Book of Abstracts

The Ninth Multidisciplinary Conference on Drug Research

Book of Abstracts: The Ninth Multidisciplinary Conference on Drug Research

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Table of Contents

Welcome

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Programme

Sunday, 11 May

Rejestracja Uczestników
Sunday afternoon, 11 May, 17:00

Kolacja
Sunday evening, 11 May, 18:30

-
Sunday evening, 11 May, 20:00

Monday, 12 May

Otwarcie Konferencji
Monday morning, 12 May, 8:15
Sala konferencyjna

Sesja wykładowa I
Monday morning, 12 May, 8:30
Sala konferencyjna
Chair: O.Achmatowicz, J.Ostrowski

8:30 Invited oral

Ethical and generic drugs

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Today 60% of prescriptions filled in Europe up to more than 80% in USA are for generic drugs. In Poland generics represent almost 85% of whole drug market. Nevertheless, a large section of the polish public (including physicians) consider generics to be not as effective as brand name drugs. However, generic drugs are required to have the same active ingredient, strength, dosage form, and route of administration as the brand name products. When a generic drug product is approved, it has met rigorous standards established by the EMA with respect to identity, strength, quality, purity, and potency. Moreover, the generic drug manufacturer must prove its drug is the same (bioequivalent) as the brand name drug. All generic manufacturing, packaging, and testing sites must pass the same quality standards (GMP) as those of brand name drugs, and the generic products must meet the same exacting specifications as brand name products. In fact, many generic drugs are made in the same manufacturing plants as brand name drug products. When it comes to price, there is a big difference between generic and brand name drugs. On average, the cost of a generic drug is often 80 to 85 percent lower than the brand name product. It is possible, because generic manufacturers are not required to repeat the costly clinical trials of new drugs and generally do not pay for costly advertising, marketing, and promotion. This creates competition in the market place, often resulting also in lower prices of the brand name drugs.

9:00 Invited oral

Genetic basis of brain diseases

Jacek Kuźnicki

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Ageing is one of the most important risk factors for development of so-called age-related diseases. Brain disorders are especially devastating since they affect individual's identity and ability to function independently; among such diseases neurodegeneration of Alzheimer's (AD) or Parkinson's (PD) type and psychiatric diseases are the most common. There has been a significant progress in understanding the causes of these diseases and it is well established that genetics plays an important role in their pathogenesis. In some familial cases the mutation in a single gene is responsible for the symptoms and early onset of the disease. For example, mutation in the parkin or alfa-synuclein gene induces familial PD, and mutations in presenilins or Amyloid Precursor Protein (APP) - induce familial AD (FAD). However, the majority of cases of neurodegenerative diseases as well as of psychiatric disorders have less obvious genetic background. In fact many genes are involved in the pathogenesis of these diseases, and their effects depend on the environment. Recent Genome Wide Association Studies (GWAS) in which genomes of thousands of patients and healthy individuals were sequenced, identified single nucleotide polymorphisms (SNPs) in genes likely responsible for brain diseases. Notably, the same SNPs were identified in five major psychiatric diseases. Despite of these achievements, the progress in understanding how the healthy brain functions and what happens in the diseased organ are far from being understood. The challenge of the XXI century is now to use the knowledge generated by genomic research towards treatment by novel innovative medicines and to prevent, reverse, or delay brain pathologies.

9:45 Invited oral

AMD – the retinal disease with an unprecised etiopathogenesis: in search of effective therapeutics

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AMD (age-related macular degeneration) is a progressive vision-thretening ocular disease, affecting central region of the retina – the macula – and manifesting in the elderly, usually over 60. It leads to

loss of central vision and in some patients to legal blindness. Clinically, the disease is classified as: atrophic – dry AMD (80-85% of all AMD cases), with advanced stage named „geographic atrophy" (35%), and neovascular – wet AMD (10-15% of all AMD cases) with newly formed subfoveal blood vessels originating from the choroid, known as choroidal neovascularization (CNV). Pathogenesis of AMD is complex, multifactorial and only poorly recognized. Main risk factors include: advanced age, genetic predispositions, environmental determinants, history of exposure to intensive light and smoking. At least four molecular processes contribute to the develop-menyt of AMD pathology: lipofuscinogenesis (in retinal pigment epithelium – RPE; linkage to oxidative stress and lipid peroxidation), drusogenesis (formation between RPE and Bruch's membrane of heterogenous and insoluble material called drusen), inflammation (atypical and chronic) and CNV (in wet AMD). CNV results from disbalanced pro- over anti-angiogenic factors. Since vascular endothelial growth factor (VEGF) is a predominant proangiogenic factor in CNV, the wet AMD can be treated with intravitreous application of „anti-VEGF" agents (Lucentis, Eylea – approved drugs; and Avastin – used off-label). Till now, there is no approved therapy for dry AMD; currently there are several agents in clinical trials showing different mechanism of action, including: drugs that decrease oxidative stress, visual cycle modulators, neuroperotectants, drugs that suppress inflammation, among the latter group are anti-complement agents. The lecture will provide current knowledge on AMD pathogenesis and will summarize latest efforts on potential therapeutics for AMD, with emphasis on therapies for dry AMD.

Przerwa kawowa

Monday morning, 12 May, 10:30

Sesja wykładowa II

Monday morning, 12 May, 10:50
Sala konferencyjna
Chair: R.Hołyst, D.Gryko

10:50 Invited oral

Cancer genome: perspectives for the practical use of next-generation sequencing

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Carcinogenesis is rooted in inherited genetic variations and evolves through a series of somatic mutations acquired over time. In a consequence, neoplasm arises as a multi-step process of successive cellular clone selection and expansion leading to progressive cytological and architectural derangement of an affected tissue.

The rate of point mutations within normal cells varies by more than 100-fold within the genome; in tumor cells this variation can be higher and may affect also whole genome regions. While the first cancer "gatekeeping" mutation provides a selective growth advantage of the pre-neoplastic cells to their normal counterparts, subsequent "driver" mutations further confer this advantage. Rates of cancer mutations are variable and range from one base substitution per exome in some pediatric neoplasms to even thousands of mutations per exome in malignancies induced by mutagens, including lung cancers and melanomas. The number of tumor mutations developing in the self-renewing tissues (like in gastrointestinal tract) correlates with age. Point mutations of cancer genes are more frequent than chromosomal rearrangements.

Although cancer originates from a common progenitor, four types of genetic heterogeneity, intratumoral, inter- and intrametastatic, and interpatient, are relevant to carcinogenesis. Therefore, determining somatic mutations is the most common aim of cancer genome-sequencing studies. Current medicine employs elements of molecular diagnostics, usually on the scale of single genes. Next-generation sequencing (NGS) is moving testing from single genes or small panels of genes to large multi-gene disease-targeted panels, expanding clinical applications of molecular profiling. However, the benefit of NGS in cancer studies will be realized when it improves understanding of basic cancer biology. Unfortunately, point mutation frequency can only prioritize genes for further analysis but cannot unambiguously identify "driver" genes.

The question how to analyze genetic variances using NGS is a constant subject of debate. Some authors recommend the focused approach involving the sequencing of specific region of cancer genomes, while others - the whole genome approach, where sequencing of the entire genome is the method of choice. Each approach has its advantages and limitations; of the latter, the cost of sequencing and of storing and analyzing high volumes of data from whole genomes causes that it may not be accessible to most researchers. Based on the background knowledge of the cancer genome, the lecture will present aims of the use of NGS regarding the focused and whole genome sequencing approaches.

11:35 Oral

Declaraton of Helsinki - code of ethical rules of human clinical reseach today

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The Declaration of Helsinki is a set of ethical principles applicable to human clinical research. The Declaration is regarded as the cornerstone document on human research ethics developed for the medical community by the World Medical Association (WMA). The Declaration was originally adopted on June 1964 in Helsinki, Finland, and has since undergone seven revisions. The most recent took place during general Assambly WMA in Brasil, in 2013 .

The year of 1747 is regarded as the birth of clinical research and since this time till 1947 basically there was no generally accepted ethical rules of human research conduct. The Declaration was based on Nuremberg Code (1947) and declaration of Geneva (1948), a statement of physicians' ethical duties.

Currently it is not legally binding law, but all clinical research must

comply with its rules.

11:55 Oral

Ligand-directed receptor trafficking

Zdzislaw Chilmonczyk[1], Ingebrigt Sylte[2], Andrzej J. Bojarski[3]

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Receptors are areas of certain proteins and glycoproteins which are conveying different stimuli upon activating by specific ligands. Activating of a receptor enables a signal transduction pathway usually by activating specific proteins. Receptors which bind G proteins upon activation are called G protein-coupled receptors (GCPRs). According to the traditional two-state model of receptor theory, GPCRs were considered as operating in equilibrium between two functional conformations, an active and inactive state. It also was thought that GPCRs were to activate only G proteins to induce response. More recent data show that numerous other signalling proteins, such as β-arrestins and phosphorylating enzymes, may interact with GPCRs and activate different intracellular signalling pathways. This also may involve pathways that are independent of G proteins. Various ligands that affect GPCR in a different way and activate specific signalling pathways have been discovered that gave rise to a concept called (among others) ligand-directed receptor trafficking. A ligand might act as an agonist for one signalling pathway while behaving as an antagonist, partial agonist, or have no effect for another signalling pathway. Side effects can arise not only because of the drug binding to different receptors but also by activating different signalling pathways of the same receptor. Challenge for new drug development may therefore not only be to discover compounds with high receptor specificity, but also compounds that can distinguish between the signalling pathways of a particular receptor.

The work was partially supported by the Polish Norwegian Research Program grant Pol-Nor/198887/73/2013.

12:15 Oral

Shimadzu solutions for the pharmaceutical science; A. High speed drug screening and quantification. B. Evaluation of pills by compression and splitting tests

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A. In the different phases of drug development, metabolites need to be screened on a regular basis. In the early stage where the number of samples screened is still relatively low, the focus is mainly on sensitivity. However, it shifts in general to higher throughput with the demand for moderate sensitivity at a later stage. In order to be able to fulfill this demand, the analytical solution needs to be reliable on the HPLC as well on the MS whereas a one hand supplier solution has a number of advantages. Fast separation, resulting in narrow peak width, requires the acquisition of enough data points over a peak to be able to generate accurate information for quantification as well as generating product ion scans for further confirmation. In addition to the required fast scan speed the polarity switching is an important feature as well to shorten run time without sacrificing the level of information generated. The demand of low dwell and pause time may be important if a large number of MRM transitions (Multiple Reaction Monitoring) are relevant to screen. In this context, a low cross talk between the different MRMs is important to avoid contamination between the different identification steps as well as an error in the quantification.

26 pharmaceutical compounds were analyzed using Shimadzu's Synchronized Survey Scan (SSS). In this mode full scan measurement is rapidly followed by automated product ion scanning. High-speed polarity switching (15 msec.) and rapid scan rates (15,000 u/sec.) allow multiple collision energies to be employed for unknowns even with narrow peak widths. This enables molecular weight confirmation from the Q3 scan data and also generates structural fragmentation information from the same peak. A total of 26 pharmaceutical compounds were evaluated. All compounds were detected in either positive mode, negative mode or both, demonstrating the LCMS-8030's effectiveness for drug discovery and synthesis confirmation.

B. Many tablets have groove in the center, allowing them to break accurately in half. The tests performed on the universal testing machine – Shimadzu's EZ-TestX with the appropriate 3-point bending jig, made possible to obtain the force parameters connected with breaking the tablet. Test force measurement and visual inspection of the specimen after the breakage have crucial meaning in optimization of groove depth. Another important use of the EZ-TestX machine is the test performed by pushing the tablet out of PTP package using spherical test jigs. Maximum test force needed to push the tablet out is important parameter for quality control and product development.

12:35 Oral

Glucuronidation of antitumor agents - detoxification, mechanism of drug resistance or the prodrug design? Studies on acridine antitumor agents in the light of clinical therapeutics

Zofia Mazerska, Anna Mróz, Anna Bejrowska, Agnieszka Potęga, Ewa Augustin

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Glucuronidation, representative of II phase of metabolism, is a crucial pathway of metabolism and excretion of endogenous compounds and xenobiotics. UDP-glucuronyltransferases (UGT; EC 2.4.1.17) catalyse transformation of bilirubine, steroids and thyroid hormones, bile acids as well as exogenous compounds, including drugs, carcinogens, environmental pollutants and nutrient components. Deactivation of xenobiotics and the following excretion of hydrophilic conjugates should be a main task of glucuronidation.

However, one can found glucuronides of comparable or even higher reactivity than that of the native compound. Nearly 35% of all drugs are metabolized by UGTs. Major sites of these reaction include the liver, intestine and kidney. There were found 22 functional UGT isoforms that belong to 5 subfamilies (UGT1A, 2A, 2B, 3A and 8A). Among variety of drugs conjugated by UGTs, anticancer agents are of special interest, because of the reported differences in UGT expression in normal and tumor tissues. On the other hand, glucuronidation may also represent a mechanism of intrinsic drug resistance, as it was observed for irinotecan and metotrexat glucuronides in colon and breast cancer, respectively. It has also been shown that new types of glucuronides would play a role of prodrugs, that are hydroxylysed selectively in tumor cells. Earlier studies of our group indicated that triazolo- and imidazoacridinone antitumor agents are glucuronidated in human liver and intestine *in vivo* and selectively to UGT1A10 isoform *in vitro.* Furthermore, glucoronide of one of them gave higher cytotoxicity than parent drug. Our current results indicated that our compounds are also able to modulate enzymatic activity of UGT. Summing up, the obtained results could be exploited for the design of analogs of clinical and pharmacological importance with the aim of increasing and/or decreasing the biological response of UGT and/or eliminating any undesired side effects of glucuronidation.

Przerwa obiadowa

Monday afternoon, 12 May, 13:30

Sesja wykładowa III

Monday afternoon, 12 May, 14:45
Sala konferencyjna
Chair: J.Kuźnicki, T.Brodniewicz

14:45 Invited oral

Nano/micro drug carriers

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Recently there is a growing interest in development of novel nano/microstructural drug delivery systems. They are expected to ensure safe and efficient transport of biologically active substance to the defined tissue, where it can be released at the required rate. Ideally, such systems should allow the targeted drug delivery and it controlled release. Various systems of that kind have been proposed. They include: liposomes, nano/microspheres, nano/microcapsules, nanostructural films or micellar systems. These carriers are usually prepared from lipids and polymeric or hybride materials. Their structure and chemical composition can be adjusted to the type of the drug and to its therapeutic profile.

Recent advances in that research area with the emphasis on the contribution of the author's research group to that field, will be described. The following systems will be presented:

A) Polymer supported liposomal nanosystems increasing bioavaiability of highly hydrophobic molecules - curcumin as a model drug

B) Microparticulate hydrogel systems obtained based on natural polymer and conjugates allowing the controlled, long term release of unfractionated heparin

C) Polymeric nano/microparticles ensuring protection and delivery of protein - alkaline phosphatase as a model protein

D) Polymeric micelles and nanospheres formed by self assembly of macromolecules as drug carriers

E) Multifunctional nano/microparticles for targeted delivery

F) Polymeric gene delivery systems

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15:30 Invited oral

Lysophospholipids - mechanisms of action and perspectives of the use in therapy

Maria Koziołkiewicz, Przemysław Rytczak, Anna Grzelczyk, Anna Drzazga, Edyta Gendaszewska-Darmach, Andrzej Okruszek

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During recent 20 years new biological functions of phospholipids have been discovered. It has been evidenced that some of them act, like hormones, as signaling molecules activating cellular receptors and, as a result, the corresponding signaling pathways. So far, the best known phospholipid mediator is sphingosine-1-phosphate (S1P) which acts as a ligand of membrane receptors belonging to GPCR family (G protein-coupled receptors).

To the group of phospholipid mediators belong also lysophosphatidic acid (1-acyl-2-hydroxy-*sn*glycero-3-phosphate; LPA) and cyclic phosphatidic acid (1-acyl-sn-glycerol-2,3-cyclic phosphate; cPA) which act as ligands of nine membrane receptors and at least one nuclear receptor and influence morphology of many types of cells as well as their proliferation, migration, differentiation, viability and apoptosis. Because of its chemical structure cPA is considered as naturally occuring, cyclic analogue of LPA.

Recently the phospholipid mediator family has been extended by some new members: lysophosphatidylcholine, lysophosphatidylinositol and lysophosphatidylserine have been identified under *in vivo* conditions. Results of preliminary studies indicate that these compounds are involved in pathophysiological processes related to cancers, type 2 diabetes, obesity and atherosclerosis. So far, lysophospholipid mediators and their receptors have been only partially recognized. Therefore, synthesis and biological activities of these compounds will be very attractive for scientific community of molecular biologists, pharmacologists and chemists.

16:15 Oral

New generation of topoisomerase I inhibitors from camptothecin family spontaneously covalently binding to DNA oligomers.

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The subject of the lecture are water-soluble derivatives of camptothecin, inhibitors of topoisomerase I, their physical properties and use. These compounds exhibit preferable biological properties, useful in anti-neoplasm therapy. Topoisomerases are important enzymes that convert the chemical energy of molecules with a superhelical structure. These enzymes unwind the DNA double helix weakening the twisting tension of the DNA molecule, making the template accessible to replication or transcription enzymes[1,2].The inhibition of topoisomerase I DNA is thus an important method of fighting cancer using chemotherapy. There are a number of effective bioorganic strategies of use in achieving this goal, such as the noncovalent binding of the poison at the site of degradation,[8,11,12] cross-linking using binding metals,[13] DNA alkylation[14,15] or photochemical DNA damage.[16] Derivatives belonging to the camptothecin family play a significant role, such as Hycamtin[TM] or irinotecan, or Camptosar[TM], are used in clinical treatment as anti-cancer agents. So far, all known derivatives that participate in the inhibition of topo I merely form complexes with the nicked DNA. In conjunction with the abovementioned molecular complex structure, these compounds poorly bind tumour DNA.

There is little data regarding the photochemical transformation using compounds of the camptothecin family (CPT). There is also a lack of other possible mechanisms of the interaction of camptothecin derivatives with tumour DNA. Extant publications concentrate only on the inhibition of the enzyme topo I by way of forming tripartite complexes containing an oligomer of DNA/ topo I /organic ligand. There is thus a large need to deliver a new solution, ensuring the formation of a stable complex using camptothecin derivatives.

The presented data relates to the synthesis of novel, water-soluble derivatives exhibiting unexpected preferable properties in terms of covalent DNA binding.

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16:35 Oral

Iron chelators in photodynamic therapy

Anna Mrozek-Wilczkiewicz[1], Maciej Serda[2], Robert Musiol[2], Jaroslaw Polanski[2], Alicja Ratuszna[1]

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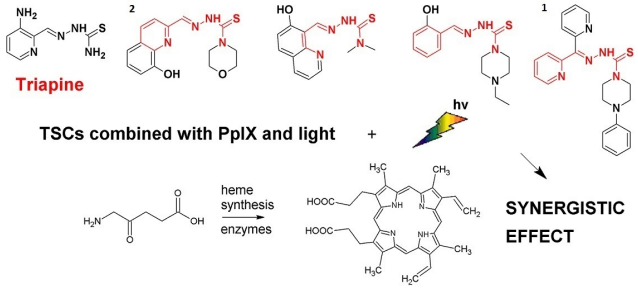
In photodynamic therapy (PDT), a non-invasive anticancer treatment, visible light is used as a magic bullet selectively destroying cancer cells by a photosensitizer that is non-toxic in the dark. Protoporphyrin IX (PpIX) is a natural photosensitizer synthesized in the cell which is also a chelating agent that if bonded to Fe^{2+} forms heme, a central component of hemoglobin. Therefore, xenobiotic iron chelators can disturb iron homeostasis, increasing the accumulation of PpIX, obstructing the last step of heme biosynthesis, and enhancing PDT efficiency. However, the attempts to use this promising idea have not proved to be hugely successful. Herein, we revisited this issue by analyzing the application of iron chelators highly toxic in the dark which should have higher Fe^{2+} affinity than the non-toxic chelators used so far. We have designed and prepared thiosemicarbazones (TSCs) with the highest dark cellular cytotoxicity among TSCs ever reported. We demonstrate that compound 1 and 2 exert powerful PDT enhancement when used in combination with 5-aminolevulinic acid (ALA), a precursor of PpIX.



Przerwa kawowa

Monday afternoon, 12 May, 16:55

Sesja posterowa I

Monday afternoon, 12 May, 17:15
Sala posterowa

17:15 Poster 1

N-terminal amidinated derivatives of the cyclic 1,4-ureido-deltorphin analogues: the synthesis and receptor binding studies

Krzysztof Bańkowski[1], Olga M. Michalak[1], Anna A. Leśniak[2], Katarzyna E. Filip[1], Jan Izdebski[3]

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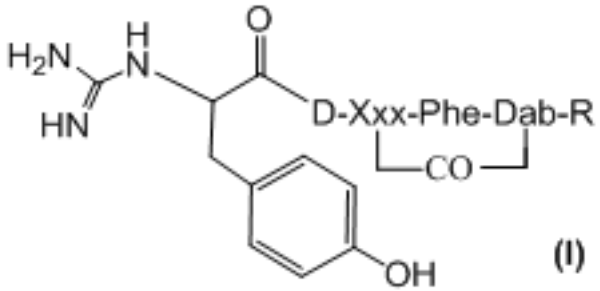
Cyclic ureidopeptides, analogues of deltorphine tetrapeptide (Formula I), were amidinated in hope of preparing new derivatives with high resistance to enzymatic degradation, interesting opioid receptor binding profile and potentially improved blood-brain barrier permeability.



Xxx = Lys, Orn

R = OH, OCH_3, NH-CH_2-CH_2-NH-CO-NH_2

Amidination of corresponding cyclic ureidopeptides was performed using two reagents: *N*,*N*'-bis-Boc-*S*-methyl-isothiourea and 1*H*-1-pyrazole-1-carboxyamidine (with the latter generally giving better results). The new analogues were purified by reverse-phase HPLC and their purity and structural identity were established by analytical HPLC, HR-MS, and NMR studies.

Binding affinities for μ and δ opioid receptors of new amidinated analogues as well as of their no-amidinated precursors were determined by competitive binding assaysin the presence of the selective radioligands: [3H]DAMGO (selective μ receptor agonist) and ([3H]Ile5,6 deltorphin II) ([3H]DELT II (selective δ agonist). Most of amidinated and no-amidinated cyclic ureidopeptides possess mixed μ agonist/δ agonist profile and have therapeutic potential as analgesic.

17:15 Poster 2

The induction of apoptosis by novel dinuclear platinum(II) complex used with anti-MUC1 in human MCF-7 breast cancer cells

Anna Bielawska[1], Agnieszka Gornowicz[1], Robert Czarnomysy[2], Magda Jadeszko[1], Krzysztof Bielawski[2]

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Mucin 1 (MUC1) is a highly glycosylated type I transmembrane glycoprotein, which is overexpressed in various cancer cells especially in breast cancer cells. The aim of the study was to investigate the proapoptotic properties Pt_2 (4-ethylpyridine)$_4$(berenil)$_2$ (Pt12) with anti-MUC1 using flow cytometry assessment of annexin V binding, analysis of mitochondrial membrane potential and defragmentation of DNA by TUNEL assay in MCF-7 breast cancer cell line. The cell death was measured by flow cytometric analysis after annexin V-FITC and propidium iodide staining. The incubation of MCF-7 breast cancer cells with Pt12, cisplatin, Pt12 with anti-MUC1 and cisplatin with anti-MUC1 induced the visible phosphatidylserine exposure after 24 and 48 hours of treatment. The apoptotic effect of Pt12 was found to be stronger than that caused by cisplatin. The ratio of early and late apoptotic cells was increased after 24h of combined treatment with Pt12 with anti-MUC1 (10 μM + 10 μg/mL) in MCF-7 from 3.0 to 19.0%. After combined treatment with cisplatin with anti-MUC1 used in the same doses as Pt12 with anti-MUC1 (10 μM + 10 μg/mL) in MCF-7 for 24 h the ratio of early and late apoptotic cells was increased from 3.0% to 12.0% . The ratio of necrotic cells was, however, also increased after treatment with the Pt12 with anti-MUC1 for 24 h (from 2.3% to 12.2%) compared to treatment with cisplatin with anti-MUC1 (from 2.3% to 6.7%). The dissipation effect of Pt12 with anti-MUC1 on mitochondrial membrane potential ($\Delta\Psi m$) is particularly conspicuous, the red-to-green fluorescence ratio at 10 μM reaches to 16% of the control value in MCF-7 cells, suggesting that the mitochondrial membrane has been severely damaged. These results are in accord with those obtained in the Annexin V/PI assay and indicate that the apoptosis induced by Pt12 with anti-MUC1 may go through the mitochondrial pathway. To detect the yield of DNA strand breaks, which are intimately associated with an apoptotic response, the TUNEL assay was performed after treating MCF-7 cells with compounds for 24h and 48h. A highest increase in the percent of TUNEL positive cells was observed after incubation of Pt12 with anti-MUC1 in comparison to cisplatin with anti-MUC1 after 24 and 48h. Control cells cultured in normal growth media showed minimal DNA fragmentation.We have found that the apoptotic effect of Pt12 with anti-MUC1 was stronger than evoked by cisplatin, anti-MUC1, Pt12 and cisplatin used with anti-MUC1.

17:15 Poster 3

Effect of the degradation of poly(L-lactide-co-glycolide) (85:15) matrices containing risperidone on thermal and morphological properties

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Risperidone (RSP) as a medical product is available in the formulation of a long-acting injection administrated as aqueous suspension of encapsulated microspheres of poly(D,L-lactide-co-glycolide). Currently offered alternative solutions, i.e. nano-, microparticles or implants, include copolymers of lactide and glycolide with various composition and chain structure. An alternative formulation has been developed. Decisive in RSP release is the rate of copolymer degradation. Determination of degradation changes in copolymer e.g. thermal and morphological properties seem to be helpful for analysis of drug release. The aim of this study is to determine thermal and morphological properties in the course of degradation process.
The matrices were obtained from poly(L-lactide-co-glycolide) (L-PLGA) (85:15) (100000 Da) by solution casting method. Matrices containing 5 wt-% of RSP were incubated in phosphate buffered saline (pH 7.4) at 37ºC under constant agitation for 0, 7, 14, 48, 58 and 105 days. Subject to performance were the tests of thermal characteristics by differential scanning calorimetry (DSC) using the TA DSC 2010 apparatus (TA Instruments, New Castle, DE); the morphological study by a scanning electron microscope (Quanta 250, FEI Quanta FEG).
The thermal analysis revealed the gradual decrease of glass transition temperature during observation period. Morphological study revealed that non-degraded matrix possessed a solid structure with single pores. The degradation influenced porosity significantly, also the widening of pores' diameter and their deformation.
The performed thermal and morphological analyses of L-PLGA (85:15) matrices reveal their suitability in the interpretation of degradation processes. Matrices show that gradual degradation makes prolonged release of RSP possible.
This work was financially supported by the National Centre for Research and Development, grant RYSPCONT no. PBS1/A7/2/201.

17:15 Poster 4

Validated LC-MS/MS method for the complementary determination of free and total genistein in human plasma

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Genistein is used for treatment of the menopause symptoms and reduces risks of heart disease, osteoporosis and some cancers. Its potential activity against psoriasis is under investigation. Genistein is present in the body as unconjugated form (free genistein) and conjugated with glucuronide and sulfate. The aim of the study was to develop and validate a bioanalytical method to determine free and total genistein in plasma, i.e. sum of free genistein and hydrolyzed metabolites.

Application of LC-MS/MS technique enabled method's sensitivity of 250 pg/ml and 20 ng/ml for free and total genistein, respectively. Enzymatic hydrolysis, applied during sample preparation, was highly optimized. The first complementary method for the determination of free and total genistein was developed and validated in compliance with European and U.S. regulatory guidelines [1,2].

The developed method enables rapid and reliable determination of free and total genistein in plasma and was successfully applied in the pilot bioavailability study in humans.

Genistein was synthesized in the Pilot Plant Department and certified in the Quality Control Department of the Pharmaceutical Research Institute. The presented study was supported by the National Centre for Research and Development under the Programme of Applied Research, contract No. PBS1/B7/7/2012.

