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SECTION OF BIOLOGICAL SCIENCES

EVALUATION OF CYTOTOXICITY OF SELECTED CHOLINESTERASE MODULATORS

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One of the key parts of the development process for new drugs is an evaluation of their toxicity using selected *in vitro* or *in vivo* models. The aim of the study was to evaluate the cytotoxicity of selected compounds, which modulate the activity of the enzyme acetylcholinesterase (AChE, EC 3.1.1.7). AChE reactivators are drugs used as antidotes against poisoning by organophosphorus compounds like organophosphate pesticides and nerve agents. On the other hand, AChE inhibitors are used as medicaments in certain types of neurodegenerative diseases such as Alzheimer's disease and as prophylactics to neurotoxic poisoning by organophosphorus compounds. The cytotoxicity of the tested substances was evaluated on the human hepatocellular cell line HepG2 after 24 h of treatment. Two methods for testing were employed and compared. The spectrophotometric MTT assay (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) was used for determination of viability of metabolic active cells. The xCELLigence system (Roche Applied Science, Prague, Czech Republic) was applied for monitoring of cell viability of the treated cells in real time. Using nonlinear regression analysis, IC_{50} values of newly prepared compounds were calculated and compared with the toxicity of currently used drugs.

THE ENERGY EXPENDITURE IN CRITICALLY ILL PATIENTS IN THE ICU

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The aim of this study was to find relations between resting energy expenditure (REE) and selected parameters in 14 polytraumatic patients in the ICU of University Hospital in Hradec Králové. The parameters are easily measurable and they were tested for possibility of REE prediction.

In this study 7 men (age 36 ± 18 years) and 7 women (age 58 ± 28 years) with polytrauma were examined. The assessment of REE was measured *via* indirect calorimetry (IC) method. The examination also included bioimpedance analysis (BIA). BIA was useful especially for obtaining values of overhydration (OH), lean tissue mass (LTM) and metabolically active body cell mass (BCM).

Average REE-IC (measured by IC) was 2116 ± 516 kcal day^{-1} in men and 1450 ± 407 kcal day^{-1} in women ($P = 0.018$). Statistically significant difference between men's and women's population was also found in these relations: calculation of basal energy expenditure according to Harris-Bennedict equation without ($P = 0.001$) and with deduction of OH from body weight ($P = 0.001$), at "breathing energy expenditure" (REE related to respiratory rate) ($P = 0.018$) and at (REE related to heart rate) "heart rate energy expenditure" ($P = 0.038$). REE-IC related to kilogram of BCM with and without deduction of overhydration was shown as statistically significant parameter too ($P = 0.017$).

Statistically significant was also demonstrated correlation between REE-IC and respiratory rate ($P = 0.027$). This relation shows equation $y = -10.681x^2 + 499.97x - 3655$, derived by polynomial regression analysis.

The metabolism of polytraumatic patients is highly individual state and it's very hard to predict it using current equations. Punctual determination of REE is possible only by indirect calorimetry. When hospitals can't use this method, derived predictive can be used for prediction of REE, very important for application accurate doses of parenteral and enteral nutrition that contribute to decrease mortality and morbidity rate of polytraumatic patients.

The study was supported by the project MH CZ – DRO (UHHK, 00179906), PRVOUK P40 and Faculty of Pharmacy (SVV 260 064).

THE FIELD EFFICACY OF TWO ANTHELMINTICS FROM SALICYLANILIDE GROUP AGAINST THE GIANT LIVER FLUKE (*FASCIOLOIDES MAGNA*) DETERMINED IN RED DEER POPULATIONS

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Extensive areas, especially in South Bohemia, suffer currently from infection of giant liver fluke (*Fascioloides magna, F. magna*). This parasitosis affects breeds of both farm and wild ruminants. High prevalence of fascioloidosis in red deer populations, repeatedly diagnosed in South Bohemian areas as Šumava, Novohradsko, Boletice, was the reason for (1) efficacy verification of traditionally used veterinary product with activity against flukes, for (2) realization of original evaluation of a new anthelmintic in Czech conditions.

The subjects of study were Rafendazol premix (formerly registered in Czech Republic, premix based on combination of rafoxanide and mebendazole) and closantel (first time used in Czech Republic). The study was held in five overwintering objects with wild red deer in Boletice region. The extensive and intensive infection of round worm *Elaphostrongylus cervi* (*E. cervi*) was found during pretreatment coprological examinations, therefore the activity of tested drugs against this parasite were verified as well. The efficacy of used anthelmintics was determined by comparing of coprological findings reached in ovoscopy (*F. magna*) and larvoscopy (*E. cervi*) examinations of fecal samples collected in overwintering objects 6, 4 and 2 weeks before and at the same range after the drugs administration.

The summary efficacy of tested drugs (administered in several dosage schemes) reached the values 18.7–100.0% against *F. magna* and 0.0–100.0% against *E. cervi*. Sufficient simultaneous activity against both parasites was found in Rafendazol administered in three consecutive days only, officially recommended shorter administration (in two days) was effective partially. Closantel was highly effective after two-day administration against *F. magna* infection only, round worm *E. cervi* was not affected at all.

CELL-FREE EXPRESSION OF A VOLTAGE-GATED SODIUM CHANNEL FOR FUTURE 2D IR SPECTROSCOPY STUDIES

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Voltage-gated sodium channels (Na_vs) are membrane proteins from the superfamily of voltage-gated ion channels, and are present in every excitable cell where they participate

in the propagation of action potentials by changing Na^+ permeability of the cell membrane. Eukaryotic Na_vs are pseudo homotetrameric polypeptides, comprising four repeats of six transmembrane segments (S1–S6), where S1 to S4 form the voltage-sensing domain and S5 and S6 create the pore domain with the selectivity filter on the extracellular site. Whereas the ion selectivity of the voltage-gated potassium channels has been elucidated on the molecular level in great detail, little is known about this for the voltage-gated sodium channels.

To allow future studies of the selectivity filter of Na_v by the means of 2D IR spectroscopy, a technique able to provide bondspecific structural information on the picosecond to millisecond time scales, large quantities of the purified channel are needed. Eukaryotic Na_vs are difficult to obtain in these amounts. Therefore we have chosen a prokaryotic pore-only Na_v from *Silicibacter pomeroy* (Na_vSp1p) as a model system. Furthermore, 2D IR studies require site-specifically isotope labeled samples which can only be produced in cell-free expression systems.

In this work, we report the first cell-free expression of a voltage-gated sodium channel and its subsequent purification. To prepare the isotope labeling, three amino acids, which are most likely involved in the ion selectivity of Na_vSp1p, were identified and mutated to amber codons. This will allow 2D IR spectroscopy studies and will help to reveal the principle of sodium ion selectivity in Na_vs.

ALKALOIDS OF SELECTED *GALANTHUS, LEUCOJUM* AND *NARCISSUS* SPECIES AND THEIR BIOLOGICAL ACTIVITY

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The most cases of dementia are nowadays caused byAlzheimer's disease (AD). AD is a progressive neurodegenerative disease and it causes gradual memory loss, disorientation and behavioral disorders which affect patient's social and occupational life. AD is characteristic by loss of neurons in some regions of brain – for example hippocampus and cortex. Pathogenesis of this disease is not completely known, but formation of β-amyloid deposits in brain tissue plays an important role. β-amyloid is a protein and creates extracellular plagues around neurites which degenerate and die. Intracellular tangles are made up of the changed τ-protein. These tangles also cause death of the neuronal cell. The degeneration of neurons is supported by reactive oxygen radicals too. In patients with AD the acetylcholine (ACh) production is damaged. ACh is a neurotransmitter and its lack participates in the

development of AD. ACh is physiologically decomposed by enzyme acetylcholinesterase (AChE). The second enzyme taking part in ACh degradation is a butyrylcholinesterase (BuChE). In severe forms of AD, levels of AChE and choline acetyltransferase are decreased by as much as 90% compared with normal condition, while the concentration of BuChE increases. That's why the new inhibitors with dual enzymatic activity against AChE and also BuChE are sought.

Galanthus, Leucojum and *Narcissus* species belong to Amaryllidaceae family. Plants of this family produce specific chemical substances called Amaryllidaceae alkaloids. Selected species and varieties were tested *in vitro* for their biological activity. Their ability to inhibit erythrocytic AChE (HuAChE) and serum BuChE (HuBuChE) was measured using Ellman's method. Alkaloid extracts were analysed by GC/MS. Alkaloids were identified from their mass spectra, retention times and retention indexes.

As ongoing studies of *Zephyranthes robusta* one alkaloid was isolated in pure form using preparative TLC, identified as 9-*O*-demethylgalanthine and tested for its biological activity.

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VASCULAR REACTIVITY OF CAROTID AND RENAL ARTERIES TO NATRIURETIC PEPTIDES: ALTERATIONS DUE TO DIABETES

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Diabetes is associated with increased prevalence of hypertension, cardiovascular and renal disease. Atrial natriuretic peptide (ANP) and B-type natriuretic peptide (BNP) play an important task in cardiovascular pathophysiology and are considered to have cardioprotective and renoprotective effect in patients with diabetes.

The aim of this work was to study the response of rabbit carotid and renal arteries to atrial and brain natriuretic peptides and whether this response is altered in diabetes.

Six weeks after diabetes induction by alloxan, the renal and carotid arteries were isolated from the body and each segment was tested for isometric tension in an organ bath. All segments were preconcentrated with phenylephrine and then with the cumulative addition of doses of ANP and BNP (10^{-12}–10^{-7} M) to the organ bath, the concentration-response curves to ANP and BNP were measured.

In all cases, natriuretic peptides produced a relaxation of the carotid and renal arteries and showed a hyporeactivity in carotid and renal arteries of diabetic rabbits. Although this hypoactivity was not present in all cases, it would be clearly observed in the case of an increase in sample size.

BIOLOGICAL ACTIVITY OF ALKALOIDS FROM *FUMARIA OFFICINALIS* L. (FUMARIACEAE)

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Alzheimer's disease (AD) is chronically progressive neurodegenerative disorder of the central nervous system causing dementia, characterized by a loss of cognitive functions. According to EBM, the current approved therapy includes anticholinesterase inhibitors (galanthamine, rivastigmine, donepezil) and the NMDA blockers (memantine). Anticholinesterase inhibitors affects acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE), of which activities are increased in AD brain, causes higher availability of acetylcholine (ACh). Prolyl oligopeptidase (POP) is an enzyme that hydrolyzes small peptides such as vazopressine, substance P and thyrotropine-releasing hormones that are involved in the process of learning and memory. Therefore, the inhibition of POP could be an important supporting tool in the treatment of AD.

Alkaloids from Fumariaceae family showed *in vitro* AChE inhibitory activity[1], which should be further examined for a potential application in therapy of AD. *Fumaria officinalis* herbs were extracted by ethanol. The obtained diethyl ether mixture of tertiary alkaloids was fractionated in alumina chromatography column using step gradient elution with petrol, chloroform and ethanol. Repeated column chromatography, preparative TLC and crystallization led to the isolation of protopine, cryptopine, fumaricine, parfumidine and fumariline. Alkaloids were identified by GC/MS, ^{1}H and ^{13}C NMR analyses. Isolated compounds were tested for inhibition activity towards human erythrocyte acetylcholinesterase, serum butyrylcholinesterase and prolyl oligopeptidase. IC_{50} values of these alkaloids were compared to IC_{50} values of standards galanthamine, huperzine A and eserine on cholinesterase inhibition and Z-Pro-prolinal and baicaline on POP inhibition.

