxygen species formation, the role of oxidative damage in ANT cardiotoxicity has been critically reviewed and is widely discussed. Both ANT and DEX also interact with topoisomerase II, which is present not only in cancer cells, but also in cardiomyocytes. Therefore, the aim of this study is the synthesis of structural analogues of DEX with changes to both the piperazine rings and connective chain with subsequent study of their biological activity. Currently, 20 new analogues have been synthesized and evaluated for their antiproliferative activity using human promyelocytic leukaemia cell line HL-60 and cardioprotection in the *in vitro* model of ANT cardiotoxicity on isolated neonatal rat cardiomyocytes, several analogues have been studied also for their ability to chelate free catalytically active iron ions and also for their ability to inhibit the catalytic activity of topoisomerase II or modulate its content in isolated neonatal cardiomyocytes.

The study was supported by the Czech Science Foundation (13-15008S).

STUDY OF EXTRACELLULAR VESICLES PRODUCTION AND THEIR ROLE IN PATHOGENESIS OF *CANDIDA ALBICANS* INFECTION

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Candida albicans (C. albicans) is a polymorphic fungus causing infections that range from superficial infections of the skin to life-threatening systemic infections. Although several virulence factors have recently been revealed, further investigations on molecular basis for the purpose of understanding the pathogenicity mechanism of *C. albicans* are required.

Within the last decade, special attention is paid to a new secretion system in gram-negative bacteria consisting in outer membrane vesicles secretion. This transport mechanism allows releasing molecules with various biological functions into host environment. These may include molecules carrying key functions for pathogenesis of microbial infections. In case of pathogenic yeasts, only the extracellular vesicles of one pathogenic yeast, *Cryptococcus neoformans,* were revealed and analyzed until now.

C. albicans yeasts (model strain, clinical isolates) were cultivated in Sabouraud broth (optimal nutrition composition) and in chemically defined minimal growth medium supplemented with limited amount of glucose (nutrition stress induction). *C. albicans* were cultivated into late exponential growth phase in 37 °C with gentle shaking. Concentrated sterile filtrates obtained after cultivation of different *C. albicans* strains in different conditions were ultracentrifuged and acquired pellets were submitted to observation *via* flurescent microscopy and tranmission electron microscopy. Preparation of samples for MS analysis and identification of protein cargo of *C. albicans* putative extracellular vesicles is now in process.

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BETA-NAPHTHOFLAVONE INDUCTION: ACTIVITIES, PROTEIN AND RNA LEVELS OF DRUG METABOLIZING ENZYMES

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Aryl hydrocarbon receptor (AhR) is a ligand-activated transcriptional factor and belongs to the family of proteins that mediates a variety of biological responses to environmental pollutants. AhR activates several metabolic and detoxification pathways, among

other xenobiotic metabolizing enzymes (XME). In our study, we examined time dependence of response of the mRNA, protein levels and activity of cytochrome P450 isoforms 1A1/2 (CYP1A1/2), glutathione S-transferase alpha (GSTA) and NAD(P)H:quinone oxidoreductase 1 (NQO1) to inducer and their correlation. To achieve this objective, the rat hepatocytes were exposed to β-naphthoflavone (BNF), one of the inducers of drug-metabolizing enzymes acting via AhR.

Rat hepatocytes were incubated with BNF for 2, 4, 12 and 24 hours. Activity, protein and mRNA levels of CYP1A1/2 were in good accordance and they continuously rise for 24 hours. Gradual increase was observed in the activity of NQO1 for 24 hours, while protein content was almost the same and mRNA level was highest two hours after treatment and it was slowly reduced to the control level during 24 hours. Activity of GSTA in BNF treated hepatocytes increased from 65% (4 hours) to 76% of controls during 24 hours. The level of GSTA mRNA in BNF treated hepatocytes was significantly increased two hours after treatment. During the rest of the experiment, determined levels of GSTA mRNA were close to levels in control hepatocytes. Protein levels of GSTA in treated cells are similar to the control ones throughout the whole experiment.

Taken together, each enzyme showed different response and correlation between levels of protein and mRNA expression and activity after BNF treatment, although they all are regulated via AhR.

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IN VITRO EVALUATION OF NON-AGREGATING (AZA)PHTHALOCYANINES – INFLUENCE OF PERIPHERAL SUBSTITUTION ON PHOTODYNAMIC ACTIVITY

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Photodynamic Therapy (PDT) and Vascular-Targeted Photodynamic Therapy (VTP) are two similar ways to treat localized non metastatic solid tumors. Both are using the same fundamental elements – photosensitizer (PS), molecular oxygen and visible red light. In PDT, PSs require time to be taken up by tumor cells before the irradiation. Red light, that has the highest permeability in tissues, is absorbed by PS. From the excited state, PS relaxes back to the ground state by emitting a photon (i.e. *via* fluorescence) or forming reactive oxygen species (ROS). The major pathway of cell demise depends mostly on the structure of PS and its subcellular localization. In PDT, subcellular structures of cancer cells are the primary targets of generated ROS. In VTP light activation starts while PS is localized in tumor vasculature causing damage of endothelium surface that leads to vascular injury and deprivation of nutrients and oxygen to the tumor. PSs involved in this study are

highly hydrophilic nonaggregating phthalocyanines[1,2] and azaphthalocyanines,[1,2,3] with absorption in near infra-red area (around 780 nm). Their photodynamic effect was studied on several cell lines – human cervical carcinoma (HeLa), melanoma (SK MEL 28), brest adenocarcinoma (MCF-7), lung adenocarcinoma (A549) in PDT or human immortalized endothelial cell line (EA.Hy926), human primary endothelial cells (HUVEC) and HeLa in VTP protocol respectively. Dark toxicity (without activating light) was also established on HeLa, EA.hy926, HUVEC and 3T3 (mouse nonmalignant fibroblast) cells. All the examined compounds were efficient PSs after irradiation with red light ($\lambda > 570$ nm, 12.4 mW/cm^2, 11.2 J/cm^2) reaching exceptionaly high photodynamic activity up to nanomolar concentrations while showing low inherent toxicity in the absence of light to both malignant and nonmalignant cells. PSs damage lysosomal (PDT) or plasma (VTP) membranes and are spread throughout the cell where they cause additional damage to other organelles. This leads to quick and efficient cell demise.

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GLUTATHIONE S-TRANSFERASE IN RUMEN FLUKE *CALICOPHORON DAUBNEYI*

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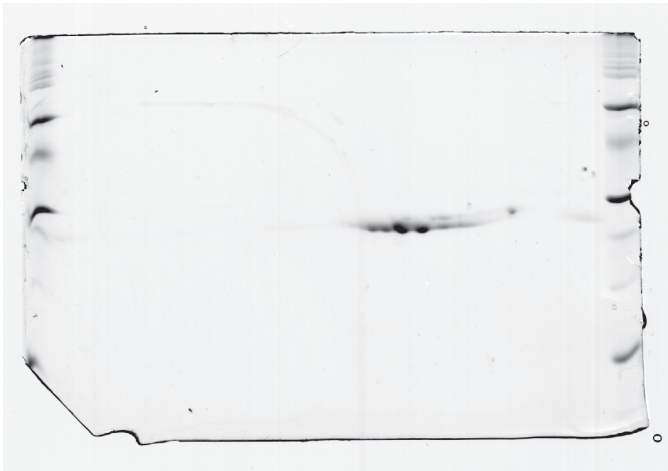
Flukes are diverse group of parasitic helminths, that live in various parts of animal or human bodies and causing harm to animals and considerable economic losses.[1] *Calicophoron daubneyi* is rumen fluke parasiting in gastrointestinal tract of various ruminants, e.g. sheep and cattle. Small numbers of adult parasites cause little or no damage but severe damage of the intestine is caused by juvenile flukes traveling through digestive system and can be dangerous in case of weakened animals or heavy parasite burdens. Although its danger is not so grave as in the case of liver flukes the prevalence of rumen flukes has increased in previous years.[2] Our research was focused on glutathione S-transferases (GST), group of common eu- and xenobiotic metabolizing enzymes.[3] GST is also posible candidate for future vacines against flukes.[4] First extracts were prepared from rumen flukes, purified and activities assesed, then the samples were analyzed *via* 2D electrophoresis with coomassie staining, imunodetection and finally spots were analyzed by mass spectrometer.

It was found that GST activities in extract (cytosol-like) were about 0.7 µmol/min/mg and specific activity was increased with purification about 10 times. Glutathione agarose was more effective in GST purification than S-hexyl glutathione agarose. Several spots reacted with anti-Mu-GST and anti-Sigma-GST antibodies from other flukes. Finally the MS experiment confirmed similarities to the Sigma class GST of liver fluke *Fasciola hepatica.*



The study was supported by Charles University, research project SVV 260 186.

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IRON METABOLISM OF THE TUMOUR-INITIATING CELLS

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An important role of iron in cancer growth and progression has been documented by studies reporting higher iron uptake due to altered metabolism in cancer cells. The con-

cept of tumour-initianting cells (TICs), also known as cancer stem cells (CSCc), claims that only a small portion of these cells is able to initiate the tumour growth and cause the formation of secondary tumours. The presence of TICs within tumour might be the reason for the failure of many conventional cancer therapies because of their higher resistance to anti-cancer drugs and/or apoptotic stimuli. It is known that there is higher iron requirement in proliferating cancer cells compared to normal ones and targeting iron metabolism with iron chelators can lead to apoptosis and cancer cell death, while supplemeting iron can block induction and promote their growth, but there are no data concerning TICs.

In our study we are using several specific culture conditions which enable cancer cell lines to grow in spheres exhibiting of 'stemness' markers – ABCG2, CD44, CDH2 and CD133. Our preliminary data show considerable differences in iron metabolism and in the expression of genes related to iron metabolism in TICs for example *ACO1, GLRX5, TFRC*, *QSOX1* and *TMPRSS6*.

We have generated genetically modified cell lines that can inducibly overexpress these genes upon doxycycline addition. This provide us with a tool to define the role of differentially expressed iron metabolism-related genes on cellular proliferation, migration and contribution to stem cell phenotype. Furthermore, the most significantly altered genes will be tested also *in vivo* model by using xenotransplantation in nude mice.

The study was supported by the Czech Science Foundation 13-28830S to J.T. and BIOCEV CZ.1.05/1.1.00/02.0109 from the European Regional Development Fund.

RIBOCICLIB AND AZD5438 MODULATE ABC TRANSPORTER-MEDIATED MULTIDRUG RESISTANCE *IN VITRO*

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Drug transporters from the ATP-binding cassette (ABC) family actively efflux wide range of substrates including drugs out of the cells diminishing thereby their intracellular concentration under cytotoxic levels. Overexpression of ABC transporter members ABCB1, ABCG2 and ABCC1 leads to development of multidrug resistance (MDR) in cancer cells resulting in treatment failure.[1] The aim of our study was to characterize the ability of ribociclib and AZD5438, the novel anticancer cyclin-dependent kinase inhibitors (CDKi) to modulate MDR caused by ABCB1-, ABCG2- and ABCC1-mediated efflux.

In order to investigate the inhibitory potential of CDKi to the ABC transporters, we performed Hoechst 33342 and daunorubicin (DNR) accumulation assays in MDCKII cells stabily overexpressing ABCB1, ABCG2 and ABCC1. Subsequently, we evaluated whether the studied CDKi affect sensitivity of overexpressing cells to the anticancer substrates DNR and mitoxantrone (MIT) by employing XTT proliferation assay.