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17:15 Poster 5

Synthesis and rating of biological activity of 3,4-dichloro-2(5H)-furanone derivatives

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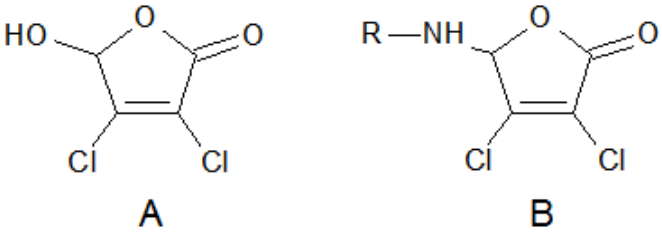
3,4-dichloro-5-hydroxy-2(5*H*)-furanone (mucochloric acid) is a substrate for synthesis of compounds with interesting chemical and biological properties. The derivatives of 2(5*H*)-furanone are currently a large group of heterocyclic compounds, among which are natural substances, medications exhibiting various biological activity and compounds used in chemical synthesis [1-8]. The 2(5*H*)-furanone subunit is a leading structure in many biologically active natural substances such as antimicrobial rubrolides or basidalin. The antic-

ancer properties in milimole concentrations of several mucochloric acid were also reported [1].



Structure of 3,4-dichloro-5-hydroxy-2(5*H*)-furanone (A) and tested derivatives (B).

We synthesized set of differently substituted 2(5*H*)-furanone derivatives and determine their anticancer activity against model cell lines (HCT 116 wt, NHDF).

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17:15 Poster 6

Antiproliferative activity of novel acetylenic derivatives of betulin against G-361 human melanoma cells

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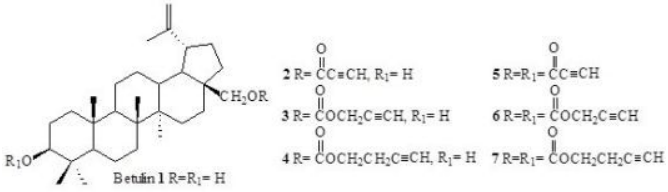
Malignant melanoma is highly resistant to chemotherapy and radiotherapy, particularly in the metastatic or relapse phase. Median survival time of metastatic patients is 6-9 months and the 5-year survival rate does not exceed 1-2%. Therefore, new possible treatment strategies are still searched for. Inhibition, delay or reversal of carcinogenesis using natural or semisynthetic compounds seems to be a promising approach [1]. Betulin (**1**) is a natural compound with cytotoxic activity against cancer cells. It can be easily converted to various semisynthetic derivatives [2-3].

The aim of the study was to investigate the effect of acetylenic derivatives of betulin (**2-7**) on cell proliferation in G-361 human melanoma cell line.

To assess cell proliferation, G-361 cells were fixed with 10% trichloroacetic acid and stained with sulforhodamine B. The range of concentrations of the tested compounds was from 1 to 20 µg/mL and the cells were treated for 72h. Monoesters **2-4,** obtained by a replacement of the hydroxyl group at C-28 position of betulin (**1**) by alkynyl groups, exhibited the most potent cytotoxicity. Instead, simultaneous esterification of the C-3 hydroxyl group (diesters **5-7**) completely abolished the cytotoxic action of the compound (in a concentration range: 1-20 µg/mL).



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17:15 Poster 7

Antiproliferative and apoptotic effects of valproic acid in human melanoma cells

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In recent years, an increase in melanoma incidence associated with rapidly growing mortality rate has been observed. The prevalence of disease doubles almost every 10 years and grows much faster than in other malignant tumors [1]. Over the years, many risk factors for melanoma, both exogenous (*eg*. UV radiation) and endogenous (genetic predispositions), have been identified. However, unraveling the etiology of this disease requires further investigation.Melanoma aggressiveness is related to the ability to rapid metastasis, high proliferative activity of cells, wide range of genetic and epigenetic changes and high resistance to conventional treatment [2]. Due to these reasons, there is a great demand for new anti-melanoma therapies.

Currently, it is considered that valproic acid (VPA) may be an effective agent in the therapy of malignant melanoma. The VPA is the histone deacetylase inhibitor (HDACi), and is involved in maintaining a balance between the acetylation and deacetylation of histones [3].

The aim of the study was to evaluate the effect of VPA on proliferation and apoptosis in human melanoma cells.

The study material was human melanoma cell line (G-361). The influence of VPA on cell growth rate was tested using XTT assay

(Sigma-Aldrich®). The ability of VPA to induce apoptosis was determined by measuring the activity of caspase-3 using "Caspase-3 Assay Kit, Colorimetric" (Sigma-Aldrich®).

It was found that higher concentrations (1, 3, 5, 10 mM) of VPA inhibited proliferation of melanoma cells. The lowest concentration (0.3 mM) of VPA was insufficient to reduce the proliferative activity of the G-361 cells. To determine the apoptotic effect of VPA in the melanoma cells, caspase-3 activity was measured after 48-hr exposure to VPA at concentrations of 1, 5 and 10 mM. The statistically significant increase in caspase-3 activity in treated cells (5 and 10 mM VPA) compared to control was observed.

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17:15 Poster 8

Method for determination of methotrexate (MTX) release from the macromolecular drug-carrier systems in plasma

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Nanoconjugates are a new class of therapeutic compounds, which may lead to a new therapeutic quality due to the combination of drugs already used in therapy, as well as new innovative substances with high molecular carriers. Synthesis of drug-carrier hybrid systems is one of the ways to implement the "magic bullet" concept propounded at the beginning of the 20th century by Paul Ehrlich, and which was aimed at achieving the selective deposition and release of therapeutic substances in target tissues.

As a model anticancer drug, we chose methotrexate (MTX) – one of the oldest antifolate drugs widely used in the treatment of cancer, rheumatoid arthritis and other diseases. In the present contribution the convenient method regarding quantification of MTX release from selected drug-carrier systems in human plasma are presented. The drug release study was investigated at 37°C in human plasma (which contains enzymes like amylases) and mineral buffers (without enzymes). The method was based on size exclusion chromatography and UV-VIS detection at the wavelength of 372 nm. Conjugates were dissolved in final solutions to a final concentration of 0.8026 mM (MTX equiv.). At selected time intervals, each reaction solution was analyzed for unbound MTX. The observed release half-time was calculated for MTX using the pseudo-first-order kinetics equation. For the method basic parameters such as linearity, range of the method, limit of detection (LOD) and limit of quantification (LOQ), reproducibility and recovery were validated.

Presented method had a linear character in the range of 2.006 – 802.6μM. LOD of the method was 0.4414 μM, and LOQ was 1.471 μM. Our drug release studies suggest that MTX may be cleaved from the conjugates both via chemical and enzymatic hydrolysis. Stability of the conjugates (half life time) in plasma is much short as the half life time in phosphate buffer at an identical pH. The release results of ester-linked conjugates indicate that a specific base-catalysis was involved in the hydrolysis of the ester conjugate, which is usually observed in weak alkaline solution. The conjugates differing in terms of structure demonstrate significantly different drug release characteristics.

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17:15 Poster 9

Methods for determination of pemetrexed in macromolecular drug-carrier systems

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Pemetrexed (PMX) is a new antifolate drug utilizied in the treatment of pleural mesothelioma and non-small cell lung cancer. The mechanism of its action is based on its ability to inhibit the activity of enzymes involved in purine and pyrimidine synthesis: thymidylate synthase (TS), dihydrofolate reductase (DHFR), and glycinamideribonucleotideformyl transferase (GARFT). Due to its low molecular weight PMX reveals a number of disadvantages that are typical for biologically active small molecules, such as enhanced metabolism and excretion from an organism, as well as adverse biodistribution profile. One of the possible solutions for these problems is to bind PMX with macromolecular carriers what may result in improved selectivity and pharmacological properties of PMX. During a study on potential carrier for PMX a fast and precise method is needed to determine the bounded and free drug comprised in investigated conjugates preparations. Two simple and time-efficient methods for quantification of pemetrexed in the polysaccharide conjugates are presented. The method for the analysis of a total amount of PMX in different conjugates was based on a spectrophotometry absorption. Validation was performed by measuring the absorbance of the standard solution in 0,1M sodium bicarbonate at 225nm. The curve representing drug concentration against absorption had a linear character in the range of 4.697-46.97μM. The reproducibility of this method was between 0.8544 and 5.488%. The recovery of this method was between 96.23 and 101.7%. The limit of a quantitative method was 1.070μM. The method for quantitative analysis of unbound PMX was based on size exclusion chromatography and UV-VIS detection at the wavelength of 225nm. Superdex® Peptide column (150 x 4.6 mm) and a mobile phase 0.1M sodium bicarbonate with a flow rate of 0.4ml/min were applied. In the free drug de-

termination method, the curve had a linear character in the range of 4.461-223.0µM. The reproducibility and precision of a presented method was 0.3761 to 2.452%. The recovery of the method was between 93.18 and 104.5%. The limit of a quantitative method was 2.897µM.

This project was supported by the statutory fund of Laboratory of Biomedical Chemistry (Institute of Immunology and Experimental Therapy Polish Academy of Sciences).

17:15 Poster 10

Evaluation of potential genotoxic impurities in prasugrel intermediate by HPLC chromatography

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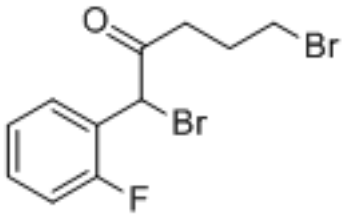
The residual impurities for active pharmaceutical ingredient may stem from starting materials, reagents, intermediates, solvents, side reactions and API degradation processes. Some impurities possess genotoxic and carcinogenic potential, therefore the determination of their acceptable limits is essential to avoid patient risk and guarantee therapeutic safety. In the course of our studies on prasugrel hydrochloride synthesis, the literature search associated with its genotoxicity was performed. Although EMEA Assessment Report discloses that prasugrel (antiplatelet drug) do not exhibit genotoxic properties when tested in a battery of standard *in vitro* and *in vivo* assays, the recent patent application suggests that the particular intermediate impurity i.e. dibrominated by-product (PU1BZ) can be finally transformed, in API synthetic sequence, to generate prasugrel-like potential genotoxic impurity. According to Muller classification which was accepted as a background to establish ICH M7 guideline proposal, above cited impurities match the group with alerting structure (primary halides), unrelated to the structure of the API and with unknown genotoxic potential.



PU1BZ

Since our prasugrel synthetic route uses secondary alkyl halide derivative, 2-bromo-1-cyclopropyl-2-(2-fluorophenyl)ethanone as a key intermediate to generate the basic skeleton of prasugrel, we synthetized and NMR characterized both revealed impurities. In further studies the HPLC chromatography was applied to examine the presence of potential genotoxic impurities in substrate and final prasugrel samples.

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17:15 Poster 11

Determination of formic acid in organic solvents by GC-TCD

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This method describes the determination of formic acid in organic solvents, which are frequently used in the gas chromatography analysis of pharmaceutical substances. Residual solvents in pharmaceuticals are defined as organic volatile chemicals that are used or produced in the manufacture of drug substances or excipients, or in the preparation of drug products. The residual solvents are not completely removed by practical manufacturing techniques.
This method allows to directly determine the formic acid in the organic solvents, without a necessity of the derivatization reaction. Formic acid was analyzed by high-resolution capillary gas chromatography with thermal conductivity detector (GC-TCD) and it was measured in organic solvents like dimethylsulphoxide (DMSO), N,N-dimethylformamide (DMF), N,N-dimethylacetamide (DMA). The lowest measured signal for formic acid was 10 ppm. Acetone and equivalent solvents were mixed to prepare standards and test solutions. The results for the determination of formic acid by GC-TCD confirmed, that the method can be used to routine control of the residual formic acid.

17:15 Poster 12

Evaluation of the lipophilic properties of betamethasone and its related compounds

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Betamethasone
,21-trihydroxy-1,4-pregnadiene-3,20 (9α-fluoro-16β-methyl-11β,17α-dione) is a synthetic glucocorticoid (GC). Some esters and salts of this compound are widely used as anti-inflammatory, immunosuppressive and antiproliferative agents, and also in dermatological therapy of different skin diseases. Despite minor differences in chemical structures, betamethasone derivatives may have different pharmacological effects. From the available literature it is obvious that the lipophilicity is one of many parameters which involved in biological activity of drugs including skin preparations [1,2]. This is a very important factor for their dermal permeation. Therefore, the main aim of this work was to determine the

lipophilic properties of betamethasone and its derivatives: 17,21-dipropionate, betamethasone-17-valerate, betamethasone-21-valerate and also betamethasone disodium phosphate by means of reversed phase high performance thin-layer chromatography RP-HPTLC (R_{MW}) under different conditions, and also with the use of calculations (logP). All obtained results indicate that regardless of the applied method the biggest similarity in lipophilic properties show betamethasone-17-valerate, betamethasone-21-valerate and also betamethasone 17,21-dipropionate. Other betamethasone compounds indicate differences in lipophilicity parameters in mutual comparison.

Our study confirms that RP-HPTLC is a reliable, precise and cost-effective analytical tool which allows determining the lipophilicity of betamethasone and its related compounds.

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17:15 Poster 13

TLC-Densitometric method for qualitative analysis of betamethasone and its related compounds in pharmaceutical preparations

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Pharmaceutical preparations containing corticosteroid salts and esters such as betamethasone and its related compounds have been recently applied in cream, injections and lotions in dermatological [1]. The official method for identification and assay of betamethasone derivatives in the presence of other ingredients including antibiotics and preservatives is high performance liquid chromatography (HPLC) [2,3]. This method is highly efficient for the determination of betamethasone but very tedious and time consuming because it requires repeated solvent extraction of multicomponent pharmaceutical preparations. Therefore we tried to develop a rapid and inexpensive TLC-densitometric method for separation and identification of betamethasone and some betamethasone derivatives (salts and esters). To achieve satisfactory separation of examined betamethasone and betamethasone compounds various chromatographic conditions (different adsorbents and mobile phases) in normal and reversed phase system were applied (NP-TLC and RP-TLC).

The results of this study might be an inspiration for further investigations concerning the quantitative analysis of examined betamethasone compounds.

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17:15 Poster 14

New analgesics with adjuvant anticancer properties.

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Substance P plays an important role in pain signals generation and transmission from periphery to CNS. In contrast, opioids suppress pain signals mainly through suppression of substance P release. These mechanisms of neurophysiological actions were fundamentals for invention of hybrid compounds that act as both antagonists of substance P to reduce postsynaptic activation of NK1 receptors as well as opioid agonists to activate presynaptic opioid receptors that result in decrease substance P release. It is known that on the surface of tumor cells an overexpression of NK-1 receptors is observed. Literature has reported that antagonists of substance P inhibit proliferation and migration of tumor cells, and reduced tumor angiogenesis. Therefore, to the list of already synthesized and characterized compounds we appended new series of compounds that combine peptide opioid pharmacophore with 3,5 bis-trifluoromethyl-benzyl derivative, responsible for antagonist at NK1 receptor. In literature we can found bifunctional peptides which can act through both opioids and NK 1 receptors . A new compound, synthesized our laboratory, was constructed from one part of biphalin and Z- Trp on the C-terminus (1). In the other team was synthesised similar hybrid which was modified on C-terminus by fragment with 3,5- bistrifluoromethyl-benzyl group (2). On this basis we design, synthesized and tested new bifunctional peptides.

New compounds exhibit high affinity to opioid receptors and decrease viability of human melanoma cells. In in vivo studies hybrids exhibit analgesic activity also.

Therefore peptide analogues as a new type of effective analgesic es-

pecially designed for chronic cancer pain treatment has been developed.

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17:15 Poster 15

Comparison of ultraviolet detection and charged aerosol detection methods for liquid-chromatographic determination of protoescigenin

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Charged aerosol detection (CAD) has been widely employed as an alternative detection system in HPLC. This detection approach was also applied in new UHPLC methods for the determination of protoescigenin (olean-12-ene-3β,16α,21β,22α,24,28-hexaol) assay. Protoescigenin, the main aglycone of escin – a saponin complex from horse chestnut seeds (Aesculus hippocastanum L.) was obtained in scalable, validated manufacturing processes (large laboratory and pilot technical scale) designed and developed in Pharmaceutical Research Institute [1].

In the present work the influence of individual parameters on CAD response and sensitivity was studied. The detection was performed using both CAD and PDA (diode array detector, 200 nm) simultaneously and the results were compared with reference to linearity, accuracy, precision and limit of detection (LOD).

Acknowledgement:
Research Project has been supported from Innovative Economy Operational Programme 2007-2013 funds, under project POIG.0101.02-14-072/09-00 Research on innovative endothelium medicine among novel escin analogues (www.ifarm.eu/poig/escyna/).

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17:15 Poster 16

Development and validation of LC-MS method for determination of lapatinib in human plasma

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Lapatinib is a novel, orally administered protein-tyrosine kinase inhibitor used in a treatment of patients with advanced or metastatic breast cancer. Considering the need for low-cost cancer treatments, it is essential to develop reliable and sensitive bioanalytical methods for lapatinib determination in human plasma for conducting bioequivalence studies of new generic drug products.

The aim of the study was to develop the first single quadrupole bioanalytical method for the determination of lapatinib in human plasma, which can be applied to pharmacokinetic studies after administration of 250 mg oral dose. The method was validated according to European Medicines Agency (EMA) [1] and Food and Drug Administration (FDA) [2] guidelines, in compliance with the principles of Good Laboratory Practice (GLP), providing its reliability. The sample preparation procedure was based on a liquid-liquid extraction with methyl tert-buthyl ether. Chromatographic analysis was carried out using C18 column and isocratic elution with mixture of acetonitrile, methanol and formic buffer. Positive electrospray ionization mass spectrometry in single ion monitoring mode was applied as a detector. Isotope labelled lapatinib was used as an internal standard.

All of the validation parameters met acceptance criteria. Calibration curve, prepared using freshly spiked plasma samples, was linear within the range of 5.00-800.00 ng/mL. The method was found to be sufficiently accurate and precise over the studied range of concentrations.

Lapatinib was synthesized in the Chemistry Department and Minisynthesis Department than certified in the R&D Analytical Chemistry Department of the Pharmaceutical Research Institute. The study was supported by the European Union (European Regional Development Fund) under the Innovative Economy Operational Programme 2007–2013 (Project No. UDA-POIG.01.03.01-14-069/08-00).

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17:15 Poster 17

Pharmacological evaluation of interaction of selected imidazol(in)e compounds with alpha2-adrenergic receptor by application of the eye mydriasis model in rats

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The imidazol(in)e compounds produce pupillary dilation after systemic application to the rats. This action is assumed to be connected with the stimulation of postsynaptic alpha2-adrenoceptors within the Edinger-Westphal nucleus in the brain. Despite numerous studies, knowledge on the involved receptors structure and on their selective ligands it is still incomplete. In this study the mydriatic effect of clonidine, rilmenidine and moxonidine on the pupil size in anaesthesized rats after or without alpha2-adrenergic receptor antagonists pretreatment was examined. The method of testing mydriasis in rats was used as a model system for assessment of selective alpha2-adrenergic activity evoked by potentially pharmacologically active compounds with the imidazol(in)e structure. In the model, experiments were carried out *in vivo* in the whole animal with testing particular imidazol(in)e agents in a wide range of doses, regarding both the agonistic and the antagonistic properties and assessing the strength of action of the ligands studied.

The aim of this study was to comparatively determine the mydriatic effect in rats of clonidine, rilmenidine and moxonidine. In order to confirm the contribution of alpha2-adrenoceptors (and their subtypes, especially alpha2D), in the observed pharmacological effects, the animals were pretreated with the following alpha2-adrenoceptor antagonists: yohimbine, RS 79948, RX 821002. A selective I1-imidazoline receptor antagonist AGN 192403 was also applied to eliminate the possible involvement of this receptor in mydriatic effects of the agents studied. Additionally the Western blot technique was used to confirm the presence of immunoreactive receptor protein corresponding with alpha2-adrenoceptor type in the brain medulla tissue of the rats. The results obtained confirm that as compared with other *in vivo* and *in vitro* pharmacological methods of testing of interactions of ligands with imidazol(in)e structure with central alpha2-adrenergic receptors, this model seems to be especially useful for the preclinical *in vivo* studies aimed at identification of the potential cardiovascular drugs acting through alpha2-adrenoceptors.

17:15 Poster 18

AGN192403 and 2BFI as pharmacological tools for determination of the engagement of I1/I2-imidazoline receptors in cardiotropic effects of imidazol(in)e compounds on isolated rat heart atria

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Imidazoline receptors represent the binding sites that have high affinity for compounds containing an imidazol(in)e structure. The functional role of I1/I2-imidazoline receptors in physiological and pathological processes is not yet fully elucidated. The receptors of I1 type are involved in the central control of blood pressure and I2 receptors have been associated with various neurobiological states, including analgesia, depression or neurodegenerative disorders. Recently, many attempts have made to explain the role of imidazoline receptors in pathophysiology of the heart (arrhythmia, ischemia). However, no systematic comparative studies were performed nowadays regarding the effect of imidazole(in)es on imidazoline receptors in isolated rat heart atria. In the our previous experiments we observed that some imidazol(in)e agents, regarded on the base of radioligand studies as imidazoline/α2-adrenergic receptor ligands, exerted positive ino- and chronotropic effects on isolated rat heart atria. Those effects were not fully antagonized neither by idazoxan - the classical I2/I1-imidazoline receptor antagonist nor by yohimbine – the classical α2-adrenoceptor antagonist.

The aim of the study was to determine the effect of selected imidazol(in)es: highly specific I1- (moxonidine, rilmenidine) and I2-receptor (RS 45041-190, BU226) ligands as well as guanabenz having “mixed” affinity for imidazoline/α2-adrenergic receptors on the amplitude and rate of contraction of isolated rat heart atria. To determine the potential engagement of I1/I2-receptors in cardiotropic activities of the compounds studied the two selected antagonists: AGN192403 (imidazoline I1-receptor) and 2BFI (imidazoline I2-receptor) were used the first time. The results obtained lead to the conclusion, that I1-imidazoline receptors are only partly involved in positive inotropic effect of moxonidine and rilmenidine which depend mainly on α2-adrenergic receptors. RS 45041-190, BU226, and guanabenz increase the rate of contraction of the atria acting mostly via imidazoline receptors of I2 type. Moreover AGN192403 and 2BFI could be useful compounds in experimental pharmacology for the determination of I1/I2-receptor selectivity of imidazol(in)e agents in search of potential antiarrhythmic/cardiotropic drugs.

17:15 Poster 19

The impact of genistein and phytic acid on the viability of nasal polyps cells in an in vitro model

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In developed countries, chronic rhinosinusitis with polyps is one of the several diseases that diminish patients' quality of life most significantly. The treatment of that often incurable disease is mainly based on the intranasal steroids and surgery in patients who had failed thorough conservative management. The frequent recurrence of inflammatory process triggers a need to repeat the operation. It appears that the introduction of new treatment substance that inhibits fibroblasts' proliferation, decreases pro-inflammatory stimulation and suppress inflammation process in the tissue (i.e. eosinophils and mast cells reduction, decreased level of inflammatory cytokines, such as IL-5, IL-6 and IL-8) would be a valuable therapeutic option. Currently, steroids are the most commonly used for that purpose what is associated with the occurrence of problematic side effects forcing many patients to discontinue therapy. Increasing interest in this field raise natural substances such as flavonoids. In addition to its antioxidant properties, they often have a capacity to influence the course of the cell cycle, inhibit cell division, induce apoptosis and change various metabolic pathways.

The aim of the present study was to evaluate the in vitro effects of genistein and phytic acid (IP6) on the viability and proliferative activity of cells derived from nasal polyps. Material for the study was obtained from patients with chronic rhinosinusitis with polyps surgically treated in the Department of Otolaryngology, Medical University in Wroclaw. The patients included into the study were free of any medication at least 4 weeks prior to surgery. The study used cells from IV-VI passage.

Cells viability and proliferation activity were analyzed in cultures in the presence of various concentrations of genistein (0.1-500 uM) and IP6 (0.1-20 mM). WST-1 test (Roche Molecular Biochemicals) and the measurements of LDH concentration in the medium were used for that purpose. An immunocytochemical assay was performed to demonstrate the possible presence of apoptotic or necrotic cells.
For both of the tested substances - stronger for genistein than IP6, concentration-dependent effect on the parameters analyzed was demonstrated.

17:15 Poster 20

Antiproliferative and cytotoxic effect of vitamin D_3 and its derivatives on the selected cells with a higher proliferative potential

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There are many reports in the literature on multidirectional activity of vitamin D and its analogs. Recently, the role of vitamin D derivates as potential anti-proliferative agents in addition to the well-known application in the treatment of rickets and osteoporosis has been revealed. Studies on the mechanisms of action of vitamin D and its analogs demonstrated that calcitriol, hormonally active form of vitamin D, has a regulatory effect toward cells' proliferation, division and differentiation. Numerous researches revealed also the influence of those substances on apoptosis and inhibition of cell proliferation in certain types of malignant tumors.
The aim of the present study was to evaluate the *in vitro* effect of new derivatives of vitamin D_3 on the divisions and viability of the cells with increased proliferative activity. The effect of calcitriol, tacalcitol and three new derivatives of vitamin D_3 synthesized at the Pharmaceutical Research Institute in Warsaw, coded as PRI-1890, PRI-1906 and PRI-2205 was examined.
All the tests were performed on tumor cell lines (SNB-19, C-32 and SH-4), cultures derived from nasal polyp tissues and cultures of normal skin fibroblasts (HFF-1). Cultures were set and carried out in 75ml Nunc cell culture *vessel*, then the cells were seeded into 96-well plates and after 24h incubation calcitriol, tacalcitol, RPI-1890, RPI-1906 and RPI-2205 at a concentration of 0.025, 0.25, 2.5 and 25ug/ml were added to the medium. After subsequent 72h the number of cells was determined in cultures using the WST-1 assay (Roche Molecular Biochemicals). We analyzed both the survival and proliferative activity of cells treated with the above derivatives of vitamin D_3. RT-QPCR technique was used to determine the expression of anti-apoptotic Bcl -2 gene and pro-apoptotic BAX gene.
Tested compounds show the most significant effects at the concentrations above 0.25ug/ml. Calcitriol had the smallest impact on both the proliferation and cell viability. Contrary, the strongest inhibition of the proliferation and decrease in cell viability was observed after the use of vitamin D analog PRI-1890.

17:15 Poster 21

Novel 2-(2-phenalkyl)-1H-benzo[d]imidazoles: synthesis, characteristics, tuberculostatic and cytotoxic activities

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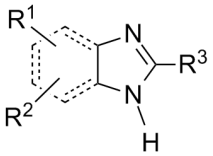
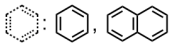
Previously we described a significant tuberculostatic activity of some 2-phenalkyl- and 2-cyclohexylalkylbenzimidazoles [1]. Then we reported synthesis and activity of 2-(2-cyclohexylethyl)-1*H*-benzo[*d*]imidazoles analogues with benzimidazole type of structure. Their tuberculostatic activity *in vitro* was at the level appropriate for the administrated chemotherapeutics [2]. Here we disclosed the synthesis of novel 2-(2-phenalkyl)-1*H*-benzo[*d*]imidazoles. We synthesized structures with different substituents at the benzene ring of benzimidazole system, styryl, phenethyl or 3,5-dichlorophenethyl moiety at C-2 position.





R^1: Me, F, NO_2 ; R^2: H, Me, Cl
R^3: −CH=CH−Ph, $-CH_2-CH_2-Ph$, $-CH_2-CH_2-(3,5\text{-}Cl_2)-Ph$

Target benzimidazoles were obtained by two different methods. In one of them appropriate carboxylic acids were heated with *o*-phenylenediamine derivatives in metal bath. In the another synthesis result was reached in two steps, substitution in order to get the amid and cyclization to benzimidazole system. All the newly synthesized compounds were characterized by IR, ^{1}H NMR, and ^{13}C NMR spectra. They have been also tested for tuberculostatic activity *in vitro* against *M. tuberculosis* strains. Their cytotoxic activity towards eukaryotic cells was also evaluated.

As a result of the synthesis twelve novel derivatives of 2-(2-phenylalkyl)-1*H*-benzo[*d*]imidazole have been obtained. Some of the compounds exhibited very good activity towards *M. tuberculosis* sensitive and resistant "wild" strains and the standard strain. Cytotoxicity studies indicated differential toxicity of these compounds against eukaryotic cells. Obtained results suggest that some of the synthesized compounds are good candidates for tuberculosis drugs. These compounds have a good therapeutic potential.