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THE EVALUATION OF DNA OXIDATIVE DAMAGE IN POLYTRAUMATIC PATIENTS

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The aim of this study was to observe levels of oxidative DNA damage in patients with multiple injuries in correlation with the nutritional support that the patients have received during their hospital stay. Oxidative DNA damage was evaluated in two periods of time, first evaluation was performed during standard nutritional support according to the ESPEN guidelines. Second evaluation was performed after a change in nutrition according to individual parameters of metabolism and utilization of nutritional components based on indirect calorimetric measurements.

This study included 6 patients with multiple injuries hospitalized in the Intensive Care Unit 1 at the Department of Surgery, University Hospital in Hradec Králové. In this experiment DNA isolated from peripheral lymphocytes was used to evaluate oxidative DNA damage. This DNA was analyzed using the Comet Assay method. The enzymatic version of the Comet Assay was used to determine the oxidative damage of purines and pyrimidines, and the alkaline version was used for detection of single strand breaks. Mann-Whitney test was used for statistic evaluation the difference between both measurements, correlation analysis for relationships between Comet assay results and clinical parameters.

Significant correlations between a total amount of nutrients given parenterally and detected oxidative DNA damage were found, as well as between the individual amount of saccharides, proteins and lipids and oxidatively damaged purines, with lipids showing the most importance. The results indicate that the levels of oxidative DNA damage are significantly higher in patients before a change of nutrition in comparison to the control group. However, the levels of oxidative DNA damage in patients after a change of nutrition are not significantly higher than the control group.

Described results of this pilot study show possible effect of accurate nutritional support on reduction of oxidative DNA damage in polytraumatic patients. Other study is needed for evaluation on large sample of patients.

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HPLC-MS DETERMINATION OF ACTIVE BIOLOGICAL COMPOUNDS IN ELBE RIVER

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Insufficient removal of pharmaceutically active compounds by water plants leads to the increased levels of such compounds in the surface waters. Chronic exposure by these compounds may lead to severe consequences on the water organisms, balance of the ecosystems and biodiversity[1].

HPLC-MS (Triple quadrupole mass spectrometer WATERS Premier XE, UPLC WATERS Acquity) was used to determine the levels of biologically active compounds in surface water. The samples were collected from different locations of Elbe River and its tributaries. The compounds assessed in this study were chosen with respect to their consumption and therefore probability with which they might occur in the surface waters[2].

Chromatographic conditions were optimized with respect to the analyzed compounds. The samples were analyzed after previous filtration without further modification. Limits of detection for each individual compound were ranging from 5 ng/l to 100 ng/l.

Pharmaceutically active compounds found in the highest levels were betaxolol (highest concentration 803 ng/l), gabapentin (highest concentration 747 ng/l), carbamazepine (highest concentration 153 ng/l), acetaminophen (184 ng/l).

Interestingly the highest concentrations of above mentioned compounds were found in smaller streams and rivers. Elbe River itself is not so polluted by pharmaceutics compared to its smaller tributaries.

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ANTIPROLIFERATIVE ACTIVITY OF NOVEL DEXRAZOXANE ANALOGUES AND THEIR EFFECT ON ANTITUMOR EFFECTIVENESS OF ANTHRACYCLINES

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Anthracycline antibiotics (such as daunorubicin, doxorubicin or epirubicin) belong to the most common and also the most effective chemotherapy regimens of the treatment of both solid tumors and hematological malignities. The clinical usefulness of anthracyclines is nevertheless hampered by their adverse effects, mainly most serious and dose-limiting cardiotoxicity. The possible pharmacological protection of anthracycline cardiotoxicity in the clinics is so far limited to the only approved cardioprotective substance – dexrazoxane. Traditionally, dexrazoxane is believed to act as through its iron-chelating hydrolytic metabolite ADR-925, but besides that, dexrazoxane acts as a catalytic topoisomerase II inhibitor and its precise mechanism of cardioprotection has not been elucidated yet. Moreover, dexrazoxane use is very limited mainly due to its relatively high price, concerns of side effects and hampering anthracycline antineoplastic effect. The synthesis of dexrazoxane analogues with different iron-chelating and topoisomerase II activity effects could therefore provide us with the knowledge of dexrazoxane mechanism of action together with the possibility of finding an analogue with better pharmacological properties.

At the Department of Inorganic and Organic Chemistry, Faculty of Pharmacy in Hradec Králové, the two novel analogues of both dexrazoxane (MK-15, ES-5) and ADR-925 (JR-159, KH-TA4) were synthesized and then we assessed their antiproliferative activity and their effect on antitumor effectiveness of daunorubicin using leukemia cell line HL-60. Cells were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum and 1% penicilin-streptomycin solution at 37 °C in a humidified atmosphere of 5% CO_2. For proliferation assays, cells were plated on 96-well plates at a density of 10,000 cells per well and incubated with the novel analogues alone and in combination with daunorubicin. Cellular proliferation was assessed using MTT assay. After 72 h of incubation, the MTT solution was added to the cell suspension to a final concentration of 0.6 mg/ml and incubated for 2 h. Then the cells were lysed with lysis buffer (isopropanol, 0.1 M HCl, 10% Triton X-100) overnight in room temperature with shaking. After dissolving, the optical density of reduced formazan was measured at 570 nm, subtracting the 690 nm background. The proliferation rates of the experimental groups were expressed as percentages of the untreated controls (100%). Moreover, we also assessed the rate of iron displacement from its complex with anthracyclines by the novel analogues and their interaction with topoisomerase II in solution.

All the studied substances caused statistically significant drop in the HL-60 proliferation, but the effect was marked enough to determine the IC50 only in dexrazoxane (IC50 = 25 μM) and KH-TA4 (IC50 = 39 μM). Other studied analogues reduced the proliferation of HL-60 cells for less than 50% of control value and therefore the IC50 value could not

be established. The novel agents (10 and 100 μM) did not compromise the antiproliferative action of 15 nM daunorubicin (IC50 value). In contrast, all the studied substances significantly enhanced the toxicity of daunorubicin to leukemic cells in both concentrations used. The relaxation activity of topoisomerase II was inhibited by 100 μM dexrazoxane, but none of the novel analogues showed in the same concentration any inhibition activity towards this enzyme isoform. From the novel analogues, only JR-159 was able to at least partially displace iron from its daunorubicin complex.

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STUDY OF EFFECTS OF PROLONGED ADMINISTRATION OF TENOFOVIR DISOPROXIL FUMARATE AND EMTRICITABINE ON EXPRESSION OF ABC EFFLUX TRANSPORTERS IN MATERNAL AND FETAL ORGANS

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The most common way of spreading HIV infection is mother-to-child transmission (MTCT). Nowadays, combination of nucleotide/nucleoside inhibitors of reverse transcriptase tenofovir disoproxil fumarate (TDF) and emtricitabine (FTC) is widely recommended by World Health Organization to prevent MTCT of HIV. To guarantee safe therapy with TDF/FTC, it is important to know interactions of both drugs with efflux drug ATP-binding cassette (ABC) transporters. Recent studies have shown that pharmacokinetics of TDF and FTC can be affected by two important ABC transporters – P-glycoprotein (MDR1, ABCB1) and Breast Cancer Resistance Protein (BCRP, ABCG2). The influence of TDF or FTC on MDR1 and BCRP expression level has not been sufficiently investigated yet. Using a model of pregnant rat (Wistar) we aimed to analyze expression of rat orthologs *Mdr1a*, *Mdr1b*, and *Bcrp* in the placenta, maternal organs (brain, liver, kidneys and intestine) and also in fetal organs (brain, liver, kidneys and intestine). TDF or FTC (saline was used in the control group) has been administered to subject rats intramuscularly from 12th to 21st day of gestation. *Mdr1a*, *Mdr1b*, *Bcrp* have been analysed from isolated RNA using qRT-PCR. We have observed that long time therapy with TDF or FTC during gestation does not cause any statistically significant changes in mRNA expression of the genes tested. Therefore, it can be concluded that the pharmacokinetics of MDR1 and BCRP substrates should not be changed in mother on TDF/FTC therapy or her newborn child. These data enhance knowledge on the safety profiles of TDF/ FTC.

IN VITRO ASSESSMENT OF THE ANTIPROLIFERATIVE ACTIVITY OF NEW SET OF SIH ANALOGUES

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Iron (Fe) is an essential element for cell growth and metabolism. In comparison to normal cells, neoplastic cells require higher amount of Fe to facilitate their rapid proliferation. Hence, the new strategy for cancer treatment has focused on Fe withdrawing from cancer cells by Fe chelators. SIH is an intracellular iron chelator with optimal lipophilicity for oral administration. Earlier studies have demonstrated its promising antiproliferative potential. However, there is a limitation represented by relatively short biological half-life of SIH due to its labile hydrazone bond that makes it prone to hydrolysis in plasma. Therefore new aroylhydrazone chelators derived from aromatic methyl- and ethylketones and naphtohydrazide and biphenylcarbohydrazide were recently developed at the Department of Inorganic and Organic Chemistry of our faculty in order to improve their stability by the methyl or ethyl group adjacent to hydrazone bond and boost their membrane permeability by increased lipophilicity.

The aim of this study was to evaluate antiproliferative effects of the six newly synthesized Fe chelators on MCF-7 human breast adenocarcinoma cell line and their toxicities towards rat-derived cardiomyoblast cell line H9c2.

Cellular viability was evaluated using neutral red uptake assay. The chelation activity in solution was studied using weak iron chelator calcein. The effect of the chelators on Fe uptake and mobilization from cells was evaluated through studies with ^{59}Fe. The redox activities of the Fe complexes of the tested chelators were investigated using the ascorbate oxidation assay. The MCF-7 cellular morphology and mitochondrial inner membrane potential were observed by phase contrast and epifluorescence microscopy. All the tested agents showed significant and dose-dependent decrease of either MCF-7 or H9c2 cells proliferation or viability, respectively. The examined chelators were generally more toxic to cancer cells and less or equally toxic to normal cells as compared to SIH. Fe complexes in a 2:1 chelator:Fe(III) ratio of all of the evaluated substances, except for H26, showed decreased antiproliferative effect after 72-h incubation with MCF-7 cells. On the other hand, the formation of Cu(II) complexes enhanced the antiproliferative activities. In solution all the studied substances showed lower ability to form Fe(III)-chelator complexes in comparison with SIH. The determination of the ability of the studied chelators to prevent ^{59}Fe uptake from $^{59}Fe_2$transferrin and to mobilize ^{59}Fe from MCF-7 cells showed that in general the naphtohydrazide analogues were more active in preventing ^{59}Fe uptake and promoting ^{59}Fe mobilization than the biphenylcarbohydrazide derivates. From the studied substances only H26 showed significant increase of ascorbate oxidation, in contrast to the reference agent SIH that prevented the ascorbate oxidation. The assessment of cellular

morphology and inner mitochondrial membrane potential showed, that all the tested new chelators displayed high potential to induce cell death and induced a rapid mitochondria depolarization on MCF-7 cells, whereas chelator SIH, in the same 3μM concentration, did not cause any change compared to untreated control.