Our accumulation studies revealed AZD5438 as inhibitor of all three studied ABC transporters, with highest inhibitory potency to ABCC1. Ribociclib was shown as inhibitor

of ABCB1 and ABCG2, not affecting ABCC1 activity. Proliferation assays further demonstrated that ribociclib possesses the ability to sensitize ABCB1 and ABCG2 overexpressing cells to DNR and MIT, respectively, while AZD5438 potentiated DNR cytotoxic properties in MDCKII-ABCC1 cells. We therefore confirm the studied CDKi as modulators of *in vitro* ABC transporter-mediated MDR.

The study was supported by GAUK 344315/C/2015 and by SVV/2015/260-185.

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A PROMISING ROLE OF HUMAN DHRS7 IN THE METABOLISM OF RETINOIDS AND STEROIDS?

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In humans, 75 short-chain dehydrogenases/reductases (SDR) have been identified, so far. Some of them have received considerable attention because of their crucial role in the metabolism of various compounds such as steroids and retinoids that are involved in a broad spectrum of physiological processes. However, significant portion of SDR members remains without any assignment of function.

Dehydrogenase/reductase (SDR family) member 7 (DHRS7) belongs among such poorly characterized SDR proteins belongs. The enzyme is a member of cluster 3 of "classical" SDR; such members are considered to be retinoid and steroid metabolizing enzymes. The aim of this study is to provide evidence for DHRS7 to be a player in the metabolism of these signalling molecules. Recently, we determined DHRS7 to be an integral membrane protein of the endoplasmic reticulum facing the lumen which has shown at least *in vitro* NADPH-dependent reducing activity toward physiologically important substrates such as androstene-3,17-dione, cortisone and all-*trans*-retinal as well as several xenobiotics bearing a carbonyl moiety. Expression patterns of DHRS7 at the mRNA as well as protein level were evaluated in a panel of various human tissue samples. DHRS7 is expressed in tissues such as prostate, adrenal glands, liver or intestine, which could correspond with proved *in vitro* catalytic activities. These results will lay the foundation for an understanding of DHRS7 role in human (patho)physiology.

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SECTION OF CLINICAL AND SOCIAL PHARMACY

STORE MEDICAMENTS AND PHARMACEUTICAL INGREDIENTS IN GHETTO TEREZÍN DURING SECOND WORLD WAR

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Theresienstadt Ghetto, also known as Theresienstadt concentration camp was established by the Nazis in n the fortress and garrison city of Terezín. More than 150,000 other persons were held in Theresienstadt before being seen sent by rail transports at Treblinka and Auschwitz extermination camps in occupied Poland, where they killed. Tens of thousands of people died there, some killed outright and others dying from malnutrition and disease.

Terezín's main hospital was located in a large barrack which was built in 1780 to service military and civilian populations and during World War II became a Jewish hospital. Terezin was a pharmacy, too. It was located in building Q412. In the archives of the Jewish Museum there are several documents related to this pharmacy. They are: daily report on labour deployment in Terezín 1943–1945 (the back of the first document contains the numbers of prominent Jews in the various sectors), graph showing the expenses of the pharmacy in L 305 in Terezín for February 1944, graph showing the expenses of the pharmacy in L 305 in Terezín for February 1944, inventory of the Central Medical Supplies Warehouse in Terezín and notifications of receipt of goods 1942–1945 and notifications of receipt of goods from the central medical supply warehouse to the Economics Department in Terezín 1944.

Most important of them is an inventory of the Central Medical Supplies Warehouse in Terezín and notifications of receipt of goods 1942–1945. This list of medicaments is an important source of knowledge about equipment pharmacies in Terezín. A number of Jewish apothecaries from the Czech lands worked in Terezín pharmacy, but also from abroad.

From the Czech Jewish pharmacists I can mention PhMr. Josef Freund, PhMr. Karel Kürschner, PhMr. Marie Sandová, pharmacy student Ilsa Steinbergová, PhMr. Leo Duschak, student pharmacy Hanna Pachnerová and numerous other. From Germany, for example, it was PhMr. Fritz Silten, owner of pharmacy Kaiser Fridrich Apotheke in Berlin. PhMr. Karel Kürschner was a manager of pharmacy from 1941 to 1944.

In 1944 (23th June) International Committee of the Red Cross visited Terezín. During his inspection, they check the pharmacy and surprised them equipment of pharmacy. Pharmacy, as well as the whole city was just a fake backdrop Nazi prepared for the visit of the Commission. The truth was completely different.

The study was supported by SVV 260 187.

ANTIBIOTIC USE PRACTICES BY PHARMACY STAFF: A CROSS-SECTIONAL STUDY IN SAINT-PETERSBURG, RUSSIAN FEDERATION

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The global emergence and spread of antimicrobial resistance remain a major infection control challenge and a predominant reason for therapy failure. Non-prescription access to antimicrobials is common and self-prescribing is increasingly popular in Russian society. The aim of this study was to assess attitudes of community pharmacists related to antibiotics use and self-medication.

We conducted a cross-sectional study of community pharmacists in Saint-Petersburg and Leningrad region, Russia (n = 410). Personal interviews were conducted using a self-administered questionnaire. The data were analysed using logistic regression and Pearson chi-squared tests.

Of the total of 316 pharmacists (77.07%) who completed the questionnaire, 241 (76.3%) self-medicated with antibiotics. Antibiotics were mostly used to self-treat upper (53.3%) and low (19.3%) respiratory tract infections relying on own knowledge (81.5%), previous treatment experience (49%) and patients' prescriptions (17%). The most commonly used antibiotics were macrolides (33.18%). Characteristics such as age, education and experience were shown to be related to antibiotics use and self-medication.

The study confirms that self-prescribing of antibiotics is a common practice amongst pharmacists in Saint-Petersburg. Pharmacists' personal and professional traits strongly associated with self-medication were identified.

The study was supported by SVV 260 187.

SAFETY OF DIRECT ORAL ANTICOAGULANTS IN PATIENTS WITH CHRONIC RENAL FAILURE

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Introduction: For many decades, the vitamin K antagonists (VKAs) have been the only oral anticoagulant drugs available for clinical use for the primary and secondary prevention of venous and arterial thromboembolic events. More recently, direct oral anticoagulants (DOACs), namely the direct thrombin inhibitor dabigatran etexilate and the direct factor Xa inhibitor rivaroxaban and apixaban, have been approved for clinical use. Because of their greater specificity, rapid onset of action, and predictable pharmacokinetics, DOACs address several limitations VKAs in day-to-day clinical practice. However, a range of practical questions relating to DOACs including topics such as patient selection, treatment of patients with renal failure.[1]

Objective: The aim of the project was to determine safety of DOACs in patients with renal failure.

Methods: We conducted an observational analysis of 25 patients with chronic kidney disease, eGFR < 60 ml/min.Patients were > 18 years and had stable renal function, and they were > 3 months after renal transplantation, the use of DOACs.

Results: Patients were screened between Januar 1 and December 30, 2015. A total of 25 patients were screened. By three patients had dose reduction during treatment due to a change renal function. Only one patient had dose reduction due to bleeding.

Conclusion: All DOACs are excreted by the kidneys, meaning that in patients with renal failure, the concentration of anticoagulant and its effects increase. Fluctuating kidney function may explain the increased bleeding with DOACs. Monitoring of adverse effects is essential during therapy by patients with renal failure.

The study was supported by 2015 SVV 265 005.

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PATIENT ACCESS GAP: THE DELAY BETWEEN MARKETING AUTHORIZATION AND REIMBURSEMENT DECISION ON NEW DRUGS

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In the Czech and other European healthcare systems, most innovative treatments, including drugs, are being made available to patients only after their inclusion on the list of products or services covered (reimbursed) by the public health care insurance. The national processes on drug pricing and reimbursement are usually strictly regulated, and local legislations must also meet the Transparency Directive 89/105/EEC. In the real world, however, there are still significant challenges to the availability of new and innovative treatments given the complexity and stringency of the drug pricing and reimbursement procedures and also due the attractiveness of the local market itself for the drug manufacturer.

The aim of the present study is thus to evaluate the current availability, i.e. positive reimbursement status, of recently authorized new/innovative drugs in the Czech Republic (CZ), and assess the delay between authorization date and reimbursement decision date.

All centralized procedures with positive decisions on new orginal drugs issued by European Commission between January 2007 and December 2015 were collected. Consequently, the inclusion of the respective drugs on the national CZ reimbursement lists and/or positive reimbursement decision availability were checked, including the timelines.

Of the 348 authorized new drugs issued by European Commission, 164 drugs (47%) have ever been reimbursed in CZ. The average length of the CZ national pricing and reimbursement procedure is 302 (±194) days, while the average overall delay between EU marketing authorization date and CZ reimbursement decision is 585 (±294) days.

The length of the pricing and reimbursement procedure apparently exceeds the limits set by the national legislation and Transparency Directive 89/105/EEC. The next step will be benchmarking CZ and other EU countries concerning the time delay between marketing authorization and reimbursement granting.

THE EFFECT OF NUTRITIONAL SUPPORT ON THE BODY FLUID VOLUMES AND ON CLINICALLY SIGNIFICANT ANTHROPOMETRIC PARAMETERS IN CRITICALLY ILL PATIENTS

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Many hormonal and metabolic changes are developed in critically ill patients and result in disruption of fluid balance, which is a risk factor for morbidity and mortality in these

patients. Therefore evaluation of fluid balance is one of the basic approaches of correct care in these patients. However the effect of nutritional support on the fluid balance is not specifically known. Another risk factor for prolongation of the period of ICU stay and higher mortality is the loss of muscle mass. The aim of this study was to evaluate the influence of administered nutritional support on those selected parameters in critically ill patients. The study included 14 patients in which was performed 32 examinations using indirect calorimetry and bioelectrical impedance spectroscopy. The average age of the patients was 45.29 ± 18.34 years. Using correlation analysis there has been demonstrated the effect of nutrition on the total fluid output and urine volume. Nutritional intake of energy in kcal d^{-1} ($p = 0.0045$; $r = 0.4890$), carbohydrate intake in g d^{-1} ($p = 0.0099$; $r = 0.4490$) and intake of lipid in g d^{-1} ($p = 0.0208$; $r = 0.4200$) significantly increased production of urine in ml d^{-1}. Only intake of protein ($p = 0.0010$; $r = 0{,}5540$) and carbohydrate ($p = 0.0070$; $r = 0.4676$) decreased the muscle degradation. Application of this knowledge can increase the effectiveness of parenteral and enteral nutrition and contribute to decrease the risk of morbidity and mortality in patients. The effects of the composition of the nutritional support will be validated in following validation study.

The study was supported by MH CZ - DRO (UHHK, 00179906), PRVOUK P40 and SVV/2015/260187.

THE EFFECT OF ENERGY AND NUTRITIONAL SUBSTRATES INTAKE ON THEIR OXIDATION AND THE ENERGY EXPENDITURE IN POLYTRAUMA PATIENTS

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The optimal amount of supplied energy and nutritional substrates is currently the subject of many professional debates and it is not well known. It is because the metabolism of these patients is completely different from healthy people and varies significantly throughout the critical state. The aim of this project was to find out how the intake of energy and nutritional substrates may affect energy expenditure (EE) and substrate oxidation (SO) in these patients.

The study was performed on 14 polytrauma patients (11 men and 3 women) with 32 examinations. Their mean age was 45.29 ± 18.34 years. Results were obtained after at least 4 hours nutritional support asdministration. REE and SO were measured by indirect calorimetry.