This study is supported by the National Science Centre, Cracow (grant no. *2011/0 1/B/NZ4/01187).*

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17:15 Poster 22

Novel N-substituted 2-(2-phenalkyl)-1H-benzo[d]imidazoles: synthesis, characteristics and tuberculostatic activity

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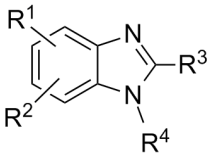
Benzimidazoles have a wide spectrum of biological activity. The direction of their activity is closely associated with the individual elements of the structure. We have already described a significant tuberculostatic activity of some 2-phenalkyl- and 2-cyclohexylalkylbenzimidazoles [1]. We also reported synthesis and activity of 2-(2-cyclohexylethyl)-1*H*-benzo[*d*]imidazole analogues with benzimidazole type of structure. Their tuberculostatic activity *in vitro* was at the level appropriate for the administrated chemotherapeutics [2]. Here we disclosed the synthesis of novel 2-(2-phenalkyl)-1*H*-benzo[*d*]imidazoles substituted at the N-1 position. We synthesized structures with methylsulfonyl or phenylsulfonyl substituent at the N-1 position from benzimidazoles possessing different substituents at the benzene ring of benzimidazole system, styryl, phenethyl or 3,5-dichlorophenethyl moiety at the C-2 position.



R^1: Me, F; R^2: H, Me
R^3: −CH=CH−Ph, $-CH_2-CH_2-Ph$, $-CH_2-CH_2-(3,5\text{-}Cl_2)-Ph$
R^4: H, $-SO_2Me$, $-SO_2Ph$

Benzimidazoles in the presence of triethylamine were treated with methane- and benzenesulfonyl chloride in anhydrous dioxane, giving the corresponding sulfonamide derivatives. As a result of the synthesis twelve novel derivatives of 2-(2-phenylalkyl)-1*H*-benzo[*d*]imidazole have been obtained. All the newly synthesized compounds were characterized by IR, ^{1}H NMR, and ^{13}C NMR spectra. They have been tested for tuberculostatic activity *in vitro* against *M. tuberculosis* sensitive and resistant "wild" strains and the standard strain H_{37}Rv. Their activity was lower than determined for benzimidazoles unsubstituted at the N-1 position.

This study is supported by the National Science Centre, Cracow (grant no. *2011/0 1/B/NZ4/01187).*

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[1] H. Foks, D. Pancechowska-Ksepko, W. Kuźmierkiewicz, Z. Zwolska, E. Augustynowicz-Kopeć, M. Janowiec: Synthesis and

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[2] K. Gobis, H. Foks, K. Bojanowski, E. Augustynowicz-Kopeć, A. Napiórkowska: Synthesis of novel 3-cyclohexylpropanoic acid-derived nitrogen heterocyclic compounds and their evaluation for tuberculostatic activity, Bioorg. Med. Chem. 20 (2012) 137-144.

17:15 Poster 23

Effects of 300 mT static magnetic field on IL-8 secretion in normal human colon myofibroblasts

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Intestinal subepithelial myofibroblasts, located in the lamina propria under the epithelial cell layer, have the specific functions in the gastrointestinal tract. Many of the factors are secreted by the activated myofibroblasts in the intestine in various disease states: experimental colitis, small bowel injury, gastric ulcer models or disease and also in naturally occurring inflammatory bowel disease (IBD). IL-8, one of the factors secreted by the activated myofibroblasts in the intestine, plays sequential role in neutrophil recruitment in the inflamed tissue. In the last few years, the increasing production of electromagnetic (EMF) and static magnetic fields (SMF), due to the expanding use of electronic devices in everyday life, has led to a number of studies on the effects of these fields on living organisms. EMF therapy, because of its anti-inflammatory properties, may be used in medicine in IBD treatment. This mechanism has not been elucidated yet.

In the present work normal human colon myofibroblasts CCD-18Co were exposed to SMF with a flux density of 300 mT. After 24 and 48 hours incubation TNF-α-dependent IL-8 secretion was determined with ELISA kit (R&D Systems) and the cell viability was determined with TOX-2 (In Vitro Toxicology Assay Kit XTT Based, TOX-2, Sigma).

It was shown that SMF has no effect on TNF-α-dependent IL-8 secretion and cell viability after 24 and 48 hours. This work was supported by the Medical University of Silesia (KNW-2-017/N/3/N).

17:15 Poster 24

Effect of Gly-His-Lys and its copper complex on TGF-β1 secretion in normal human dermal fibroblasts

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Background. Transforming growth factor beta (TGF-β) is a cytokine involved in a wide variety of biological processes such as cell growth, differentiation and proliferation, apoptosis and regulation of the immune response. It has an important role in wound healing process, fibrosis and scar tissue formation. Similarly to TGF-β1, insulin growth factor (IGF) family is expressed locally in response to tissue injury. Treatment of dermal fibroblasts with IGF-1 caused a substantial induction of TGF- β1 mRNA. Not a great deal of research so far has focused on IGF-2. Much attention has been focused on the tripeptides such as Gly-His-Lys (GHK) and their copper complexes, which have a high activity and good skin tolerance. Recent data suggests their physiological role has been related to the process of wound healing, tissue repair and skin inflammation.

Purpose. In the present study, the influence of 1 nM solutions of GHK, GHK-Cu and $CuCl_2$, on IGF-2 – dependent TGF-β1 secretion in normal human dermal fibroblasts cells was investigated.

Methods. Fibroblasts were cultured in 24-well plates. Total TGF-β1 protein was evaluated using the ELISA kit. The Bradford Reagent was used to determine the total quantity of cellular protein.

Results. Treatment of fibroblasts with 100 ng/ml IGF-2 resulted in a significant increase in TGF-β1 secretion. GHK and its copper complex and free copper ions decreased IGF-2 - dependent TGF-β1 secretion.

Conclusions. Our observations provide some new information on the potential use of that peptide contained in cosmetics to treat and prevent the formation of hypertrophic scars.

17:15 Poster 25

Fullerenes as the carriers of compounds with amide bond

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Fullerenes are molecules created solely of carbon and have a highly unusual attributes. The smallest structure in the fullerenes family is conformation C_{20} consists of pentagonal rings only what results in its instability. The first fullerene containing 12 pentagonal and 20 hexagonal rings, in which no two pentagons share an edge what results in good stability of carbon cage, is C_{60}. The structure of C_{60} is a truncated icosahedron, with two different bond lengths: between two hexagons are shorter then between a hexagon and a pentagon and average of bond length is 1.4 Å (angstroms). The attributes like structure, geometry, size and shape of carbon cages create the possibility to use them as vehicles to carry on substances with pharmacological activity.

The compounds of fullerenes are classified into following categories because of their functionalization: endohedral fullerenes when active molecules occur inside the carbon cage and exohedral when wide variety of both inorganic and organic groups, which exist outside, are added to the fullerenes exterior.

The main field of our investigation was to determine, with the use of molecular modeling techniques, the energy of conformation where a molecule which contains an amide bond is trapped inside the carbon cage of fullerene C_{60}. The formamide derivatives and their phosphorus analogues were studied in silico calculations as

models of bigger molecules containing peptide bonds. The absolute minimum energy of selected substances and their endohedral C_{60} compounds was calculated. Then the deformation and inclusion energies were delimited respectively with the use of DFT (*Density Functional Theory*) method. The results of calculations show the possibilities of use of fullerene C_{60} as the carrier of the compounds with amide bond that do not undergoes change inside fullerenes cage.

17:15	Poster	26

Selective protection of protoescigenin as the key step in synthesis of escin analogs

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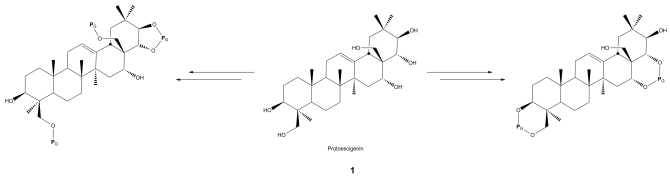
Escin, an active pharmaceutical ingredient of innumerable pharmaceutical preparations, consists of a complex mixture of some tens of triterpenoid saponins with similar structures, practically inseparable and rather poorly characterized. Herbal material, which is isolated from seed of the horse chestnut (*Aesculum hippocastanum* L.) has good clinical opinion as a remedy for chronic venous insufficiency and vain varicose. Yet, from the scientific point of view, the escin mixture is not compatible with modern principle of evidence based medicine, postulating API with strictly defined physical, chemical and pharmacological properties.

Our study of escin has been focused on a switch from multicomponent mixture of natural saponins to properly characterized, high purity individual chemical entities, with preserved pentacyclic triterpene scaffold. It has been already demonstrated [1] that such a change is feasible, based on technical scale preparation of protoescigenin (PES) – the main aglycone (genin) of the escin complex. Following application of semi-synthesis can have multidirectional design, but critically depends on reactivity and selectivity of the six hydroxyl groups present in the olean-12-en structure, which have practically not been explored before. Thus, systematic and critical survey of PES derivatization, with application of acylating, alkylating, and silylating reagents have been carried out – the most desirable feature being the ability to obtain a derivative in a state of reasonable chemical purity, without resort to chromatographic methods of isolation. In our hands, only ketalization has met such criteria, which in turn severely limited a scope of follow up derivatization, among which chemical glycosylation has been considered a priority. In search for a compromise between chemical feasibility and functional needs, “click chemistry” dipolar cycloaddition based on azide - alkyne ligation has been selected and evaluated as suitable synthetic tool. Necessary propargyl ethers of PES were obtained by standard method and according to expectation their tagging with variety of azides allowed for introduction of desired functionality, e.g. sugar conjugation or solubility enhancing moiety.



Acknowledgement:

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17:15	Poster	27

Isolation and application of β-galactosidases in the synthesis of 2-deoxy-β-D-galactosides

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The biological significance of glycosides and glycoconjugates has stimulated many works on the development of methods for glycosidic bond formation. They occur widely in natural products 2-deoxyglycosyl moieties are present in many biologically active compounds like anthracycline antitumour antibiotics, and aureolic acids, highly potent antimicrobial agents. The anomerically selective enzymatic synthesis of 2-deoxy-β-D-glycosides is a very interesting approach in contrast to multi-stage chemical synthesis requiring the use of temporary groups equatorially disposed at C(2) which must be removed in later steps, often lowering reaction yields.

In this communication we report strategy to isolated β-galactosidase from different strains of yeasts and application in the synthesis of 2-deoxy-β-D-galactosides. Finally their fungitoxic activity were tested for yeasts (*Candida albicans, Candida dubliniensis, Candida tropicalis, Candida glabrata, Candida krusei, Candida parapsilosis*) isolated from clinical materials in the Laboratory of Microbiology, Silesian Center for Heart Diseases in Zabrze. tested for antifungal activity in cooperation with Silesian Centre of Heart Disease.

Acknowledgement

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17:15 Poster 28

Isolation of galactosyltransferases from swine tissues. Screening of enzymatic activities

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Glycosyltransferases are very important proteins. They play a crucial role by participating in biosynthesis of glycolipids, glycoproteins, oligosaccharides and other glycoconjugates. They are responsible for synthesis of cell surface glycoconjugates. Galactosyltransferases assist in biosynthesis such important glycoconjugates as blood group antigens, Lewis antigens and lipopolysaccharides, therefore they are promising target for antiviral and anticancer drugs Study of enzymatic activities and inhibition of GTs are of considerable interest of many works in this field of science. Such study indicate possible way towards a suitable tool to measure the specific activity of potent antiviral or antitumour agents towards the mammalian source of GTs.

This communication describes the isolation of soluble glycosyltransferases from swine tissues. In our experiments, various tissues were tested – lymph nodes, thymus and liver. Performed study allow to reduce the isolation procedure time, offering promising prospects for preparation of this kind of enzyme from mammalian source – swine tissue.

Acknowledgement

Research studies part-financed by the European Union within the European Regional Development Fund (POIG.01.01.02-14-102/09).

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17:15 Poster 29

Modified isoxazolidinyl nucleosides

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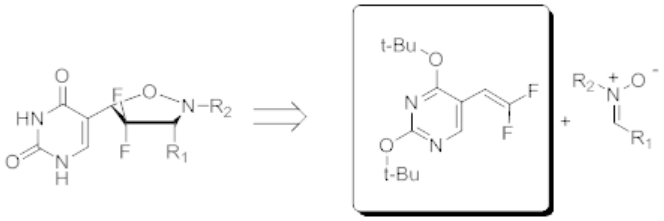
Research for new organic compounds which may show potential antitumor and antiviral activity leads to the discovery of effective medications. Modified nucleosides are significant class of compounds showing therapeutic activity. Recently a number of studies describing modified heterocyclic nucleosides with isoxazolidinyl ring instead of sugar moiety has appeared [1]. The most convenient method of synthesis modified isoxazolidinyl nucleosides are reactions of N-vinyl derivatives of nucleic acid bases with corresponding nitrone. However there are only few studies reporting synthesis of isoxazolidinyl and izoxazolyl C-C nucleosides.



We would like to present results of studies on synthesis of modified C-C nucleosides in which sugar moiety has been replaced with fluorinated isoxazolidinyl ring. Fluorinated analogues with potential biological activity attract more attention due to their unique properties, which are so important in biochemistry and medical chemistry.

Synthesis of fluorinated 5-vinylpyrimidine as diporalophile in 1,3-dipolar cycloaddition with selected nitrones will be described in

some details [2].

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17:15 Poster 30

Microarray analysis of CYP's gene expression in human dermal fibroblasts with cyclosporine A

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Cyclosporine A is an immunosuppressive drug used not only to prevent organ or tissue rejection, but also in curing dermal diseases such as atopic dermatitis, pyoderma gangrenosum or pemphigus. Cyclosporine is metabolized by the cytochrome P450 isoform CYP3A4 mainly, but also works as an inhibitor of CYP3A4, CYP2C19 and CYP2D6. At the current state of our knowledge, we do not exactly know how cyclosporine A influences healthy dermal cells.

The aim of this study was to estimate how cyclosporine A acts on gene expression of cytochrome P450 isoforms in normal human dermal fibroblasts (NHDF) using microarray analysis.

Normal human fibroblasts cells (CC-2511, Lonza) were cultured in the FGM medium (Fibroblast Growth Medium; Lonza, Basel, Switzerland) on 25 cm^2 plates with Nunclon surface, equipped with bacteriological filters (Nunc, Wiesbaden, Germany). After the confluence state, there was added cyclosporine A (sigma Aldrich) at a concentration of 100 ng mL^{-1}. Next with the use of TRIZOL® the full cell RNA had been extracted accordingly to the producer protocol (Invitrogen Life Technologies, USA). Extracted RNA was purified and used to cDNA and cRNA synthesis and next was hybridized with the HG-U133A 2.0 microarrays accordingly to the protocol (Affymetrix Inc. California, USA). The array plate was scanned by the GeneArray Scanner 3000 7G (Affymetrix Inc.) obtaining fluorescence signal of 22277 mRNA. Statistical analysis was performed using t-test. The significance level was set at $p \leq 0.05$.

After comparing fluorescence signals for 91 mRNA isoforms of cytochrome P450 in NHDF culture with and without cyclosporine A were stated that 31 mRNA of CYP's over-expressed in the presence of cyclosporine and 15 down-regulated. Between all of them the eight mRNA had statistically significant changes, but only CYP1B1 and CYP19A1 characterized highest expression.

We observed changes in the gene expression of CYP's connected with steroids metabolism after an eight-hour cyclosporine A exposure in human dermal fibroblasts.

This work was supported by Medical University of Silesia grants KNW-1-013/N/3/0 and KNW-1-060/K/3/0.

17:15 Poster 31

The effect of cyclosporine A on Wnt signaling in human dermal fibroblasts

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Xenobiotics such as medicines distinctly affect the expression of different genes as well as modulate signaling pathways in cells. One of the most important pathways, which activate processes such as proliferation, differentiation, inflammation and more, is the Wnt signaling pathway. Wnt factors can activate several signaling pathways such as the main strictly related to β-catenin - the canonical and also two non-canonical, namely the Wnt/Ca^{2+} dependent and the planar cell polarity pathway. All of these start when a specific Wnt molecule binds to a frizzled receptor on the cell membrane and activates the dishivelled protein. Cyclosporine A is a immunosuppressant drug which acts as a calcineurine inhibitor, which regulates gene expression via NF-AT transcription factor which is also a part of the non-canonical Wnt/Ca^{2+} dependent signaling pathway. Thus, the aim of our study was to estimate if cyclosporine can affect the Wnt signaling in healthy dermal fibroblasts.

Normal human dermal fibroblasts (NHDF, CC-2511, Lonza) were cultured in the liquid growth medium (Lonza, Basel, Switzerland) in standard conditions. After the confluence state there was added cyclosporine A (Sigma Aldrich) at a concentration of 100 ng/ml. RNA had been extracted from cells after 8, 24, 48 hours by the use of TRIZOL® reagent accordingly to the producers protocol (Invitrogen Life Technologies, USA). The extracted RNA was purified and next used to synthesize cDNA and cRNA which was hybridized with the HG-U133A_ 2.0 Affymetrix microarray accordingly to the protocol (Affymetrix Inc. California, USA). Then the array was scanned with GeneArray Scanner 3000 7G (Affymetrix Inc.) obtaining fluorescence signal of 22277 mRNA. Statistical analysis were performed by the use of GeneSpring software (Agilent Technologies, USA), and only $p < 0.05$ were accepted.

We performed an ANOVA test for all culture times comparing them to the control without cyclosporine A and obtained 146 mRNA. The differences between all three culture times were checked with t test. In results there were only 9 mRNA common for all culture times. But also we noticed changes of Wnt molecules in different times.

After 24 h of human dermal fibroblasts exposition to cyclosporine A

the *Wnt5A* expression had been over-expressed which suggest that via this pathway are trying to stimulate NF-AT signaling.

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17:15 Poster 32

Ebselen analogues: synthesis and antimicrobial activity

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Ebselen and its organoselenium analogues are well known as active immunostimulants, inhibitors of enzymes, antioxidants, anti-inflammatory, antitumor, antiviral and antimicrobial agents[1-6].
In this work we report synthesis and results of biological evaluation of a series of C substituted ebselen analogues (Fig.1).

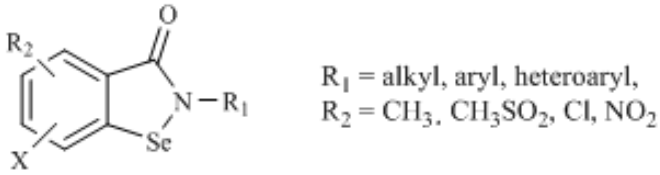

Fig. 1

The general strategy for the synthesis of C-substituted benzisoselenazol-3(2*H*)-ones is based on the conversion of commercially available 5 substituted 2-chlorobenzoic acids or 2-aminobenzoic acids into 5-substituted 2 (chloroseleno)benzoyl chlorides and finally on the tandem selenenylation-acylation of primary amines with these reagents. Prepared compounds were tested towards inhibition of the viability of gram positive (*Bacillus subtilis*), gram negative (*Escherichia coli*) bacteria and pathogenic fungus *Candida albicans*. The preliminary results indicate the promising, anti-bacterial and anti-mycotic activity of tested compounds in low concentrations.

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17:15 Poster 33

Application of the new data processing method for photodiode array detector in the analysis of drug substances

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Intelligent Dynamic Range Extension Calculator (i-DReC) is a new data processing method for photodiode array detector available in LabSolutions software by Shimadzu. It allows to extend the linear range of the chromatographic method by shifting the chromatographic profile to the wavelength where target signal is not saturated.

In the analysis of active pharmaceutical ingredients (API's) it is necessary to determine both assay of the drug substance itself and content of impurities in drug substance. As impurities must be determined at relatively low levels, it is often not possible to select such analytical conditions which allow to determine both API assay and content of impurities in one chromatographic run. Concentration of drug substance in method for determination of its purity is often outside the linear range of applied detector and additional method for drug substance assay must be developed.

Temozolomide is a drug which is used for the treatment of primary brain tumours. Impurity profile of temozolomide substance manufactured at Pharmaceutical Research Institute imposed the necessity to use separate methods for determination of assay and purity of the API. Application of i-DReC allows to determine assay of temozolomide using the method for purity determination although temozolomide signal is saturated. Comparison of results of assay determination with the use of i-DReC with the results obtained from the separate assay method revealed good coherence between them. Thus it is possible to use only one analytical procedure instead of two which allows to reduce the solvent consumption, lower the costs of analysis and save time.

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17:15 Poster 35

Evaluation of genistein ability to modulate the protein expression of CTGF and the genes expression of TGF β1, β2 and β3 isoforms in keloid fibroblasts in vitro

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Keloids characterize overgrowth of connective tissue in the skin that arises as a consequence of abnormal wound healing. Normal wound healing is regulated by a complex set of interactions within a network of profibrotic and antifibrotic cytokines that regulates new extracellular matrix (ECM) synthesis and remodeling. These proteins include transforming growth factor-β (TGF-β) and connective tissue growth factor (CTGF). TGF-β stimulates fibroblasts to synthesis and contraction of ECM and acts as a central mediator of profibrotic response. CTGF is induced by TGF-β and is considered as a downstream mediator of TGF-β action in fibroblasts. CTGF plays a crucial role in keloid pathogenesis by promoting prolonged collagen synthesis and deposition and in a consequence sustained fibrotic response.

Genistein (4,5,7-trihydroxyisoflavone) exhibits multidirectional biological action. Genistein shows numerous intracellular targets of actions in live cells such as estrogen receptors (ERs), tyrosine-specific protein kinases and transcription factors etc. NFKB, Akt and AP-1 engaged in cytokines expression.

The aim of the study was to investigate the effect of genistein on the expression of CTGF and TGF β1, β2 and β3 isoforms in keloid fibroblast cultured *in vitro*. Normal dermal fibroblasts were used as a control cells.

Natural soybean genistein in: 370 μM, 185 μM, 92,5 μM, 37μM, 18,5 μM, 3,7 μM, 1,85 μM and 0,185 μM concentrations were used in the study. Real time RT-QPCR was used to estimate transcription level of *CTGF* and *TGF β1*, *β2* and *β3* isoforms in normal and keloid fibroblasts treated with genistein.

Secreted/cell-associated CTGF was evaluated in cell medium by ELISA. Total protein quantification was evaluated by fluorymetric assay in cells lysates (Quant-iT TM Protein Assay Kit).

Results: *TGF β1*, *β2* and *β3* genes expression are modulated by genistein in concentration dependent manner. Genistein suppresses the expression of mRNA *CTGF* and its protein.

17:15 Poster 36

The effect of soybean genistein on the expression of selected genes regulating cell cycle in fibroblasts derived from keloids

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Keloids are bening fibroproliferative tumors considered to be scars that results when wounds heal abnormally. The underlaying pathophysiological mechanism is unclear. However disturbed cell cycle regulation is observed in keloids. The process plays an important role in maintaining genetic integrity of the cell. P53 can arrest human cells with damaged DNA in G1 phase of cell cycle, that allows DNA repair before S-phase. If the DNA damage is severe and cannot be repaired, apoptotic pathways are activated to eliminate damaged cells. P53 as a transactivator of transcription can induce apoptosis by up-regulation of pro-apoptotic gene expression such as *BAX* and down-regulation of antiapoptotic genes expression such as *BCL-2*.

Genistein (4,5,7-trihydroxyisoflavone) exhibits multidirectional biological action. The concentration of genistein is relatively high in soybean. Genistein has been shown as effective antioxidant and chemopreventive agent. Genistein can bind to estrogen receptors (ERs) and modulate estrogen action due to its structure similarity to human estrogens. Genistein also inhibits transcription factors NFKB, Akt and AP-1 signaling pathways, that are important for cell proliferation, differentiation, survival and apoptosis.

The aim of the study was to investigate genistein as a potential regulator of *TP53*, *CDKN1A*, *BAX* and *BCL-2* in normal fibroblasts and fibroblasts derived from keloids cultured *in vitro*.

Natural soybean genistein in: 370 μM, 185 μM, 92,5 μM, 37μM, 18,5 μM, 3,7 μM, 1,85 μM and 0,185 μM concentrations were used in the study. Real time RT-QPCR was used to estimate transcription level of selected genes in normal and keloid fibroblasts treated with genistein.

Results: *TP53* and *CDKN1A* genes expression are modulated by genistein in concentration dependent manner. The agent also modulates *BAX/BCL-2* ratio in examined cells.

17:15 Poster 37

Inhibition of inducible nitric oxide synthase expression by inositol hexaphosphate in cultured human colon cancer cells exposed to proinflammatory agents

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Inflammatory bowel diseases (IBDs) are chronic inflammatory con-

ditions associated with increased risk in developing to colorectal cancer. A number of mediators of inflammation, such as pro-inflammatory cytokines, prostaglandins and nitric oxide have been involved in carcinogenesis, especially in the promotion and progression stages. NO is synthesized from L-arginine by constitutively expressed endothelial and neuronal nitric oxide synthases (eNOS and nNOS, respectively) and an inducible NOS (iNOS) isoform expressed under inflammatory conditions. Therefore, selective inhibitors of iNOS could be considered to be good candidates as chemopreventive agents against colon cancer. One of the promising dietary phytochemicals with preventive and therapeutic potential is inositol hexaphosphate (IP6), which demonstrated anti-cancer and anti-inflammatory properties in in vivo and in vitro studies.

In this study the effect of IP6 on the mRNA expression of iNOS stimulated with bacterial lipopolysaccharides (*Escherichia coli* and *Salmonella typhimurium*) and IL-1β in intestinal cells Caco-2 for 3, 6 and 12 h was investigated. A transcription level of iNOS was performed on cells after treatment with 1 and 2.5 mM IP6 by the use real time QRT-PCR technique.

Caco-2 exposed to IP6 only revealed an increase in iNOS gene expression after 3 h, but longer exposition (12h) caused a significant reduction in the level of mRNA. Stimulation of cells with pro-inflammatory factors (LPS and IL-1β) resulted in an up-expression of iNOS mRNA at 3, 6 and 12h. IP6 enhanced *E. coli* LPS-stimulated transcription of this gene after 3 h. At a concentration of 1 mM it affected down-expression of iNOS in cells treated with IL-1β or endotoxin of *Salmonella*. A decrease in iNOS transcription by IP6 following the gene induction by proinflammatory agents in 6 and 12 h lasting cultures was also determined.

The findings of this study suggest that one of the anti-cancer and anti-inflammatory abilities of IP6 can be realized by suppressing the expression of gene encoding inducible nitric oxide synthase isoform at the transcriptional level.

17:15 Poster 38

The effect of inositol hexaphosphate on the transcriptional activity of genes encoding cyclin D1 and histone H3 in human intestinal cells stimulated with interleukin 1β

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Loss of proliferation control is one of the major features of malignant cells. Proliferation rate is useful prognostic factor for their survival and response to treatment with anticancer drug. In proliferating cells, histone synthesis is functionally and temporally coupled with replication of DNA during the S phase. Determination of replication dependent histone H3 can be applied as a proliferative marker. Cyclin D1 (CCND1) regulates the cell cycle by participating in the control of the G1/S phase transition. IL-1 is known to act as a tumor growth factor which stimulates the proliferation and tumor cell survival. Inositol hexaphosphate (IP6), a naturally occurring phytochemical, exhibits anticancer activity in a wide range of cancers. IP6 activity has been reported to involve several processes like proliferation, apoptosis, metastasis, however, the mechanism of its anti-carcinogenic effect is still under investigation. IP6 induced cell cycle arrest in G1 phase and a significant decrease of the S phase of human cancer cells.

The aim of the present study was to examine the influence of IP6 on the expression of genes coding for proliferation markers, i.e., CCND1 and histone H3 in IL-1β-stimulated intestinal cell line Caco-2. Quantification of genes expression was carried out using real time RT-QPCR technique in Caco-2 cells after treatment with 1 ng/ml of IL-1β, 1 and 2.5 mM of IP6 for 3, 6 and 12h. In separate cultures, cells were incubated with 1 ng/ml IL-1β for the indicated times. The untreated Caco-2 cells were used as the control.

In a time course experiment, stimulation of cells with IL-1β only resulted in an overexpression of both CCND1 and histone H3 mRNAs as compared with control. IP6 had no influence on IL-1β-stimulated CCND1 expression for 3 and 6 h. After 12 h, statistically significant decrease in mRNA CCND1 was observed in cells exposed to IL-1β and IP6 (1 and 2.5 mM) in relation to cells treated with IL-1βonly. The levels of H3 mRNA in IL-1β-stimulated cells and cells treated with IL-1β and IP6 revealed no statistically significant differences after 3 h. IP6 at concentration of both 1 and 2.5 mM enhanced IL1β-stimulated transcription of H3 gene after 6 h. Subsequently (12 h), the combination of IP6 and IL-1β decreased H3 mRNA level compared to IL1β-treated cells.