Results of this study indicate that in this new set of more lipophilic SIH analogues may be potent and selective anticancer agents. However, future research is needed to assess the effect in more cell lines, *in vivo* cancer models and further examine the involved mechanisms.

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DETERMINATION OF RESTING ENERGY EXPENDITURE AND NUTRITION SUBSTRATE UTILIZATION IN PATIENTS WITH ADVANCED FORM OF CHRONIC OBSTRUCTIVE PULMONARY DISEASE

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Chronic obstructive pulmonary disease (COPD) is the name for lung diseases including chronic bronchitis, chronic obstructive airways disease and emphysema. COPD usually develops over the years and is not fully reversible. Main symptoms of COPD are frequent infections of chest, persistent cough with phlegm and shortness of breath. Besides the respiratory symptoms there are also described changes in body metabolism, which could lead to the development of serious metabolic syndrome called cachexia.

The main aim of this study was to compare the resting energy expenditure and nutrition substrate utilization in 12 patients with advanced form of COPD from the Czech Multicentre Research Database of COPD (5 females and 7 males, mean age 68 ± 6 years) and in 9 patients of control group without respiratory impairment (5 females and 4 males, mean age 62 ± 4 years).

Body metabolism was determined by means of an indirect calorimeter with ventilated hood system at basal conditions (subjects were measured after an overnight fast, in the supine position, completely at rest and in thermally neutral environment). The values of resting energy expenditure (REE) were calculated from measured values of oxygen consumption and carbon dioxide production in exhaled air. Measured REE was then compared with prediction based on Harris-Benedict equation. The utilization of main nutrition substrates (saccharides, lipids and proteins) was determined from the respiratory quotient and urea nitrogen loss in urine. Results are presented as mean ± standard deviation.

We found that measured REE in COPD patients was about 22% higher than in control group (1913 ± 456 and 1567 ± 238 kcal/day or 122 ± 15 and 102 ± 14% of predicted values, respectively). Similar results were observed also if values of REE were adjusted for

body weight, amount of fat-free mass or body surface area. When we separated patients to three categories according to their level of metabolism, we found that 83% of COPD patients were hypermetabolic (more than 110% of predicted values of REE), 17% normometabolic (90-110% of predicted values of REE) and none of them hypometabolic (less than 90% of predicted values of REE). By contrast in control group were 50% of patients normometabolic, 38% hypermetabolic and 12% hypometabolic. While we found no statistical significant differences in total amounts of utilized nutrition substrates between COPD and control group, the relative proportion of utilized proteins was significantly lower in COPD patients in comparison with control group (16 ± 4 and 21 ± 5%, respectively).

This study approved the increase in REE and changes of substrate utilization in patients with advanced form of COPD.

The study was supported by the project MH CZ-DRO (UHHK, 00179906), PRVOUK P40, UNCE 204026/2012 and Faculty of Pharmacy (SVV 260 064).

THE EFFECT OF 5-*TERT*-BUTYL-*N*-(4-CHLORBENZYL)PYRAZINE-2-CARBOXAMIDE ON FLAVONOLIGNAN PRODUCTION *SILYBUM MARIANUM (L.) GAERTN.* CULTURES *IN VITRO*

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The subject of this study is the evaluation of secondary metabolites production in *Silybum marianum (L.) Gaertn.* cultures *in vitro* after elicitor treatment. The aim of the study was to find if an abiotic elicitor 5-*tert*-butyl-*N*-(4-chlorbenzyl)pyrazine-2-carboxamide increases the flavonolignan production *S. marianum* cultures *in vitro.*

Experiment was carried out in callus and suspension cultures of *S. marianum* using Murashige – Skoog nutrient medium supplemented with 10 mg/l α-naphtylacetic acid as growth regulator. The elicitor was added in the form of solution in three different concentrations ($C_1 = 3.292 \times 10^{-3}$ mol/l, $C_2 = 3.292 \times 10^{-4}$ mol/l and $C_3 = 3.292 \times 10^{-5}$ mol/l) and it was affecting 6, 12, 24, 48, 72 and 168 hours. The content of flavonolignans and taxifolin was determined by HPLC. Flavonolignan release into nutrient medium was also a part of this study.

The maximum flavonolignan production (0.280 mg g^{-1} DW) in callus culture was observed after 24 hours of elicitor application in concentration of $C_2 = 3.292 \times 10^{-4}$ mol/l, when the highest content of silychristin was detected. The second significant increase in flavonolignan production (0.271 mg g^{-1} DW) in callus culture was noticeable after 12 hours of elicitor treatment in concentration of $C_3 = 3.292 \times 10^{-5}$ mol/l, when the highest increase in silydianin and silybin B accumulation was found. The maximum content of flavonolignan (1.871 mg g^{-1} DW) in suspension culture was detected after 48 hours of

elicitor treatment in concentration of $C_2 = 3.292 \times 10^{-4}$ mol/l, when the maximum production of silychristin and silydianin was observed.

Flavonolignan release into the nutrient medium was also detected. In the case of callus cultures, the silydianin release was obvious especially after 168 hours of elicitor addition in concentration of $C_2 = 3.292 \times 10^{-4}$ mol/l and silybin A after 24 hours of elicitor treatment in concentration of $C_1 = 3.292 \times 10^{-3}$ mol/l. The significant increase of flavonolignan release from suspension culture into nutrient medium was found after 12 hours of elicitor treatment in concentration of $C_3 = 3.292 \times 10^{-5}$ mol/l, when the presence of silychristin, silydianin and silybin A was detected.

The elicitor 5-*tert*-butyl-*N*-(4-chlorbenzyl)pyrazine-2-carboxamide is able to increase the flavonolignan production in *S. marianum* cultures *in vitro*.

The study was supported by SVV 260065 and the project implementation: "Support of Establishment, Development and Mobility of Quality Research Teamsat Charles University", the project number CZ.1.07/2.3.00/30.0022, supported by the Education for Competitiveness Operational Programme (ECOP) and co-financed by the European Social Fund and the state budget of the Czech Republic.

RATING OF BUTYRYLCHOLINESTERASE ACTIVITY IN THE CZECH POPULATION

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Butyrylcholinesterase, BChE (EC 3.1.1.8) is non-specific enzyme which hydrolyses many different types of choline esters. This enzyme plays important role in metabolism of drugs such as depolarizing neuromuscular blockers (succinylcholine), non-depolarizing neuromuscular blockers (mivacurium), local anesthetic (procaine) and other groups of drugs. Mutation or damage of gene encoding BChE results in slowed drugs metabolism.

BChE is synthesized in liver. It's released into plasma and its plasma level may indicate liver function. The BChE activity is also used in some pesticide poisoning evaluation.

The main goal of our study was to determine levels of BChE activity in the Czech population according to age, sex and smoking. Activity of BChE was found out in population of healthy blood donors (n = 400). Blood samples of healthy persons were achieved in cooperation with Transfusion Department, University Hospital, Hradec Králové. Samples were divided into three groups according to the age (until 25, more than 25 until 35, more than 35 until 45) and these samples were subsequently statistically evaluated. Supposition that BChE can be useful as liver function test was laboratory verified in population (n = 68) of patients with leukemia and its hepatotoxic treatment.

Measuring was based on modification on Ellman's method for determination of cholinesterase in blood[1].

Comprehensive results will be presented oraly during conference.

Study was supported by Internal Grant Agency (IGA) of Ministry of Health (Czech Republic), grant No. NT-12062.

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THE EFFECT OF FLAVONOIDS ON SELECTED BIOTRANSFORMATION ENZYMES

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Catechins belong to the flavonoids and they are the main polyphenolic compounds of green tea. Catechins are considered to be very beneficial for the human organism. The popularity of dietary supplements with high levels of flavonoids is still increasing, but contrary to the positive effects they can bring lots of risks. A whole range of biotransformation enzymes can be modulated by high levels of catechins and thus can affect both desirable and undesirable effects of many drugs. Polyphenon 60 (POE) is a defined green tea extract that is consisted of catechins.

The aim of our study was to evaluate the effect of POE and its constituents – catechin, epicatechin, epigallocatechin (EGC), epicatechin-3-gallate and epigallocatechin gallate (EGCG) on the cell viability and on the activity of selected biotransformation enzymes. The effect of POE and individual catechins was studied on the Caco-2 cell line.

Two different cell viability tests were used – MTT assay and NR-assay. There was no significant viability decrease in the non-proliferating cells in the presence of catechins. The viability of the proliferating cells can be decreased by high concentrations of several catechins (especially: EGC, EGCG and POE).

After the incubation of the CaCo-2 cell line with POE and several catechins the subcellular fractions (cytosol and microsomes) were prepared. The activities of glutathion Stransferase and sulfotransferase were detected in cytosol but no effect of catechins was found. The activities of aldo-keto reductase 1A1 and 1C were at the detection limit. The activity of carbonyl reductase was not detected. The activity of UDPglucuronosyltransferase and cytochrome P450 isoforms (1A1, 1A2, 3A) was measured in microsomes but no activity was detected.

The obtained results showed that the selected catechins have no effects on the activities of the selected biotransformation enzymes in comparison with the control samples. Normal

consumption of green tea seems to be safe, but extremely high doses of green tea extracts in dietary supplements could decrease viability of the proliferating cells.

The study was supported by the Charles University, SVV 260 065.

SECTION OF CHEMICAL SCIENCES

NMR SPECTROSCOPY IN STRUCTURAL ANALYSIS OF UNKNOWN SUBSTANCES

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Series of alkaloids from the plant *Berberis vulgaris* (Berberidaceae) was isolated at the Department of Pharmaceutical Botany and Ecology Faculty of Pharmacy in Hradec Králové.

The aim of this project was identification of an unknown chemical formula (code Ckr) employing NMR analysis. The structure of the unknown compound was determined on the basis of the NMR experiments (^{1}H NMR, ^{13}C NMR, gHSQC, gHMBC, gCOSY, NOESY).

The result of NMR elucidation is:

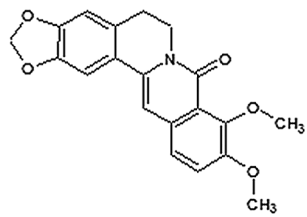

Fig. 1.

Structure of the compound (Fig. 1) was named 8-oxoberberine which belongs to the group of alkaloids of protoberberine type[1].

The project was supported by SVV UK 260 062.

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SYNTHESIS OF ROSCOVITINE ANALOGS BASED ON 1,2,4-TRIAZOLO[4,3-*A*]PYRAZINES

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Roscovitine, also known as seliciclib, is a synthetic purine-based experimental inhibitor of cyclin-dependent kinases (CDKs), i.e., serine/threonine kinases, which participate in cell cycle control and are frequently deregulated in cancer cells. Roscovitine (Fig. 1) is currently undergoing the clinical trials and is being evaluated for the treatment of lung or nasopharyngeal cancer.[1]

The success of roscovitine facilitated the development of related CDK inhibitors *via* the optimization of the substituents on the purine, changing the positions and ratios of nitrogen and carbon atoms in the heterocyclic core or by a combination of both. Several series of analogs with 2 to 5 heterocyclic nitrogens were developed and many of these bioisosteres showed similar activity to roscovitine.[1]

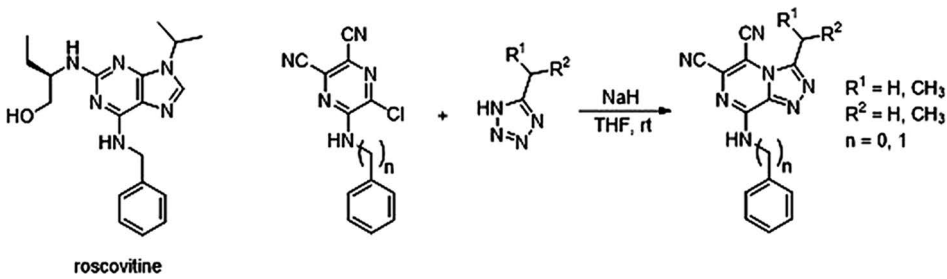

Fig. 1. Structure of roscovitine and its analogs studied in this work.