The intake of energy in kcal kg^{-1} d^{-1} ($p = 0.0084$; $r = 0.4582$), carbohydrates in g kg^{-1} d^{-1} ($p = 0.0185$; $r = 0.4141$) and lipids in g kg^{-1} d^{-1} ($p = 0.0208$; $r = 0.4200$) sig-

nificantly influenced EE in kcal kg^{-1} d^{-1}. Only the lipids intake in g kg^{-1} d^{-1} increased the oxidation of lipids in g kg^{-1} d^{-1} ($p = 0.0041$; $r = 0.5085$).

It was demonstrated that the amount of administered nutritional substrates was not proportional to the organism needs. This knowledge can be applied in clinical practice for optimization of the nutritional support composition and thus help to reduce the risk of morbidity and mortality in critically ill patients.

The study was supported by MH CZ – DRO (UHHK, 00179906), PRVOUK P40 and SVV/2015/260187.

SAFETY ASSESSMENT OF FOOD ADDITIVES IN TOP-SELLING DIETARY SUPPLEMENTS IN THE CZECH REPUBLIC

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Food additives find use not only in the food industry but also in the pharmaceutical sector. They are present mainly in medicines and in dietary supplements (DS). Adverse reactions of food additives are in the general population 0.01–0.23%, higher in atopic individuals (2–7%).[1]

The aim of this study is to evaluate the frequency of potentially harmful excipients in the best selling dietary supplements in the Czech Republic and to consider their adverse effects. In total, 418 DS were identified, including 54.8% DS for children under 12 years of age. Of these, 66.7% contained at least one additive known to have a negative health effect. On average, there were five additives per DS. The most frequently reported additives were glycerol (28.7%) and titanium dioxide (26.3%). The most commonly found potential adverse effects caused by additives as reported in the literature were gastrointestinal symptoms (50.96%), hypersensitivity reactions (31.34%), or attention-deficit/hyperactivity disorder (6.5%). Food additives should be safe if the acceptable daily intake is complied with; however, some individuals can experience immediate effects (headaches etc.) or long-term effects.[2]

The study was supported by SVV 260 187.

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MEDICATION ADHERENCE TO SEVERE CHRONIC OBSTRUCTIVE PULMONARY DISEASE (COPD)

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Inhalation drugs are the basics of pharmacological treatment of COPD. Sufficient adherence to the treatment, including proper application technique and choice of a suitable inhalation system are key factors in the effectiveness of the treatment. The major problem is failure to adhere to application technique. The objectives of the study are to assess adherence to inhaled medication with emphasis on application technique (inhalation adherence) and to study correlates of the adherence among patients with severe COPD in clinical practice.

An observational multicentre study with the participation of 12 centres in the Czech Republic was conducted in cooperation with the Czech Multicentre Research Database of COPD. Eligibility criteria were as follows: diagnosis of COPD and post-bronchodilator FEV1 $\leq$ 60%. The assessment was structured into five steps to be followed while using an inhaler. Adherence to each step was assessed in a dichotomous manner.

A total of 234 patients were available for the analysis of adherence. The assessment of the adherence to application technique revealed that less than 32% of the cohort adhered properly to each of the five steps. For most types of inhalation systems, the highest rate of failure was observed in step 3 (failure to breathe out completely in one breath before taking the medicine with the next breath). Errors in application technique were associated with depression, exacerbations and inspiratory fraction (IC/TLC).

Only one third of patients with COPD was fully adherent to inhalation therapy. The most common error was incomplete breathing out before taking the medicine.

The study was supported by project SVV 260 187. The COPD project is registered in ClinicalTrials.gov with the identifier NCT01923051.

NEGATIVE EFFECTS OF ANTIEPILEPTIC DRUGS ON BONE HEALTH

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Negative effects of antiepileptic drugs (AEDs) on bone health are one of the most significant and discussed side effects. More than half of the adult patients with epilepsy treated with "older" antiepileptic drugs have abnormal values of bone mineral density (BMD). Osteopenia was found in many of these cases, osteoporosis was diagnosed to lesser extent. The effect of new generation of AEDs is unknown. Especially in children with epilepsy there is a lack of reliable data on the effects of AEDs on bone. When compared to older AEDs newer AEDs in general have better safety profile with comparable efficacy. However some evidence exists on association between AED medication and decreased BMD in children. Since AED medication usually means long time treatment it is necessary to bring clear information on the effects of AEDs on bone.

The aim of this study was to measure BMD in children treated with AED monotherapy, compare the effects of three commonly used AEDs on BMD and consider some life style aspects with possible influence on bone health. Children between 3–18 years who were receiving either topiramate (TPM) or lamotrigine (LTG) or valproic acid (VPA) for at least 12 months and who were not treated with any other AEDs in the past were enrolled in the study. BMD was examined by dual roentgen absorptiometry (DEXA). Daily calcium intake, physical activity and time of staying outside were assessed by questionnaire and interview.

In the group of 28 children (mean age 179 months, mean treatment duration 38 months) 10; 10 and 8 children were treated with VPA; LTG and TPM, respectively. Osteopenia was found in 8 (28.6%) children – 4 with VPA, 2 TPM and 2 treated with LTG. Z score indicating osteoporosis was not found in any of them. BMD was significantly correlated only with age and BMI (Spearman coef. 0.78, resp. 0.49, $p < 0.05$). Decreased z score was found in children with low physical activity.

According to the cross-sectional design of the study and low number of participants we cannot estimate the risk of particular AEDs on decreased BMD. Further investigation is necessary to find out the impact of AEDs on bone health. However we suggest that regular screening of BMD in children treated with AED therapy should be recommended.

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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.

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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.

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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.

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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.

The study was supported by the Charles Univerzity, SVV 260 294.

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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.

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

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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.

The study was supported by Charles University (project GA UK No. 772216), Faculty of Pharmacy (SVV 260 295) and PRVOUK P40.

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.

The study was supported by SVV 260 293.

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.

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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.

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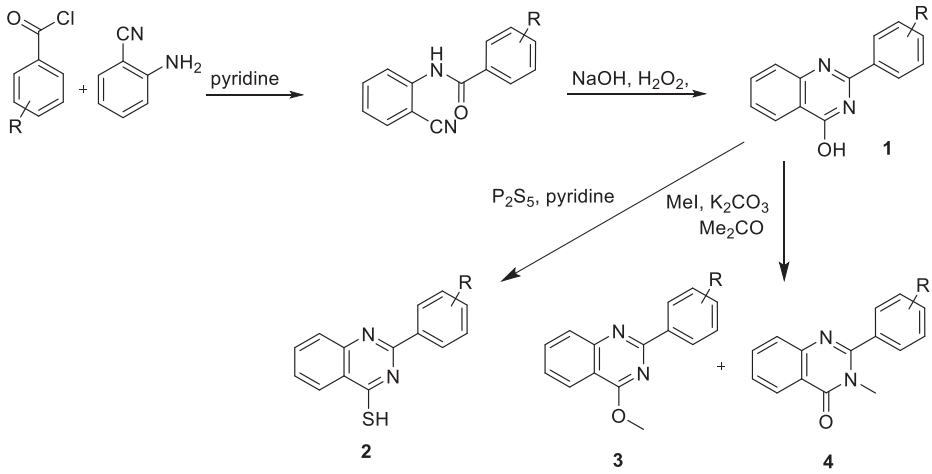
This project deals with a synthesis of 2,4-disubstituted quinazoline derivatives with the affinity to CAR receptor. CAR receptor (constitutive androstane receptor) is a nuclear recep-

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).

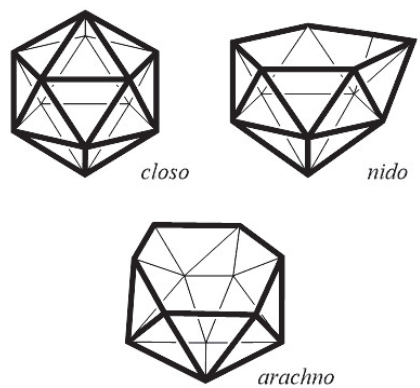

Fig. 1. Structures of boron clusters compounds.

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.

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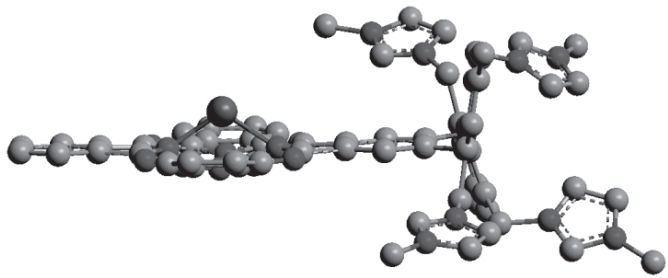
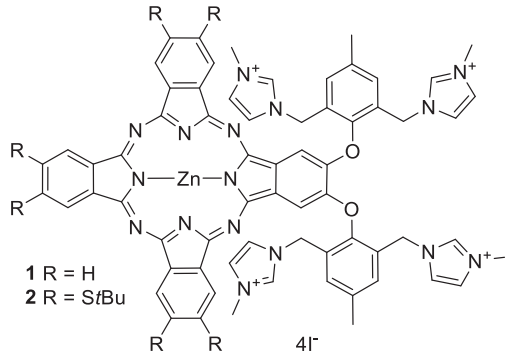
The photodynamic therapy is a curative method of cancerous and non-cancerous diseases. It uses light, oxygen and photosensitizer to destroy the cancer cells. Photosensitizer absorbs energy of light and produces singlet oxygen. Singlet oxygen attacks cellular structures like cellular membrane, lysosomes,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

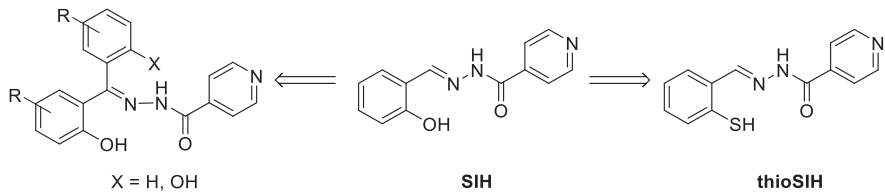

Fig. 1. Structure of SIH and its analogues studied in this work.

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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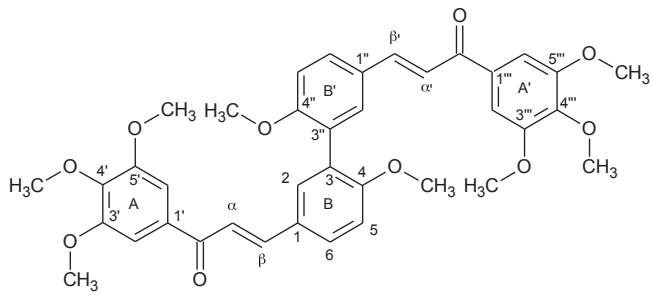
With more than 3 million new cases and 1,7 million deaths each year, cancer represents the second most important cause of death and morbidity in Europe.[1] Search for new anticancer agents is one of the most important ways towards significant advancements in cancer therapy. At present, 50% of anticancer 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.

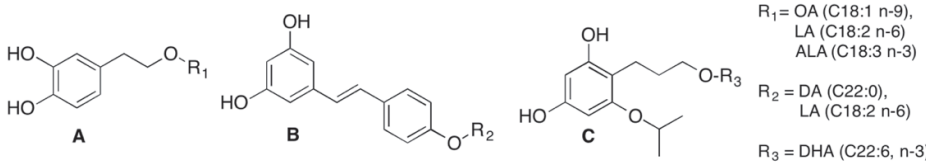

Fig. 1. Target lipophenolic derivatives of hydroxytyrosol (A), resveratrol (B) and phloroglucinol (C).