In conclusion, proinflammatory cytokine IL-1β upregulates CCND1 and histone H3 mRNAs expression in colon cancer epithelial cells Caco-2. These results suggest that the ability of IP6 to inhibit colon cancer cells proliferation may be mediated through downregulation of genes encoding cyclin D1 and histone H3 at the mRNA level.

17:15 Poster 39

Synthesis of chiral triazine coupling reagents based on esters of N-alkylproline and their application in the enantioselective incorporation D or L amino acid residue directly from racemic substrate

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The concept of traceless enantioselective coupling reagents is based on chiral 1,3,5-triazine derivatives with the chiral fragment departing after the activation of a carboxylic component. According to this concept, such reagents are prepared by temporary attachment of the chiral fragment to a classical peptide-bond-forming agent. Thus, after selection of the enantiomer at the activation stage and departure of the chiral component, the activated intermediate is converted to a well-known form of a conventional acylating species [1]. It has been shown that configuration and enantiomeric enrichment once established remain intact and independent on the structure of acylated counterpart. Furthermore, as anticipated, parameters like configuration, optical purity of the product, and the efficiency of coupling

were predictable on the basis of a single experiment of the model reaction. Application of chiral *N*-triazinylamonium salts derived from alkaloids (brucine, strychnine, quinine) yielded peptides within up to 99% enantiomeric purity from racemic substrate, with expected configuration, obtained in high yield under coupling procedure typical for other triazine coupling reagents [2]. Until now, the main limitation in the broad application of the traceless coupling reagents are toxicity of the alkaloids and limited access to both enantiomeric forms of the chiral component of coupling reagent.

Thus, to open access to the representative collection of non-toxic chiral coupling reagents readily available in both enantiomeric forms the systematic studies were undertaken to develop versatile procedures for transformation of proline readily available in d and l form into versatile chiral component. Herein are presented results of application of *N*-methyl or *N*-allilproline esters for the synthesis of chiral tracelles coupling reagents and their application in the synthesis of peptide directly from racemic amino acids.

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17:15 Poster 40

1,3-Oxazolidin-5-ones as chiral components in the synthesis of traceless enantioselective reagents

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Traceless chiral coupling reagents for enantioselective peptide synthesis directly from racemic substrates, developed at Lodz University of Technology, are composed from two fragments: achiral, substitued 1,3,5-triazine derivatives and chiral, quaternary amonium salts. In the previous studies it has been confirmed that application of traceless enantiodifferentaiating coupling reagent leads from racemic substrates to enantiomerically homogenous products with the purity, configuration and the efficiency of coupling predictable on the basis of a single model experiment. This outcome is achived by designing modular structure of enentiodifferentiating reagent with a chiral fragment of reagent acting as stereoselector only in the activation step and its departure after fulfilling its stereoselective function what makes possible to exact prediction of the stereochemical outcome. Thus, all further reaction steps proceeds only in the presence of the achiral module, which is exactly the same as in well known standard achiral triazine reagents [1]. Until now the most impressive stereoselectivities were achieved for reagents derived from alkaloids such as strychnine and brucine. This permit, incorporation of the amino acid into peptide chain in high yields (in the range 85-95%) and high enantiomeric enrichment reaching ee 98-99%. However chiral reagents with alkaloid moiety posses also several drawbacks: alkaloids are highly toxic and occur in only one enantiomeric form, what limits an access to only to one enantiomeric form of the final product (the second one is discriminated). In order to overcome those limitations we attempt to use as a chiral component of traceless coupling reagent, prepared from amino acids derivatives available in both enantiomeric forms. For the best enantioselectivities those derivatives should contain a stereogenic center (except for α-carbon) located on chiral, configurationally stable nitrogen atom (bridged position). In our studies these requirements was reached by applying bicyclic proline derivative in which the amine and carboxyl functions are engaged in the formation an additional ring stabilizing configuration on bridgehead nitrogen atom. Among many bicyclic derivatives, the most promising were 1,3-oxazolidin-5-ones warranted stability of the stereogenic center on proline α-carbon. This particular advantage feature of 1,3-oxazolidin-5-ones was utilized in chirality transfer and chirality selfregeneration processes [3]. Herein, we present the results of our attempts to obtain of 1,3-oxazolidin-5-ones derived from L- and D-proline with a variety of aldehydes and to evaluate their efficiency in the synthesis of new chiral traceless coupling reagents.

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17:15 Poster 41

Novel antifungal peptide-inhibitor conjugates

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The common use of antibacterial antibiotics and several modern medical technologies giving rise to attenuation of human immunolo-

gical system, have caused a substantial increase in frequency of disseminated mycoses. These infections are nowadays considered a serious clinical problem, due to the high mortality rate, limited number of effective antifungal drugs and high level of antifungal drug resistance [1]. Search for new antifungals, especially targeting novel molecular targets in fungal cells, is therefore an urgent need.

The fungal enzyme, glucosamine-6-phosphate (GlcN-6-P) synthase, catalysing a crucial reaction in the cell wall biosynthesis, is known as potential target for antifungal chemotherapy and N^3-(4-methoxyfumaroylo)-*L*-2,3-diaminopropanonic acid (FMDP) is an effective and selective inhibitor of this enzyme [2]. Unfortunately FMDP is a highly hydrophilic molecule and is poorly taken up by fungal cells.

In the present communication we present construction of highly effective antifungal agents by incorporation of FMDP into oligopeptide carriers composed of 5-10 proteinogenic amino acid residues. These oligopeptide carriers are amphipatic and contain basic amino acids (Lys and Arg) and amino acids with hydrophobic side chains. FMDP-oligopeptide conjugates exhibit strong antifungal in vitro activity, at least two orders of magnitude better than FMDP alone. Evidence confirming that the conjugates obtained effectively penetrate fungal cells, are splitted inside to generate the active inhibitor of GlcN-6-P synthase and finally their action causes destruction of fungal cells, are presented.

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17:15 Poster 42

Bioequivalence study of ondansetron film-coated tablets in healthy volunteers

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Ondansetron is a selective 5-HT_3 receptor antagonist administered as an antiemetic during chemotherapy and radiotherapy.

The aim of the study was to investigate the bioavailability of a generic product of 8 mg ondansetron film-coated tablets (test) as compared to that of a branded product (reference) at the same strength to prove determine bioequivalence and to apply for regulatory approval.

A double blinded, randomized, crossover, two-period, single-dose, comparative study was conducted in healthy white volunteers in fasting conditions in compliance with the Good Clinical Practice principles. A single oral dose of the test or reference product was followed by 7-day wash-out period. Ondansetron concentration was determined by validated HPLC-UV method in compliance with the Good Laboratory Practice. The products were considered bioequivalent if the 90% CI of the geometric mean ratios (test/reference) for $AUC_{(0-t)}$, $AUC_{(0-\infty)}$ and Cmax were within the range 80.00 – 125.00% [1,2].

20 healthy male and female volunteers completed the study. There were no significant differences in pharmacokinetic parameters between products. The results of the study indicate that Ondatron 8 mg film-coated tablets manufactured by Tarchomińskie Zakłady Farmaceutyczne Polfa S.A. (test product) are bioequivalent to Zofran 8 mg film-coated tablets manufactured by GlaxoSmithKline Export Ltd (reference product). Both products were well tolerated [3].

The study was supported by Tarchomińskie Zakłady Farmaceutyczne Polfa S.A.

Instytut Farmaceutyczny

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17:15 Poster 43

Synthesis and characterization of 3-indolylmethanol imprinted polymers

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Molecularly imprinted polymers (MIPs) are modern materials specifically designed for selective and specific recognition of target analytes. MIPs are primarily used as sorbents in bioanalysis and medical diagnostics, as microreactors in organic chemistry, and as carriers used in modern drug delivery systems [1,2].

The synthesis of MIPs involves formation of polymer by combining the template, the functional monomer and solving them in porogenic system prior to copolymerisation that occurs in the presence of cross-linking agent. Once the polymer is formed, the template molecule is then removed from the polymer matrix to create three dimentional cavities that are complementary in shape to the template [1].

3-indolylmethanol (I3C) is a phytochemical that naturally occurs in cruciferous vegetables. After oral administration I3C undergoes dimerization in stomach acid to 3,3'-diindolylmethane (DIM). Both I3C and DIM display multidirectional antitumor activity and are used as a dietary supplements in prevention of tumors [3].

The aim of our work is to produce and characterize 3-indolylmethanol imprinted polymers suitable for selective recognition of I3C. The conducted research will provide a significant contribution to the characterization of imprinting materials of compounds containing indole structure as well as allows selective separation of I3C from biological matrix.

The polymers were prepared by the radical bulk thermal polymerisation. The impact of functional monomer and porogenic solvent was investigated. As functional monomers, we examined acidic monomers (methacrylic acid), basic monomers (allylamine, 4-vinylpyridine), neutral monomers (1,1,1,3,3,3-hexafluoroisopropyl methacrylate).

Our preliminary results show that the highest selectivity (imprinting factor) was achieved for the polymers prepared from 1,1,1,3,3,3-hexafluoroisopropyl methacrylate as well as from allylamine as the functional monomers in carbon tetrachloride as the porogenic solvent. The full set of results and physico-chemical characterization of selected polymers will be presented during the conference.

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17:15 Poster 44

Prediction of the extracellular loops in thirteen G-protein-coupled receptors

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G protein-coupled receptors (GPCRs) play key roles in a variety of signaling cascades that control many cellular processes and are related to numerous diseases, thus much effort is devoted to obtain their functional structures. Construction of applicable GPCR models requires accurate structure determination of extracellular elements, especially the second extracellular loop (ECL2). We applied a well-established protein modeling tool – the CABS model – for structure prediction of three extracellular loops in thirteen GPCRs. The CABS method proved to be accurate during single loop reconstruction [1]. In this study, the long ECL2 loops (of length between 13 and 34 residues) were predicted in an environment of other extracellular loops being fully flexible and transmembrane domain fixed in its X-ray conformation. The modeling procedure utilized theoretical predictions of ECL2 secondary structure and experimental constraints on disulfide bridges. Our approach yielded ensembles of low-energy conformers and the most populated conformers, that contained models close to the available X-ray structures [2]. The modeling results are comparable to other state-of-the-art modeling methods and provide a benchmark of lately reported GPCR structures for assessing other algorithms.

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17:15 Poster 45

Fucosyl derivatives in the synthesis of uridine glycoconjugates - potential glycosyltransferase inhibitors

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Glycosylation is highly sensitive to alterations in cellular function and abnormal glycosylation is diagnostic of a number of diseases. The control of glycosylation by the cell affords a means of putting the same recognition markers on quite different proteins without having to code the infor mation into the DNA of that protein. Glycosyltransferases (GTs) are family of enzymes that are responsible for the biosynthesis of glycoconjugates such as glycolipids and glycoproteins as well as oligo- and polysaccharides which are crucial factors in bacterial and viral infections [2]. Development of new selective inhibitors is of great importance in dealing with bacterial [3] and fungal diseases [4]. The key role played by fucose in glycoprotein and cellular function has prompted significant research toward identifying recombinant and biochemical strategies for blocking its incorporation into proteins and membrane structures [5]. In connection with our own studies in this area we became interest in the prospect of developing a simple method that would lead to various fucosyl derivatives of uridine as a potential GTs inhibitors. Utilization of fucal led us to unsaturated disaccharide building blocks, which are very useful in glycoconjugate synthesis.

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17:15 Poster 46

Unsaturated saccharides as a useful chiral synthons and their application in synthesis of polyphenol glycoconjugates - potential antiproliferative agents

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Genistein is a naturally occurring polyphenol-type product with chemopreventive and antitumor potential. For example, genistein inhibits topoisomerase I and II , 5a-reductase and protein histidine kinase, all of which may contribute to the antiproliferative or pro-apoptotic effects of genistein. Additionally, genistein has been shown to inhibit the growth of various cancer cells through the modulation of genes that are intimately related to the regulation of cell cycle and programmed cell death (apoptosis) [1]. Our previous experiments, among genistein modified at C7 by different unsaturated sugars, revealed that several compounds appeared more active than parent compound in preliminary screening for inhibition of cancer cell proliferation [2, 3]. The development of improved methods for carbohydrate synthesis and particularly glycosidic bond formation is crucial. Therefore, utilization of glycals as building blocks for the total synthesis of various natural products is of great interest in bioorganic and medicinal chemistry. We optimized an effective method for synthesis of new disaccharide building blocks from glycals, which are very useful chiral synthons in glycoconjugates synthesis.

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17:15 Poster 47

Synthesis and biological activity of synthetic β-D-glucose and β-D-galactose conjugates with various acyclic nucleosides

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Glycosyltransferases (GTs) are enzymes responsible for the biosynthesis of glycoconjugates such as glycolipids and glycoproteins as well as oligo- and polysaccharides which are crucial factors in bacterial and viral infections. They are also responsible for tumor metastasis. Selective inhibition of the GTs may influence the composition of glycoconjugates and oligosaccharides on the cell surface so that they cannot be recognized by virus or bacteria during infection. Therefore GTs are interesting molecular targets [1-3]. Development of effective inhibitors of these enzymes is very important because it can lead to better understanding of biological pathways implicating glycosyltransferases and also to potential therapeutic applications.

Glycosyltransferase donor type inhibitors are generally designed based on analogies between the three different moieties composing the NDP sugar natural substrates, mimicking either the carbohydrate part [4], the diphosphate linkage, the nucleoside moiety or combination of these [5], while modifications of the uridine sugar have not previously been explored and there is lack of reports on derivatives containing acyclonucleosides.

After studies on modification of sugar motif, pyrophosphate mimicking linker and conformation at anomeric center of the transferred sugar our attention has been directed toward the versatility of the developed methodology in the synthesis of a novel group of analogues of natural donor type substrates of GTs which will be complement to the set synthesized earlier. Herein we present synthesis and biological evaluation of β-D-glucose and β-D-galactose conjugates connected by an amide bond acyclic derivatives of uracil.

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17:15 Poster 48

Synthesis of glycosyltransferases natural substrate analogues from 1,6-anhydrosugars and acyclic uridine derivatives

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Glycosylation is one of the most important post-translational modification of gene products and is often critical to specific cellular biological functions. It is well proven that sugars binding to lipids and proteins on the cell surface participate in various intercellular and intracellular events such as cell-cell interactions, cell adhesion, inflammation, immune system response, they take part in tumor metastasis and both viral and parasitic infections [1]. Because of the importance of glycosylation in biological systems glycosyltransferases are interesting targets for the development of their specific inhibitors which might have the potential to precisely modify the structures of any class of cell-surface glycoconjugate. For this reason, considerable effort has been directed toward the design of glycosyltransferase inhibitors [2, 3]. The development of inhibitors of glycosyltranferase may lead to discovery of novel therapeutics for the treatment of certain diseases in which carbohydrates-protein interactions are involved [4].

Synthesis of GTs donor type natural substrate analogues, in which carbohydrate moiety is connected to aromatic aglycon (nitropyridine derivative) via 1-α-thioglycosidic bond is very challenging and purification of final products can cause many difficulties. Our recent research led us to utilize 1,6-anhydrosugars in the synthesis of optically pure 1-α-thioglycosides and their use as glycoconjugates building blocks. The anomeric centre configuration of the synthesized compounds is identical to the anomeric centre of the natural substrate of β-1,4-galactosyltransferase [5]. Final glycoconjugates are expected to control and modulate activity of investigated enzymes.

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17:15 Poster 49

Determination of the viscosity and density of veterinary vaccines

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Determination of viscosity and density plays a significant role in assessing the stability of veterinary vaccines. Analysis of these parameters are often a critical indicator, which determine the quality of product, its appearance as well as the expiry date. The aim of the study focused on determination and validation of dynamic viscosity and relative density method devoted for practical usage of veterinary vaccines quality monitoring at national market. Immunological veterinary medicinal product (ivmp) for four different animal species poultry, cats, cattle and rabbits were tested. The viscosity and density were measured using a rotational viscometer SVM 3000 combined with a density measurement unit, Anton Paar (USA), with a fixed shear rate at 20°C. The measurements were performed under the following environmental conditions: ambient temperature of + 18 to + 25°C and relative humidity of 20 to 80 %. The optimization of the minimum quantity, 2 ml of a sample dispensed into the measuring cell was estabilished. The three different types of matrices: emulsions, suspensions and solvents were tested, also viral as bacterial vaccines. The maximum scatter of results (CV) for the viscosity (3.87 %) and for the density (0.261 %) were found for the measurements of the suspensions. It was relevant for the only bacterial vaccine in this study. The method of dynamic viscosity was linear in the range 3,902 – 1110 mPa·s , with r^2 = 1,000000. The method of relative density was linear in the range 0,81213 – 0,84262 g/cm^3, with r^2 = 0,999995. The method was used for vaccines market monitoring in 2011, 2012, 2013 and it is still in operation.

17:15 Poster 50

Identification of degradation products of cilostazol drug substance

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Cilostazol, 6-[4-(1-cyclohexyl-1*H*-tetrazol-5-yl)butoxy]-3,4-dihydro-2(1*H*)-quinolinone, is used to improve the symptoms of blood flow problem in the legs (intermittent

claudication). Cilostazol can increase blood flow and the amount of oxygen that gets to the muscles. It works by preventing platelets blood from sticking together and by widening blood vessels in the legs. This helps the blood to move more easily and increases blood flow [1].

The crude cilostazol obtained using the synthetic route developed in PRI contains four main impurities. The impurities were identified. Additionally, stress testing studies were performed to determine the stability of the drug substance under different conditions (neutral, acidic, basic hydrolysis and oxidative degradation) and identify the main degradation products. Attempts to make structural assignment of these compounds by HPLC-MS-MS was the main assumption of this work. Based on the HPLC-MS-MS analyses the potential structures of the degradation products were proposed.

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17:15	Poster	51

Validation of HPLC methods for analyzing the chemical purity of cilostazol

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Two selective HPLC methods with UV detection were developed to analyse cilostazol and its related substances. Due to great difference between spectral properties of the analytes, the analysis was divided into two independent parts. Separation of cilostazol and its potential impurities was achieved on Kinetex C18 chromatographic column (100 mm x 4.6 mm x 2.6 µm) by gradient elution with ammonium acetate buffer and acetonitrile. Chromatograms were acquired at 254 nm. The second method for the quantitative determination of CX-1 (starting material in the synthetic route) assay was investigated on Synergi MAX-RP chromatographic column (150 mm x 4.6 mm x 4 µm) with the use of H_2O:CH_3CN (1:1, v/v) mixture and isocratic elution. Detection was carried out at 193 nm.

Both methods were validated for linearity, specificity, precision, accuracy, limit of detection, limit of quantification and robustness. Suitability of HPLC methods for the pharmaceutical control of cilostazol samples and the validation according to requirements of Q2(R1) International Conference of Harmonization scientific guideline were demonstrated.

Acknowledgement:
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17:15	Poster	52

Evaluation of anti-denaturation activity of C-5' - substituted uridine derivatives.

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Denaturation of tissue proteins is one of the well-documented causes of inflammation. Agents that can prevent protein denaturation would be worthwhile for anti - inflammatory drug development. Currently used anti - inflammatory drugs (salicylic acid, diclofenac, etc.) have shown dose dependent ability to inhibit heat induce protein denaturation. Inflammation which is a pattern of response to injury, involves the accumulation of cells and exudates in irritated tissues, that allows protection from further damage. This process has been studied in an attempt to combat its effects on the body [1].

In our research anti - denaturation study was performed by using several albumin (e.g. BSA, HSA, OVA) as it is practiced in research [2]. It is known that nonsteroidal anti - inflammatory drugs such as dicolfenac sodium and salicylic acid prevent denaturation of these albumins. We have observed that some new C-5'- substituted uridine derivatives prevent heat-induced albumin denaturation at the level similar to known anti – inflammatory drugs. Studies on anti-denaturation effect were carried out at 50 -70°C in various buffer systems (e.g. HEPES, Tris, phosphate).

The highest effect was observed at pH below 5 in HEPES buffer. Comparison of effects of different compounds and IC_{50} values will be presented.

Acknowledgement

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17:15 Poster 53

Method development for the determination of class II C residual solvents by GC

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The purpose of the study was a development of a new method for the determination of class IIC residual solvents in following organic solvents: dimethylsulphoxide (DMSO), N,N-dimethylformamide (DMF), N,N-dimethylacetamide (DMA) by gas chromatography (GC). In this analytical method we used helium as the carrier gas and high polarity column together with FID as a detector. This GC method is intended to be used for identifying and quantifying residual solvents in DMSO, DMF, DMA. These solvents are used as dissolvents for analysis of residual solvents in drug substances, excipients or drug products. They must meet the requirements of the maximum allowable amounts of residual solvents determined by special guidelines included in ICH (Q3C) and pharmacopeias (USP 467, European). The organic solvents are constantly present in the pharmaceutical production and they are difficult to be completely removed in the manufacturing processes of pharmaceuticals. A low assay of these solvents, which may remain in the final product. They are called residual solvents or they are known as organic volatile impurities (OVI). The class II residual solvents are described as non-genotoxic animal carcinogens. The content of these solvents should be evaluated, justified and limited in the final drug products because of their inherent toxicity. In our research popular diluents like: DMSO, DMF DMAc were used as test samples. It was difficult to determine following class IIC impurities (in accordance with USP): N,N-dimethylacetamide, N,N-dimethyl-formamide, 2-ethoxyethanol, 2-methoxyethanol, ethyleneglycol, formamide, N-methyl-pyrrolidone, sulfolane in these liquid samples. The peaks from these dissolvents were co-eluated with the peaks from analities in the chromatograms obtained with test samples. The established method can be used in routine control of class II C residual solvents.

17:15 Poster 54

Capecitabine molecular structure in the liquid state as predicted from NMR measurements and theoretical calculations.

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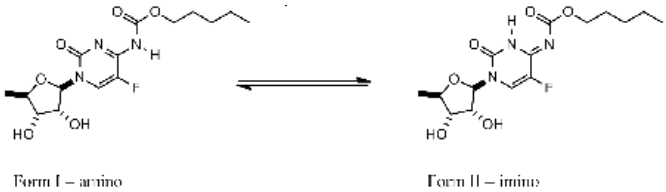
In spite of enormous literature (over 6800 papers) reporting biochemical, pharmacological or medicinal data, the molecular structure of capecitabine was rarely described. Scarce spectral and crystal data are not consistent with respect to the tautomeric forms occuring in solid or liquid phases. This has prompted us to study capecitabine in DMSO-d_6 (at room temperature) and THF-d_8 (at various temperatures) solutions. Based on ^{1}H, ^{13}C, ^{15}N and ^{19}F NMR (HSQC, HMBC) spectra it was found that at least two tautomeric forms (I-amino, II-imino) exist in equilibrium



Form I – amino Form II – imino

At T=218 K form I-amino dominates. It was also found (^{1}H, ^{19}F spectra) that tautomeric equilibrium is temperature dependent in various solvents. Density functional theory quantum mechanical calculations suggested that tautomeric form I-amino is prefered for series of molecules related structurally to capecitabine and posessing the central moiety reminding 5-fluorocytosine. However, if the intramolecular and intermolecular hydrogen bond can be formed (due to conformational changes or interactions with neighbouring molecules) tautomeric form II-imino can be prefered.

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The computational part was performed at the ICM Warsaw University computer center within the G18-6 grant.

17:15 Poster 55

Histone H3 expression – a potential cell proliferation marker for molecular pharmacology

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Polymeric biomaterials originally designed for regenerative medicine are increasingly used in pharmacy *eg.* in preclinical trials or in drug delivery systems. In the case of preclinical studies, the use of

polymeric scaffolds may reduce the need for animal testing and the risk of early treatment failure *in vivo*. These improvements are the results of restoration of naturally occurring cell interactions. Previously used 2D cultures allowed the cell attachment to polystyrene surfaces but didn't allow the formation of spatial interactions between cells. Scaffolds, made of polymeric biomaterials, ensure proper both cell-cell and cell-matrix interactions that mimic *in vivo* conditions. To ensure the optimal growth of cells, scaffolds are usually placed in suitable bioreactors which provide the proper flow of oxygen, nutrients and waste materials. These 3D cultures could be useful in drug discovery and enable the evaluation of the drug efficacy and cytotoxicity which among others corresponds with proliferative activity of cells. The reliable assessment of these properties is necessary in order to detect unsafe drugs and choose the best therapeutic candidate for clinical trials. The majority of manufactured scaffolds are characterized by structural heterogeneity. This property limits the ability to assess the proliferative status of cells using standard assays based on *eg.* dye detection. Due to these limitations, novel cell proliferation marker for molecular pharmacology is needed. The aim of the study was to evaluate the usefulness of replication-dependent subtype of histone H3 (H3.b) as a novel molecular marker of proliferation. The expression of replication-dependent subtypes of histone H3 is tightly correlated with cell cycle status. The experiment was carried out using normal human articular chondrocytes from knee – NHAC-kn. The results of the expression of histone H3.b (real time RT-PCR) were compared to results of the XTT and resazurin reduction. The obtained results were promising and indicated that H3.b may be a potential molecular marker of cell proliferation.

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17:15 Poster 56

Degradation process of L-lactide/glycolide/trimethylene carbonate terpolymer in cell culture environment

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Degradable polymeric carriers for controlled release of drugs and other bioactive substances (growth factors, hormones) are of growing importance in regenerative medicine and tissue engineering [1]. Terpolymers containing glycolidyl, lactidyl and carbonate units have a great potential for applications in these fields, as their properties (degradation rate, mechanical features) can be tailored by changes in the terpolymer composition as well as changes in the chain microstructure [2]. Kinetics of polymer degradation is usually studied in vitro by incubation of polymeric specimens in a buffered medium (e. g. phosphate buffered saline - PBS). However, in cell culture environment some factors augmenting polymer degradation/erosion (enzymes, free radicals) can be present. They can derive from cultured cells or bovine serum – a typical component of the cell culture medium. The aim of the study was to investigate the degradation process of a novel terpolymer of L-lactide, glycolide and trimethylene carbonate, incubated in cell culture with human fibroblasts (11Lu cell line). Polymeric specimens were incubated for 30 and 90 days. Characteristics of the terpolymer were studied by means of ^{1}H and ^{13}C NMR spectroscopy, gel chromatography and differential scanning calorimetry. During the first 30 days, a considerable decrease in molecular weight (Mn) was observed without any change in the weight of polymeric specimens. After 90 days, Mn continued to decrease and some loss of the specimens weight was noticed. The content of glycolidyl, lactidyl and TMC units remained relatively stable through the whole degradation period. In conclusion, degradation of the studied terpolymer proceeded regularly, therefore it can be appropriate for various medical applications.

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17:15 Poster 57

The transcripional activity of genes encoding MMPs and TIMPs in breast cancer cells treated by genistein and in normal cancer-associated fibroblasts - in vitro studies

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Breast cancer is one of the most common cancers among women. Thus, the scientists are still looking for the new efficient therapeutic methods of treatment.

Phytoestrogens are currently in the center of attention because of their therapeutic and preventive proprieties. Genistein is a naturally occurring plant compound from the group of phytoestrogens which possess many therapeutic activities. Its anticancer effects occur at multiple levels, including cell cycle regulation, induction of programmed cell death and inhibition of angiogenesis and metastasis.

The aim of this study was to examine the impact of genistein on genes involved in MMPs and TIMPs synthesis in breast cancer cells and normal fibroblasts stimulated by medium derived from T-47 D cultures. We used slightly invasive hormone-dependent T-47D (ATCC) breast cancer cells, subtype Luminal A and normal fibro-

blasts HFF-1 (ATCC) isolated from skin. Quantitation of the genes encoding MMP-1, -2, -3 -9, -13, -14, -15 and tissue inhibitors of metalloproteinase : TIMP-1, TIMP-2, TIMP-3 was done using RT-QPCR.

We demonstrated that genistein has the influence on the expression of MMP-1, -3, -14, -13 -15 and TIMP-1 in T47D and HFF-1 cells in the way that implies its capacity to inhibit the process of angiogenesis and metastasis. Though this effect *in vitro* is not unequivocal, we presume it can be helpful in clinical practice to decrease the risk of angiogenesis and increase survial of patients with breast cancer.

17:15 Poster 58

Molecular effects of newly synthesized derivatives of phenothiazine in C-32 and MDA-MD-231 cancer cells

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Cancer and cardiovascular diseases are recently the most major health problems in the society of industrialized countries. As a result of increase of average lifetime, pollution of environment, inappropriate diet and lifestyle, the frequency of incidences of these diseases is still increasing. The difficulties with early diagnosis and the lack of efficient therapy leads to increased mortality. Clinitians and scientists alike are constantly looking for new therapy approaches, new molecules and new delivery vehicles of already approved drugs to improve the efficiency of current therapeutic tools in malignant tumors.