In this work we designed and prepared several roscovitine analogs derived from 1,2,4-triazolo[4,3-*a*]pyrazines. In the first step, 5-(benzyl/phenylamino)-6-chloropyrazine-2,3-dicarbonitriles were prepared from commercially available 5,6-dichloropyrazine-2,3-dicarbonitrile and aniline or benzylamine. These 6-chloropyrazines underwent a nucleophilic substitution with 5-alkyl-1*H*-tetrazoles. These reactions proceeded with spontaneous elimination of nitrogen and rearrangement to yield the final products (Fig. 1).

The study was supported by Charles University in Prague (SVV 260 062).

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SYNTHESIS OF AZAPHTHALOCYANINE CONTAINING ANIONIC GROUPS

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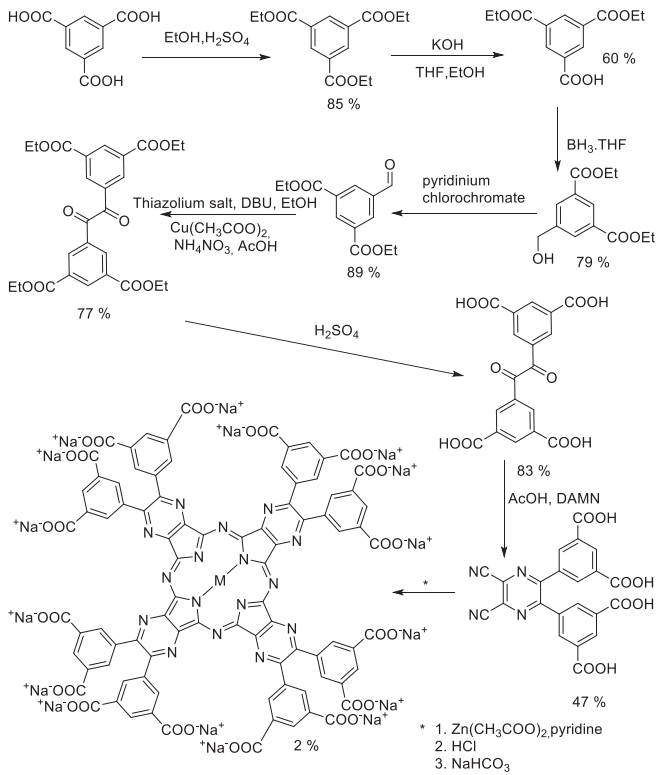
Photodynamic therapy (PDT) is type of therapy that can be used in the treatment of cancer. It consists of 3 essential components: photosensitizer (PS), light, and oxygen. None of these is individually toxic but together they initiate a photochemical reaction that culminates in the generation of a highly reactive product termed singlet oxygen.

Research in Azaphthalocyanine group focuses on synthesis, photophysical and photochemical behaviour of phthalocyanines and azaphthalocyanines (AzaPc) in respect to the application in PDT for a long time. Aim of this work is a synthesis of new PS from the group of water-soluble AzaPc with future potential to be used in PDT. The synthesized compound should contain peripheral carboxylic groups that can be turned into anionic by formation of salt. Presence of anionic groups is expected to lead to inhibition of undesired aggregation of the AzaPc in water medium.



Scheme

The starting compound for synthesis was trimesic acid that was esterified by ethanol (see Scheme). The triester was partially hydrolysed to mono carboxylic acid. Subsequently, the free carboxyl group was selectively reduced to hydroxyl and followed by oxidation to aldehyde. Benzoin condensation of this aldehyde gave acyloine that was oxidized to diketon. This product was hydrolyzed to diketon with four free carboxylic groups. Substituted pyrazine-2,3-dicarbonitrile, a precursor for AzaPc, was obtained by condensation of diaminomaleonitrile with this vicinal diketon. AzaPc substituted with sixteen free carboxylic groups was synthesized in a template reaction with zinc(II)acetate in pyridine. AzaPc was converted into the sodium salt and product was then purified by gel chromatography. The photodynamic activity of the final product and its butoxy ester was then tested *in vitro*.

The work was supported by SVV 260 062 and by Czech Science Foundation (13-27761S).

SYNTHESIS OF PRECURSORS OF 4-QUINOLONES ACTIVE AGAINST *TRYPANOSOMA BRUCEI* FOR ^{18}F-LABELLING

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Human African trypanosomiasis (HAT), also known as a sleeping sickness, is a parasitic disease caused by two subspecies of *Trypanosoma brucei (T. b. gambiense* and *T. b. rhodesiense*). This parasite is transmitted by the bite of infected tsetse flies, therefore people living in rural areas with an occurrence of this insect are at high risk. The sleeping sickness occurs in two clinical stages. The first one is characterized by the multiplication of parasite in the blood and lymphatic system. Very nonspecific symptoms, like fever, swollen lymph nodes, joint pain and headache are present. After a few weeks, a parasite crosses the blood-brain barrier and typical neurological symptoms like behavior changes, confusion, apathy, attacks of aggression and disruptions of sleep cycle appear. A coma and death results, if untreated.[1]

Nowadays, there are only five drugs used for the medical treatment of HAT. Suramine and pentamidine are used for the first stage, melarsoprol and eflornithin in combination with nifurtimox for the second (neurological) one. A life threatening side-effects and a developing of resistances are the reasons, why new compounds are urgently needed. Since it was discovered, that quinolones such as ciprofloxacin show antitrypanosomal activity, a quinolones-type library was synthesized and studied. *In vitro* evaluations and structure-activity relationships analysis have shown, that 4-quinolones with a benzylamide function in position 3 and cyclic or acyclic amines in position 7 possessed high antitrypanosomal activity. According to studies, one compound from a library, *N*-benzyl-1-butyl-6-fluoro-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxamide (Figure 1) exhibited promising *in vitro* activity against *T. b. gambiense* (IC_{50} = 47nM) and *T. b .rhodesiense* (IC_{50} = 9nM) together with a low cytotoxicity against macrophages.[1]

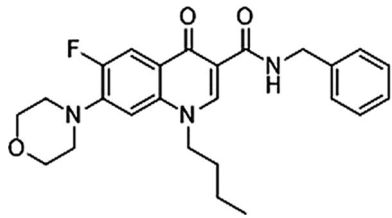

Fig. 1. N-benzyl-1-butyl-6-fluoro-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxamide.

Nevertheless, ^{18}F-radiolabeled compound is needed for *in vivo* testing. Measurements using positron emission tomography-computed tomography would clarify, whether the compound is able to cross blood-brain barrier to be a potential drug against the second stage of HAT. In this study, I focused on the synthesis of a precursor of *N*-benzyl-6-fluoro-1-[3-(fluoro-^{18}*F*)propyl]-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxamide and its nonradioactive ^{19}F.

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DEVELOPMENT AND VALIDATION OF SFC-UV METHOD FOR THE DETERMINATION OF AGOMELATINE AND ITS IMPURITIES

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Agomelatine is the first antidepressant from a new group of melatonin agonists and selective serotonin antagonists. Agomelatine is a synthetic analogue of hormone melatonin, which regulates circadian rhythms and is indicated for the treatment of depression disorders in adults. The aim of this study was to develop and validate a new analytical method for the determination of agomelatine and its six potentials impurities: (7-methoxynapht-1-yl) ethylamine hydrochloride, (7-methoxynapht-1-yl)acetonitrile, bis[2-(7-methoxynaphtalen-1-yl)-ethyl]amine, (7-methoxynapht-1-yl)acetamide, (7-methoxynapht-1-yl)acetonitrile, (7-methoxynapht-1-yl)acetic acid. Their separation and quantification was accomplished by supercritical fluid chromatography with PDA detection.

The impurities of agomelatine include compounds with basic, acidic and also neutral properties. Therefore, one of the main goals was to compare the retention and selectivity on different stationary phases (BEH, BEH-2-EP, CSH PFP and HSS C18). Using additives such as, 20 mM ammonium acetate, 20 mM ammonium formate and 20 mM ammonium formate with 5% of water, to CO_2/MeOH based mobile phase was necessary to ensure symmetrical peak shapes. These separations were performed at following conditions: flow

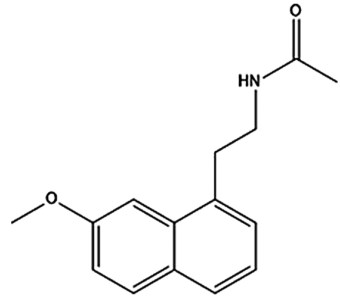

Fig. 1. The structure of agomelatine.

rate 2ml/min, column temperature 40 °C, UV detection at 275 nm and BPR 2000psi. The gradient program started at 5% of MeOH with 20mM additive and was increased up-to 30% within 3 minutes.

The column BEH-2-EP and gradient elution with 20 mM ammonium formate with the addition of 5% of water were chosen due to the best selectivity and resolution results.

During method development the solubility of tablets and the recovery of agomelatine in pure methanol and in mixtures of methanol and water in the ratio 1:1 and 1:3 was tested. The samples of the tablets or tablet's powder were dissolved in one of these solvents, and then diluted 10x with tetrahydrofuran to guarantee the compatibility of solvent and SFC mobile phase. Based on recovery results (99.7% for pure MeOH, 86.4% for ratio 3:1 and 77.5% for ratio 1:1) and RSD (4.8% for pure MeOH, 8.6% for ratio 3:1 and 20% for ratio 1:1) the pure methanol was finally chosen.

The developed method was validated in terms of linearity, limit of detection, limit of quantification, accuracy and precision. The method demonstrated good linearity with correlation coefficient values > 0.9992. Detection limit for impurities was 0.25 μg/ml – 0.36 μg/ml, quantification limit 0.83 μg/ml – 1 μg/ml. The accuracy and precision were measured at four concentration levels: 10, 40, 50 and 60 μg/ml for agomelatine and 1, 2, 2.5 and 3 μg/ml for impurities. The results of accuracy were between 97.6% and 100.4% for agomelatine and 90.5% – 103.5% for impurities, respectively. Method precision demonstrated RSD < 0.77% for agomelatine and < 3.44% for impurities. Subsequently the measurement of real samples was performed. The advantages of newly developed SFC-UV method are its environmental friendliness due to mobile phase used, a good resolution, selectivity and high speed of analysis (total time of separation is 4.1 min). In conclusion this allows for the widespread use of this method in quality control in pharmacy.

The study was supported by SVV/2014/260063.

CAMPHORQUINONE-DERIVED AZAPHTHALOCYANINE: FORMATION OF AN UNEXPECTED SIDE PRODUCT.