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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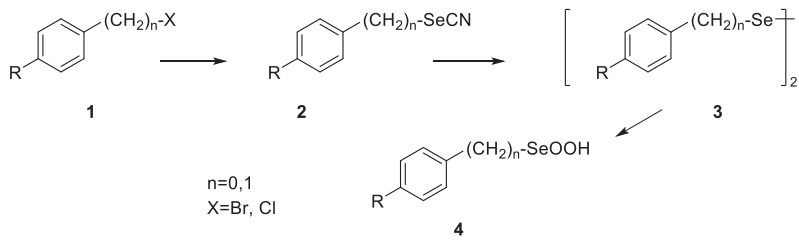
Selenium containing organic compounds have been known to possess promising *in vitro* antiparasitic activity against *Leishmania* infection. Various selenocyanate **2** and diselenide **3** derivatives with different functional groups in the aryl ring and in the aliphatic chain, displaying promising 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.

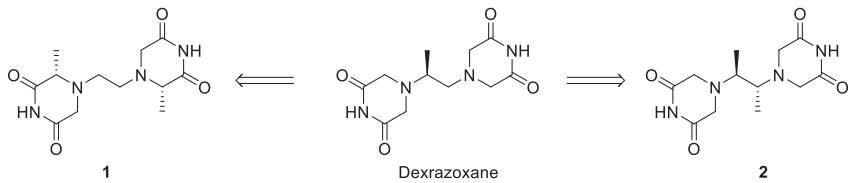

Fig. 1. Structure of DEX and its intended analogues **1** and **2**.

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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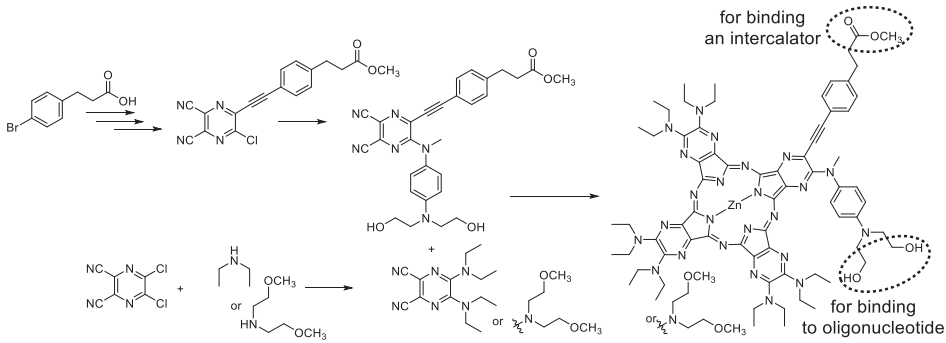
Azaphthalocyanines (AzaPc) are planar macrocyclic compounds. The alkylamino substituted derivatives can be used as dark quenchers in DNA-hybridization assays. The probe usually contains a quencher and a fluorophore. If these two moieties are close enough, the emitted fluorescence is quenched by the quencher. 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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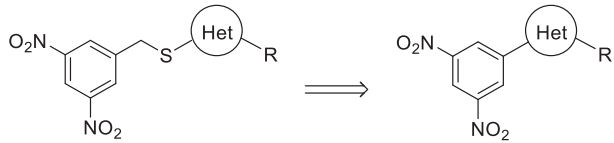
Tuberculosis (TB) is widespread infectious disease predominantly caused by *Mycobacterium tuberculosis* (*M.tb*.). According to World Health Organization, estimated 9.6 million new TB cases and 1.5 million deaths from TB were registered worldwide only in 2014.

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.

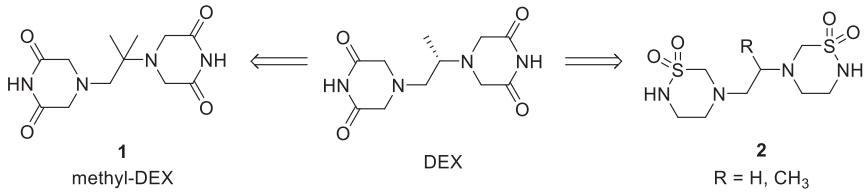

Fig. 1. Structure of dexrazoxane (DEX) and its analogues **1** and **2**.

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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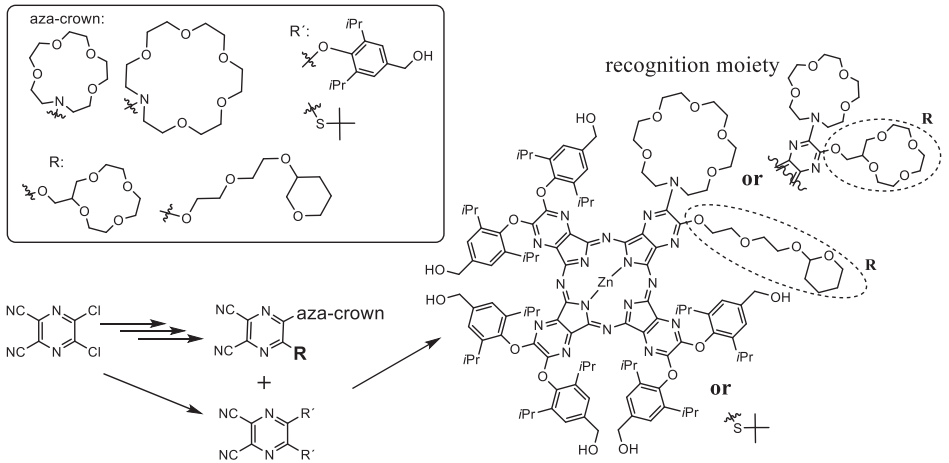
The impact of azaphthalocyanines (AzaPc) bearing aza-crowns on the periphery to be used as fluorescence sensors for metal cations has already been demonstrated in our previous works.[1] The great advantage of these compounds is the emission in the red part of the spectrum because such light is not absorbed 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.



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SYNTHESIS OF PHENOL-BASED PH SENSORS

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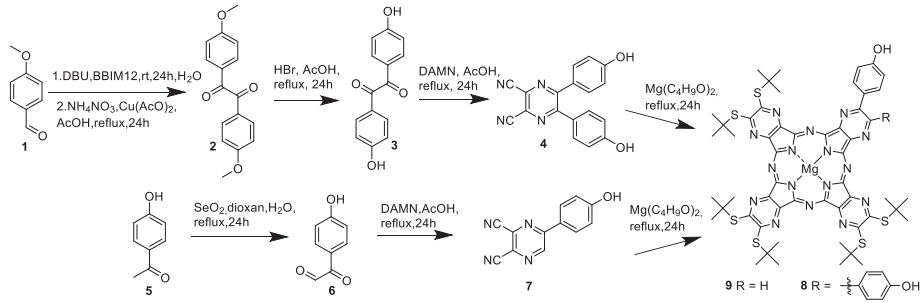
Tetrapyrazinoporphyrazines (TPyzPzs) bearing suitable phenol moieties were found to be potentially useful pH sensors.[1] In this study, we focused on expanding the series of phenol derivates synthesized previously. The main reason of our effort was the fact, that pK_a of previous compounds (12.5–12.7) 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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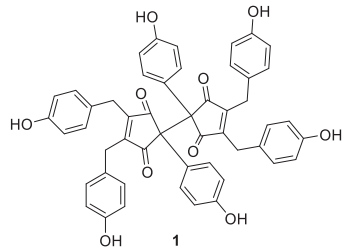
Growing demand for novel highly effective antimicrobial agents is one of the prevailing trends in current medicinal research due to increasing antimicrobial resistance. Particularly promising anti-infectives have been found among naturally occurring products providing us with a broad spectrum of lead 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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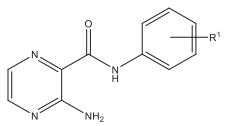
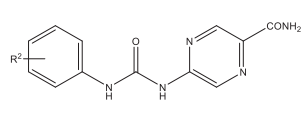
Tuberculosis (TB) is a major global health problem. In 2014, there were 1.5 million TB deaths.[1] Resistant tuberculosis-forms, namely multi-drug resistant (MDR) constitute a serious problem and emphasize the need for novel antitubercular drugs. Pyrazinamide (PZA) is the first-line antituberculotic drug, 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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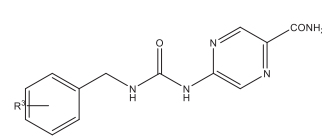

(3)

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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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).

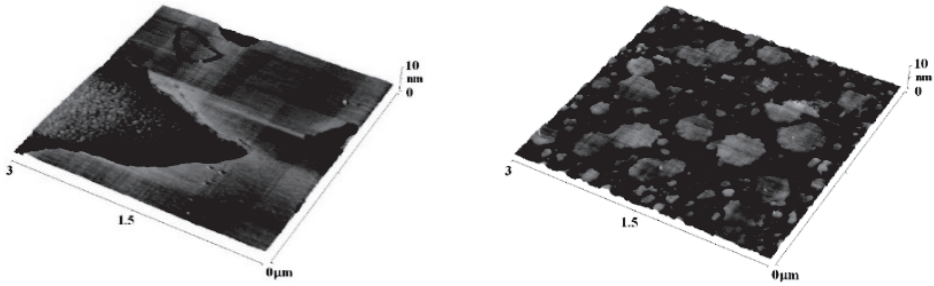

Fig. 1. Model of SC without GluCer (left) and separated domains of the model with 75% GluCer (right).

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.

The study was supported by SVV 260 291.

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]

The study was supported by SVV 260 291.

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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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MLADĚNKA, P., HRDINA, R., FILIPSKÝ, T., ŘÍHA, M., PALIČKA, V.: Is a Highly Linear Relationship Between the Dose of Quercetin and the Pharmacological Effect Possible? – A Comment on Liu, et al. Evaluation of Antioxidant and Immunity Activities of Quercetin in Isoproterenol-Treated Rats. Molecules 2012, 17, 4281–4291. Molecules, 19 (7), 2014, 9606–9609.

PROCEEDING PAPERS

KUNEŠ, M., KVĚTINA, J., TACHECÍ, I., KOPÁČOVÁ, M., BUREŠ, J., NOBILIS, M., KREJCAR, O., KUČA, K.: Imaging and Evaluating Method as Part of Endoscopical Diagnostic Approaches. In: Inteligent Information and Database Systems: 6th Asian Conference, ACIIDS 2014, Bangkok, Thailand, April 7–9, 2014, Proceedings, Part II. Cham: Spriger, 2014, 605–614. ISBN 978-3-319-05458-2.

MONOGRAPHIES

CAHLÍKOVÁ, L., MACÁKOVÁ, K., BENEŠOVÁ, N., CHLEBEK, J., HOŠŤÁLKOVÁ, A., OPLETAL, L.: Natural Compounds (Small Molecules) as Potential and Real Drugs of Alzheimer‘s Disease: A Critical Review. In: Studies in Natural Products Chemistry. Oxford/Amsterdam: Elsevier, 2014, 153–194. ISBN 978-0-444-63281-4.

DOHNAL, F.: Etablování, hlavní rysy vývoje a přední osobnosti vědecké práce v podmínkách československého vojenského zdravotnictví let 1918-1938 (Establishment, the main features of the development and leading personalities of scientific work in the conditions of the Czechoslovak military health service in the years 1918-1938). In: Věda a technika v českých zemích mezi světovými válkami. Prague: National Technical Museum, 2014, 401–413. ISBN 978-80-7037-245-6.