Phenothiazine and its derivatives are wildely used as antipsychotics. It is well known that they have antiprion, antiviral, antibacterial and antiprotozoan properties. Futhermore, their ability to inhibit proliferation and to induce apoptosis in tumor cells implies their potential utilization in cancer treatment.

The aim of this study was to define the influence of derivatives of phenothiazine on cancer cells.

Two cells lines purchased from American Type Culture Collection (ATCC) were used: amelanotic melanoma C-32 and breast cancer MDA-MB-231 cells. We analysed the impact of two newly sythethized derivatives of phenothiazine prepared in Department of Organic Chemistry of School of Pharmacy with the Division of Laboratory Medicine in Sosnowiec which were considered the anticancer agents. To mesure their cytotoxic and antiproliferative effects we performed assays on cell cultures treated by examinated molecules and on cells after incubation with approved cytostatic drugs: cisplatine and doxorubicine (reference semples). We analyse the expression of genes involved in regulation of cell cycle (*TP53, CDKN1A)* and genes controlling intrinsic pathway of apoptosis (*BAX BCL-2*). The influence of these derivates on activity of antioxydative enzymes were studied as well.

We demonstrated that the examined molecules have the cytotoxic and antiproliferative properties on two tested cells lines. The incubation of these cells with derivatives of phenothiazine at the concentration of 0,5 ug/ml leads to change in amount of mRNA encoding regulators of cell cycle: *TP53, CDKN1A*, genes involved in mitochondrial pathway of apoptosis: *BAX i BCL-2* and genes encoding enzymes SOD, CAT i GPX. Increased *BAX/BCL-2* ratio in MDA-MB-231 treated with studied drugs and cisplatin suggests that these substances act via intrinsic pathway of apoptosis.

17:15 Poster 59

Folate targeted vitamin C based liposomal formulation of Epirubicin – novel nano drug delivery system, which ensures fast drug release

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Liposomes are one of the best drug delivery systems. They can favourably alter the pharmacokinetics and pharmacodynamics of the encapsulated drugs. There are many factors like liposome composition, drug-bilayer interactions and encapsulation method which have a direct influence on drug leakage from liposomes and indirect on antitumour activity of the drug [1].

In this study we decided to prepare liposomal formulations of epirubicin (EPI) loaded in the lipid vesicles via ascorbic acid and ammonium ascorbate gradients [2]. We investigated physical state of the drug inside the vesicles using circular dichroism and Cryo-TEM microsopy and correlate it with in vitro cytotoxic activity of the prepared liposomal formulations of EPI on l breast cancer cell lines. We also show that developed in our laboratory gradient based on vitamin C protect healthy cells against Epirubicin cytotoxicity. To increase anticancer activity of developed liposomal forlmulation we used folic acid to active targeting of liposomes. The folate receptors over-expressed in many tumours are being intensively studied as a target to enhance the selectivity of drug delivery to cancer cells [3]. In vitro activity of the liposomal formulation was studied on 4T1 murine mammary cancer cells, which are highly physiological mouse model that closely resembles breast cancer in human. Prepared liposomal formulation showed great anticancer activity because of the active targeting and physical state of the drug inside the vesicles which allows to fast drug release from the liposomes and kill cancer cells.

The research was supported by Wroclaw Research Centre EIT+ under the project „Biotechnologies and advanced medicaltechnologies ”– BioMed (POIG.01.01.02-02-003/08) financed from the European Regional Development Fund (Operational Programme Innovative Economy, 1.1.2).

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17:15 Poster 60

New analog of sulforaphane: 2-oxoheptyl isothiocyanate as a compound with selective effect on the normal and colon cancer cells.

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According to epidemiological data, diet rich in isothiocyanates reduces the risk of cancers, including colorectal cancer [1]. Isothiocyanates reinforce endogenous cellular mechanisms that protect normal cells against endogenous and exogenous carcinogenic factors. It takes place among others by induction of multidrug resistance-associated protein (MRP). Simultaneously, MRP's induction in cancer cells contributes to the multidrug resistance (MDR) phenomenon [2,3]. On the other hand, isothiocyanates exhibit cytotoxic effects on tumor cells [4].
The aim of this study was to find a compound characterized by selective action - greater cytotoxicity against tumor cells and concurrent least influence on the viability of normal cells. Because the cytotoxic effect of anticancer drugs decreases due to the MDR, for this reason we are looking for compound that will selectively induce total MRP protein activity in normal cells, and inhibit it in the tumor cells. Such double action could contribute to greater sensitization of tumor cells to anti-cancer drugs and ensure proper protection of cells against toxic effects of these drugs.
Isothiocyanates with sulfinyl group (sulforaphane and alyssin) and isothiocyanates with acetyl group (2-oxohexyl isothiocyanate, 2-oxoheptyl isothiocyanate) were objects of this study. It has been shown that changes in the structure of isothiocyanates strongly influence their biological potency [5].
In order to investigate selective action of isothiocyanates we determined their effect on the survival and transport activity of total MRP protein in CRL1790 normal and HT29 colon cancer cells.
Cytotoxicity of isothiocyanates was examined with MTT assay. SigmaPlot program was used to determine IC50 parameter. IC50 allows for comparison of potency of tested compounds. Selectivity index (SI , SI = IC50 normal cells/IC50 colon cells) was defined to determine selectivity of the compounds. SI > 3 shows the high selectivity of tested compound between normal and tumor cells. Total MRP transport activity test was performed using calcein assay (Calcein AM).
It was found that isothiocyanates with acetyl group acted more cytotoxically against both cell lines than isothiocyanates with sulfinyl group. Isothiocyanates with acetyl group had higher selectivity than sulforaphane and alyssin. It was found that sulforaphane was least selective (SI=2,1). In contrast, 2-oxoheptyl isothiocyanate had the highest selectivity (SI=4.1).It was found that 2-oxoheptyl isothiocyanate induced transport activity of total MRP in CRL1790 normal cells, and had no effect on the activity in HT29 cancer cells.
Studies have shown that 2-oxoheptyl isothiocyanate has high selectivity. It also induces the endogenous protective mechanisms in normal cells. Such action of 2-oxoheptyl isothiocyanate could be used to increase the effectiveness of anticancer drugs and reduce side-effects of therapy.

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17:15 Poster 61

Improved manufacturing process of bosentan monohydrate.

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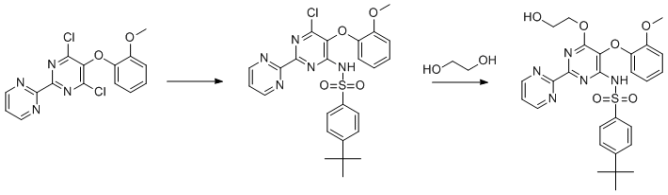
Bosentan is first oral dual endothelin A and B receptor (ETA/ETB) antagonist. The drug is indicated for the treatment of pulmonary arterial hypertension (PAH). In patients with systemic sclerosis and ongoing digital ulcer disease it is used for the reduction of new digital ulcers.

The synthesis of bosentan was carried out according to the scheme shown below, described in the patent EP 0526708. The main problem in this synthesis was obtaining pharmaceutical grade bosentan, due to the presence of three impurities in the final product. All these impurities were very difficult to remove. Improved methods of bosentan synthesis or purification of bosentan are described in many patent applications however, the problem of the obtaining pharmaceutical grade bosentan with a good yield had to be reconsidered.



We have investigated all steps of this process, and we have developed the best conditions in the last key step of the synthesis. The synthesis of bosentan was optimized, all chemical and degradation

impurities were isolated or synthesized and characterized by NMR, MS and HPLC techniques. Moreover, the efficient method of bosentan purification was developed. We obtained bosentan with a proper polymorphic form and particle size favorable for a pharmaceutical formulation. Furthermore, X-ray single-crystal investigation of bosentan unambiguously proved its structure.

These allowed us to develop the large scale efficient synthesis of pharmaceutical grade bosentan containing < 0,1 % of known impurities and < 0,5 % of total impurities.

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17:15	Poster	62

Effect of 5-amino-3-methyl-4-isoxazolecarboxylic acid hydrazide on primary humoral immune response in mice

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5-Amino-3-methyl-4-isoxazolecarboxylic acid hydrazide was used for the synthesis of a series of semicarbazides and thiosemicarbazides with immunotropic activity. In *in vitro* studies the compound showed no cytotoxic activity in murine cell lines and stimulated proliferation of murine lymphocytes isolated from peripheral lymphatic organs and murine peritoneal macrophages stimulated with mitogens [1]. The aim of this study was to determine the influence of 5-amino-3-methyl-4-isoxazolecarboxylic acid hydrazide on primary humoral immune response in sheep red blood cells (SRBC)-immunized mice. All experiments were carried out on Balb/c mice, 7-8 weeks of age, each weighing 18-20 g. The animals were immunized intraperitoneally (i.p.) with 0.2 ml of 10% SRBC suspension. The 5-amino-3-methyl-4-isoxazolecarboxylic acid hydrazide was administered intraperitoneally (*i.p.*) four times at 24 h intervals at three different doses of 10, 1 and 0.1 mg/kg, before or after antigen stimulation. The measurements included number of plaque forming cells (PFC) in spleen and anti-SRBC haemagglutinin titre in the serum. The splenocytes producing hemolytic anti-SRBC antibodies (PFC) was determined by a local hemolysis technique in agar gel as described by Mishell and Dutton [2]. The total and 2-mercaptoethanol-(2-ME) resistant serum agglutination titres were defined by active haemagglutination test carried out on microplates [3]. The 5-amino-3-methyl-4-isoxazolecarboxylic acid hydrazide increased primary humoral immune response in SRBC-immunized mice, which resulted in an elevated number of splenocytes producing hemolytic anti-SRBC antibodies (PFC) and increased total and 2-mercaptoethanol-resistant serum hemagglutinins titers. Those modulating effects were dependent on the dose applied. Hydrazide administered before as well as after stimulation with antigen increased the number of PFC only at the lower doses- 1 and 0.1 mg/kg. On the other hand, four injections of the agent before immunization elevated the 2-ME resistant anti-SRBC antibody titers at all used doses. Total anti-SRBC haemagglutinin titre was increased only by the dose of 10 mg/kg of the hydrazide. Administration of the compound after priming changed only 2-ME resistant anti-SRBC antibody titre which was increased at the dose of 1 and 0.1 mg/kg.

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17:15	Poster	63

Immunomodulatory activity of new isoxazole derivatives

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Searching for derivatives with immunomodulatory properties we synthesized new series: MZ, MZO and MZA isoxazole derivatives. The described derivatives were synthesized by reaction of cyclization of 5-amino-3-methyl-4-isoxazolecarboxylic acid benzylamides with orthoesters or sodium nitrite. The derivatives showed significant immunological activity in several assays in mice and humans blood cells. The compounds were non-toxic and exhibited differential, dose-dependent property to suppress phytohemagglutinin A (PHA) - induced proliferation of human peripheral blood mononuclear cells (PBMC) and a weak ability to suppress lipopolysaccharide (LPS) – induced production of tumor necrosis factor alpha (TNFα) in whole blood cultures. The compounds were virtually devoid of toxicity against PBMC in 24h culture with exception of MZA-1 and MZO-1 which were moderately toxic at 100μg/ml. All compounds showed differential, dose-dependent, suppressive effects on PHA-induced proliferation. Among them MZO-2 was most active (statistically significant suppression already at 1μg/ml). The effects of the compounds on LPS-induced TNFα production were weak. The best inhibitory action was observed in the case of MZA-1 (25ug/ml). MZO-2, which demonstrated strong antiproliferative action, did not inhibit TNFα production. The differences in the observed immunosuppressive properties of the studied derivatives of isoxazole are a good reason for the theoretical investigations. The performed *ab initio* calculations provided useful information on the electron charge distribution in described molecules. The isoxazole

ring is common part of all studied compound and can be considered as the reference molecular subunit. The charge distribution of the isoxazole ring should be related with the electronic structure of whole molecule.

The study was supported by grant of Polish National Science Centre nr N N405 682840.

17:15 Poster 64

Hplc study of Cilostazol tablets: assay and release profile determination

Marta Majewska, Edyta Pesta, Michał Tynderynda, Katarzyna Badowska-Rosłonek, Magdalena Kossykowska, Anna Ostaszewska

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The aim of this work was to develop analytical methods for studying cilostazol tablets. Cilostazol is an antithrombotic drug, used to extend painless walking in intermittent claudication.Medium recommended for *FDA* didn't produce good results for the release profile Cilostazol tablets *[1]*. A method for the release profile determination was developed using 0.5% sodium dodecylsulphate in water as the medium (900 mL volumes, rotations at 50/min). The active substance was released by the spatula method. The obtained release profiles were similar to the profiles of the reference product (Pletal 50 mg and Pletal 100 mg purchased from Brecon Pharmaceuticals Ltd.). Over 80% of the active substance was released after 15 min in both 50 mg and 100 mg tablets obtained in the Pharmaceutical Research Institute. A repeatable HPLC method for the API assay in tablets was developed. The method is characterized by short analysis time, about 9 minutes. For the purity study, a method was developed which was characterized by good HPLC parameters: high separation, favorable peak symmetry, numerous theoretical plates. The developed methods are in accordance with the Pharmacopea's *[3]* requirements regarding HPLC methods.

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17:15 Poster 65

Glutathione depletion and reactive oxygen species generation by thiosemicarbazones in cancer therapy.

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Thiosemicarbazones are important compounds in biological application. Their broad spectrum of biological properties includes antiviral, antifungal and antiproliferative activity. Due to the ability to chelate heavy metals, these compounds have been used extensively in cancer research to study the effects of their inhibition of cell growth. Their mechanism of action includes generation of reactive oxygen within cancer cells, iron chelation, inhibition of the enzyme ribonucleotide reductase (RR) necessary in the synthesis of DNA [1]. Iron is essential for cancer cell proliferation and can also participate in the Fenton reaction to generate reactive oxygen species (ROS).

The generation of reactive oxygen species (ROS) by drugs is associated with loss of intracellular redox potential. Cellular redox potential is largely determined by reduced glutathione (GSH). GSH is important for many cellular biochemical functions including the regulation of gene transcription, as well as the modulation of apoptosis [2]. Reduced GSH is the biological active form that is oxidized to glutathione disulfide (GSSG) during oxidative stress. Changes of the ratio of GSH to GSSG determines the cells fate. Decreases in cellular GSH levels leads to initiation of the Fas receptor activation or mitochondrial apoptosis pathway [3].

Obtained series of novel derivatives thiosemicarbazones, showing the highest antiproliferative activity against human colon carcinoma (HCT116 +/+) have been tested for effects on mitochondrial GSH. The results demonstrated the significant reduction in the cellular levels of glutathione, which is expected effect of cancer therapy.

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17:15 Poster 66

The interaction of new Piroxicam analogues with lipid bilayers – a calorimetric and fluorescence spectroscopic study

Jadwiga Maniewska[1], Berenika Szczęśniak-Sięga[1], Andrzej Poła[2], Kamila Środa-Pomianek[2], Wiesław Malinka[1], Krystyna Michalak[2]

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Piroxicam, from the group of the oxicams, is mainly known as a non-steroidal anti-inflammatory drug (NSAID), used in the treatment of chronic rheumatic diseases. The molecular target of NSAIDs is cyclooxygenase (COX). There are three isoforms of the enzyme (COX-1, COX-2 and COX-3).

Most solid tumors express the cyclooxygenase-2 (COX-2) protein, which is a target of NSAIDs, and that is why those drugs are evaluated as anti-cancer. They inhibit proliferation, invasiveness of tumors, and angiogenesis and overcome apoptosis resistance [1]. NSAIDs particulary decrease the incidence of and mortality from colon cancer, and therefore those drugs have been a major advance in chemoprevention [2].

Lenard Lichtenberger *et al* think that one of the alternative mechanisms by which NSAIDs can be effective is by interacting with cellular membranes and altering their biophysical properties. Those drugs can induce changes in the fluidity, permeability and biomechanical properties of cell membranes [3].

To optimize and modulate the biological effects of piroxicam, some efforts are made to synthesize the derivatives of this oxicam. In our present work we studied the influence of two new piroxicam analogues PR17 and PR18, synthesized in our department, on lipid bilayers.

We studied the thermal effects of these compounds in dipalmitoylphosphatidylcholine (DPPC) membrane bilayers. The addition of PR17 and PR18 to DPPC resulted in a decrease in the lipid main transition temperature and the broadening of the transition peaks.

In spectroscopic experiments we assessed the influence of the oxicams under consideration on Laurdan and Prodan (two fluorescent probes localized in different membrane segments) fluorescence in liposomes made of lecithin from egg yolk (EYPC). PR17 and PR18 quenched the fluorescence of both Laurdan and Prodan.

The results presented allow the conclusion that the studied oxicams interact with the studied lipid bilayers and may penetrate the membranes.

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17:15 Poster 67

Effect of the new derivatives of oxicams on efflux pumps overexpressed in resistant human colorectal adenocarcinoma cell line

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Oxicams belong to a class of non-steroidal anti-inflammatory drugs (NSAIDs). Antitumor potential of commonly used non-steroidal anti-inflammatory drugs is currently often reported in literature. NSAIDs are inhibitors of cyclooxygenase-2 (COX-2), the enzyme expressed in the most of solid tumors. COX-2 is also involved in multidrug resistance - the expression of COX-2 is well correlated with overexpression of multidrug resistance transporter MDR1/P-gp.It has been suggested that COX-2 inhibitors can sensitize cancer cells to anticancer drugs by inhibition of MDR transporters, such as P-gp, MRP1 and BCRP.

The aim of our studies was to obtain two new piroxicam derivatives: PR17 and PR18 possessing anticancer and/or multidrug resistance reversing activity. Compounds should inhibit both COX-2 enzyme and MDR transporters' activity. Human colorectal adenocarcinoma cell line LoVo and its doxorubicin-resistant subline Lovo/Dx were chosen to study multidrug reversal activity of newly synthetized oxicams. Expression of MDR transporters in LoVo and LoVo/Dx human colon adenocarcinoma cell lines was studied. The effect of new oxicams on expression of mRNA of P-gp, MRP1 and BCRP was determined by RT-PCR. The effect of the compounds on protein expression was checked by Western-Blot. The influence of oxicams on transport activity of P-gp was studied by flow cytometry using Rhodamine 123 as fluorescent substrate for P-gp. Compounds cytotoxicity and cytotoxicity of doxorubicin in the presence of oxicams were determined by SRB assay using microplate reader.

Molecular modeling (geometry optimization in vacuum, ab initio HF) was applied to describe electronic and structural parameters andhydrophobicity of the oxicam derivatives, andthese features were compared with ability of the compoundsto reverse MDR phenotype and with their cytotoxicity in adenocarcinoma cells.

Two new synthesized compounds from oxicam group of drugs,

PR17 and PR18 are able to modulate MDR phenotype in human adenocarcinoma cells. Both compounds, PR17 and PR18 restore accumulation of fluorescent P-gp substrate in resistant adenocarcinoma cells and they reduce Rh123 efflux much more efficiently than verapamil, well known P-gp inhibitor.

17:15	Poster	68

Simultaneous CE determination of counterion and possible impurity from synthetic route in pharmaceutical substance prasugrel hydrochloride.

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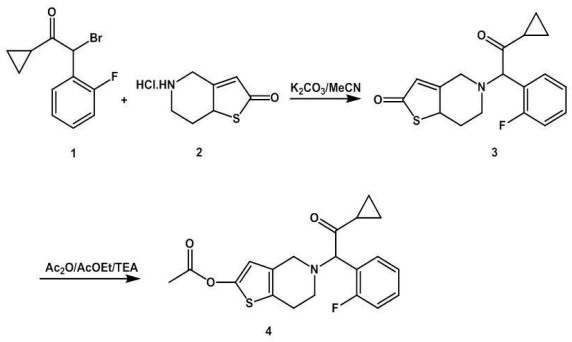
The determination of small organic and inorganic ions is an important part of pharmaceutical analysis. Many drug molecules are charge and are manufactured with counterion. Hence, it is important to analytically characterize the drug stoichiometry to ensure that the potency of the batch of drug substance is known. On the other hand small organic and inorganic ions could be a contaminant impurities coming from the synthetic route and the task of impurity determination in drug is of principle importance.

Prasugrel is the most recent member of the thienopyridine class of antiplatelet agents[1]. Similarly to other thienopyridines, it is commonly used in a therapy of choice for patients with acute coronary syndrome (ACS) undergoing percutaneous coronary intervention (PCI) with stent implantation[2,3]. To date, the registered medication brands with prasugrel contain the active substance (API) in the form of hydrochloride salt. The last steps in our synthetic procedure comprise the *O*-acetylation of 5-(2-cyclopropyl-1-(2-fluorophenyl)-2-oxoethyl)-5,6,7,7a-tetrahydro thieno[3,2-c]pyridin-2(4H)-one (**3**) to the compound **4**, in basic reaction conditions and a presence of acetic anhydride, then the formation of prasugrel salt. During coupling reaction workup the acetic anhydride may convert to acetic acid or its salts followed the acetic acid formation as an impurity in final procedure for prasugrel hydrochloride obtaining.



The determination of small organic and inorganic ions in pharmaceutical analysis include mainly ion chromatography (IC), flame atomic absorption spectrometry and flask-based methods e.g. titration which are expensive, time, reagent and cost consuming. Because such ions usually have little or no chromophore can be analyzed by indirect UV detection. This detection mode could be performed using capillary electrophoresis technique (CE). During the past decade many original and review papers as well as monographs mention about the couterions or impurities determination in drugs. But there was not found the work described validated method for simultaneous conterion and impurity ion determination in pharmaceutical substance. This work present the potential advantages and validation of CE method for the simultaneous determination of counterion and possible impurity from synthetic route in prasugrel hydrochloride.

Acknowledgements:

Research project has been supported by European Union (under European Regional Development Fund) UDA-POiG.01.03.01-14-062/09-00 **"Innovative technologies of cardiovascular medicines of special therapeutic and social importance"**

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17:15 Poster 69

Synthesis of 2-(2,4-dihydroxyphenyl)thieno[1,3]thiazin-4-ones and their antiproliferative activity against cancer cells

Joanna Matysiak[1], Małgorzata Juszczak[2], Monika M. Karpińska[3], Ewa Langner[2,4], Katarzyna Walczak[4], Marta Lemieszek[2], Wojciech Rzeski[2,5], Andrzej Niewiadomy[1,3]

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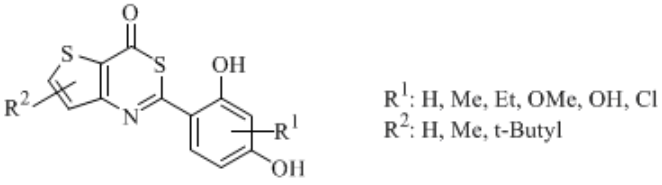
Heterocyclic derivatives with 2,4-dihydroxyphenyl moiety are compounds of documented *in vitro* and *in vivo* anticancer activity [1]. We reported the synthesis and antiproliferative activity of 1,3,4-thiadiazoles, 4*H*-3,1-benzothiazines and benzimidazoles [2]. Taking into account their valuable biological properties we developed an efficient synthesis of novel differently substituted thieno[1,3]thiazin-4-ones. We obtained a series of compounds modified at positions 3 and 5 of a resorcinol moiety to find the most active structures.

The compounds were prepared by the reaction of aryl-modified sulfinylbis[(2,4-dihydroxyphenyl)methanethione]s with a corresponding aminothiophenecarboxamides. Their structures were identified using elemental, IR, ^{1}H NMR, ^{13}C NMR and mass spectra analyses. The developed method offers short reaction times, relatively large-scale synthesis, easy and quick isolation of the products, and good yields.



Figure

Compounds in Figure were evaluated for their antiproliferative activity against human non-small cells lung cancer (A549), human colon adenocarcinoma (HT-29) and rat glioma (C6) cells. Cisplatin was used as a reference drug. For comparison, cytotoxicity of compounds was determined in normal human skin fibroblast (HSF) primary culture with use LDH method.

Proliferation of all tested cancer cells was decreased in the cultures exposed to a compounds in a concentration-dependent fashion as measured by means of MTT assay. The activities of compounds were varied and evidently depended on the type of substitution on both rings.

This study was supported by the National Science Centre (Poland), decision No. DEC-2011/01/B/NZ4/05005.

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17:15 Poster 70

Synthesis and biological activity of novel N,N-cyclic-2,4-dihydroxythiobenzamide derivatives

Joanna Matysiak[1], Alicja Skrzypek[1], Urszula Głaszcz[1], Joanna Wietrzyk[2], Elżbieta Krajewska-Kułak[3], Andrzej Niewiadomy[1]

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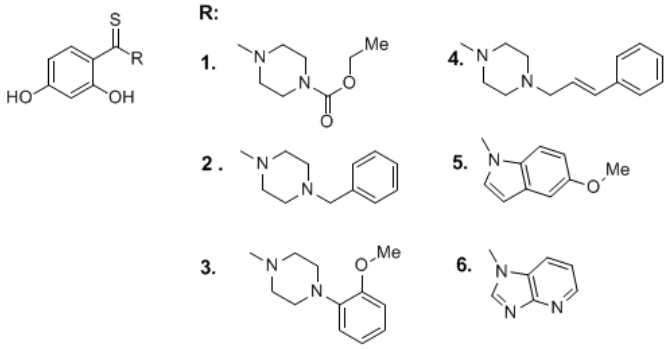
Benzanilide (N-phenylbenzamide) derivatives possess a broad spectrum of biological activities. They have been found to exhibit antimalarial, antibacterial and antifungal properties [1-3]. Derivatives of N-(2-hydroxyphenyl)benzamide have been studied for the last few years as the possible metabolites of the antibacterial active benzo[*d*]oxazole derivatives [4]. Some benzanilide derivatives have been reported to inhibit the c-Met tyrosine kinase receptor, which is a potentially important target for the treatment of cancer [5].

The aim of presented work was the synthesis of new N,N-cyclic-2,4-dihydroxythiobenzamide derivatives with potential antifungal and antiproliferative activity against human cancer cells. The synthesis of the desired compounds were achieved by reacting of the sulfinylbis((2,4-dihydroxyphenyl)methanethione (STB) with secondary amines. Purity of compounds was monitored by the reversed-phase (RP-18) HPLC chromatography (MeOH – water). The structure of compounds was confirmed by IR, ^{1}H NMR, ^{13}C NMR spectroscopy and mass spectrometry.



The tested compounds inhibited proliferation of tumor cells derived from human bladder cancer line (HCV29T). Cisplatin was used as a reference drug. The cytotoxic activity *in vitro* was expressed as IC_{50} and was at the level of 10.51 – 33.98 $\mu g \cdot ml^{-1}$. Antifungal properties were evaluated against different strains of *Candida*.

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Czas wolny
Monday evening, 12 May, 18:45

Uroczysta Kolacja
Monday evening, 12 May, 20:00

-
Monday night, 12 May, 23:00

Tuesday, 13 May

Śniadanie
Tuesday morning, 13 May, 7:00

Sesja wykładowa IV
Tuesday morning, 13 May, 8:30
Sala wykładowa
Chair: M.Koziołkiewicz, J.Z.Nowak

8:30 Invited oral

Synthesis of Indoles via Nucleophilic Substitution of Hydrogen in Nitroarenes

Mieczyslaw Mąkosza

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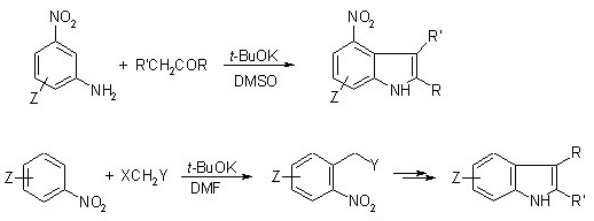
Nucleophilic substitution of hydrogen in nitroarenes, S_NArH, proceeds via fast addition of nucleophiles at positions occupied by hydrogen to form σ^H adducts that are subsequently converted into products of substitution of hydrogen on a few ways. It should be stressed that S_NArH proceeds much faster than conventional nucleophilic substitution of halogens, S_NAr.

Facile introduction a variety of functionalized carbon substituents into nitroaromatic rings via S_NArH with carbanions opens wide possibilities for synthesis of indoles as exemplified in scheme



In the lecture will be presented basic variants of nucleophilic substitution of hydrogen, main methods of synthesis of indolesvia S_NArH and examples of application of these methods.