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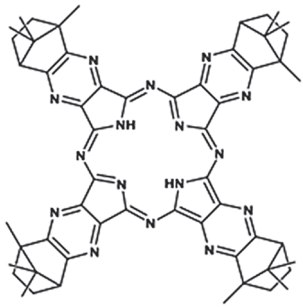
Phthalocyanines (Pc) are a group of planar synthetic macrocyclic molecules structurally similar to the naturally occurring porphyrinoids, which show some attractive photophysical properties. They have found many applications from which the most promising is singlet oxygen production. They are able to absorb the energy of light and transfer it to the surrounding molecules creating a highly reactive species, the most important being singlet oxygen. This can be used in photodynamic therapy (PDT) which is used to destroy tumor cells, kill microbes etc. PDT uses three non-toxic components: light, photosensitizer (PS) and oxygen to create reactive oxygen species. The property of the PS is determined both by the central cation and the peripheral substituents.



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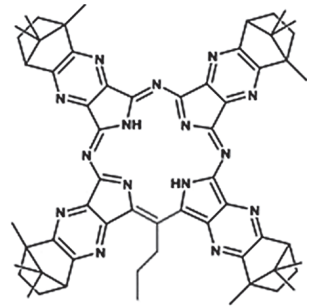

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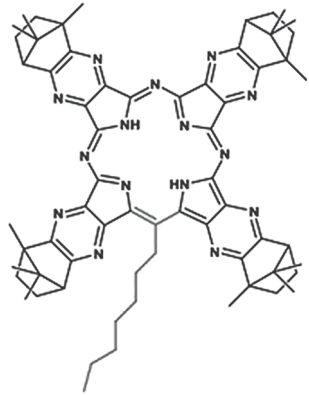

3

The original aim of this work was to synthesize compound **1**, an aza-analogue of Pc, with bulky peripheral substituents derived from camphor that could prevent the aggregation of the compound. Subsequently, a series of various metal complexes of **1** were planned. The precursor of **1**, substituted pyrazine-2,3-dicarbonitrile, was prepared by condensation reaction between diaminomaleonitrile and camphorquinone. However, an interesting side product of different color and with a slightly higher R_f than the targeted compound **1** occurred upon cyclotetramerization of the precursor in butanol with lithium. Cyclotetramerization in octanol with lithium led to the similar by-product with even higher R_f. Mass spectra (MALDI-TOF) revealed that the structure corresponds with unusual products **2** and **3** with an aliphatic chain instead of one nitrogen atom on the central porphyrazine ring of. The aliphatic side chain most likely originated from the solvent the reaction was carried in.

The study was supported by SVV 260 062.

SYNTHESIS OF POTENTIAL ORGANOCATALYSTS BASED ON QUINAZOLINE ALKALOIDS

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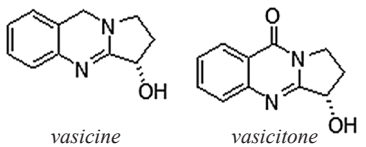
A series of substances derived from vasicine-type alkaloids (Fig. 1) was synthesized. The compounds were prepared using different α-hydroxy carboxylic acids (lactic and mandelic) (Fig. 2) and α-amino carboxylic acids (phenylglycine and alanine) (Fig. 3). These derivatives are currently being tested for their organocatalytic activity on a series of reactions, such as asymmetric enamine catalyzed aldolisation and conjugate addition of aldehydes to nitroalkenes.



vasicine *vasicitone*

Fig. 1.

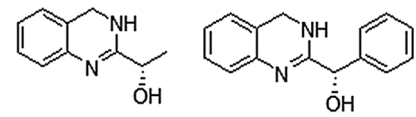


Fig. 2.

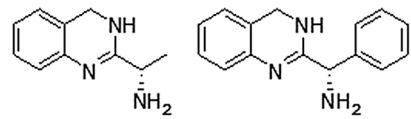


Fig. 3.

The study was supported by GA UK (No. 5671/2012), GA ČR (No. P207/10/2048) and Charles University Research (SVV-260-062).

DERIVATIVES OF CARBAZOLE AND THEIR INFLUENCE ON MELTING POINT OF DNA DUPLEXES

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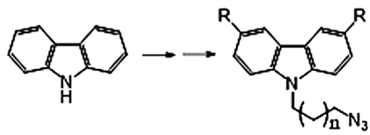
It is known that some molecules able to bind double-helix DNA by inserting into the Minor Groove increase the bond strength of complementary strands of DNA. Such molecules are referred as MGB (Minor Groove Binders). Thanks to binding in the minor groove they complicate the transcription and subsequent translation and multiplication of unwanted cells or tissues, which can be used in anti-infective therapy or as antineoplastics.

More recent MGB applications of great significance for the practice is their employment for the nucleic acid duplexes stabilization, emerging during molecular biological and genetic methods used for the detection of target DNA sequences, mutations, etc.

The aim of my work was to synthesize new molecules with MGB activity.

I chose the molecule of carbazole as a starting material for preparing the MGB because it meets the criteria: it is small, planar and the suitable basic substituents attached at positions 3 and 6 would fit into the small curvature of the groove (1). Nitrogen in position 9 could be used for introduction of a spacer to attach the modified carbazole molecule to the 5′-end of oligonucleotide (2).



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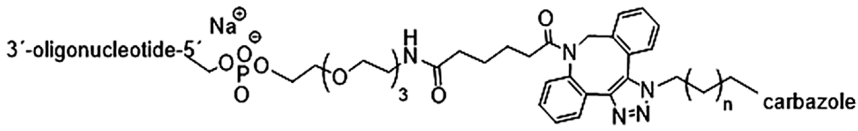

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The study was supported by SVV 260 062.

QUANTIFICATION OF LIPOPHILICITY BY HPLC

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Lipophilicity is one of many properties, which are determined in drug development. During the development process it is very important to find out some of the physicochemical parameters like lipophilicity to predict behaviour of the drug in human body. This property could be determined as a partition coefficient in octanol-water system, but because of its significant disadvantages, various attempts try to find a better way, how to estimate the partition coefficient and its logarithm (logP).

Currently reversed phase high performance liquid chromatography (RP-HPLC) is a promising method for the determination of logP. The partition coefficient is determined by a proportion of the concentration of compound in the octanol phase and its concentration in the aqueous one. In RP-HPLC the retention factor *k* of the compound in chromatography system of a non-polar stationary phase and a polar mobile phase is always used to quantify lipophilicity.

During the experiment, we tried to find out the simplest way, how to determine lipophilicity. All of indirect separation methods are based on construction of a correlation model[1]. To determine log*k* and then logP, we used the extrapolation of the measured *k* to zero organic solvent (in our case methanol).

ZORBAX Eclipse XDB-C18 was chosen as a suitable surface chemistry for our purpose. The original method, serving as a starting point, had a serious disadvantage. Because of long column used retention times were too high, especially for very lipophilic compounds. This can be fixed by rising flow rate and shorting the column like in our case. Compared to the original method, we used 5 times shorter column with particle size 1.8 µm against 5 µm, so the retention time get 2.5 times shorter. Then it was easier to determine log *k* with high accuracy, which was tested by measurement of reproducibility. Retention factors of studied substances were measured at five different compositions of mobile phase (methanol/water). According to the literature[2] plot of log *k* versus composition of mobile phase is linear. Consequently for getting the most accurate results, the values of *k*, measured in proportion of the organic phase to water 70:30 to 30:70, were plotted and extrapolated to zero methanol content.

Using RP-HPLC to determine partition coefficient contribute to get quick results, which may be used for example in drug development thanks to small amount of chemical entity, which is needed.

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SYNTHESIS AND BIOLOGICAL EVALUATION OF DIMEFLURONE AND BENFLURONE DERIVATIVES

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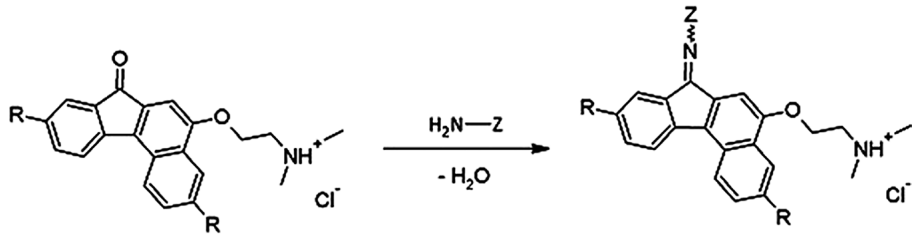
Dimeflurone and benflurone are unsaturated polycyclic compounds with a carbonyl group on the central five-membered cycle. Water solubility is enabled by a tertiary ammonium salt group on the side chain. Although both of them are effective against proliferating carcinomas, they are also toxic and undergo fast metabolic deactivation.

The oxo function, the main target of tumor cell reductase, is essential for therapeutic effect. Therefore we focused on as yet unreported structural modifications keeping the double bond intact. For this purpose, nucleophilic addition-elimination was the easiest procedure to consider.



R = OMe or H

To this end, we prepared eight derivatives of dimeflurone and benflurone with different nucleophiles, their cytostatic activities were tested on mice. We have also optimized the large-scale synthesis of dimeflurone with special focus on Stobbe condensation and Williamson synthesis.

SYNTHESIS OF AZAPHTALOCYANINES BEARING ONE 2,6-DI(*TERT*-BUTYL) PHENOL SUBSTITUENT

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Azaphtalocyanines (AzaPc) are planar macrocyclic dyes, which can find applications as the quenchers of fluorescence, as the sensors emitting in the red part of the spectrum and as the photosensitizers in the photodynamic therapy.

AzaPc peripherally substituted with aromatic amines are known to deactivate excited states very fast. The deactivation is caused by intramolecular charge transfer (ICT) from donor (amine) to acceptor (AzaPc macrocycle). The ICT in these molecules was shown to be blocked, e.g. by protonation donor nitrogen in acidic media[1].

In this work, AzaPc bearing one 2,6-di(*tert*-butyl)phenol substituent was synthesized. Phenolic OH in this moiety is a weak donor for ICT. However, this group can be ionized in basic media and the resulting phenolate anion is very strong donor. This will cause ICT and quench fluorescence of this compound.

The precursors for AzaPc were prepared *via* two-step reaction of 5,6-dichloropyrazine-2,3-dicarbonitrile with 2,6-di(*tert*-butyl)phenolate followed by nucleophilic substitution by *tert*-butylthiolate. Resulting AzaPc was prepared by cyclotetramerization of two precursors initiated by magnesium butoxide. Congener (in metal-free form) bearing one 2,6-di(*tert*-butyl)phenol substituent was isolated from the mixture of six congeners and converted to zinc complex.

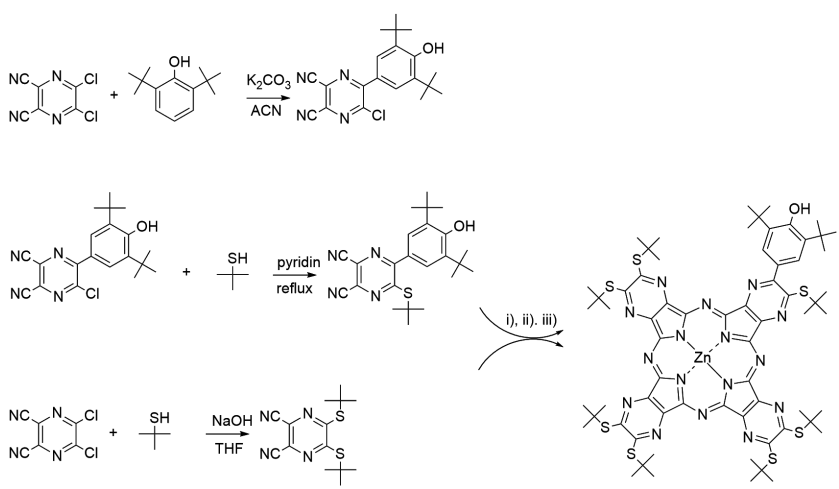

Fig. 1. Synthesis and structure of AzaPc. Reaction conditions: i) Mg, BuOH, reflux, ii) p-toluenesulfonic acid, THF, iii) $Zn(CH_3COO)_2$, pyridine, reflux.