DOHNAL, F.: V předvečer první světové války. Přeměny vojenského zdravotnictví Rakousko-Uherska v letech 1866-1914 (On the Eve of the World War I. Transformations of the Military Health Service of Austria-Hungary in the 1866-1914 Period). In: Zdraví a nemoc v dějinách člověka a zvířat. Brno: Technical Museum in Brno, 2014, 32–38. ISBN 978-80-87896-09-9.

DOSEDĚL, M., LÁDOVÁ, K., MALÝ, J.: XVI. Sympozium klinické farmacie René Macha (XVI. Symposium of clinical pharmacy of René Mach). Hradec Králové: Faculty of Pharmacy, 2014, pp. 78. ISBN 978-80-260-7216-4.

KUCHAŘ, M., ANTOŠOVÁ, Z., DURDIL, P., FELTL, L., FUSEK, M., HAMPL, F., JIRÁT, J., KODÍČEK, M., KOLAŠÍNOVÁ, A., KRAUS, J., PACÁKOVÁ, V., RÁDL, S., SOUČEK, R., SUCHÝ, V., TOMKOVÁ, H., TREJTNAR, F.: Farmaceutický encyklopedický slovník (Pharmaceutical Encyclopedic Dictionary). Prague: University of Chemistry and Technology, 2014, pp. 830. ISBN 978-80-7080-876-4.

MACÁKOVÁ, K., KOLEČKÁŘ, V., CAHLÍKOVÁ, L., CHLEBEK, J., HOŠŤÁLKOVÁ, A., KUČA, K., JUN, D., OPLETAL, L.: Tannins and their influence on health. In: Recent advances in medicinal chemistry. Sharjah: Bentham Science Publishers, 2014, 159–208. ISBN 978-1-60805-797-9.

VLČEK, J., VYTŘÍSALOVÁ, M., BERAN, J., ČERVENÝ, P., DRIÁK, D., DUŠEK, P., KELLER, O., KRČMOVÁ, I., KROUPA, R., LUKÁŠ, M., MARKOVÁ, J., PALIČKA, V., ROTH, J., RUDOLF, K., SALAJKA, F., SLÁMA, O., ŠEDO, J., TŮMA, I., VORLÍČEK, J., VYZULA, R.: Klinická farmacie II (Clinical pharmacy II). Prague: Grada, 2014, pp. 255. ISBN 978-80-247-4532-9.7

ŽÁČKOVÁ, P. (Editor): Folia Pharmaceutica Universitatis Carolinae, Praha, Karolinum, 42, 2014, pp. 158, ISBN 978-80-246-2417-4.

TEXTBOOKS

DOHNAL, F.: Studijní texty k dějinám farmacie (Study texts for History of Pharmacy). Prague: Karolinum, 2014, pp. 154. ISBN 978-80-246-2608-6.

HRONEK, M.: Nutriční potřeba ve zvláštních situacích (Nutritional requirement in specific situations). In: Vnitřní lékařství. Prague: Galén, 2014, 1006–1014. ISBN 978-80-7492-145-2.

ZADÁK, Z., HRONEK, M.: Klinická fyziologie výživy (Clinical nutrition physiology). In: Vnitřní lékařství. Prague: Galén, 2014, 969–985. ISBN 978-80-7492-145-2.

DEGREES

Lecture for the Professorship Appointments, Faculty of Pharmacy in Hradec Králové (CZ), Charles University (CZ), 2014

Doc. Pharm.Dr. PETR PÁVEK, Ph.D.: Associate Professor, Department of Pharmacology and Toxicology, Faculty of Pharmacy, Charles University, Hradec Králové
Discipline: Humánní a veterinární farmakologie, MŠMT 24 295/2007-30/1
Inauguration: 15. 10. 2013
Continuation: 11. 3. 2014
Title of Lecture: Regulace exprese biotransformačních enzymů – klinické aspekty a význam ve vývoji léčiv (Regulation of Biotransformation Enzymes gene Expression – clinical Aspects and Importance for Drug Development) 11. 3. 2014
Appointment: 19. 9. 2014

Doc. Pharm.Dr. MILAN NOBILIS, CSc.: Associate Professor, Department of Pharmaceutical Chemistry and Pharmaceutical Analysis, Faculty of Pharmacy, Charles University, Hradec Králové
*Discipline:*Pharmaceutical Chemistry MŠMT 24 295/2007-30/1
Inauguration: 11. 3. 2014
Continuation: 10. 6. 2014
Title of Lecture : Studium osudu léčiv v organismu z pohledu bioanalytika (Study of the fate of drugs in the body from the perspective of bioanalyst) 10. 6. 2014
Appointment: 1. 5. 2015

Doc. Ing. BARBORA SZOTÁKOVÁ, Ph.D.: Associate Professor, Department of Biochemical Sciences, Faculty of Pharmacy, Charles University, Hradec Králové
Discipline: Biochemistry, MŠMT 24 295/2007-30/1
Inauration: 24. 11. 2014
Continuation: 9. 12. 2014
Title of lecture: Metabolismus anthelmintik (Metabolism of Anthelmintics) 9. 6. 2015
Appointment:

Habilitation Thesis (Associate Professor), Faculty of Pharmacy in Hradec Králové (CZ), Charles University (CZ), 2014

Pharm.Dr. IVA BOUŠOVÁ, Ph.D.: Senior Lecturer, Department of Biochemical Sciences, Faculty of Pharmacy, Charles University, Hradec Králové.
*Discipline:*Biochemistry, MŠMT 24 295/2007-30/1
Inauguration: 15. 10. 2013
Continuation: 11. 3. 2014
Title of Lecture: Neenzymová glykace proteinů jako faktor ovlivňující jejich biologickou aktivitu (Non-enzymatic Glycation of Proteins as a Factor influencing their Biological Activity) 11. 3. 2014
Appointment: 1. 5. 2014

Pharm.Dr. PŘEMYSL MLADĚNKA, Ph.D.: Senior Lecturer, Department of Pharmacology and Toxicology, Faculty of Pharmacy, Hradec Králové.
Discipline: Humánní a veterinární farmakologie, MŠMT 24 295/2007-30/1
Inauguration: 15. 12. 2013
Continuation: 10. 12. 2013
Title of Lecture: Současné klinické užití chelátorů železa a jejich další účinek ve stadiu experimentu (Current Clinical Use of Iron Chelators and their other Effect in Experiments) 10. 12. 2013
Appointment: 1. 4. 2014

Pharm.Dr. MIROSLAV MILETÍN, Ph.D.: Senior Lecturer, Department of Pharmaceutical Chemistry and Pharmaceutical Analysis, Faculty of Pharmacy, Charles University, Hradec Králové.
Discipline: Pharmaceutical Chemistry, MŠMT 24 295/2007-30/1
Inauguration: 15. 10. 2013
Continuation: 10. 12. 2013
Title of Lecture: Nukleové kyseliny a jejich analogy nebo deriváty jako therapeutika (Nucleic Acids and their Analoques or Derivatives as therapeutic Agents) 10. 12. 2013
Appointment: 1. 4. 2014

Ing. LUCIE CAHLÍKOVÁ, Ph.D.: Senior Lecturer, Department of Pharmaceutical Botany and Ecology, Faculty of Pharmacy, Charles University, Hradec Králové.
Discipline: Pharmacognosy, MŠ MT 29 593/2011-M3
Inauguration: 19. 11. 2013
Continuation: 10. 6. 2014
Title of Lecture: Přírodní látky jako potencionální léčiva v terapii neurodegenerativních onemocnění (Natural Compounds as a potential Drugs in the Therapy of neurodegenerative Diseases) 10. 6. 2014
Appointment : 1. 10. 2014

Pharm.Dr. VERONIKA NOVÁKOVÁ, Ph.D.: Senior Lecturer, Department of Biophysic and Physical Chemistry, Faculty of Pharmacy, Charles University, Hradec Králové.
Discipline: Pharmaceutical Chemistry, MŠMT 24 295/2007-30/1
Inauguration: 9. 9. 2014
Continuation: 14. 10. 2014
Title of Lecture: Fluorescenční senzory pro biologicky relevantní analyty (Fluorescenze Sensors for biologically relevant Analytes) 9. 12. 2014
Appointment: 1. 3. 2015

Pharm.Dr. RADIM KUČERA, Ph.D.: Senior Lecturer, Department of Pharmaceutical Chemistry and Pharmaceutical Analysis, Faculty of Pharmacy, Charles University, Hradec Králové.
Discipline: Pharmaceutical Chemistry, MŠMT 24 295/2007-30/1
Inauguration: 15. 9. 2014
Continuation: 14. 10.2014
Title of Lecture: Alternativní stacionární báze využívané v analýze biologicky aktivních látek (Utilization of alternative stationary Phases in the Analysis of biologically active Compounds) 9. 12. 2014
Appointment: 1. 3. 2015

Pharm.Dr. LUDMILA MATYSOVÁ, Ph.D.: Senior Lecturer, Department of Analytical Chemistry, Faculty of Pharmacy, Charles University, Hradec Králové.
Discipline: Analytical Chemistry, MŠMT 24 295/2007-30/1
Inauguration: 18. 11. 2014
Continuation: 9. 12. 2014
Title of Lecture: Validace chromatografických metod– legislativa a provedení (Validation of Chromatografic Methods- Legislation and Implementation) 3. 3. 2015
Appointment: 1. 5. 2015

MUDr. KOPEČNÁ, EVA Sc.:Volně prodejná léčiva, jejich regulace a bezpečnost (Regulation and Safety of Non-prescription Medicines). 02. 09. 2014.

Mgr. NOVÝ, ZBYNĚK: Studium interakcí radionuklidy značených monoklonálních protilátek s receptorem pro epidermální růstový faktor (*In Vitro* Study of Interactions of radiolabeled Monoclonal Antibodies with Epidermal Growth factor).

PharmDr. RAMOS MANDÍKOVÁ, JANA: Interakce antimikrobiálních látek s lékovými ledvinnými transportními systémy *in vitro* (Interactions of Antimicrobial Agents with Drug renal Transport Systems *in vitro*). 12. 09. 2014.

Mgr. KREJČOVÁ, JINDŘIŠKA: Historie léčby diabetu v českých zemích (History of DiabetesTreatment in the Czech countries). 12. 12. 2014.

PharmDr. Ing. KOSTŘIBA, JAN:Analýza vybraných determinant lékové politiky v České Republice (Analysis of Selected Determinants of Drug Policy in the Czech Republic). 12. 12. 2014.

PharmDr. KLIMEŠ, JIŘÍ: Zdravotní ekonomie a outcomes research jako součást procesu hodnocení zdravotních technologií v České republice (Health Economy and Outcomes Research in the Health Technology Assessment in the Czech Republic). 12. 12. 2014.

PharmDr. MVDr. VRÁNOVÁ, VILMA: K dějinám farmaceutického marketingu a reklamy v ČSR v letech 1918–1938 (Contributions to the History of the Pharmaceutical Industry, Marketing and Advertising in the Czechoslovak Republic in 1918–1938). 12. 12. 2014.

PharmDr. HENDRYCHOVÁ, TEREZA: Analýza vybraných faktorů ovlivňujících farmakoterapii diabetu mellitu (The Analysis of Selected Factors that Influence the Pharmacotherapy of Diabetes Mellitus). 13. 03. 2014

PharmDr. PETŘÍKOVÁ, ALENA: Farmakoekonomický pohled na léčbu revmatických onemocnění (Pharmacoeconomic view on the treatment of rheumatic diseases). 15. 12. 2014.