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9:15 Invited oral

New tetrapyrrole derivatives as NO-independent sGC activators

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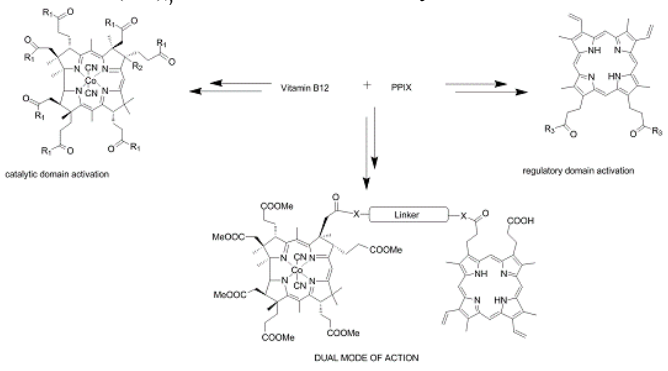
Over the past decade there has been much interest in NO-independent soluble guanyl cyclase (sGC) activators. sGC operates via activation of nitric oxide (NO) and plays an important role in cardiovascular homeostasis, platelet function, angiogenesis and neurotransmission.[1] Currently, sGC regulation is achieved by using various NO releasing organic nitrates or nitrovasodilators such as nitroglycerine, trinitrate, isosobide dinitrate and isosobide-5- mononitrate. NO-activators although effective, are known to rapidly induce tolerance and have a limited mode of delivery. However, it has been discovered that natural tetrapyrroles, such as protophorphyrin IX (PPIX) and vitamin B_{12} derived cobainamide ($(CN)_2$Cbi), can act as NO-independent sGC acitivators.[2-4] Moreover, modes of activation differ for each compound; PPIX activates through the regulatory domain while $(CN)_2$Cbi activates via the catalytic domain.[4]



Hence our goal was to synthesise novel hydrophobic and hydrophilic cobinamide derivatives via aminolysis of cobalamin and examine their potential as sGC activators. Moreover, since both PPIX and vitamin B_{12} derivatives can stimulate sGC enzyme via different domain activation. In order to achieve a synergistic effect a series of hybrid molecules were obtained using two synthetic strategies: copper (I) catalyzed alkyne–azide 1,3 dipolar cycloaddition (CuAAC, 'click' reaction) and selective amide formation between vitamin B_{12} and PPIX derivatives.

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10:00 Oral

Efficacy of Viburnum opulus fruit juice and its components against herpesvirus

Bogumiła Litwińska[3], Beata Wileńska[2], Aleksandra Misicka[2], Krzysztof Rozycki[1], Barbara Nowicka[1], Aleksandra M. Szczucińska[1], Andrzej W. Lipkowski[1,4]

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Viburnum opulus that belongs to the *Caprifoliaceae* plant family is widespread in eastern and central Europe. In folk medicine *Viburnum opulus* bark extracts have long tradition of use as antispasmoticum and antihaemorrhagicum, especially in menstrual problems as well as miscarriage. Lipophilic wax-like material components (amyrins) are likely responsible for these properties. Some personal information reported (B. Baranowska, unpublished) effectiveness of *Viburnum opulus* fruit juice in treatment of herpes virus treatments. Pilot, *in vitro* studies, indeed, confirmed low but significant activity. This initiated systemic studies of various juice fractions, with hope to identify active component(s). Application of various methods of extraction combined with size exclusion chromatography allowed to separate fractions containing different components. The anti HSV-1 activities have been evaluated in vitro using CV1 or A459 cells. The cytotoxicity, virucidal activity assay, antiviral activity, the attachment as well as the penetration assays have been applied. Primary hypothesis predicted that particular flavonoid or catechin derivatives are active components. However, searching for particular active compound did not resulted with success. The tests indicated that antiviral activities spread out on various fractions suggesting that for enhanced activity combination of flavonoids is advantegous. In addition, we noticed that isolated pectins express also significant anti HSV activity. The remixing of isolated fractions resulted in a mixture with the highest anti-HSV properties.

In conclusion we may hypothesize that flavonoids, catechins and pectins form natural mixture which in combination provides concerted interactions, resulted in anti HSV activities observed on various stages of viral infections (attachment, penetration and replication).

Acknowledgement:

This work was supported by the Polish National Science Center (NCN), grant N N405 304436

Przerwa kawowa
Tuesday morning, 13 May, 10:20

Sesja wykładowa V
Tuesday morning, 13 May, 10:40
Chair: Z.Chilmonczyk, A.Leś

10:40 Invited oral

On the border line of concerted and non-concerted mechanisms in the [2+3]-cycloaddition reactions

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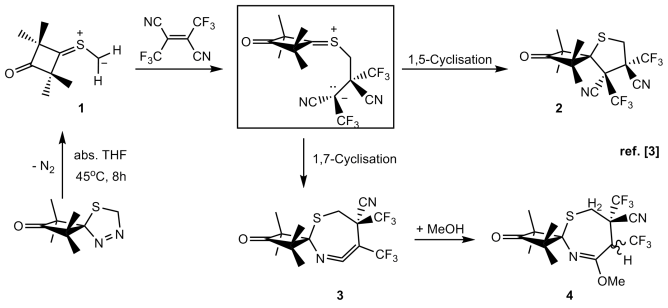
The [2+3]-cycloaddition reactions, known also as 'Huisgen reactions', relate to the interaction of a 1,3-dipole molecule with a dipolarophile and in typical cases they result in the formation of a five-membered cycloadducts. It is well known, that they are isoelectronic with the Diels-Alder reactions ([2+4]-cycloadditions) [1]. Both types of cycloaddition reactions are of fundamental importance for the synthesis of natural products and biologically active compounds, including drugs [2]. According to classical presentations, mechanisms of these reactions are interpreted as concerted processes, which lead to the formation of five-, and six-membered products, respectively, in a stereoselective/stereospecific manner [1].

In recent three decades diverse [2+3]-cycloadditions with relatively less known thiocarbonyl ylides of type **1**, classified as '*S*-centered', electron rich 1,3-dipoles, have extensively been studied. The *in situ* generated 1,3-dipoles of type **1** react smoothly with electron deficient dipolarophiles, e.g. tetracyanoethylene (TCNE), 1,2-bis(trifluoromethyl)-1,2-dicyanoethylene (BTE) or dimethyl dicyanofumarate (DCFM), following the non-concerted (stepwise) mechanism. The zwitterionic intermediate formed therby undergoes competitive 1,5- or 1,7-cyclisation, leading to the expected tetrahydrothiophene derivatives **2** and seven-membered products, like keteneimines of type **3**, respectively (Scheme) [3].



Scheme. The non-concerted pathway for the reaction of sterically crowded thiocarbonyl ylide **1** with 1,2-bis(trifluoromethyl)-1,2-dicyanoethylene.

Another examples of stepwise 1,3-dipolar cycloadditions ([2+3]-cycloadditions) with cyclic nitrones, derived from imidazole [4], as well as with azomethine ylides [5] generated via thermal ring opening of aziridines, will be discussed.

Acknowledgement:

Author acknowledges the National Science Center (PL-Cracow) for a generous support (Grant Maestro-3, Dec-2012/06/A/ST-5/00219).

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11:25 Oral

Separation of octopamine on 2-amino-1-phenylethanol imprinted polymer - theoretical and experimental investigation

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4-(2-Amino-1-hydroxyethyl)phenol (octopamine) is a biogenic amine, gained well importance as neuromodulator. This amine plays a certain role in various human disorders such hepatic encephalopathy, schizophrenia, and renal diseases. Due to its stimulating properties, octopamine has been prohibited in elite sports by the World Anti-Doping Agency (WADA) as a specified substance, and several adverse analytical findings (AAFs) of octopamine in doping control samples have been reported during recent years [1]. The determination of octopamine is very difficult because of its presence in very low concentrations in complex samples (plasma or urine).

Molecular imprinting is a synthetic procedure that become an increasingly important technique for the construction of new polymeric materials to selective adsorption of biogenic amines. During this process cavities which are complementary to the template structure are produced. The molecularly imprinted polymers have a great variety of applications in analytical technology (chromatography, solid phase extraction, sensors), drug delivery systems, and organic synthesis.

In presented experiment, 2-amino-1-phenylethanol was selected as the structural analogue of the target analyte, octopamine in the polymerization process. The strategy that used structural analogues during imprinting process is very useful because it enable to avoid the bleeding of target analyte from the polymer matrix during the analysis, what could be the source of overestimated results [2]. We synthetized ten imprinted polymers using different functional monomers. After preliminary estimation of the affinity factors towards octopamine we have chosen polymer with the highest affinity factor for the further analysis. The main part of our experiments were the selectivity measurements using eight different biogenic compounds: octopamine, synephrine, tyramine, N-methyltyramine, hordenine, serotonine, tryptamine, L-tyrosine.

In the theoretical analysis we have created the model of 2-amino-1-phenylethanol imprinted polymer cavity using DFT, molecular mechanics and molecular dynamics methods. Next we have analyzed binding energies between eight different analytes and the polymer matrix to get insight into the selectivity of obtained polymer matrix.

We have obtained good correlation between theoretical (binding energies) and experimental (affinity factors) values. The model of polymer cavity can be used to evaluate recognition properties of the polymer matrix. The proposed procedure could be a very useful theoretical tool for screening the molecularly imprinted systems in the experiment-free way.

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11:45 Oral

Synthesis, structure and anticancer activity of alkynyl derivatives of betulin

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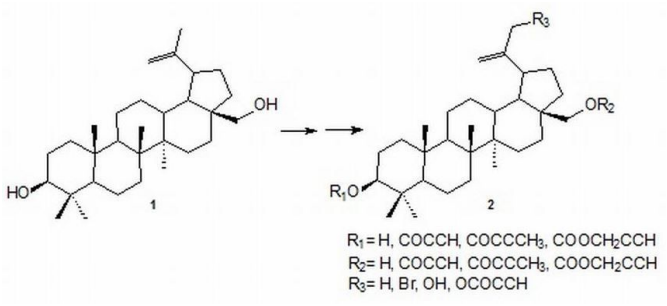
The high content of betulin (up to 30%) in white birch bark and the ease of its isolation in almost any amount, make it important starting material for synthesis of new compounds with various interesting medical properties. Betulin (**1**) has three available sites for simple chemical modification, namely: secondary hydroxyl group at position C-3, primary hydroxyl group at position C-28 and isopropenyl side chain at position C-19 [1,2]. In the last few years a large number of betulin derivatives have been reported to possess anticancer, anti-inflammatory, anti-HIV and anti-leishmanial activity [2,3].

In this paper we present the synthesis of new series of derivatives of betulin containing one, two or three pharmacophores bearing an alkynyl function at the C-3, C-28 and/or C-30 positions. This interest has resulted from the recognition of the value of such compounds in a wide range of biological and chemical synthetic aspects. Synthesis of alkynyl derivatives of betulin (**2**) has been described in scheme presented below.



The structure of obtained compounds were determined on the basis of their spectroscopic and crystallographic data. All compounds were tested for cytotoxic activity against human: breast cancer (T47D), leukemia (CCRF/CEM), colorectal adenocarcinoma (SW707) and murine: leukemia (P388), (Balb3T3) cancer cell lines. The presented studies demonstrates that simple modification of the parent structure of betulin (**1**) can produce new potentially interesting anti-cancer agents. The presence of an alkynyl group offers the possibility for further functionalization of betulins (**2**) and construction of new heterocyclic systems.

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12:05 Oral

Phase Transfer Catalyzed Dialkylation of Sterically Hindered Arylacetonitriles

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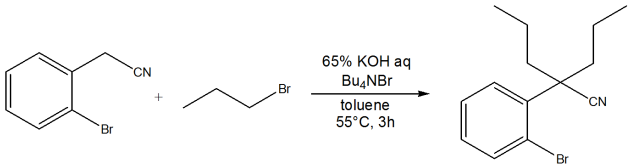
Phase transfer catalysis (PTC) seems to be the most general, efficient and environment friendly methodology of performing reactions in which organic anions (particularly carbanions) react with organic substrates[1]. PT catalyzed reactions are carried out in two phase systems containing two mutually immiscible phases. One phase (inorganic) is a source of base for generation of carbanions. The second (organic phase) contains organic reactants, usually neat or sometimes in appropriate solvent. Upon the introduction of a catalyst - tetraalkylammonium (TAA) salt - continuous transfer of reacting anions produced in the interfacial region, into the organic phase in the form of lipophilic ion pairs with the catalyst cation takes place. All the further reactions occur in the organic phase.

It is well established that the monoalkylation of arylacetonitrile carbanions with primary alkyl bromides carried out in the presence of 50% aqueous sodium hydroxide and TAA catalyst results in high yields. However under these conditions the yields of reactions with *sec*-alkyl bromides seldom exceed 50%, introduction of the second alkyl group to the 2-arylalkanenitriles proceeds with difficulty, yields of 1-aryl-1-cyanocyclobutanes in reactions with 1,3-dibromopropane are low and finally cyclopropanation of phenylacetonitrile with 1,2-dichloro- or 1,2-dibromoethanes does not proceed - dehydrohalogenation of the dihaloalkane is the only observed process. Such alkylations are often important steps in the syntheses of biologically active compounds (Verapamil, Sibutramine, Anastrozole etc.).

In all the cases mentioned, a simple replacement of 50% sodium hydroxide solution for 60-75% potassium hydroxide results in a significant increase of the yields and purity of products or allows the reaction to proceed[2,3].

Now we found that sterically hindered arylacetonitriles, for instance 2-bromophenylacetonitrile, can be efficiently dialkylated using alkyl bromide in the presence of TAA salt and concentrated aq. KOH solution. An example is given below.



1, 92%

Such reactions occur very slowly or not at all when carried out in the presence of concentrated NaOH solution. A possible explanation of the facts presented above involves a kinetic effect, since for more concentrated solutions of hydroxides alkylation proceeds much faster and dominates over side reactions.

2-(2-Bromophenyl)-2-propylpentanenitrile (**1**) is an important intermediate in the synthesis of benzocyclobutenones, which in turn are powerful synthetic intermediates in organic synthesis, leading to lactones, lactams, cyclopentanones, benzodiazepines etc. [4].

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12:25 Oral

Neuroprotective effects of biphalin in a mouse model of mild traumatic brain injury (mTBI)

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Traumatic brain injury (TBI) concussion is often a result of traffic accidents, contact sports as well as battlefield or terrorist explosions. TBI is classified based on severity. A mild form of traumatic brain injury (mTBI), often results in the post-concussion syndrome (PCS). Unfortunately, PCS is usually underestimated, because the immediate physical symptoms decrease rapidly and conventional neuroimaging studies of the brains of most mTBI victims often do not express any radiologicalevidence of brain lesions. However, cognitive impairments persist for weeks, months or even years after the incident. A mouse weight drop model mirrors well the mTBI-induced long-lasting learning and memory impairments observed in humans [1]. Recent results indicate that opioids, especially biphalin show promising anti-neurodegenerative properties [2]. Therefore, we decided to assess if an immediate post-injury injection of biphalin provided any benefits in mTBI behavioral impairments. After a systemic administration of biphalin we observed an improvement in spatial and recognition memory in the Morris Water Maze and Novel Object Recognition tests 7 and 30 days post-trauma. Our new data suggest that opioid receptor activation may provide neuroprotection of post-traumatic neurodegeneration processes. Further investigations will be carried out in the development of optimal post-accidental therapeutic time-window for efficacious treatment of mTBI.

Acknowledgements:

This work was supported by the Polish National Science Center (NCN), grant no. 2011/03/N/NZ4/02021 for Anna Lesniak. The technical support by Zdzislawa Kowalska is highly acknowledged.

References:

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Czas wolny

Tuesday afternoon, 13 May, 12:45

Przerwa obiadowa

Tuesday afternoon, 13 May, 13:30

Sesja wykładowa VI

Tuesday afternoon, 13 May, 14:45
Sala wykładowa
Chair: A.Kutner, K.Koziak

14:45 Invited oral

Anticancer activity of vitamin D, genistein and indoloquinoline analogs

Joanna Wietrzyk

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The natural products with various chemical structures have been identified as anticancer agents. Several potential lead molecules such as vincristine, vinblastine, camptothecin, taxol etc. have been isolated from plants and many of them have been modified to obtain compounds with optimal pharmacological properties. Among them, successful molecules like irinotecan, topotecan, teniposide, taxotere, etoposide, etc. have appeared as drugs after modification of natural leads. Therefore the studies concentrated on natural products from various sources are still intensive. Our studies were focused on two groups of plant derived compounds as well as on vitamin D derivatives. The first group represents isoflavones, such as genistein, which are phytochemicals found along with soy protein. They are supposed to be responsible for the low incidence of prostate, mammary gland and colon cancer in Asian countries. Another one plant derived phytochemical is configuration of indolo[2,3-b]quinoline which exist in nature as indole alkaloid – neocryptolepine. It was discovered in extract from African plant *Cryptolepis sanguinolenta*. This alkaloid possess, among others, cytotoxic and antimalaric activity. Synthetic analog of neocryptolepine - 5,11-dimetylo-5H-indolo[2,3-b]quinoline (DIMIQ)) is also structurally similar to elipticine – plant alkaloid with cytotoxic activity. On the other hand, serum active form of vitamin D_3, 1,25-dihydroxyvitamin D_3 (calcitriol) level is found to be inversely related to incidence of some cancers. Calcitriol levels are determined by skin exposure to ultraviolet light and, to a minor extent, nutritional uptake and subsequent conversion of the precursor vitamin D to the active hormone by the cytochrome P450 hydroxylases.

In our studies we evaluated the anticancer effect of series of analogs of these three natural compounds alone and in combined therapy with known anticancer agents in several mouse and human tumour models. In the course of these studies we selected lead compounds especially from the group of vitamin D analogs, which represents agents showing better anticancer activity with lowered toxicity in comparison to calcitriol. We also conducted the studies on its mechanisms of action.

15:30 Invited oral

What modern crystallography can add to pharmaceutical research?

Krzysztof Woźniak[1,2], Maura D. Malińska[1,2], Paulina M. Dominiak[1,2], Andrzej Kutner[3]

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More than centuary ago, Max von Laue, William Henry Bragg and William Lawrence Bragg opened the field of structural applications of X-ray diffraction. Since then, the X-ray diffraction methods have been used to establish crystal and molecular structure of more than milion compounds - among them many of pharmaceutical importance. However, modern crystallography can go far beyond routine crystal structure analysis.

In my contribution, I will discuss pros and cons of classical diffraction methods. Then will present the main ideas of modern methods based on quantitative studies of electron density in crystals. Such methods were introduced by Prof. Philip Coppens (SUNY, Buffalo, USA), who has started a new field of crystallographic and physicochemical research. Quantitave studies of electron density allow for far more detailed characterisation of properties of pharmaceutical substances in the solid state than any other classical X-ray diffraction techniques. My contribution will also cover quantitative relationships between atoms interactiong in crystals, and the role of factors influencing the quality of such relations such as: treatment of h-atoms, assumptions applied during refinement process and importance of neutron data both for charge densities and also for routine structural refinements. I will present relevant examples of such studies.

I will also discuss the idea of pseudoatom databanks - now being intensively developed in our laboratory by my co-workers - containing the smallest transferable fragments of electron density, which can be used to reconstruct electron density in small molecules and proteins. This allows to obtain energy of electrostatic interactions between drug molecules and proteins. In most pharmaceutical problems, these are electrostatic interactions which play the crucial role. Thus the methods based on reconstruction of electron density allow for optimisation of drug molecule properties and design of better drugs.

16:15 Oral

Characterization of protein structural changes: an efficient modeling approach.

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Proteins exist in solution as ensembles of structurally different conformations. These ensembles can exhibit different degrees of structural diversity, ranging from almost static to highly heterogeneous. Structural flexibility allows proteins to play important functions in living cells. Therefore, characterization of protein structural changes can provide important insights into cell mechanisms and new therapies.

Recently, we developed an efficient modeling method for the characterization of flexibility of globular proteins. The method have been made available as CABS-flex (http://biocomp.chem.uw.edu.pl/CABSflex) server [1]. The CABS-flex was shown to be a computationally efficient alternative to all-atom molecular dynamics - a classical simulation approach [2]. Moreover, we demonstrated that the relative fluctuations of protein residues obtained from CABS-flex are well correlated to those of NMR ensembles [3]. Based on a similar modeling approach, we have also developed a method for the modeling of highly dynamic protein complexes. This method allowed us to characterize the mechanism of coupled folding and binding of an intrinsically disordered protein [4]. The obtained ensemble of highly heterogeneous complexes agreed well with experimental data.

References:

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16:35 Oral

Interaction of star-shaped copolymers with fluorescein isothiocyanate and their use as a drug carriers: thermodynamic studies

Katarzyna Bernaczek, Anna Mielańczyk, Zbigniew Grzywna, Dorota Neugebauer

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Nowadays, new drugs, with increasing therapeutic efficacy, are synthesized. It often happens that the drug has a high activity at the target, but has a poor solubility in aqueous solutions or damages healthy cells. The solution to these problems is using properly selected carrier of the active substance. It was shown that star copolymers can be excellent drug carriers through their unique solution behavior and enhanced cell uptake. Copolymers having in its structure amine groups were bind to fluorescein isothiocyanate. Isothermal titration calorimetry technique was used to provide a full thermodynamic data for description of these reactions. Collected thermodynamic parameters were compared, which resulted on selecting the best drug carrier.

16:55 Oral

Bioresorbable copolymer of L-lactide and ε-caprolactone for controlled paclitaxel delivery

Monika A. Musiał-Kulik[1], Katarzyna Gębarowska[1], Janusz Kasperczyk[1,2], Małgorzata Pastusiak[1], Henryk Janeczek[1], Piotr Dobrzyński[1,3]

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Bioresorbable, aliphatic polyesters are known in medicine where serve as orthopedic devices (e.g. rods, pins and screws) or sutures and staples in wound closure. Moreover, such materials are extensively studied as scaffolds – three-dimensional structures for tissue engineering but also drug delivery systems (DDS) [1].

The aim of this study was to determine release profile of paclitaxel, one of the anti-inflammatory, antiproliferative and anti-restenotic agent [2], from biocompatible copolymer based on L-lactide and ε-caprolactone that seems to be very attractive especially for minimally invasive surgery due to its potential shape-memory property. The influence of drug on copolymer hydrolytic degradation was also analyzed.

Three types of matrices (3%, 5% of PTX and without drug) were prepared by solvent-casting method and degraded in vitro. The physicochemical changes of copolymer were analyzed by means of nuclear magnetic resonance spectroscopy (NMR), gel permeation chromatography (GPC) and differential scanning calorimetry (DSC). The amount of drug released into media was monitored with the use of high-pressure liquid chromatography (HPLC). Similar drug release profiles were obtained for matrices with paclitaxel. The drug-containing matrices degraded slightly slower than drug free matrices, regardless PTX content.

Results of this work may be helpful in designing new bioresorbable paclitaxel delivery system applied in anti-cancer therapy or drug-eluting stents technology.

Acknowledgement:

This study has been financially supported by a Grant from Polish Ministry of Science and Higher Education (N N405 682 340) and Centre of Polymer and Carbon Materials (Grant for Young Scientists 2013).

References:

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Przerwa kawowa
Tuesday afternoon, 13 May, 17:15

Sesja posterowa II
Tuesday afternoon, 13 May, 17:25

17:25 Poster 71

Preparation and physicochemical properties of crystalline forms and amorphous pemetrexed disodium

Olga M. Michalak, Kamil Jatczak, Anna B. Witkowska, Marta Łaszcz, Wiesław Pucko, Iwona Bujak, Aleksandra Groman

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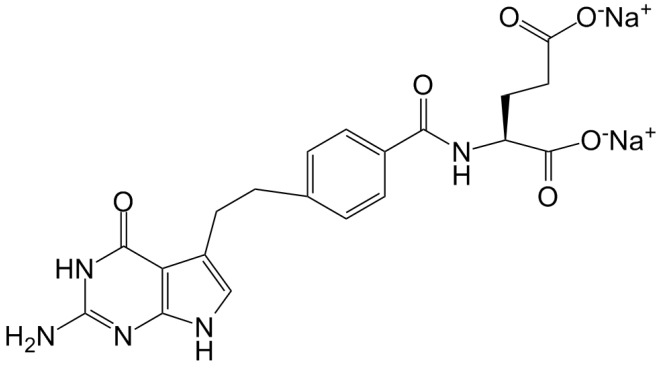
The commercial product pemetrexed disodium, a known anticancer drug, is used for the treatment of locally advanced or metastatic non-small-cell lung cancer (NSCLC).



Two hydrate forms heptahydrate and hemipentahydrate as well as amorphous pemetrexed disodium are currently well established in the literature. The heptahydrate form is used in marketed products.
We have developed processes for preparation of hemipentahydrate form and amorphous pemetrexed disodium. These developed processes are in compliance with the purity requirements for an active substance used in a medicinal product.
Two approaches for the preparation of pemetrexed disodium were used. In the first approach, hydrolysis of diethyl ester in aqueous sodium hydroxide solution led to hemipentahydrate form. In the second approach, deprotonation of pemetrexed diacid in the presence of sodium methoxide in the anhydrous conditions gave amorphous pemetrexed disodium.

The physicochemical characterization of crystalline forms and amorphous pemetrexed disodium as well as starting materials and process-related impurities was accomplished using various analytical methods (IR, NMR, XRPD, DSC, TG).

INNOWACYJNA GOSPODARKA NARODOWA STRATEGIA SPÓJNOŚCI
Instytut Farmaceutyczny
UNIA EUROPEJSKA EUROPEJSKI FUNDUSZ ROZWOJU REGIONALNEGO

17:25 Poster 72

Comparison of combined effects of 5-fluorouracil and allysin after concurrent treatment in colon and breast cancer cell lines

Małgorzata Milczarek[1,2], Katarzyna Wiktorska[2], Katarzyna Lubelska[2], Lidia Śliwka[2,3], Dariusz Matosiuk[1], Zdzislaw Chilmonczyk[2]

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5-Fluorouracil is used to treat many types of cancers, such as breast, ovarian, colon and gastric cancers. The antitumor activity of this medicine as a single agent is low (approximately 40%), therefore it is used mainly in polytherapy.

A new idea has been observed in scientific publications to enhance effectiveness of anticancer drugs through combined treatments with naturally occurring chemopreventive agents. Using natural compounds increases efficacy of anticancer treatment and decreases toxicity in normal cells. Such compounds are for example isothiocyantes, which inhibit early stages of carcinogenesis and post-initiated stages of the process. In vitro studies show that they potentiate the activity of the anticancer drugs e.g, 5-fluorouracil, oksaliplatin and doxorubicine.

The aim of this study was to investigate the type of interaction between 5-fluouracil and the synthetic isothiocyanate – allysin – in two colon cancer and two breast cancer cell lines after concurrent treatment.

The study was performed on cancer cell lines: breast one (MDA-231 and MCF-7) and colon one (Caco-2and Ht-29). The cytotoxic effects of single 5-fluorouracil or isothiocyanate treatments and their combination were evaluated by the MTT assay. There was used a concurrent scheme. The cells were treated with both alyssin and 5-fluorouracil for 72 hours. The types of interaction were determined through the median effect analysis as described by Chou and Talalay and only when cytotoxicity of the combination was more than 50%.

Synergic types of interaction between isothiocyanate and 5-fluorouracil were observed in breast cell lines. The cytotoxic effects of combination of 5-fluorouracil and alyssin were stronger than the effects of single treatments.

In one colon cell line – Caco-2 – there were observed additive effects (cytotoxicity between 50% and 60%) and antagonism (cytotoxicity of more than 60%). In the second colon cell line – HT-29 – there was noticed antagonism of the tested compounds.

In conclusion, allysin potentates the anticancer activity of 5-fluorouracil in breast cancer cells lines.

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17:25 Poster 73

Effect of MTT and CVDE evaluation methods of cytotoxic action of compounds on the results of interaction studies of new, organic selenium compound (IV) with Alyssin

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Selol is the original Polish patented discovery. It is an organic selenium compound in oxidation state of +4. Currently Selol is a subject of intensive research that will allow to evaluate its usefulness as an anticancer compound. Alyssin is a synthetic analogue of sulforaphane that is found in Cruciferous vegetables. Numerous studies have shown that ITCs increase cytotoxic activity of the compounds used in antitumor therapy. They are characterized by pleiotropic activity which is in many ways similar to that of Selol.

The aim of the study is to evaluate the usefulness of the MTT and CVDE in determination of the antiproliferative properties of Selol and Alyssin and their interactions.