The influence of properties of environment on fluorescence was subsequently studied – in particular the influence of polarity of solvent and presence of a base. The more polar solvents appeared to support the ICT most likely due to stabilization of charge separated states. AzaPc was also sensitive to bases and formation of the phenolate quenched the fluorescence leading to changes in its intensity for magnitude factor of 20. The process was found to be fully reversible.

The study was supported by the Charles University in Prague (SVV 260 062).

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SYNTHESIS OF TRICLOSAN CARBAMATES AND NEW SALICYLANILIDES

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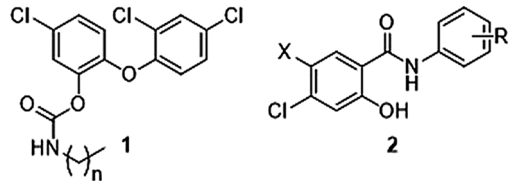
During our group's efforts for the development of the existing active molecules against *Mycobacterium tuberculosis*, rational chemical modifications on molecular structures have previously afforded derivatives with increased pharmacokinetic profile and/or activity.[1] Here in we present our dual approach for the development of existing antimicrobial and/or antimycobacterial molecules.

a) Triclosan has been reported as an inhibitor of the enoyl acyl carrier protein reductase one essential enzyme in the synthesis of fatty acids of *Mycobacterium tuberculosis*.[2] However, it is believed that the existence of the phenol group contributes to the decreased bioavailability.[3] We have previously shown that the formation of carbamates in order to protect the phenolic hydrogen of salicylanilides, enhanced the pharmacokinetic profile of the molecules.[4]
b) N-substituted 2-hydroxybenzamides, broadly known as salicylanilides, demonstrated a wide range of biological activities, including antiviral potency.[5] The structural motif of the parent salicylanilide allows the synthesis of numerous analogues and thus appears to be a good substrate for drug development. Previous Structure Activity Relationship studies revealed that lipophilicity is a major factor for the activity of these molecules.[6] On our ongoing research our intention is to explore the influence of different substitutions on the parent structure, thus the introduction of a second halogen atom at the salicylic part was examined.

Concluding, we present the synthesis of triclosan carbamates **1** derived from the conjugation of triclosan and aliphatic isocyanates, as well as the synthesis of novel salicylanilides possessing two halogens at the salicylic part at positions 4 and 5 (structure **2**). The biological activity of the synthesized molecules is currently under investigation.



General structure of triclosan carbamates (**1**, n=0-10) and salicylanilides (**2**, X=Br or Cl).

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

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AU^{I}-CATALYZED SYNTHESIS OF 4-ARYL-SUBSTITUTED NICOTINIC ACIDS

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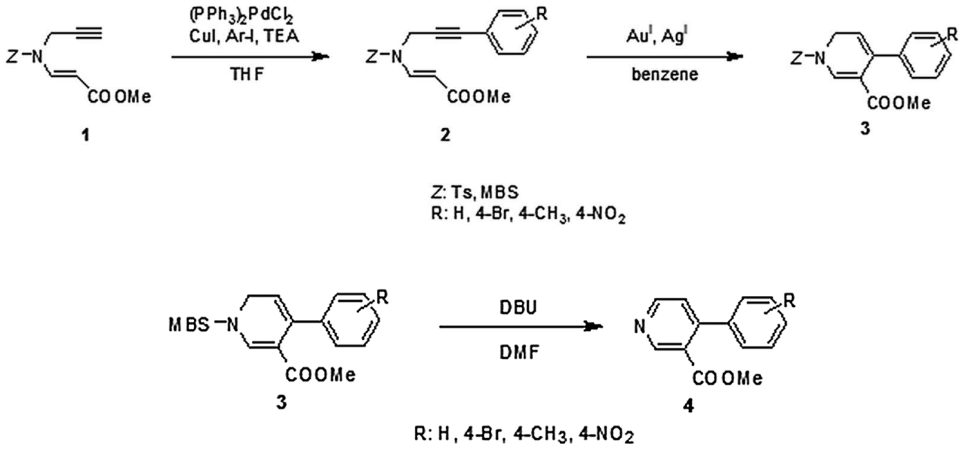
p-Toluenesulfonyl (Ts) or *p*-methoxybenzenesulfonyl (MBS) protected propargylamine reacts with methyl propiolate to form enyne **1** that undergoes Sonogashira coupling with aryl iodides. To this end, various aryl derivatives were prepared.

Substituted enynes (**2**) form cyclic derivatives (**3**) in the presence of gold (tri-(2-furyl) phosphinegold^{I}-chloride) and silver ($AgBF_4$) catalysts. MBS protection provides higher yields than tosyl.

Various types of deprotection conditions were tested. The use of DBU (1,8-diazabicyclo[5.4.0]undec-7-ene) in DMF provided the highest yields (about 50%).



This procedure leads to the preparation of 3,4-disubstituated pyridine derivatives (**4**) or more precisely 4-aryl-substituted derivatives of nicotinic acid.

The study was supported by GA UK (No. 567112) and Charles University Research (SVV260-062)

SYNTHESIS AND STUDY OF THE THIOSEMICARBAZONE ANTITUMOR AGENTS AND THEIR METABOLITES

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Iron chelators are a promising class of anticancer agents, which act through the iron deprivation of rapidly growing neoplastic cells with intensified iron metabolism. Iron is an important part of various intracellular processes as cellular respiration or DNA synthesis. The iron deprivation causes the decrease of metabolism and proliferation of cancer cells. In addition, the complexes of some chelators (e.g. thiosemicarbazones) with Fe or Cu can enter a redox cycle, leading to the release of reactive oxygen species, which further damage the DNA and cell membranes of tumor cells.

Recently, thiosemicarbazone iron chelators derived from 2-benzoylpyridine (BpT) and di-2-pyridylketone (DpT) have been studied for their high and selective antiproliferative activity.

The effective substances from this group with good *in vivo* anticancer potency are Bp4eT, Bp44mT, Dp44mT and DpC (Fig. 1).

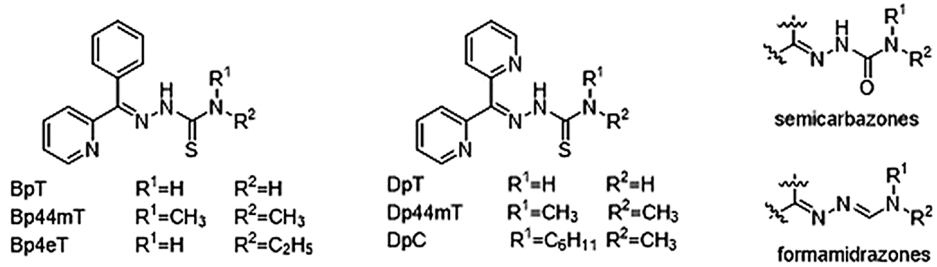

Fig. 1. Structures of thiosemicarbazone iron-chelators and their metabolites.

In this study, we prepared the parent thiosemicarbazone drugs together with their semicarbazone and formamidrazone analogs, which were used as standards in metabolic and pharmacokinetic studies. Furthermore, *in vitro* chelating properties, antiproliferative activities and toxicities of these drugs and their metabolites were studied.

This study was supported by the Charles University in Prague (SVV 260 062 and 260 065).

INFLUENCE OF CORROSION STATE OF MATERIALS ON INTERACTION WITH CELLS

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The titanium is one of the most progressive materials in dental implantology, mainly in alloys. It has very good corrosion resistance which is caused by the passivity. On the surface, there is a tin layer of oxides which stops the progress of corrosion. If the protective layer is destructed due to the aggressive conditions, the corrosion activity increases and may lead to the release of corrosion products and to the adverse reaction of organism.

In our work, we have focused on study of interaction between metal surface and cell lines and on comparation of different alloys of titanium after exposition by acid pH and presence of fluorides.

Samples from titanium, titanium grade 5 (Ti 90%hm., Al 6%hm, V 4%hm.), TiNb39 (Ti 61%hm., Ni 39%hm.) – cylinders Ø 8/3 mm, ground up to P1200, degreased, sterilized at 121 °C, 20 min. Corrosion exposure 18 hours by 37 °C in unbuffered physiological solution and buffered (phtalate buffer) physiological solution pH 4.2 with addition of 100, 200 and 1000 ppm of fluoride ions. Intensive washing by distilled water and by medium.

Adding culture of osteosarcoma cell line MG63. Cultivation 72 hours by 37 °C. Fixation with 5% glutaraldehyde and dehydration by alcohol. Preparing samples for scanning electron microscopy.

Cell line MG63 colonises researched materials differently. Influence of pH on colonization is minimal after exposition. Presence of florid ions in corrosive environment affects morphology of MG63 cells significantly.

This project has received financial support from the European Social Fund and from Government of the Czech Republic.

SYNTHESIS OF AZAPHTHALOCYANINES SUBSTITUTED WITH BULKY AROMATIC SUBSTITUENTS

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Azaphthalocyanines (AzaPc) are macrocyclic dyes, which can potentially found widespread applications in various fields of science. In particular, AzaPc are studied as a photosensitizers in photodynamic therapy, as fluorophores, fluorescence sensors or as dark quenchers.

In this work two new types of AzaPc with bulky peripheral substituents bound with stable C-C bond were synthesized. Furthermore, three complexes of each AzaPc were prepared: metal-free or coordinated with zinc or magnesium ion.

The first AzaPc, **ZIP 225**, carried eight peripheral phenolic hydroxy groups sterically hindered by adjacent *tert*-butyl substituents. These acidic hydroxy groups can be ionized and the resulting negative charge can cause an intramolecular charge transfer (ICT) and quench the fluorescence of this compound. This behavior may find its potential in sensoric applications. Second AzaPc, **ZIP 229**, carried eight peripheral methoxy groups with adjacent *iso*-propyl substituents. This AzaPc with unionizable substituents served as the ICT-negative control (Fig. 1).

The precursor for ZIP 225 was prepared *via* nucleophilic substitution of 5,6-dichloropyrazine-2,3-dicarbonitrile with 2,6-di(*tert*-butyl)phenolate. Bulky *tert*-butyls hindered the phenolate fully from *O*-substitution and strong M+ effect of the phenolate rendered the carbon in position 4 a sufficiently strong *C*-nucleophile. Such reaction is not possible in case of 1,3-di(*iso*-propyl)-2-methoxybenzene. The precursor for ZIP 229 was therefore synthesized *via* condensation of diaminomaleonitrile with vicinal diketon prepared from aldehyde by benzoin condensation and oxidation. The substituted pyrazin-2,3-dicarbonitriles were cyclotetramerized using magnesium butoxide (Fig. 1). Photophysical properties of AzaPc were studied.