PharmDr. BABICA, JAN: Racionalizace v československém lékárenství ve 20. století (Rationalization in the Czechoslovak pharmacy practice in the 20th century). 15. 12. 2014.

RNDr. SRBA, JINDŘICH: Pharmacovigilance:Spontaneous Reporting Systems (Pharmacovigilance:Spontaneous Reporting Systems). 15. 12. 2014.

Mgr. ŠTAMBERGOVÁ, HANA: Lidské membránově vázané karbonylreduktasy (Human Membrane-bound Carbonyl Reductases). 16. 01. 2014

Mgr. ZEMÁNKOVÁ, LENKA: Vliv statinů na TGF-ß1 signalizační kaskádu *in vivo* a *in vitro* (The Effects of Statins on TGF-ß1 Signaling *in vivo* and *in vitro*). 19. 06. 2014.

PharmDr. RATHOUSKÁ, JANA: Farmakologické ovlivnění aterogeneze u experimentálních zvířecích modelů ateroskleózy (Pharmacological Influence on Atherogenesis in Experimental Animal Models of Atherosclerosis). 19. 06. 2014.

Ing. KARABANOVICH, GALINA: Synthesis and structure-antimycobacterial activity relationship study of dinitrobenzylsulfanyl heteroaromates. 19. 12. 2014.

Mgr. SCHMIDT, MONIKA: Analýza membránových proteinů patogenní bakterie *Francisella tularensis* (Analysis of Memrane Proteins of Pathogenic Bacterim *Francisella Tularensis*). 20. 06. 2014.

PharmDr. ANGELINI, JINDŘIŠKA: Úloha cytoskeletu a fosfolipidů v signalizaci obranných reakcí rostlin (The Role of Cytoskeleton and Phospholipids in signaling During Plant Defense Response). 23. 09. 2014.

Mgr. STRÁSKÝ, ZBYNĚK: Možnosti ovlivnění aterogeneze u experimentálních zvířecích modelů a endoteliálních buněk (Possibilities to Influence Atherogenesis in Experimental Animal Models and Endothelial Cells). 25. 04. 2014.

Mgr. KREJČÍ, ANDREA: Alkaloidy vybraných druhů čeledi *Amaryllidaceae*, jejich toxicita a biologická aktivita (*in vitro* studie) I.(Alkaloids from Selected Species of *Amaryllidaceae* family, their Toxicity and Biological Activity (*in vitro* study) I.). 25. 11. 2014.

PharmDr. POTŮČKOVÁ, ELIŠKA: Studium antiproliferačních účinků nových chelátorů železa (Study of Antiproliferative effects of Novel Iron Chelators). 29. 09. 2014.

Rigorous Thesis (Pharm.Dr.) Faculty of Pharmacy in Hradec Králové (CZ) Charles University (CZ), 2014

Pharm.Dr. SOJKOVÁ, KRISTÝNA: Možnosti ovlivnění sekundárních látek v *in vitro* kulturách léčivých rostlin (Possibilities of affecting secondary metabolites production in *in vitro* cultures of medicinal plants). 16. 12. 2014.

Pharm.Dr. SAJKOVÁ, DENISA: Reologické vlastnosti polotuhých přípravků (Rheological behaviour of semi-solid preparations). 15. 12. 2014.

Pharm.Dr. KUŠNÍR, JAROSLAV: Identifikace hlavních degradačních produktů butan – 1,3-diolu (Identification of main decomposition products of butane-1,3-diol). 16. 06. 2014.

Pharm.Dr. PTÁČNÍKOVÁ, LUCIE: Vliv vybraných kovů a chelátorů na oxidaci přírodních látek (Influence of selected metals and chelators on the oxidation of natural substances). 16. 12. 2014.

Pharm.Dr. SOHROVÁ, BARBORA: Vývoj HPLC metody pro stanovení homatropinu a skopolaminu v očních kapkách (Development of HPLC method for the determination of homatropine and scopolamine in eye drops). 16. 06. 2014.

Pharm.Dr. TYCOVÁ, BLANKA: Vyšetření tělních tekutin na automatickém analyzátoru XT-4000i (Analysis of body fluids on the automatic analyzer XT # 4000i). 30. 06. 2014.

Pharm.Dr. SEIDLOVÁ, HELENA: Biotransformace flubendazolu a albendazolu v rostlinných buňkách (Biotransformation of flubendazole and albendazole in plant cells). 06. 03. 2014.

Pharm.Dr. MACHOVÁ, KAROLÍNA: Syntetické obměny vasicinonu vedoucí k látkám s bronchodilatační aktivitou (Synthetic studies on vasicinone leading to bronchodilatory active compounds). 09. 06. 2014.

Pharm.Dr. MICHALÍKOVÁ, ZUZANA: Redukce hmotnosti morbidně obézních diabetiků 2. typu – změny vybraných laboratorních parametrů (Weight reduction in morbidly obese patients with diabetes mellitus type 2 # changes in selected laboratory parameters). 13. 02. 2014.

Pharm.Dr. MICHALICOVÁ, ALENA: Stanovení chelatace iontů mědi u flavonů bathocuproinovou metodou (Assessment of copper chelation of flavones by bathocuproine method). 24. 09. 2014.

Pharm.Dr. KREPSOVÁ, VERONIKA: Příprava a studium spektrálních a fotofyzikálních vlastností nového ve vodě rozpustného azaftalocyaninu nesoucího cukerné jednoky na periferii (Synthesis and study of spectral and photophysical properties of a new water-soluble azaphthalocyanine bearing saccharide units on the periphery). 16. 10. 2014.

Pharm.Dr. ŠKORICOVÁ, DAGMAR: Stanovení anthokyanidinů jako potenciálních ligandů lidského konstitutivního androstanového receptoru (hCAR) *in vitro* (Investigating of anthocyanidins as the putative ligands of the human constitutive androstane receptor (hCAR) *in vitro*). 13. 02. 2014.

PharmDr. DUVANOVA, NATALIA: Používání antibiotik farmaceuty (Antibiotic use by pharmacy employees). 13. 11. 2014.

RNDr. RUDOLFOVÁ, PETRA: Vliv katechinů na toxicitu vybraných léčiv (Effect of catechins on toxicity of selected drugs). 06. 03. 2014.

Pharm.Dr. KOČÍ, MICHAL: Nemocniční pozitivní listy v České republice (Hospital Drug Formularies in the Czech Republic). 13. 11. 2014.

PharmDr. KURUCOVÁ, KRISTINA: Hodnocení klidového energetického výdeje a utilizace substrátů u pacientů s pokročilým bronchogenním karcinomem (Rating resting energy expenditure and substrate utilization in patients with advanced lung cancer). 03. 09. 2014.

PharmDr. MYŠÍKOVÁ, MARKÉTA: Ověření parazitologické aktivity hlístice *Elaphostrongylus cervi* u jelena evropského ve farmovém chovu (Verification of parasitological activities of roundworm *Elaphostrongylus cervi* in farmed red deer population). 13. 02. 2014.

PharmDr. SYNÁKOVÁ, KLAUDIE: Výskyt syptomů poruchy epileptického spektra u vybraných skupin populace (Signs of epilepsy spectrum disorder in a selected population sample). 13. 02. 2014.

PharmDr. DITTRICHOVÁ, KATEŘINA: Development and validation of an HPLC method for determination of 3-aminopropanol and stability study of pharmaceutical preparation. 16. 06. 2014.

PharmDr. HOUDKOVÁ, TEREZA:Současné medicínské využití konopí v ČR (Contemporary using of medicinal Cannabis in the Czech Republic). 19. 12. 2014.

PharmDr. KAČENKOVÁ, OLGA: Nežádoucí účinky vybraných antiepileptik (A comparison of side effects of some antiepileptic drugs). 13. 02. 2014.

PharmDr. MERTLÍKOVÁ, VERONIKA: Vliv sunitinibu na expresi iNOS u normotenzních a hypertenzních potkanů (Effect of sunitinib on the expression of iNOS in normotensive and hypertensive rats). 03. 09. 2014.

PharmDr. ŠUMBEROVÁ, KATEŘINA: Vliv sunitinibu na expresi P-selektinu u normotenzních a hypertenzních potkanů (Effect of sunitinib on the expression of P-selectin in normotensive and hypertensive rats). 13. 02. 2014.

PharmDr. KOHOUTOVÁ, LUCIE: Lymeská borrelióza – laboratorní a klinická diagnostika (Lyme borreliosis, clinical and laboratory diagnosis). 03. 09. 2014.

PharmDr. DIBLÍKOVÁ, DENISA: Studium transdermální a dermální absorpce acyklických nukleosidfosfonátů ze skupiny 2,6-diaminopurinu (Study of transdermal and dermal absorption of 2,6-diaminopurine acyclic nucleoside phosphonates). 04. 02. 2014.

PharmDr. BURDOVÁ, KATEŘINA: Stabilitu indikující metoda pro hodnocení účinné látky v přípravku (Stability indicating method for evaluation of the active substance in pharmaceutical product). 04. 12. 2014.

PharmDr. PILÁTOVÁ, KRISTÝNA: Studium formulace polyesterových nanočástic (Study of formulation polyester nanoparticles). 15. 12. 2014.

PharmDr. VOLKOVÁ, MARIE Ph.D.: Modulační účinky quercetinu a rutinu na aktivitu a expresi cytochromu P4501A ve střevních buňkách (Modulatory effects of quercetin and rutin on cytochrome P4501A expression and activity in intestinal cells). 13. 02. 2014.

PharmDr. MALÁ, MARIE:Vliv flavonoidů a vybraných chelatorů na oxidaci přírodních látek (Thesis The effect of flavoinoids and chosen chelators on the oxidation of natural substances). 19. 06. 2014.

PharmDr. KRÁČALÍKOVÁ, MARKÉTA: Studium vlivu terbinafinu na teplotu skelného přechodu polyesterových matric (The study of the influence of terbinafine on glass transition temperature of polyester matrices). 02. 04. 2014.

PharmDr. BUCHAROVÁ, VERONIKA: Vliv inkorporace léčiv na teplotu skelného přechodu větvených polyesterů (The effect of the drugs incorporation on the glass transition temperature of branched polyesters). 15. 12. 2014.

PharmDr. HLAVÁČOVÁ, JITKA: Inhibiční účinek anthokyanidinů na jaterní karbonylreduktasu (Inhibitory effect of anthocyanidins on hepatic carbonyl reductase). 06. 03. 2014.

PharmDr. GREGOR, STANISLAV: Fyzikálně chemická charakteristika mikokrystalických celulos (Physicochemical characteritazion of microcrystalline celluloses). 26. 06. 2014.

PharmDr. DROBÍK, OTO: Kolorimetrická metoda založená na imobilizované acetylcholinesterase pro stanovení účinnosti inhibitorů používaných v terapii (Colorimetric assay based on immobilized acetylcholinesterase for the determination of efficiency of inhibitors used in pharmacotherapy). 16. 10. 2014.

PharmDr. HOLUBOVÁ, KATEŘINA: Studium směsných matricových tablet (The study of dual matrix tablets). 15. 12. 2014.

PharmDr. IHNÁT, LUKÁŠ: Vývoj HPLC metody pro stanovení výbušniny diazodinitrofenolu (The metod development for determination of diazodinitrofenol explosive). 04. 12. 2014.

PharmDr. LÍBENKOVÁ, MARIE: Mukoadhezivní polymerní systémy s aciklovirem (Mucoadhesive polymeric systems with aciclovir). 26. 06. 2014.