Evaluation of antiproliferative properties of Selol, Alyssin and their combinations was conducted using two cytotoxicity assays: MTT (3 - (4,5- dimethylthiazol- 2 -yle) -2,5- diphenyltetrazolium bromide) and CVDE (crystal violet, (4 - [(4 - dimetylaminofenyl) - phenylmethyl]-N , N -dimethyl- aniline). In MTT assay, cell viability is measured using reduction of MTT to formazan. Amount of colored, reduced MTT is proportional to the oxidative activity of cell mitochondria. While crystal violet assay is based on intercalation of the dye with the nuclear DNA. For both tests, it is assumed that the amount of product formed is directly proportional to the percentage of living cells in the population. IC_{50} values (compound concentration at which the cell viability is 50%) were determined on the basis of assays. IC_{50} were then used in Chou – Talalay's interaction tests of Alyssin and Selol.

IC_{50} values for Alyssin, determined by MTT and CVDE assay, did not differ significantly. But for Selol, IC_{50} determined using MTT was much lower than IC_{50} based on CVDE's results. Interaction studies of Selol and Alyssin using the MTT method showed antagonism (weakening of the anti-proliferative effect) between the two compounds. And the results obtained by CVDE indicate different effect: additive effect of the combination of compounds.

The results indicate that the evaluation of the cytotoxic properties of the compounds may be different depending on the method used and the properties of the tested compound. Concurrently, the results of the interaction of compounds obtained using various methods may indicate different nature of the interactions taking place. When interpreting the results of antiproliferative activity of the compounds, parameter which uses a test to assess cytotoxicity should be considered.

17:25 Poster 74

Trifluoromethylation of bicyclic lactones leading to a new type of fluorinated morpholinols

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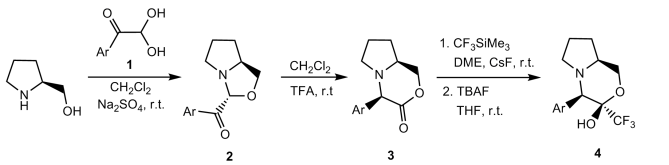
Differently substituted morpholin-2-ols are of significant importance for the preparation of diverse drugs [1], but to the best of our knowledge their fluorinated analogues have not been reported, yet. On the other hand, it is well established, that introduction of fluorine atom or a perfluoroalkyl substituent, especially trifluoromethyl group CF_3, results in substantial modification of physico-chemical properties and biological activities of organic compounds [2]. Therefore, elaboration of new protocols for synthesis of fluorinated compunds is in focus of medicinal chemistry [3].

The goal of the present study was the synthesis of bis-heterocyclic compounds starting with L-prolinol and arylglyoxals **1**. The initial formation of bis-heterocycles **2** was in line with the results of a similar study already described in the literature [4]. However, we found that they are labile compounds and smoothly undergo diastereoselective isomerisation in the presence of an acidic catalyst yielding bicyclic morpholinones **3**.



The latter were used for nucleophilic trifluoromethylation reaction via treatment with (trifluoromethyl)trimethylsilane TMS-CF_3 (Ruppert-Prakash reagent) in the presence of cesum fluoride CsF as a catalyst. Trifluoromethylated morpholinols **4** with *trans* orientation of the CF_3 and Ar groups were formed in a complete stereoselective manner.

The mechanism of the aroyl 1,2-migration leading to the formation of bicyclic morpholinones **3** and the influence of the para-substituent on the rate of isomerisation will be discussed.

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17:25 Poster 75

New camptothecin derivatives alkylating DNA oligomers: the synthesis, physicochemical data and application as Topo I inhibitors

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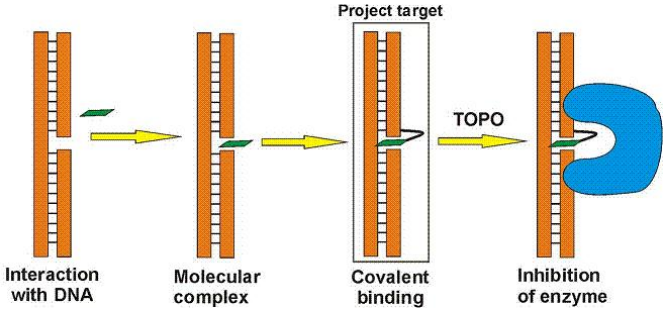
Topoisomerases are essential enzymes, which relieve the torsional stress produced in DNA during replication by cutting DNA on one strand, allowing the broken strand to rotate around the uncut strand. Inhibition of Topo I is one of the strategies in the design of anticancer drugs, whose goal is create a stable covalent ternary complex DNA-Topo I-drug. (shown below)



Derivatives belonging to the camptothecin family, such as Topotecan (Hycamtin ^{TM}) and Irinotecan (Camptosur^{TM}) are the Topoisomerase I inhibitors. They are used in clinical treatment as anticancer agents. These derivatives can intercalate into the nick site generated by Topo I and stabilise the DNA-Topo I complex, however this is weak interaction with DNA.

In this presentation will be presented new water soluble camptothecin derivatives, their synthesis and physicochemical data. It was already established that these derivatives have better biological activity against colon cancer and breast cancer cell lines than Irinotecan, which is used in clinical treatment. We have also evidence that the new derivatives alkylate DNA oligomers. This was established using the PFGSE NMR and MALDI-TOF MS techniques.

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17:25 Poster 76

Evaluation of cytoxicity of glycosyl uridine derivatives

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Glycosyltransferases (GTs) are enzymes responsible for formation of the glycosidic bond by transfer of sugar unit from glycosyl donor to glycosyl acceptor. In cells these enzymes are responsible for synthesis of glycoconjugates and they precipitate in glycosylation of cell surface proteins. Inhibition of GTs activity may contribute to decrease of cancer cell proliferation as a result disorders in biosynthesis of glycoconjugates [1,2]. Many efforts were made in different laboratories to obtain new inhibitors of GTs. Uridine derivatives are very promising compounds for potential antiviral and anticancer activity. In our department series of analogues of UDP-glucose or UDP-galactose were designed and synthesized. Indicated that some of these derivatives decrease the number of cells infected by classical swine fever virus (CSFV) and its insignificantly toxic to normal cells.

The aim of this work is determination of cytotoxicity of uridine derivatives. We indicated toxicity of the tested compounds in HCT 116 and DU 145 cell lines with use of MTT assay. Cells were plated in 96-well plate at the density 2*103 (HCT 116) or 3*103 (HCT 116) cells per well and grown for 24h. Then, the cells were treated by the tested compounds at growing series of concentrations: 0.01, 0.1, 0.5, 1.0, 5.0, 10.0, 25.0, 50.0 and 100μM for 72h. After 3h incubation with MTT substrate (3-(4,5-dimethylthiazol-2-yl)2,5-diphenyl tetrazolium bromide) the absorbance of samples was measured spectrophotometrically with a microplate reader at 570nm wavelength.

Our result indicate that described compounds did not affect proliferation of the tested cell lines.

Acknowledgement:

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17:25 Poster 77

Prediction of intestinal absorption and metabolism of biologically active genistein derivatives

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Genistein is a natural isoflavone regarded as a useful agent for prophylaxis or treatment of many diseases: i.e. osteoporosis, menopause, cardiovascular disease, and cancer. During recent years new derivatives of genistein, which show better pharmacological characteristics comparing to the parent isoflavone, were synthesized in different laboratories. The promising class of genistein derivatives, with potential anticancer properties comprise of glycoconjugates in which a sugar moiety is separated from the isoflavone skeleton via an alkyl chain containing two, three or five carbon atoms. These compounds are further divided into two classes: the derivatives substituted at C7, and the derivatives substituted at C4' position of genistein. Previous studies showed that this class of compounds inhibits proliferation of cancer cell in vitro at the concentration several-fold lower than genistein through inhibition of the cell cycle [1-2].

The aim of this work was to determine the relationship between the structure of the linker and the position of genistein substitution, and bioavailability of the glycoconjugates and their metabolism in the intestine. In our studies we used Caco-2 cell line, cultured in 24- well plates with porous membranes until the tight monolayer was obtained. The integrity of the monolayer was estimated by transepithelial electrical resistance (TEER) measurement. The concentration of genistein derivatives in medium collected from apical and basolateral chambers was determined using a Dionex UHPLC system connected to a 4000 Q TRAP triple quadrupole linear ion trap mass spectrometer. Chromatography was performed using a C18 ACE column (150×4.6 mm, 3.0 µlm). Isocratic conditions were applied: 30% of 0,1% aqueous formic acid and 70% of acetonitrile, the flow rate was set at 0,8 ml/min and sample injecion volume was 5µl. The MS conditions: the parameters dependent on the source and the compound were tuned up for individual derivatives. Final analysis was performed in selected reaction monitoring mode, using the precursor ions and the corresponding product ions. For determining the metabolites, the full scan mode was used (scanned mass ranged between 50 and 700 m/z).

Our result indicate that transport and metabolism of genistein derivatives depend on their chemical structure. Both, structure of the linker between the sugar moiety and genistein, and position of genistein molecule substitution are important determinants of bioavailability of the compounds. Moreover, we determined that genistein derivatives described in this work were transformed in Caco-2 cells into glucuronide metabolites.

Acknowledgements:
Research studies part-financed by the National Science Centre

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17:25 Poster 78

Application of 1-amino sugar in synthesis of glycoconjugates and determination of their biological activity toward β-1,4-galactosyltransferase

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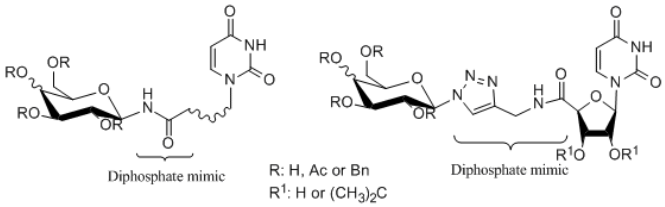
Glycosyltranferases are enzymes responsible for the formation of the glycosidic bond in living system. They transfer the sugar unit from a nucleoside diphosphate sugar (NDP-sugar e.g. UDP-glucose) to a specific hydroxyl- or amino- group of the acceptor substrate which is usually other sugar but nucleic acids, proteins, lipids and other small molecules are also encountered [1]. Modification of many structures through addition of sugars unit can have a significant impact on their physical, chemical or biological properties. Current research confirms that cell surface glycoconjugates have pivotal functions in various cellular recognition systems involving cell differentiation, development, inflammation, immune response, bacterial/viral infections, and many other intercellular communications [2]. In recent years increased attention toward GTs inhibitors has emerged due to key role of these enzymes in biological synthesis of glycoconjugates and polysaccharides. Development of new selective inhibitors is of great importance in dealing with bacterial [3] and fungal diseases [4]. Analogues of NDP-sugar can have an important role as new drug candidates. Our recent research led to analogues of GTs natural substrates in which diphosphate moiety was replaced with amide unit connecting carbohydrate to cyclic or acyclic nucleoside part. Alternative route includes 1,2,3-triazole linkage installed by dipolar cycloaddition.



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17:25 Poster 79

Synthesis and potential spectrum of biological activities of glycoconjugates derivatives of hydroxy-2-methylquinoline carboxylic acids

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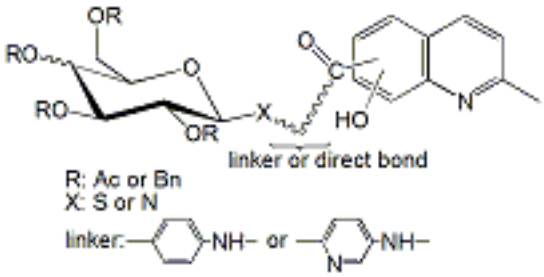
Molecules containing quinoline fragment are present in many natural and synthetic drugs covering a broad spectrum of activity of anticancer, antifungal and antiviral effects [1,2]. Unfortunately, compounds designed on the core of quinoline moiety still suffer from poor bioavailability. This encouraged us to introduce the sugar part into the backbone fragment of quinoline to improve the bioavailability of quinoline derivatives. Sugar part could be connected to quinoline derivatives by amide, ester, thioester or glycosidic bond. Based on this assumption, we received a whole range of glycoconjugates quinoline derivatives, which were subjected the biological tests.



Novel series of quinoline based compounds have been tested for antiproliferative activity. The cytotoxicity tests were performed using MTS assay with doxorubicin as the reference. They were found to be active against HCT116 p53+/+and p53 -/- cancer cell lines. Moreover we have tested their activity against human dermal fibroblast to determine their selectivity. This preliminary results have showed us a high potency of synthesized compounds for inhibitions cancer cell lines. Thus further investigations of these compounds should be conducted.

Molecular modelling and 3D QSAR COMFA studies have been performed using TRIPOS SYBYL 2.0 programme running on Intel

Pentium based machine under the Debian GNU/Linux operating system. Preliminary results have showed that biological activity of synthesized compounds might be through the competitive inhibition of acetylglucosaminyltransferase enzyme.

Acknowledgements

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17:25 Poster 80

Synthesis and biological evaluation of 5'-glycine derivatives of uridine

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Glycosyltransefrases (GTs) are enzymes responsible for transfer of activated monosaccharide units in the form of their nucleotide diphosphate derivatives (NDP-sugar) to a specific free hydroxyl group of the acceptor molecule e.g. growing oligosaccharide, a protein or a lipid. Oligosaccharides that are present on cell surface participate in many intracellular and extracellular events such as viral or bacterial infection, tumor metastasis or immune response [1]. Therefore GTs represent an interesting molecular targets for development of novel therapeutic agents.

The aim of this work was to synthesize a set of uridine derivatives as GTs donor substrate analogues. Several peptidyl derivatives of nucleosides such as nikkomycines or polioxines are known antibiotics. They are potent GT inhibitors acting against chitin synthase [2]. This motivated us to introduce aminoacid to the structure of target compounds. Glycine motif was used as a replacement of diphosphate linkage present in NDP-sugars. Such approach was based on the fact that glycine is known to form complex with divalent metal cation [3-4], so that designed compounds could interact with Mn^{2+} in the active site of enzyme. Moreover uridine part should ensure proper orientation of a compound and thus enabling binding with the enzyme active site. The general structure of target compounds is presented in Figure 1.

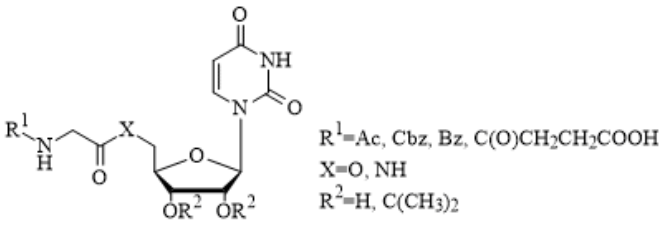

Fig. 1

All synthesized compounds were tested as potential inhibitors in a competition assay against chosen GTs and evaluated for antifungal properties against *Aspergillus fumigatus* and *Candida albicans*. Synthesis of target compounds and evaluation of their activity will be presented.

Acknowledgement

Research studies part-financed by the European Union within the European Regional Development Fund (POIG.01.01.02-14-102/09).

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17:25 Poster 81

Synthesis and biological activity of N^3-(4-metoxyfumaroyl)-L-2,3-diamiopropanoic acids (FMDP) glycolamides and lactamides

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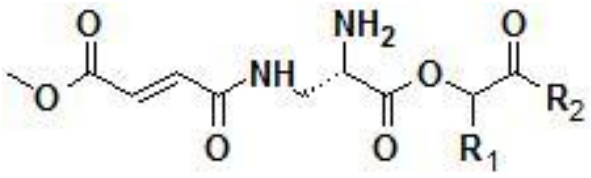
Recently, we have synthesized a group of FMDP amides displaying good inhibitory properties against glucosamine-6-phosphate synthase, an important enzyme in the biosynthetic pathway of cell - wall macromolecules.These amides also exerted reasonable good antifungal activity. To extend our studies we have decided to obtain a new group of FMDP derivatives containing amides of glycolic and lactic acids.

In our communication we present the synthesis of FMDP conjugates with amides of glycolic and lactic acid (Figure 1) and their antifungal activity.



R_1=H, CH_3; R_2 = NH_2,$NHCH_3$, $N(CH_3)_2$

Figure 1: Structure of new derivatives of FMDP.

FMDP is one of the specific inhibitors of enzyme glucosamine-6-phospate (GlcN-6-P) syntase, a potential molecular target for antimicrobial therapy.[1] This enzyme is involved in the biosynthesis of amino sugars building cell wall in microbial cells and its inhibition leads to cell lysis. FMDP is the most potent inhibitor of enzyme, however, this molecule displayed a weak activity in antimicrobial tests.[2] The polar structure of inhibitor makes free diffusion through the cell membrane difficult.

An example of the use of amide of a-hydroxy acids (AHAs) as drug carriers is using the nanoparticles made of copolymers of lactic and glycolic acid.[3] Furthermore, amides of glycolic and lactic acids associated with biologically active compounds take on a properties of the prodrug.[4]

The novel FMDP derivatives with glycolamides and lactamides increased the lipophilicity this molecule and may be a chance to find new effective antimicrobial chemoterapeutic agent.

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17:25 Poster 82

Synthesis of Zn(II)-sulfonated morin complexes: characterization and antioxidant study

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Morin (2',3,4',5,7-pentahydroxyflavone) is a natural plant dye belonging to flavonol class of the flavonoids a group of low cytotoxicity polyphenols. The natural compounds have a broad pharmacological activity: antibacterial, anticancer, antioxidant, anti-inflammatory, and antiallergic [1, 2]. The ability to chelate metal ions and scavenge free radicals renders the flavonoids very good antioxidants. Most of the studies of metal complexation were done in non-aqueous solvents or mixed solvent systems due to the limited solubility of flavonoids in purely aqueous solution. Much better solubility in water is shown by some sulfonic derivatives of flavonoids (quercetin and morin water-soluble derivative has been synthesized [3]), and, at the same time, these retain the properties of the parent compound [4]. The last studies indicated that substitution of the sulfo group at position 5' on the lateral phenyl ring enhances anti-staphylococcal activity of flavonoids [5]. Furthermore, to date, a crystal structure of flavonoids, and their metal complexes has been limited reported.

The present study we described a modified synthesis of the water soluble sodium salt of morin-5'-sulfonic acid (NaMSA hydrate), and investigate the behavior of morin-5'-sulfonic ligand (MSA) at water environment in the presence Zn(II) ions. To date, crystalization experiments carried out in other conditions have not led to obtained single crystal suitable for laboratory diffraction measurements. Herein, single crystal of zinc(II) ions with sulfonated morin from aqueous solution first time were isolated and described. New structures were confirmed with IR, UV-Vis and NMR spectroscopy, elemental and X-ray diffraction analyses. Furthermore, the aim was examination and comparison of antioxidant properties of the synthesized compounds by DPPH radical scavenging activity method.

Our results indicated that composition of the compounds Zn(II)-MSA depends on the excess of either metal cations or ligand during precipitation. If there is an excess of metal cations in the solution $Zn(C_{15}H_8O_{10}S)(H_2O)_2 \cdot 1{,}5\ H_2O$ is formed. However, in the case of MSA ligand excess, sodium salt of bischelate $Na_2Zn(C_{15}H_8O_{10}S)_2(H_2O)_2 \cdot 4H_2O$ is obtained. Moreover, the free radical scavenging activity of NaMSA and ZnMSA is higer than for morin investigated in the same conditions. In addition, the radical DPPH scavenging activity of $Na_2Zn(MSA)_2$ is the largest, about 90% (Fig. 1). This suggest that the introduction of the sulfo group and metal ions significantly change the chemical properties of the morin. In DPPH radical reaction for investigated compounds a hydrogen atom is abstracted from Zn(II)-MSA complexes to give a semiquinone complexes stabilized by the metallic center and by conjugation with the 3-OH group [6].

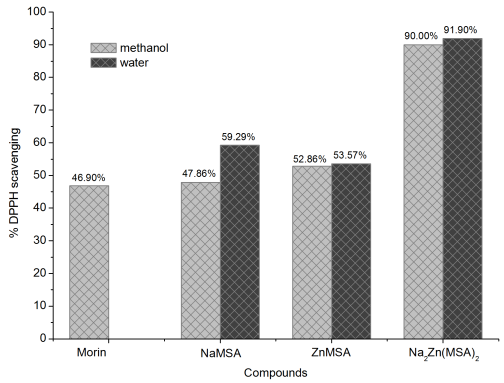

Fig. 1. Relative antioxidative potential of morin, NaMSA and Zn(II)-MSA complexes

In addition, the $Na_2Zn(MSA)_2$ bischelate complex is more effective free radical scavenging than corresponding compounds due to the acquisition of additional superoxide dismutating centres. The complexation with metal ions decrease the oxidation potential of the flavonoids; so the complexed morin-5'-sulfonic acid ligand (MSA) is relatively more effective antioxidant than the uncomplexed one.

These data have important role to a better understanding of the

chemistry of sulfonated flavonoids ligands in the presence of metal ions involved with biological system.

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17:25 Poster 83

Potentiometric study of Pd(II) complexes of some flavonoids in water-methanol-1,4-dioxane-acetonitrile (MDM) mixture

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Morin, rutin and chrysin (Fig 1.) belong to the group of flavonoids, a very important class of phenolic compounds occurring in all parts of plants. Flavonoids has received increased attention during the last years because of their wide range of biological activities. Beneficial effects of flavonoids have been described for diabetes mellitus, cancer, allergy, viral infections, and inflammations. They can bind to biomolecules, such as hormone carriers and DNA, enzymes. Hydroxyflavones catalyze electron transport and scavenge free radicals [1-3].

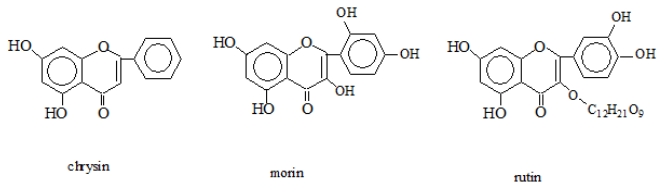

Fig.1. Structural formula of chrysin, morin and rutin

Proton-transfer reactions constitute an important class of chemical reactions and have been studied extensively over a long period of time. Many biological systems use proton transfer reactions to communicate between the extra cellular and intra-cellular media, and the rate if these reactions depend on the degree of dissociation of the species in the solution phase. On the other hand, $PdCl_2$ is widely used as a color forming reagent in spectrophotometric determinations of many drugs [4].

In this study, the stoichiometric protonation constants of chrysin, morin and rutin and the stability constants of Pd(II) complexes of those flavonoids, have been determined in 50% (v/v) water-MDM (methanol:1,4-dioxane:acetonitryle) mixed solvents using a potentiometric method at 25.0 (±0.1) ^{0}C and the ionic strength 0.2. The protonation constants and stability constants were analyzed by Hyperquad2008 computer program.

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17:25 Poster 84

Validated HPLC-UV method for determination of capecitabine in human plasma

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Capecitabine is a drug widely used in the treatment of breast and colorectal cancers which annualy caused over 458.000 and 608.000 deaths worldwide, respectively. The aim of the study was to develop the bioanalytical method to investigate bioequivalence of generic and reference medicinal products.

Sample preparation, based on simple liquid-liquid extraction [1], was optimized to reduce organic solvent consumption. HPLC analysis run time was only 8 min, comparing to 30 min reported previously [1]. On-line wavelength switching from 305 nm to 265 nm was applied for detection of low-cost and widely available voriconazole, which was first-ever used as internal standard. The method selectivity was confirmed in the presence of six main capecitabine metabolites. The stability studies of capecitabine in human plasma were preceded by the detailed stability studies of the active pharmaceutical substance [2].

The method is fully validated according to the European [3] and U.S. [4] regulatory guidelines. It is ideally suited for bioequivalence studies in humans after the administration capecitabine tablets, especially when using a large number of samples [5].

Capecitabine was synthesized in the Chemistry Department and Minisynthesis Department than certified in the R&D Analytical Chemistry Department of the Pharmaceutical Research Institute.

The study was supported by the National Centre for Research and Development of the Republic of Poland under the project No. KB/151/13364/ITI-B/U/08.

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17:25	Poster	85

Composition and properties of lecithin gels intended for parenteral implantation

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Introduction

Vesicular Phospholipid Gels (VPGs) as parenteral implants have been described for the first time 20 yaers ago [1]. From that time VPGs were quite well structurally and physicochemically characterized, but till now some practical and technological aspects about these semisolid liposome dispersions (e.g. sterilization method or stability) are not fully explained.

Aim of the study

The aim of the study was to prepare and characterize VPGs formulations composed of soy or egg-yolk lecithin (40-60%) and different aqueous phases: phosphate buffer, glycerol, macrogol or poloxamer. Production of the gels was optimized in terms of homogenization (homogenizer type, time and temperature range) and sterilization methods (time and temperature of autoclaving). Moreover, to some gels (containing 50% of lecithin) acive substances were added (e.g. ondansetron hydrochloride-OND or risperidone-RIS) and properties of these formulations were compared with VPGs placebo.

Methods

In order to prepare semisolid implants, appropriate amounts of lecithin were mixed (using Unguator or Ultra-Turrax homogenizer) with aqueous phase: water (W), phosphate buffer (PB), 2.4% glycerol (GL), 10% macrogol (PEG) or 10% poloxamer (PL). Drugs (OND or RIS), if present, were added to the aqueous phase before homogenization step and finally the gels were thermally sterilized.

Implants were examined visually and microscopically (using optical or polarized-light microscope). Physicochemical tests comprised of rheological analysis (using a texture analyzer and rheometer), size measurement of lecithin particles (light microscope and laser diffractometry method), determination of drug content and dissolution test (dialysis-tube method).

Results

Regardless of the composition and homogenization method, all obtained lecithin gels were semisolid and cream- to yellow-colored. Microscopic analysis indicated, that the gels are composed of many small, mainly multivesicular lecithin particles, which size depended on the lecithin concentration, type of aqueous phase and homogenization method. The smallest vesicles (d(0.9)<1 μm) were present in formulation composed of lecithin (60%) and PL. More stable were gels prepared by Ultra-Turrax (compared to Unguator mixer), because the morphology of their vesicular particles remained unchangeable, even after autoclaving. Both rheological tests showed, that viscosity of gels depended on lecithin concentration and type of aqueous phase. The highest viscosity was measured for systems composed of lecithin (60%) and PB or PL. Incorporation of drug substances into the gel matrix did not change their physicochemical properties significantly (slight reduction in particle size and increase in gel viscosity were noticed). For all drug formulations prolonged release of OND and RIS was demonstrated. Depending on gel composition, after 24 h, about 50-80% of the active substance was released from phospholipid implant matrix during dissolution test.

Conclusion

Lecithin (egg or soy) after dispersion in aqueous solution forms semisolid systems (gels), which can be use as implantable prolonged-release matrices. Active substances incorporated into the gels did not destroy the structure and properties of the matrix, but are released from the system in a sustained manner for at least of 24 h.

Acknowledgments

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17:25 Poster 86

Synthesis and antitumor activity of novel N'-(2-alkylthio-4-chloro-5-methylbenzenesulfonyl)-5-phenyl-1H-pyrazole-1-amidine derivatives

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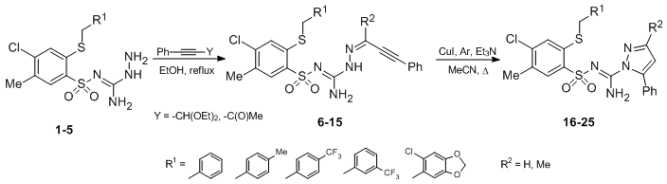
Our previous research for biological properties of 2-mercaptobenzenesulfonamides (MBSA) has revealed the significant anticancer activity of a series of *N′*-(2-benzylthiobenzenesulfonyl)-1 *H*-pyrazole-1-amidine - the MBSA derivatives [1]. Currently, we report on the novel series of *N′*-(2-alkylthio-4-chloro-5-methylbenzenesulfonyl)-3-R^2-5-phenyl-1*H*-pyrazole-1-amidine exhibiting even higher in vitro activity against different type of human tumors. As presented in the scheme below, the synthesis of the desired derivatives were achieved by reacting of the corresponding 3-amino-2-(benzenesulfonyl)guanidines (**1-5**) [2,3,4] with either ketone or acetal to give 1-(1-R^2-3-phenylprop-2-ynylideneamino)guanidines (**6-15**) which were then converted to the *N′*-(2-alkylthio-4-chloro-5-methylbenzenesulfonyl)-3-R^2-5-phenyl-1*H*-pyrazole-1-amidines (**16-25**). The structure of the obtained compounds was confirmed by elemental analyses, IR, ^{1}H and ^{13}C NMR spectroscopy and X-ray crystallography.