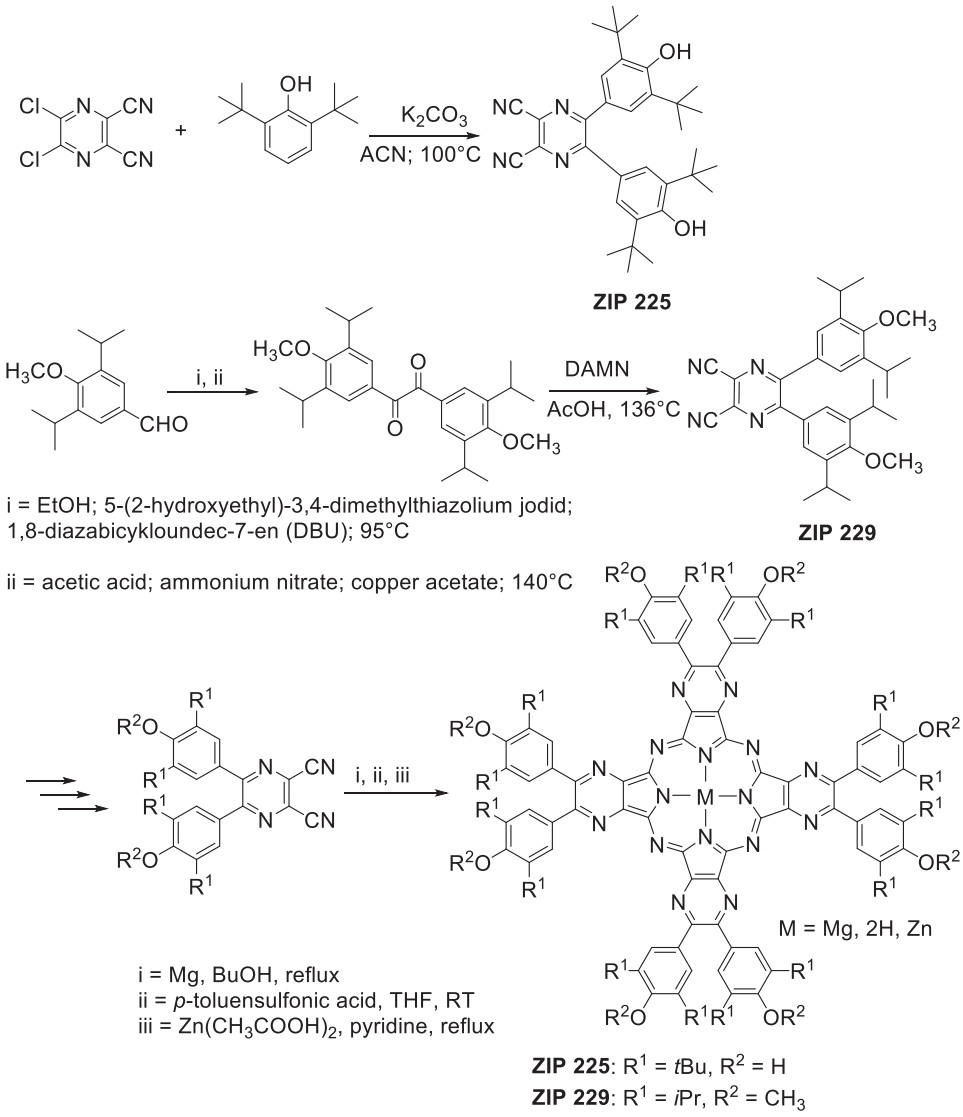

Fig. 1. Synthesis and structures of AzaPc ZIP 225 and ZIP 229.

The study was supported by the Charles University in Prague (SVV 260 062).

PREPARATION OF CATIONACTIVE TENSIDES WITH HYDROXYMETHYL MOIETY

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The work will refers about free homological series of cationic tensides bearing quarternary nitrogen in their molecule specifically 4-hydroxymethyl-1-alkylpyridinium-bromide, 3-hydroxymethyl-1-1alkylpyridinium-bromide a 2-hydroxymethyl-1-alkylpyridinium-bromide derivatives. Each of this series contained five homologues with alkyl linker length between 10 and 18 with even number of carbon atoms. Above mentioned compounds were characterised by melting point, NMR spectra and elemental analysis. Next topic of my work was evaluation of these compounds. Critical micelar concentrations were measured employing conductometric method. Compounds were further tested against several fungal and bacterial phylum. Structure- activity relationship studies of influence of substituted position and length of alkyl linker on previously mentioned properties were carried out.

The study was supported by SVV 260 062.

THE EFFECT OF BULKINESS OF NON-PERIPHERAL SUBSTITUENTS OF PHTHALOCYANINES ON THEIR ABSORPTION AND PHOTOPHYSICAL PROPERTIES

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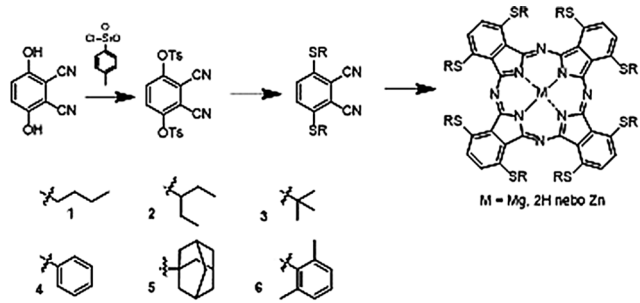
Phthalocyanines (Pc) are planar macrocyclic compounds which absorb light over 670 nm due to their large system of conjugated double bounds. Peripheral substitution of Pc significantly affects their final properties – for instance it influences the position of the main absorption band (Q band). Absorption at longer wavelengths is highly advantageous in any biological applications because such light does not interfere with endogenous chromophores. It was discovered that the desired red-shift of Q band is much more pronounced at non-peripherally substituted Pc. However, it was recently found out that presence of bulky non-peripheral substituent shifts significantly absorption maximum back to lower wavelengths.[1]



Scheme: Multistep synthesis of desired Pc **1–6**.

The aim of this study was to explain this phenomenon more in detail. For this purpose, a series of Pc bearing non-peripheral (alkyl- or arylsulfanyl) substituents of different bulkiness was prepared. Syntheses were performed according to the Scheme below and included nucleophilic substitution and cyclotetramerization with alcoholate as the initiator of the reaction. The prepared magnesium complexes Pc **1–6** were further studied from the absorption and photophysical point of view. Discussion on the unexpected shift in absorption spectra will be presented.

The study was supported by SVV 260 065.

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SECTION OF SOCIAL AND TECHNOLOGICAL SCIENCES

INHALER ADHERENCE IN PATIENTS WITH SEVERE CHRONIC OBSTRUCTIVE PULMONARY DISEASE

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Aims: Adherence to drug therapy for chronic obstructive pulmonary disease (COPD) is suboptimal and as such adds to the burden of mortality, hospitalization, and health care costs. The major problem is failure to adhere to inhaler use technique. The aims of this project were to assess inhaler adherence for different types of devices and to analyse various aspects of adherence in a cohort of patients with severe COPD.

Methods: 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 (http://chopn.registry.cz). Inhaler compliance assessment in each particular setting was performed by a pulmonologist. The assessment was structured into five steps to be followed while using an inhaler. Some steps may somewhat vary between different types of inhalers. Adherence to each step was assessed in a dichotomous manner (performed properly/improperly). After the step-by-step assessment of inhaler use technique, each respondent was asked to report how often he/she rinses his/her mouth after using the corticosteroid inhaler (every time: 75–100%, sometimes: 25–75%, never: 0–25%).

Results: One hundred and ninety patients were enrolled in the study (mean age of 67 years). They used different types of inhalers, sometimes in combination. The most often used devices were Handihaler (N = 110), pressurized metered-dose inhaler (pMDI) (N = 104), and Aerolizer (N = 89). The assessment of the step-by-step adherence to inhaler use technique revealed that less than 50% of the study cohort adhered properly to each of the five steps when using the following inhalers: Handihaler (40.9%), pMDI (35.6%), and Aerolizer (46.1%). For all types of inhalers, the highest rate of failure to adhere to inhaler use technique was observed for step 3 (failure to breathe out completely in one breath before taking the medicine with the next breath) – Handihaler (41.8%), pMDI (50.0%), and Aerolizer (44.9%). After using a corticosteroid inhaler, 63% of respondents rinse their mouth every time, 27.7% sometimes, and 8.9% never. Patients who fully adhere to inhaler use technique rinse their mouth every time after using the inhaler in 80.6% of cases while those who fail to use the inhaler properly rinse their mouth equally often in 55.7% of cases ($p < 0.001$).

Conclusion: Patients fail to adhere to inhaler use technique more often while using aerosol inhalers, but powder inhalers are also used improperly by more than half of respondents. The most common failure to adhere to inhaler use technique is that to breathe out completely in one breath before taking the medicine with the next breath. Patients who adhere better to inhaler use technique rinse their mouth after using the corticosteroid inhaler more often than those who fail to adhere to one or more steps to be followed when using a particular inhaler.

The study was supported by project SVV 260 066. The COPD project is registered in ClinicalTrials.gov with the identifier NCT01923051.

ARTERIAL HYPERTENSION AND NUTRITION: FAT-SOLUBLE VITAMINS A, D AND E.

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Arterial hypertension (AH) is a disease affecting population globally, and thus considered as a problem of public health and socioeconomic. Studies are trying to identify the connection between diet and the prevalence of arterial hypertension.

Objective of the study was to determine possible association between an occurrence of AH and fat-soluble vitamins A, D and E intake.

The nested, case-control population study investigation was grounded on database from the Spanish Hortega study, and performed on a random sample of 1,514 people (50.3% women, 49.7% men). From this sample we selected those aged ≥ 40 years old and untreated for hypertension and divided them into two groups: non-hypertensive (n = 429; 63.6%) (controls), and newly diagnosed AH (n = 246; 36.4%) (cases). Biochemical and anthropometric measurements, data on dietary intakes, education, socioeconomic status, place of residence, health habits, comorbidities, consumption of alcohol and tobacco were used for our study. Descriptive study of the data was carried out and compared by ANOVA and Chi-Square; analytical study was performed through logistic regression, calculating odds ratio and a set of adjusted models with different variables. No p value higher than 0.05 was considered significant.

The results showed that intake of vitamin A was higher in AH subpopulation (1732.77 ± 962.27 μg vs. 1655.89 ± 902.81 μg), and intakes of vitamins D and E were lower (8.13 ± 9.71 μg vs. 8.25 ± 9.52 μg and 18.79 ± 7.84 mg vs. 18.60 ± 8.20 mg, respectively), but with no statistically significant differences, neither in any of adjusted models.

This study did not identify any association between the intake of fat-soluble vitamins A, D, E and AH occurrence in any of investigated cases.