PharmDr. JUNGMANN, MICHAL: Výsledky dlouhodobého sledování parazitostatu a jeho kontroly v populaci *kozy bezoárové* (The results of long term monitoring of parazitostatus and its control in the population of *bezoar goat*). 13. 02. 2014.

PharmDr. VYSKOČILOVÁ, ERIKA: Modifikace vybraných biotransformačních enzymů v průběhu stárnutí (u potkana) (Modification of selected biotransformation enzymes during ageing (in rat)). 06. 03. 2014.

RNDr. DRESLEROVÁ, ELLEN: Rheoferetické postupy pro odstranění cholesterolu a jejich dopad na imunitní systém u pacientů s VPMD (AMD) (Rheopheresis for the cholesterol depletion and its immunological impact on patients with Age-related Macular Degeneration). 20. 06. 2014.

PharmDr. URBAN, MICHAL: Testování interakce MEK inhibitorů s cytochromem P 450 3A4 (Interaction studies of MEK inhibitors and Cytochrome P450 3A4). 13. 02. 2014.

PharmDr. Mgr. BECHNÁ, KLÁRA: Měď – chelatující vlastnosti 8 – hydroxychinolinů (Copper chelating properties of 8 – hydroxyquinolines). 03. 09. 2014.

RNDr. KRAČMAROVÁ, ALŽBĚTA: Vliv injekčního podání léčiv na vznik oxidačního stresu na modelu laboratorního morčete (The impact of an injection drug administration on an oxidative stress development in laboratory Guinea pig model). 20. 06. 2014.

RNDr. FUČÍKOVÁ, ALENA Ph.D.: Využití proteomické analýzy pro identifikaci potenciálních biomarkerů u onemocnění kardiovaskulárního systému (Use of proteomic analysis to identify potential biomarkers for cardiovascular disease). 03. 02. 2014.

PharmDr. POSPÍŠILOVÁ, BLANKA: Fytoremediace nitrolátek: toxicita dinitrotoluenu a změny v proteomu rostlin (Phytoremediation of nitro compounds: a toxicity of dinitrotoluene and a change in proteom of plants). 20. 06. 2014.

PharmDr. MUDROCHOVÁ, EVA: Kontaktní a bezkontaktní podání léčiv v oborním chovu spárkaté zvěře – výsledky porovnání účinnosti a ekonomiky léčebných zásahů (Contact and contactless administration of drugs in game park of deer – results comparing the effectiveness and economics of treatments). 13. 02. 2014.

PharmDr. ŠTICOVÁ, KATEŘINA: Osmolalita parenterálních směsí. Glukosa a mléčnan sodný (Osmolality of parenteral mixtures. Glucose and sodium lactate). 12. 02. 2014.

PharmDr. BÖHMOVÁ, LENKA: Stanovení myorelaxancií rokuronia, vekuronia a pankuronia metodou kapilární elektroforézy s bezkontaktní vodivostní detekcí (Determination of rocuronium, vecuronium and pancuronium muscle relaxants by capillary electrophoresis with contactless conductivity detection). 16. 06. 2014.

PharmDr. PRŮŠOVÁ, KRISTÝNA: Akutní intoxikace v urgentní medicíně (Acute intoxication in urgent medicine). 03. 09. 2014.

PharmDr. ŘIHOŠKOVÁ, PETRA: Interakce katechinů s ionty mědi (Interactions of catechins with copper ions). 24. 09. 2014.

PharmDr. PECHANDOVÁ, KRISTINA: Frekvence výskytu vybraných bodových polymorfismů CYP2C8 a MDR1 v české populaci a jejich vliv na působení amiodaronu (Frequency of occurrence of selected single nucleotide polymorphisms of CYP2C8 and MDR1 in the Czech population and their influence on the effect of amiodarone). 13. 02. 2014.

PharmDr. NAVRÁTIL, RADEK: Vyhodnocení aktivity potenciálně antimikrobiálních látek pomocí mikrodiluční bujónové metody (Evaluation of activity potentional antimicrobial substances through the use of microdilution broth method). 03. 09. 2014.

PharmDr. ŠTORKOVÁ, ALENA: Osmolalita parenterálních směsí. Ringer-laktátový roztok (Osmolality of parenteral mixtures. Ringer-lactate infusion). 12. 02. 2014.

PharmDr. NESVADBOVÁ, ELIŠKA: Dávkování očních kapek – charakterizace očních kapátek (Eye drop dosing – dropper tip characterization). 12. 02. 2014.

PharmDr. JANOUTOVÁ, MARTINA: Ovlivnění produkce sekundárních látek v rostlinných kulturách *in vitro* (Influencing of secondary compounds production in plant cultures *in vitro*). 19. 06. 2014.

PharmDr. MUCHOVÁ, SANDRA: Studium matricových lipofilních tablet s glyceroldibehenátem (The study of matrix lipophilic tablets with glyceryl behenate). 26. 06. 2014.

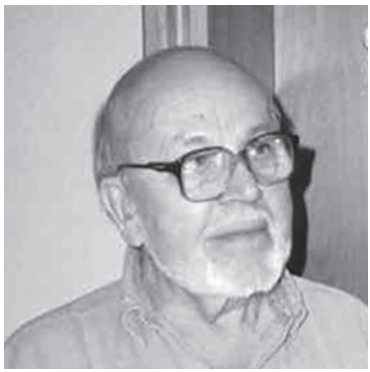
PharmDr. ŠULCOVÁ, HANA: Analýza farmakoterapie v domovech pro seniory (Analysis of pharmacotherapy in nursing homes). 01. 10. 2014.

PATENTS

SOLICH, P., SKLENÁŘOVÁ, H., CHOCHOLOUŠ, P., ŠATÍNSKÝ, D., ANDRUCH, V., ŠKRLÍKOVÁ, J.: Zařízení sekvenční injekční analýzy pro extrakci kapalina-kapalina (A device for sequential injection analysis for liquid-liquid extraction). Prague: Industrial Property Office, 2014. Patent No. CZ 304296 B6.

SOCIAL HAPPENINGS

IN MEMORY OF
Assoc. Prof. RNDr. PhMr. VÁCLAV RUSEK, CSc.



Assoc. Prof. RNDr. PhMr. Václav Rusek was born on 25th February 1928 in Komárov (nowadays a part of Opava). He graduated from the secondary grammar school in Opava in 1947. Having completed two years' pharmaceutical apprenticeship (known as tyrocinial practice), he studied pharmacy at the Masaryk University in Brno. He earned the degree Master of Pharmacy (PhMr) at the Faculty of Sciences (1949–1951) and started to work as a junior assistant at the Department of Galenic Pharmacy. Concurrently, he completed his studies in the newly established four years' course of pharmacy at the Faculty of Pharmacy in Brno (1951–1953). In 1954, when Assoc. Prof. RNDr. PhMr. J. Hladík left the faculty, V. Rusek took over management of the Institute of History of Pharmacy at the same faculty.

In 1960, the Faculty of Pharmacy of Masaryk University in Brno merged with the Faculty of Pharmacy of Comenius University in Bratislava and V. Rusek moved to Bratislava. Based on his dissertation "Development of some drugs and their production equipment", he obtained the scientific degree Candidate of Sciences (CSc. – equivalent to Ph.D.) in 1964. The degree Doctor of Natural Sciences (RNDr.) was awarded to him four years later (1968).

When a new Faculty of Pharmacy was established in Hradec Králové (1969), Dr. Rusek left Bratislava (1971) and continued his work at the Department of Organization and Management of Pharmacy. He was appointed an Associate Profesor in the field Organization and Management of Pharmacy in 1987. In addition to his academic and scientific education, he obtained the 1st and 2nd grade specializations at the Institute for Further Education of Physicians and Pharmacists in Prague.

His pedagogical activities were also rich. Moreover, he always belonged among teachers that were admired and loved by students. He was a principal reader in the subject "Introduction to pharmaceutical studies, history and organization of pharmacy". He taught this subject in Brno (1959/60), Bratislava (1960/61–1970/71), Hradec Králové (1971/72–1994/95), and also at the newly established Faculty of Pharmacy of the Veterinary and Pharmaceutical University in Brno (1992/91–1994/95). In the period 1954–2003 he supervised 162 of master

theses. He tutored also 66 rigorous (RNDr. or PharmDr.) theses (1966–2005) and 5 doctoral dissertations in the field History of Pharmacy.

He was a head of the Academic Senate of the Faculty of Pharmacy in Hradec Králové and a member of the Academic Senate of Charles University (1991–1993), head of the Ethical Committee at the Faculty of Pharmacy in Hradec Králové and a member of the Ethical Committee of the Ministry of Health of the Czech Republic, and a member of the Advisory Board of the Faculty of Pharmacy of the Veterinary and Pharmaceutical University in Brno (1991–1992). His career at the Department of Organization and Management of Pharmacy ended in 2005. Nonetheless, he continued his scientific and consulting activities and was engaged in further development of the museum, often in spite of serious health problems.

Assoc. Prof. V. Rusek is one of main representatives of Pharmaceutical History. He started to build the discipline in 1952 in Brno and continued to develop it throughout his life. He established the Institute of Pharmaceutical History within the Department of Organization and Management of Pharmacy and is also a founder of the Czech Pharmaceutical Museum (1994) in Kuks. He also significantly influenced the activities of the Czech Pharmaceutical Association, where he was an active member since 1953. For several years (1969–1975 and l977–1990) he was a head of the Section of Pharmaceutical History. Tens of seminars and conferences was held under his leadership. He presided two International Congresses of History of Pharmacy in Prague (1971 and 1991).

Since 1956, he was a member of International Society for History of Pharmacy (ISHP) and a member of its Extended Executive Committee (1967–1981). Furthermore, he was a member of Académie Internationale d'Histoire de la Pharmacie, a corresponding member of Deutsche Gesellschaft für Geschichte der Pharmazie, a member of Gesellschaft für Wissenschaftsgeschichte, and a member of the Society for the History of Sciences and Technology.

For his valuable contributions to theoretical aspects of pharmacy and extraordinary work for pharmaceutical history, Assoc. Prof. V. Rusek was appointed honorary member of the the Czech Pharmaceutical Association, the Society for the History of Sciences and Technology (Section of History of Medicine, Pilsen), the Czech Chamber of Pharmacists, the Association for the Formation of the Czech Pharmaceutical Museum and The Pharmacy Guild in Prague. He was also honoured by various awards, such as Medal of the Comenius University, Weber Prize and Medal of PhMr. V. J. Žuffa (Slovak Pharmaceutical Association), Fritz Ferchl Medal (Deutsche Gesellschaft für Geschichte der Pharmazie), Medaille Carmen Francés (Académie Internationale d'Histoire de la Pharmacie), Medal of the Faculty of Pharmacy in Hradec Králové, Johann Valentin Medal (Gesellschaft für Geschichte der Pharmazie, DDR), C. W. Scheele Award (Swedish Pharmaceutical Society), Medal of the Czech Medical Association of J. E. Purkyně. His interest in graphical art and his honorary membership in the Association of Collectors and Friends of Exlibris must be mentioned as well.

Scientific bibliography of Assoc. Prof. V. Rusek includes 64 original papers (12 international), 135 reviews and articles, 44 authorships or co-authorships of monographies and textbooks, and 55 communications such as reviews of books or news. He delivered 136 lectures on scientific conferences (27 abroad), 121 lecture on professional meetings and six times appeared in radio and TV. He was an editor of six proceedings and prepared 37 exhibition scenarios and 10 exhibition catalogues.