ACICLOVIR RELEASE FROM MUCOADHESIVE MATRICES

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Star-like low molecular weight copolymers of hydroxyl acids with polyhydric alcohols are promising drug carriers. The biodegradation rate of these polymers runs continuously over several hours to several days depending on the molecular weight parameters. Release of the incorporated drug may proceed simultaneously or just somewhat faster than their degradation. Recently, we have proved remarkable mucoadhesive parameters of substanc-

es of these types. Terpolymer of D,L-lactic acid, glycolic acid and mannitol as branching monomer was plasticized using triethyl citrate, ethyl pyruvate, methyl salicylate and ethyl salicylate. The mucoadhesive properties of these plasticized polymeric systems were determined using the Material testing machine (Zwick/Roel) with modified equipment for adhesive test. The contact time was 120 s, consolidation force 5 N, and rate of the sample detachment from the substrate 100 mm/min. Hydrated mucin from porcine stomach was used as a model substrate. The maximum force required for detachment of the adhesive material from the model substrate was recorded and related to the contact area. Aciclovir in concentration of 2% was incorporated into the plasticized oligoester carrier, and viscous matrices were applied on the model mucus substrate in glass vials, poured over with 20.0 mL of phosphate-citrate buffer pH 7.4 and placed into a shaking water bath at 37 °C. The dissolution medium was withdrawn and replaced at predetermined time intervals. The amount of Aciclovir released was analysed by spectrophotometry at wave length of 256 nm. The results show the sufficient mucoadhesion influenced by plasticizer type and concentration. The rate of Aciclovir release was the highest in case of carrier plasticized by ethyl pyruvate, and also in case of triethyl citrate, probably due to the hydrophilisation and prompt elution of plasticizer from the carrier. Ethyl pyruvate in concentration of 20% can be considered as the most suitable plasticizer of branched oligoester with respect to mucoadhesion as well as drug release.

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RHEOLOGICAL BEHAVIOUR OF SEMISOLID PHARMACEUTICAL PREPARATIONS

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From the rheological viewpoint semisolid preparations like gels and ointments are non-Newtonian systems exhibited shear-thinning with yield stress. It means the stress sufficient to disrupt a material structure must be applied to initiate significant flow. The viscosity decreases markedly (sometimes by many orders of magnitude) and the material makes the transition from an apparent solid to a free-flowing liquid. Not only a single-point test but a multi-point flow curve, over a range of shear rates will characterise rheological behaviour of semisolid preparations.

Rheological characteristics of the 1% Chloramphenicol eye ointment and common used eye ointment bases were measured on Malvern Kinexus rheometer at 25.0 °C and shear rate range from 0.100 to 10.000 s^{-1}, using cone upper geometry 2°/20 mm diameter. The Power Law (or Ostwald) Model and its two parameters, Power Law Index (or Flow Index) n, and Consistency coefficient K were used for their rheological behaviour. The Flow index n is a measure of non-Newtonian-ness. For a Newtonian system $n = 1$, for a shear-thinning systems n is between 0 and 1. Consistency coefficient K is viscosity (or stress) at

a shear rate of 1 s^{-1}. In comparison to the ointment bases the tested 1% Chloramphenicol eye ointment with K = 69 Pa.s and n = 0.40 can be based on Unguentum ophthalmicum simplex *Ph. Eur.*

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SAFETY OF THE SELECTED FOOD SUPPLEMENTS IN TERMS OF THE PRESENCE OF ADDITIVES

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Additives are commonly present not only in food, but also in food supplements. Its presence may pose a risk of side effects for some predisposed individuals. Food supplements are used by healthy people as part of preventive health care, but also by chronically ill patients – for whom, the presence of additives in not only in food supplements may mean a possible health risk. Currently, there is no study that addresses the issue of additives in food supplements. Only one study was published, which discusses the impact of dyes as additives in medicines[1].

The aim of the theses is to evaluate the safety of the bestselling supplements in the Czech Republic in the year 2011 from the viewpoint of additives and constituents.

List of best-selling nutritional supplements in the Czech Republic for the year of 2011 was obtained from one of several largest pharmacy chains. A list of the most frequently occurring substances contained in the top 100 food supplements was prepared, which was subsequently complemented with products with the same substance from the database of the Ministry of Health. As a source of information about the content and the presence of additives, the data from the Ministry of Health and the Information System decision of the Chief Hygienist (IS RoHy) were used. Given the scale of researched products, I focused on products strengthening the cardiovascular system containing omega 3 fatty acids, garlic, coenzyme Q 10 or lecithin. The safety evaluation of additives was carried out on the basis of the available methodology for consumers[2]. Food supplements were classified as safe (containing additives of categories 1 and 2 according to the above methodology) and harmful (containing additives of categories 3, 4, 5 according to the above methodology) with three degrees of harmfulness – 1st stage: mild (containing at least one additive substance of category 3, but do not contain any substances of category 4 and 5), 2nd stage: moderate – severe containing at least one additive of category 4 , but do not contain any additive of category 5), 3rd stage: severe (containing at least one additive of category 5). Information about adverse effects of additives were searched using information databases Martindale, Reprotox independent commission database JECFA Joint FAO / WHO Expert committee on Food additives)[3] or PubMed database. Descriptive statistics was used.

Of the large number of products (97), 5 products were classified as safe, and 92 as products harmful to health. In some products, I have found the same side effects in the content substance and additives contained there as well. The most common reactions were primarily gastrointestinal problems, and hypersensitivity reactions.

A pilot study of selected food supplements shows that although the majority of the products was evaluated only as slightly harmful, there are also products on the market that have been assessed as severely damaging to health and can cause side effects in predisposed individuals. Given that some of the adverse effects of additives and content substances were identical, its action may even be potentiated. Moreover, this finding raises the question whether the reported side effects of some supplements that are added to the substance content in these products can be caused or potentiated by the presence of additives with the same side effects. For these reasons, it is reasonable to continue researching additives contained in food supplements.

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INFLUENCE OF NANOPARTICLE STRUCTURES ON INTERACTIONS WITH PULMONARY SURFACTANT EVALUATED BY AF4

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Pulmonary surfactant is an essential part of lungs' function. It also forms a natural biological barrier. As of now, there is known only a little about behaviour of surfactant with nanoparticles, whilst respiratory tract is a promising way of drug delivery especially for emerging biologics bypassing the first-pass effect.

Due to its stickiness and complexity, experiments with pulmonary surfactant are often challenging, therefore state-of-the-art Wyatt Eclipse™ DUALTEC AF4 was chosen. For studying of influence on interactions of differently structured magnetite nanoparticles (PLGA, protein A coated, PEG 5000 coated, lipid coated, dextrin coated) with porcine surfactant, it was important to characterize nanoparticles by DLS, NTA, SEM, TEM and freeze-drying, develop AF4 method by determining optimal membrane, buffers and software settings and conduct experiments with nanoparticles and surfactant.

Interactions' experiments were performed at the determined optimal settings of PBS without potassium buffer, regenerated cellulose membrane 10 kDa at 20 °C. The results

revealed that PLGA, dextrin, PEG 5000 coated particles behaved similarly allowing for elution of particles and surfactant without significant disturbances of surfactant peak profile, whereas protein A and lipid coated particles appeared to interact with both membrane and surfactant heavily causing observable alterations to final surfactant peak profile.

The experiments shed light on so far neglected field of particle-surfactant interactions. The developed AF4 method appears to be well-suited for further studies in this matter. What is more, the AF4 presents an opportunity to conduct more relevant test at higher temperature and with non-magnetite nanoparticles which would otherwise be highly demanding or illusive at current state of the practice.

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QUALITATIVE ASPECTS OF ADHERENCE TO ANTIRESORPTIVE TREATMENT IN WOMEN WITH POSTMENOPAUSAL OSTEOPOROSIS

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Low compliance with oral bisphosphonates (oBIS) significantly affects effectiveness of the treatment. The study aim was to assess qualitative compliance with oBIS among Czech women in common clinical practice.

A cross-sectional multicentre questionnaire survey was performed in consecutive secondary care female patients aged ≥ 55 years. Compliance with five dosing instructions (DI) for safe use and adequate absorption of oBIS was evaluated. Score on the compliance was calculated: non-accordance with the manufacturer's guidelines or a missing answer to an individual item scored 0 point. The correct technique scored 1 point.

Response rate of the survey was 95%. As much as 363 self-reported questionnaires were used for analysis. Respondents (mean age 68.9 years) were treated with alendronate, alendronate + vitamin D in the fixed combination, risedronate (N = 36.6%) – once a week dosing interval and ibandronate (N = 63.4%) – once a month dosing interval. Only 44% of respondents were compliant to all five dosing recommendations. Compliance with DI that depends on time interval was 71% in weekly and 52% in monthly subgroups, respectively ($P < 0.001$). Compliance with dosing instructions (score) correlated positively with education ($P = 0.009$) and negatively with number of concomitant prescription drugs ($P = 0.010$).

Most patients do not comply with DI. Reduced bioavailability, particularly of monthly ibandronate, can be expected in clinical practice. All patients should be monitored for compliance with DI emphasizing the length of the time interval during each visit to the health facility. Particular attention should be given to patients with polypharmacotherapy and lower education level.

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A STUDY OF THE COMPACTION PROCESS AND THE PROPERTIES OF TABLETS WITH HYPROMELLOSE AND α-LACTOSE MONOHYDRATE

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Thesis studied the co-processed dry binder RetaLac® from the aspect of his compressibility and dissolution of the active ingredient from tablets. RetaLac® contains α-lactose monohydrate and hypromellose in the identical proportion. The same parameters were tested in the corresponding physical mixtures of Flowlac®100 with various types of hypromellose (Metolose® 100 SR, Metolose® 4000 SR, Metolose® 100 000 SR) and compared with the substance RetaLac®. Compressibility was evaluated by means of the energy profile of compression and tensile strength of tablets. Salicylic acid was used as the model active ingredient. Dissolution testing was performed using the method of the rotating basket.

The values of total energy of compression and plasticity were higher in the substance RetaLac® than in the physical mixtures of lactose with various types of hypromellose; tablet strength, on the contrary, was lower. Dissolution profile of the active ingredient from tablets with RetaLac® corresponded to the dissolution profile of tablets from a physical mixture of Flowlac®100® and Metolose® 4000 SR.

The study was supported by SVV 260062 and by the firms Meggle-Pharma, Shin-Etsu Chemical Co., Ltd. and SPI Pharma which supplied the samples of the excipients tested.

IS ADVERTISING OF OTC AND FOOD SUPPLEMENTS EVIDENCE-BASED? A SURVEY OF MAJOR LIFE STYLE JOURNALS FOR WOMEN

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Advertisements are important means of communicating information to public. The quality of the pharmaceutical advertising has been continuously criticized.

The objective of the study was to assess availability of supporting scientific information for health claims stated in the food supplements and OTC (over the counter) medicines ads that were published during periods September–December 2012 and January–June 2013 in the mostly read life style journals for women. For each advertisement, the emphasized claim/-s was compared to available scientific unbiased evidence. The literature search was conducted by using Pubmed database. Further information was searched in the Scholar

Google, Natural Medicines Comprehensive Database (Consumer version) and Micromedex. Different keywords and their appropriate combinations were used to identify relevant information. Frequency analysis was used to assess frequency of a particular ad occurrence.

Totally, there were 255 ads for OTC medicines and food supplements published in the surveyed period. Consistently with food supplements prevalence among the advertised products (n = 101; 80%) most of the ads were to promote this category (n = 190; 75%). There were 25 OTC medicines (20%) advertised with 65 ads (representing 25% of all analyzed ads). Evidence was searched for 6 OTC medicines and 6 food supplements. Generally, health claims in the OTC ads were considered to be better supported with evidence (with some exceptions with either missing information or information with very high risk of bias).

Health claims in the ads for both categories – OTC medicines or food supplements – can be in some cases considered as potentially misleading with no supporting information or information of very low quality.