Assoc. Prof. Václav Rusek died on 30th January 2016 in Brno. He will be missed by all pharmacist who knew him as a colleague, teacher, passionate lover of art and life, and versatile Renaissance personality.

R. I. P.

J. Solich, V. Opletalová

TO THE ANNIVERSARY OF PROFESSOR ALEXANDR HRABÁLEK

In the 2nd of June 2016 professor PharmDr. Alexandr Hrabálek, CSc., a distinguished scientist and teacher of the Faculty of Pharmacy of the Charles University (FaF UK) and leading representative of the Czech pharmacy, celebrates his 60th birthday.

Alexandr Hrabálek is closely connected with the FaF UK since his studies there. He graduated in 1980 and two years later he gained the title of the doctor of pharmacy. For his scientific work at the department of inorganic and organic chemistry he gained the title of CSc., then he was appointed associate professor and in 2009 full professor of pharmaceutical chemistry. Since 2002 prof. Hrabálek led the department of inorganic and organic chemistry and a year later he became the vice-dean of the faculty for research. His long-term scientific and pedagogical activities and above all his merits for the development of the faculty and whole pharmacy made him became the dean of FaF UK since 2006–2014. After finishing his deanship he stays in the management of the faculty, at present he works as the vice-dean for public relations and technology transfer.

Prof. Hrabálek is not only the leader of the department of inorganic and organic chemistry but also, and above all, its essential member. He guarantees the subjects of Organic chemistry and Constitution of organic compounds, he works with students during practical exercises in Chemical laboratory technique and he also is a supervisor of pre-graduate and postgraduate students. He also leads a research group, which initially focused on the synthesis and study of transdermal permeation enhancers and synthesis of nitrogen heterocycles. Now, his scientific work focuses on potential antitubercular drugs. He founded and coordinates a wide interdisciplinary team which deals with chemical synthesis, microbiological evaluations and *in vivo* studies of promising compounds (in cooperation with Biological defense department in Těchonín). During his career he published more than 80 publications and gain more than 20 national and international patents, he was principal investigator of many scientific grant and EU funded projects. Prof. Hrabálek is also a holder of many prestigious awards and prizes – e.g. the Silver Medal of UK or the Medal of D. I. Mendělejev

(Saint-Petersburg state technological institute, Russian federation). In 1997 he was awarded by gold medal on Brussels Eureka ’97.

In the last years prof. Hrabálek was involved in the construction of the first building of the campus for Faculties of Pharmacy and Medicine. We must also mention a very important project, which is now under negotiations – a complete renovation of fortification object called “Pajkrova flošna” and establishing of the university students club in it. To illustrate the wide spectrum of his activities we must mention the realization of the permanent exhibition of Vladimír Renčín’s drawings and paintings. Thanks to his contacts and initiatives we can admire this exhibition in Czech Pharmaceutical Museum in the Hospital Kuks near Dvůr Králové nad Labem.

Jaroslav Roh, Kateřina Vávrová, Věra Klimešová, and Tomáš Šimůnek

OBITUARY FOR Assoc. Prof. PharmDr. MILOŠ MACHÁČEK, CSc.

The week starting with February 1 was a genuine black week for the School of Pharmacy, Charles University in Hradec Králové. As soon as all came to terms with the loss of Assoc. Professor Václav Rusek, another sad event happened – a long-time teacher of the School – Assoc. Professor Miloš Macháček deceased.

Miloš was an employee of the School for almost 40 years, and most of our readers have thus passed "through his hands". Having been a former student of Professor Waisser, his research dealt with mathematical evaluation of the relationships between structure and activity of potential antituberculotics, and Miloš accomplished important achievements in this field.

As regards teaching, he was involved in teaching courses in General and Inorganic Chemistry, Chemometry and Biostatistics, in both Czech and English.

As mentioned above, biostatistics and application of statistical methods to quantitative analysis of the structure-activity relationships were among his scientific interests. He also became the first teacher at the School to deal with practical problems in NMR spectroscopy, although the machine available back in 1980s did not allow him to do so in proper depth. However, his interest in analytical instrumental methods, above all spectroscopies of various kinds, led him to membership in the Jan Marcus Marci Spectroscopic Society, of which he was a prominent and active member especially in the 1990s.

Miloš always liked, and his students would certainly confirm this, expressing himself in an exact and correct fashion. It is therefore no wonder that chemical nomenclature was yet another of his hobbies. Based on his grasp of nomenclature principles, he served as the Chairman of the Czech Pharmacopoeial Committee, and as a representative of the Czech Republic on the European Pharmacopoeial Committee.

Membership (and later chairmanship) in the Academic Senate of the School must also be mentioned among his noteworthy activities. Again, thanks to his ability to express himself clearly and succintly, major parts of the current Statutes of the School which originated in the 1990s were formulated under his leadership.

The School has lost an experienced teacher, his colleagues at the Department have lost a friend. Please, stop for a quiet moment in memory of Miloš Macháček.

Colleagues from the Department of Inorganic and Organic Chemistry

Prof. RNDr. FRANTIŠEK BARTOŠ, DrSc. is Ninety Three

Professor Bartoš was born on 21 December 1923 in Nový Bydžov. After finishing the primary school, he moved to Hradec Králové and was educated at Rašín State Czechoslovak Grammar School where he graduated in 1943. After the end of World War II, he started studying biology and geography at the Faculty of Science at Charles University in Prague.

After passing the first state exam, he received an offer from professor Malý to become a research assistant at the Anthropological Institute. He started working there in 1948, successfully defended his diploma thesis and finally graduated in February 1950. Then professor Bartoš left Prague and moved to Hradec Králové where he worked as an assistant at the Department of Biology at the Faculty of Medicine in Hradec Králové. At that time the leader of the Department was the associate professor Hluchovský (a pre-war student of professor Růžička). He profiled the Department especially at the cell study, cell cultivation and cell behaviour *in vitro*. František Bartoš was involved in these studies and started to deal with regeneration and healing processes. Particular attention was paid to the intercellular filamentous fibre structures, collagen and elastin. At the same time, he studied their physio-chemical properties, and was mainly focused on the changes during ageing. The healing of skin wounds was also the topic for his candidate dissertation paper which he successfully defended in 1962 at ČSAV and obtained CSc. degree.

His scientific progress continued. He was habilitated at the Faculty of Medicine at Charles University in Hradec Králové in 1962 and became an associeate professor. Thanks to the influence of the political situation in 1968–69, he was forced to leave the Faculty of Medicine. He started working at the newly established Faculty of Pharmacy at Charles University in 1971 at the position of a research assistant, and later as the head of the Department of Pharmacological Propedeutics (currently the Department of Biological and Medical Sciences) until his retirement in 1992.

During his academic career he published more than 100 scientific papers in domestic and world scientific magazines. In 1987 he successfully defended his academic disserta-

tion at the Faculty of Science at Charles University in Prague and obtained DrSc. degree. Owing to the political unreliability he got the professor degree after the Velvet Revolution in 1993. He was named by then president Václav Havel.

Professor Bartoš addicted himself to the general education and the education of molecular biology and genetics with a great interest. He passed the knowledge on his students and colleagues in an unforgettable and peculiar way. His lectures were very popular among students because they were full of humour, hyperbole and experience of research work. Professor Bartoš was demanding on himself as well as others. Fairness, honesty and constant humour are the characteristic features of this pedagogue who had the rare gift of passing the knowledge on others.

His workmates from the very-democratically-led department often think back on this period, e.g. their common "department trips". General knowledge and the relationship to culture and art gave him new dimension in his directive work and the functioning of the whole department. Professor Bartoš devoted all his scientific and pedagogical career to cytology study and the ageing process in relation to fibrous proteins, collagen and elastin. He collaborated with some clinics of Teaching Hospital in Hradec Králové (Orthopedical, Dental and Ophthalmological clinic). He also realized fruitful cooperation with numerous theoretic departments of Faculty of Medicine and Faculty of Pharmacy at Charles University in Hradec Králové (Biochemistry, Pathological physiology, Pharmacology, Physics etc.).

Professor Bartoš was for many years the scientific secretary of research field committee of Gerontology at the Ministry of Health. He participated in organizing of state gerontology research with professor MUDr. Groh, the head of the committee. In 1960s and 1970s he was also a member of Czechoslovak Gerontology Association.

He was famous for tens of scientific lectures in various scientific associations and in public.

His interest in public events, culture and the science results accompany him even nowadays. He just started the tenth decennium of his fruitful life.

dr. Petr Jílek

INSTRUCTIONS FOR AUTHORS FOR FOLIA PHARMACEUTICA UNIVERSITATIS CAROLINAE

Manuscripts should be submitted on the A4 paper, in English, typed in editor Microsoft Word, format Times New Roman 12 normal.

Manuscript should be divided into sections:

Title – (14, left alignment, (SPECTROPHOTOMETRIC DETERMINATION OF …). Write the title in lowercase letters and then format it using Font – All caps (Písmo – Všechna velká).

Names of Authors – first name and surname with the reference to institution's name (Times New Roman 12, center alignment) (JIŘÍ GASPARIČ[1], MILENA ČERMÁKOVÁ[2]). Write the names in lowercase letters and then format them using Font – All caps (Písmo – Všechna velká).

Names of Institutions – (Times New Roman 10, centre alignment) (1 Department of …, Faculty of Pharmacy in Hradec Králové, Charles University in Prague, Czech Republic).

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The text should be written continuously in Times New Roman 12, normal, left alignment with line space 1.5, without indent, only using left alignment, and starting a new paragraph by "enter". Bold and Italic may be used. Manuscript should be divided into sections:

Headings of individual sections for original Papers:
Abstract – 12, bold, left alignment (ABSTRACT)
Keywords – maximum 5 keywords – 12, bold, left alignment (KEYWORDS: extraction – spectrophotometry)
Introduction – 12, bold, left alignment (INTRODUCTION)
Experimental – 12, bold, left alignment – (EXPERIMENTAL)

Text can be further divided:
Main Chapter (12 bold, left alignment), Subchapter (Italics, 12 bold, left alignment) and Further (Italics, 12, left alignment).
If it is not absolutely necessary, do not use more than three levels of headlines.
Results and discussion – 12, bold, left alignment (RESULTS AND DISCUSSION).

Figures must be submitted in the best quality and original size (not more than 12.5 × 18 cm) separately as a supplement. Indicate the placement of the figure in the text. Captions and notes are placed below (10, centre alignment).

<Koukal_Fig2.jpg>

Fig. 1. Structures of the studied compounds

Tables are placed in the text. Values in the table are written in columns without frame. Title of the table (Table 1. Antifungal activities of the studied compounds.) (10, left alignment) is above the table. Notes are below the table. The layout of the table must be submitted separately.

Chemical structures should be drawn with a suitable drawing program.

Acknowledgements – 12 italic, left alignment (Acknowledgements)
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References must be numbered continuously and indicated as an upper index in the text.
References from journals:

1. Agrawal, Y. K., Patel, D. R.: Spectrophotometric Determination of Clioquinol. Indian J. Pharm. Sci., 47, 1985, 207–209.

References from books:

1. Němcová, I., Čermáková, L., Gasparič, J.: Spectrophotometric Reactions. New York, Marcel Dekker Inc., 1996.

Manuscripts should be submitted in one copy (on a paper and on CD) to the editor (Assoc. Prof. RNDr. Pavla Žáčková, CSc., Department of Pharmacology and Toxicology, Faculty of Pharmacy, Charles University, Hradec Králové, Heyrovského 1203, 500 05, Czech Republic; e-mail: folia.@faf.cuni.cz).

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