Anticancer in vitro screening was performed at the Department of Biotechnology, Intercollegiate Faculty of Biotechnology UG-MUG with using three cancer cell lines. Some of them were tested at the National Cancer Institute (USA) using 60 cell lines derived from 9 types of human tumors. The obtained results showed the high anticancer activity of the tested compounds. The distinctive derivative, i.e.
N′-(2-benzylthio-4-chloro-5-methylbenzenesulfonyl)-5-phenyl-1 *H*-pyrazole-1-amidine showed remarkable activity against 17 of human tumor cell lines representing leukemia, lung, colon, melanoma, ovarian, renal and breast at low nanomolar GI_{50} level in the range of 265 – 870 nM, whereas for the other cell lines GI_{50} were in the range of 1.16 - 3.15 μM.

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17:25 Poster 87

Effect of doxorubicin on selected oxidative stress markers in adenocarcinoma cell line A549

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Doxorubicin - DOX (adriamycin), belongs to the group of anthracycline antibiotics. The molecule of this substance contains aglycone and sugar moiety. Doxorubicin and its anthracycline derivatives are involved in free radical reactions, lipid peroxidation and changes in cellular structures. The mechanism of toxicity of doxorubicin and its anthracycline derivatives relies on a number of free radical reactions that start lipid peroxidation processes in the cell, which in turn can cause damage to cellular structures and functions. Target molecules of the reactive forms of oxygen are, in the first place, DNA, proteins and phospholipids present in the structures of cell membranes. The degree of cell damage by oxidative stress is a result of both its intensity and reduced antioxidant potential. The aim of the study was to demonstrate the impact of doxorubicin, in the used dose and within selected time interval, on cancer cells of the studied line in vitro and to evaluate the dynamics of changes in the activity of enzymes belonging to the main prooxidant/antioxidant.systems : superoxide dismutase and its isoenzymes (mitochondrial, MnSOD and cytoplasmic Cu/ZnSOD) and peroxidase glutamate. As a marker of lipid peroxidation, serum malondialdehyde (MDA) was used. Cultures were grown on the A549 adenocarcinoma line. The culture medium was Dulbeco's with L-glutamine, fetal bovine serum enhanced. Breeding was conducted under standard conditions of temperature and carbon dioxide-enriched atmosphere. Cells were treated with three concentrations of doxorubicin: D1=0,125mg; D2 = 0,25mg/ml; D3 = 0,5mg/ml. After 24-hour exposure in cell supernatant, activity of studied enzymes and the concentration of serum malondialdehyde was measured. The research found that doxorubicin in the used doses and within selected time limit interferes with the activity of major oxidative stress systems of cancer cells of the studied line in in-vitro conditions. The effect depends on dose and duration of ac-

tion of cytostatic drugs. The study also demonstrated a relationship between the chemoresistance of the cancer cells of the line A549 in vitro and the activity of mitochondrial superoxide dismutase isoform.

17:25 Poster 88

Evaluation of lactase dehydrogenase activity as a marker of carbohydrate metabolism in melanoma cells line Me45 under the influence of cisplatin

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Cisplatin - CPL (cis-diaminodichloroplatin), a platinum derivative, belongs to the group of alkylating medicines. The mechanism of action of alkylating agents is based on damaging the biological activity of the deoxyribonucleic acid, DNA, through the formation of cross-links between adjacent DNA strands and within the same strand. The formation of such strands prevents DNA replication and cell division. It also affects the metabolic processes and can trigger cell apoptosis. The aim of the study was to evaluate the activity of lactase dehydrogenase, LDH [EC 1.1.1.27] in cell supernatants as the survival indicator of melanoma cells line Me45 exposed to cisplatin. Lactate dehydrogenase is a marker of malignant diseases, cardiac and liver diseases. The metabolism of a cancer cell is based on the process of glycolysis. LDH is an enzyme that catalyzes the last step of glycolysis, i.e. the oxidation of pyruvic acid to lactic acid, so the activity of the total LDH may indicate the degree of cancer cell damage. Cultures were grown on the human melanoma Me45 cell line. The culture medium was Dulbeco's with L-glutamine, fetal bovine serum enhanced. Breeding was conducted under standard conditions of temperature and carbon dioxide-enriched atmosphere. Cells were treated with three concentrations of cisplatin: C1 = 0.125 mg/ml; C2 = 0.25 mg/ml; C3 = 0.5 mg/ml, the control group were cells with no cytostatic in the culture medium. The cell supernatant was collected after 24-hour exposure and lactate dehydrogenase activity was determined. The research found that the activity of the enzyme in the supernatants from the studied groups decreased compared to the control group. The results suggest that cisplatin, depending on the applied cytostatic dose, acts as a brake on the process of cell glycolysis, as shown by the observation of a decrease in LDH activity in cell supernatants of Me45 melanoma line treated with chemotherapy in in-vitro conditions.

17:25 Poster 89

Mercapturic acids as a naturally occurring isothiocyanates prodrug form with site-directed delivery system built-in based on an oxidative environment of the tumor

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Background. Naturally occurring isothiocyanates are a well-known constituents present in cruciferous vegetables exhibiting high anticancer activity *in vivo* which is associated with relatively low toxicity. Their mode of action involves multiple mechanisms of which glutathione depletion appears to be the most important. These process proceeds though mercapturic acids pathway that start from isothiocyanate conjugation with glutathione and ends once its final product – dithiocarbamate (mercapturic acids) is excreted. Outside the cell it can undergo slow hydrolysis resulting in isothiocyanate's moiety release which can re-enter the cell *via* passive diffusion. These metabolic cycle appears to play a pivotal role in isothiocyanates biological activity, thus, enhancement of these process in the vicinity of cancer cells (tumor) might be an important mechanism of their selective killing.

Among many metabolic, phenotypic and genetic changes involved in malignant transformation one can find increased reactive oxygen species production. These feature is responsible for higher mutation rate and is generally recognized as beneficial for cancer growth. However, such attribute might be useful as a mechanism of prodrugs activation specificity in the vicinity of a tumor tissue. We believe that the stability of dithiocarbamate moiety is correlated with environment redox status, thus, mercapturic acids might be recognized as a redox sensitive prodrugs for isothiocyanates.

Aim of the study. To investigate a potential role of reactive oxygen species (ROS) and oxidative stress in mercapturic acids decomposition to isothiocyanates.

Methods. A series of mercapturic acids were synthesized from parental isothiocyanates using well established methods. The stability of representative compounds in water with or without hydrogen peroxide added was assessed using HPLC system with UV or MS/MS detection methods. Antiproliferative activity *in vitro* was analyzed with SRB (sulforhodamine B) assay. Caspase-3/7 activity was used as an indicator of apoptosis and was detected by the analysis of the sample proteolytic activity using Ac-DEVD-AMC as a fluorogenic substrate.

Results. A series of mercapturic acids was analyzed for the antiproliferative activity after 72 hours of drug treatment using a series of concentrations. Activity observed for almost all tested compounds (expressed as IC_{50} value) was similar to the activity exhibited by corresponding parental isothiocyanates. The most active compounds were mercapturic acids, derivatives of previously not tested

3,4-dimetoxybenzyl isothiocyanate (**1**), 6-benzoyloxyhexyl isothiocyanate (**2**) and 6-hydroxyhexyl isothiocyanate (**3**) as well as previously tested benzyl (**4**) and phenethyl isothiocyanate (**5**). The stability of three representative compounds (**2**, **4** and **5**) was significantly reduced (2-3 times, expressed as $t_{1/2}$) when hydrogen peroxide was present (5 molar equivalents). For example for **5** $t_{½}$ dropped down from 306 minutes to 126 minutes (k = 0.3252 and 0.1320, respectively). For all analyzed compounds, parental isothiocyanate and *bis*-*N*-acetyl-cystine were recognized as a final breakdown products both in sample with or without H_2O_2 added. Interestingly, when hydrogen peroxide was present, additional intermediates were observed indicating a striking differences in the mechanism of decomposition. These intermediates were identified as likely oxidized form of dithiocarbamate (M + 16) and desulfurized form of oxidized dithiocarbamate (M + 16 – 32). Addition of hydrogen peroxide (non-toxic concentrations) to the culture medium enhance mercapturic acids decomposition leading to faster induction of apoptosis – significant caspase-3/7 activity was observed after 6 hour and 15 hour of treatment with or without H_2O_2 added, respectively (for parental isothiocyanates such activity occurred after 4-5 hour).

Conclusions. The results of our studies clearly indicates that mercapturic acids stability is influenced by the presence of hydrogen peroxide. *In vitro* studies provide evidence that the feature observed using HPLC method is probably associated with a more rapid mercapturic acids activation (caspase-3/7 assay). Moreover, mercapturic acids showed high antiproliferative activity comparable to parental isothiocyanate's activity, thus, the addition of the selectivity mechanism does not interfere with biological activity. As the result of the ROS overproduction by the cancer cells, the accumulation of isothiocyanates should occur preferentially inside the cancer cells. Therefore, mercapturic acids could be consider as form of the prodrug for natural isothiocyanates responsible for the site-directed delivery of isothiocyanate directly to the cancer cells.

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17:25 Poster 90

Administration of isothiocyanate-derived mercapturic acids inhibits solid tumor growth of 4T1 murine mammary carcinoma cells in BALB/c mice

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Background. Mercapturic acids are a metabolites of naturally occurring isothiocyanates – well-known constituents present in cruciferous vegetables exhibiting high anticancer activity *in vivo*. Isothiocyanates enter the cells *via* passive diffusion and are rapidly conjugated with glutathione, main intracellular thiol acting as a redox guardian. Next, they undergo a successive rearrangements - mercapturic acid pathway, which ends when dithiocarbamate (mercapturic acid) is excreted outside the cell. There, it can undergo slow hydrolysis resulting in isothiocyanate's moiety release which can re-enter the cell. Due to these metabolic cycle, mercapturic acids can be treated as isothiocyanate's intracellular reservoir. Moreover, recent findings indicates that they can directly modulate cancer cells metabolism, growth and proliferation acting as histone deacetylase inhibitors. Application of the isothiocyanates as an inhibitors of tumor growth *in vivo* in difficult, because of their pungent taste and smell, low water solubility and potential stomach irritating effect. Mercapturic acids lack these adverse effect, thus, they application *in vivo* should be possible.

Aim of the study. The comparison of mercapturic acids and their parental isothiocyanates activity *in vivo*.

Methods. A series of mercapturic acids were synthesized from parental isothiocyanates using well established methods. Balb/c, female mice were inoculated with 4T1 cancer cells grown *in vitro*. Tested compounds were administered 5-times a week (300umol/kg b.w., i.p.), tumor growth and body weight change was evaluated 3-times a week. In the 31th day of the experiment animals were sacrificed, organs were weight, blood was collected for further morphological and biochemical analysis.

Results. All five isothiocyanate-derived mercapturic acids (benzyl **(NAC-1)**, phenethyl **(NAC-2)**, allyl **(NAC-3)**, 3,4-dimetoxybenzyl **(NAC-4)**, 6-benzyloxyhezyl **(NAC-5))** showed anticancer activity. The highest activity was observed for **NAC-4** and **NAC-5** (49% and 40% of tumor growth inhibition (TGI), respectively), the lowest for **NAC-3** (17% TGI). No significant differences between the activity of mercapturic acid and the corresponding isothiocyanate was observed. However, mercapturic acid had less pronounce influence on body weight change than corresponding isothiocyanate (*e.g.* 9.5% and 1.9% of body weight decrease (BWD) for **ITC-1** and **NAC-1**, respectively), thus, they might be safer as anticancer drugs in further applications. Morphological blood analysis showed increased level of erythrocytes, hepatocyte and hemoglobin in all groups treated with isothiocyanates/mercapturic acids. Interestingly, leucocytes level was positively correlated with anticancer activity (the highest level was achieved for **NAC-4**), indicating their possible immunostimulating activity. Analysis of LDH, ALTL, ASTL enzymes, as well as creatinine, urea, albumin and bilirubin showed no significant differences, however level of albumin and bilirubin was decreased in groups treated with tested compounds.

Conclusions. Isothiocyanates and the corresponding mercapturic acids showed comparable acticancer activity against 4T1 murine mammary gland tumor. Significant tumor growth inhibition was observed especially for **NAC-4**, **NAC-5** and the corresponding isothiocyanates. All results obtained during the present studies indicates that isothiocyanates might be replaced by mercapturic acids in further *in vivo* studies.

This work was supported by a grant of National Science Centre, grant no. 2011/01/N/NZ4/03361.

17:25 Poster 91

Fungicidal activity of thionocarbamates derivatives of alcohols

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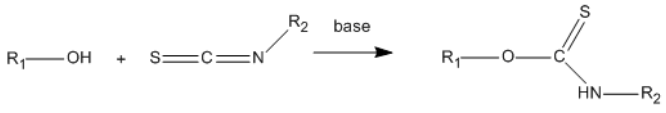
N-Allyl and N-aryl thionocarbamates are an important class of compounds that have received considerable attention in the literature [1]. Such compounds are of interest due to their numerous biological effects including anesthetic, fungicidal, bactericidal, pesticidal, and antiviral [1-5]. Thionocarbamates as fungicides have been extensively studied since 1960 [4]. However, little attention had been given to N-allyl and N-aryl substituted thionocarbamates derivatives alcohols until Thorne [5] studied a wide variety of derivetives of thiocarbamic and carbamic acids. He found that phenyl carbamates, alkyl-phenyl carbamates, and alkyl thiophenyl carbamates have low fungitoxicity and that some thionocarbamates showed activity. To study the possibility of improving the fungicidal activity of the known thionocarbamates, in the present work a series of N-alkyl, N-phenyl and N-benzyl thionocarbamates derivatives alcohols were synthesized (Figure 1), and their fungitoxic activity was tested for yeasts (*Candida albicans, Candida dubliniensis, Candida tropicalis, Candida glabrata, Candida krusei, Candida parapsilosis*) isolated from clinical materials in the Laboratory of Microbiology, Silesian Center for Heart Diseases in Zabrze. Results presented in this paper reveal good fungicidal activity of certain N-allyl, N-phenyl and N-benzyl thionocarbamates.



R_1 - various alcohols

R_2 - allyl
- Ph
- Bn

Figure 1

Acknowledgement:

Research studies part-financed by the European Union within the European Regional Development Fund (POIG.01.01.02-14-102/09).

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17:25 Poster 92

N-Phenyl thionocarbamates derivatives of alcohols as donors protecting groups in carbohydrate chemistry

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The synthesis of complex molecules, e.g., natural products, is still a challenge despite tremendous progress in organic synthesis achieved over the last decade's [1]. Most of multistep syntheses still require many protection and deprotection procedures. In response to the increasing complexity of the molecular structures synthesized, numerous protecting groups have been developed, as well as methods for their introduction and their deprotection [2-4]. Nevertheless, new and more selective protecting groups are still required [5] while milder and more selective conditions are actively pursued [11-14]. The benzyl group like 4-methoxybenzyl (PMB), 3,4-dimethoxybenzyl (DMB), 4-nitrobenzyl and others has been used extensively as a protecting group in carbohydrate chemistry. Benzyl ethers are frequently used as protecting groups indispensable to the general pursuit of oligosaccharides. Traditionally, preparation of arylmethyl ethers from alcohols is accomplished by Williamson ether synthesis protocol under basic conditions, by treating substrate with benzyl halide and a strong base, such as potassium hydroxide [1-8].

In this communication we report the novel method protection of sugars as the corresponding 4-methoxybenzyl, 2,4-dimethoxybenzyl and 3,4-dimethoxybenzyl and other aryl groups using N-phenyl thionocarbamates as donors protective groups. These compounds are readily obtained from corresponding alcohols by reaction with commercially available N-phenyl isothiocyanate [9].

Acknowledgement:

Research studies part-financed by the European Union within the European Regional Development Fund (POIG.01.01.02-14-102/09).

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17:25 Poster 93

Free radicals in thermally sterilized Acidum Boricum and optimization of this proccess

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Electron paramagnetic resonance (EPR) spectroscopy was used to examine free radicals in thermally treated *acidum boricum* (AB). Thermal treatment in hot air as sterilization process was tested. Sterilization is expected to exterminate microorganisms in *acidum boricum* [1]. Conditions of thermal sterilization are described in the pharmaceutical Norms [2]. It is expected that thermal sterilization related breaks of chemical bonds and free radicals formation in *acidum boricum samples* depend on temperature. Our previous works reported formation of free radicals in organic pharmaceutical substances at high temperature [3-5].The aim of this work was to determine concentration and free radical properties of thermally sterilized *acidum boricum,* which is used as a 3% solution to wash infected wounds and burns.[1].

There is no literature information about free radicals in AB samples. EPR results for AB were compared to results obtained for the others thermally sterilized drugs [6].

EPR analysis for AB was done 15 minutes, 2, 8, 10, 13, 16, 22, 32 and 40 days after sterilization. EPR measurements were done at room temperature. EPR spectra were recorded in the range of microwave power of 2.2-70 mW. g-Factor, amplitudes (A), integral intensities (I), and linewidth (ΔB_{pp}) of the spectra were determined. The shape of the EPR spectra was analysed.

EPR spectra were not obtained for the non heated AB. As was expected free radicals do not exist in the original AB sample. EPR spectra were detected for the all thermally sterilized samples. The lowest free radicals concentration was found for the AB sterilized at temperature 170 ^{o}C during 60 minutes. Free radicals concentrations in sterilized AB change during storage as the result of oxygen interactions with the samples.

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17:25 Poster 94

Immunorestoring activity of isoxazole derivative R-13

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Immunoreconstituting effects of R-13 compound (3,5-dimethylisoxazole[5,4-e]6H-triazepin-4-one) on immune status of mice treated with cyclophosphamide (CP) were evaluated. In this study we evaluated effects of R-13 administration on: phenotypic changes of cells in lymphoid organs in normal mice, leukocyte and splenocyte numbers and blood cell composition in CP-treated mice, spontaneous and mitogen-induced splenocyte proliferation as well as humoral and cellular immune responses. In normal mice R-13 significantly elevated percentages of $CD3^+$ and $CD4^+$ cells in the spleens and lymph nodes, accompanied by reduction of $CD19^+$ cells. In CP-treated mice, R-13, administered in five doses, increased number of blood leukocytes and splenocytes, spontaneous and Con A-induced splenocyte proliferation (day 15 after CP). Blood picture analysis showed decreases of neutrophil and eosinophil levels and appearance of lymphocyte immature forms. The number of circulating lymphocytes increased by 2-fold whereas absolute numbers of neutrophils remained unchanged. The cellular response to OVA (day 15 following CP) was completely restored using five R-13 doses. The humoral immune response, determined 38 days after CP administration, was also significantly restored by using ten R-13 doses. In conclusion, we presented characteristics of isoxazole derivatives R-13 which exhibits selective T-cell-tropic activity and accelerates restoration of the cellular and humoral immune responses. Such a property and other features such as lack of toxicity and bioaccessibility at oral administration are desirable for use in immunocompromised patients. It is also conceivable that R-13 could find application in states of T-cell deficiencies. This study was supported by a statuary grant from the Polish Ministry of Education, No 4/2009, for the Institute of Immunology, Polish Academy of Sciences, Wrocław, Poland, and by the State Committee for Scientific Research, grant No. KBN 3PO5F 01224.

17:25 Poster 95

Sex differences in the opioid component of swim stress-induced analgesia in mouse lines selected for high or low sensitivity to stress

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This study searched for sex-specific differences in the contribution of μ- , δ- and κ-opioid receptors in analgesic response evoked by stress. Opioid receptors are widely known to influence stress-induced analgesia. With the use of mouse lines selected for high and low swim stress-induced analgesia we show that males and females from both lines display similar analgesic responses. Antagonism of the 3-min swim-induced analgesia with prototypic opioid antagonist naloxone (10mg/kg i.p.) was less effective in females in these stress conditions. Further analysis using selective –mu (cyprodime), –delta (naltrindole) and –kappa (nor-binaltorphimine) opioid receptor antagonists (all 10mg/kg, i.p.) show that differences in the sensitivity to naloxone inhibition of SSIA may be caused by a higher involvement of the –delta opioid system and decreased functionality of –mu opioid analgesia. No sex differences in neurochemical mechanism of analgesia were also showed in the naloxone-resistant LA mice. We postulate that studies performed on mice with divergent opioid system activity are relevant in detection of tenuous sex differences in opioid analgesia.

17:25 Poster 96

Synthesis of new 5H-indolo[2,3-b]quinoline derivatives with a high selective cytotoxic activity

Katarzyna Sidoryk[1], Marta Świtalska[2], Piotr Cmoch[1,3], Monika Kaczmarska[1], Iwona Bujak[1], Joanna Wietrzyk[2], Krzysztof Bańkowski[1], Łukasz S. Kaczmarek[1]

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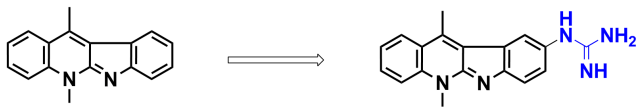
5,11-dimethyl-5H-indolo[2,3-b]quinoline (DiMIQ), the synthetic analogue of the neocryptolepine, displays high cytotoxic activity, which is comparable to the cytotoxic activity of doxorubicin, but the high toxicity of DiMIQ and its low bioavailability inspired us to look for new analogues of DiMIQ.

Numerous examples in recent literature and first results of our investigations reveal that the lowered toxicity of DiMIQ can be achieved by a construction of a conjugate composed of DiMIQ and amino acids or peptides. Also the strongly hydrophilic guanidinium group can increase the bioavailability of new conjugates, and it can modulate their cytotoxic activity, and can increase their selectivity.

Now, we designed and obtained a series of novel hybrid compounds composed of 5H-indolo[2,3-b]quinoline and the guanidinium group or a residue of a N-guanylamino acid. All the new conjugates displayed a high cytotoxic activity against cell lines: non-small cell lung cancer A549, breast cancer MCF-7, colon cancer LoVo, cervix carcinoma KB. The best compound (I) displays also >1000 – fold more potent cytotoxic activity against all the cancer cell lines than for normal mice fibroblasts BALB/3T3.



IC_{50} = 1.42±0.23 BALB/3T3
IC_{50} = 0.38±0.13 MCF-7

IC_{50} = 24,98±4,59 BALB/3T3
IC_{50} = 0,024±0,004 MCF-7

IC_{50} - compound concentration leading to 50% inhibition of cell proliferation [μg/mL]

17:25 Poster 97

Influence of the side chain on the cytotoxicity of betulin derivatives

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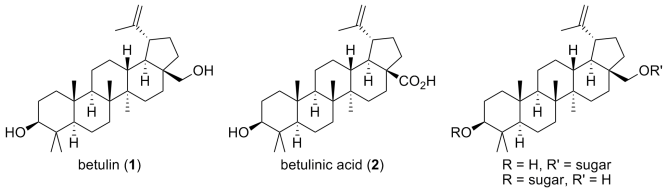
Betulin (1) and betulinic acid (2) are natural compounds with proven anticancer, anti-bacterial, antimalarial, antiviral and anti-inflammatory activities [1, 2]. Recent investigations have demonstrated that even a simple modification of the structure can significantly increase their biological activity. Lupane saponins (triterpenes bearing sugar moiety at C-3 or C-28 positions) are also considered as very promising cytotoxic derivatives [3].



In this communication, we will report on the synthesis of new lupane derivatives as well as, novel lupane-type saponins. All new derivatives were examined for the cytotoxic activity against cancer cell lines of various histopathological origins, including T-

lymphoblastic leukemia (CEM), breast carcinoma (MCF-7), and cervical carcinoma lines (HeLa) as well as normal fibroblasts (human BJ-H-tert). Our investigations demonstrate that the modifications at C-3 and/or C-28 positions of lupane triterpenes, and saponins obtained thereof, significantly influence the cytotoxic activity of the resulting new compounds. Structure-activity relationships will also be discussed.

The support from the National Science Centre (Grant No. 2012/07/B/ST5/00823) is acknowledged.

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17:25 Poster 98

Synthesis and anticancer activity of novel N-phenyl-N'-(pyridine-3-sulfonyl)urea derivatives

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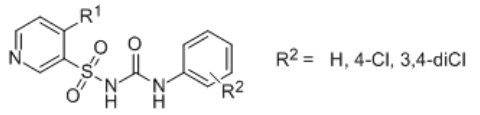
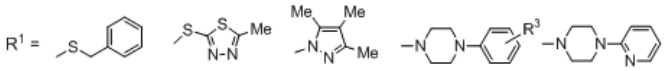
Numerous diarylsulfonylurea derivatives are well known from their anticancer properties. Our extensive study on the synthesis and biological properties of a number of 4- substituted pyridine-3-sulfonamide derivatives [1-5] prompted us to the synthesis and anticancer evaluations of novel series of *N*-phenyl-*N'*-(pyridine-3-sulfonyl)urea derivatives.

The synthesis of the target sulfonylurea derivatives were achieved by multi-step reactions starting from 4-chloro-pyridine-3-sulfonamide with *N*- or *S*-nucleophiles to produce the corresponding 4-substituted pyridine-3-sulfonamides that were subjected to reactions with suitable phenyl isocyanates to give the expected *N*-phenyl-*N'*-(4-substituted pyridine-3-sulfonyl)urea derivatives as shown in scheme below. The structure of this compounds was confirmed by IR, ^{1}H and ^{13}C NMR spectroscopy.





Several compounds were tested in vitro at the National Cancer Institute in Bethesda (USA). This sulfonylureas revealed moderate or reasonable anticancer activity. It could be noticed, that several cancer cell lines exhibit relatively high susceptibility to these sulfonylurea derivatives. Among them, there are cell lines of breast cancer (MDA-MB-468), prostate cancer (PC-3), melanoma (UACC-62 and LOXIMVI) and leukemia (RPMI-8222 and K-562) cell lines. The most active compound *N*-(4-chlorophenylcarbamoyl)-4-[4-(3,4-dichlorophenyl)piperazin-1-yl] pyridine-3-sulfonamide exhibited significant activity against 7 of human tumor cell lines representing leukemia, colon, melanoma and renal cancer at the low micromolar concentrations, i.e. GI_{50} ranged from 1.55 to 9.81μM.

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17:25 Poster 99

Synthesis of novel 2-benzylthio-4-chloro-N-(5-substituted 1,2,4-triazin-3-yl)benzenesulfonamide derivatives with potential antitumor activity

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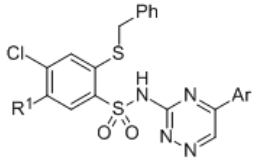
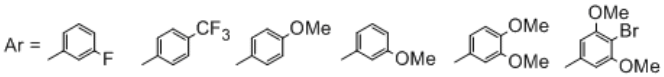
Recently, we reported on the significant anticancer activity of a series of 2-mercapto-*N*-(1,2,4-triazin-3-yl)benzenesulfonamides bearing phenyl or substituted phenyl attached to the 1,2,4-triazine moiety simultaneously at positions 5 and 6 [1]. In the present study, in search for even more potent 2-mercaptobenzenesulfonamides we elaborated the synthesis and anticancer evaluations in vitro of novel *N*-(5-substituted 1,2,4-triazin-3-yl)benzenesulfonamide derivatives as depicted in scheme below.

The synthesis of the desired 2-benzylthio-4-chloro-*N*-(5-aryl-1,2,4-triazin-3-yl)benzenesulfonamide derivatives were achieved by reacting of the corresponding 3-amino-2-(benzenesulfonyl)guanidines [2,3] with suitable phenylglyoxal hydrate in refluxing glacial acetic acid for at least 30 hours. The structure of the obtained compounds was confirmed by elemental analyses, and IR, ^{1}H and ^{13}C NMR spectroscopy.





R1 = Me, PhNHCO, 4-Cl-PhNHCO, 4-Me-PhNHCO, 4-MeO-PhNHCO

Anticancer in vitro screening was performed at the Department of Biotechnology, Intercollegiate Faculty of Biotechnology UG-MUG with using three cell lines of breast (MCF-7), colon (HCT-116) and cervix cancer (HeLa), and at the NCI (Bethesda MD, USA) using 60 cell lines derived from 9 types of human tumors. This tests revealed moderate or reasonable anticancer activity of tested compounds.

The distinctive compound, i.e. 2-benzylthio-4-chloro-*N*-

[5-(3-methoxyphenyl)-1,2,4-triazin-3-yl]-5-methylbenzenesulfonamide showed remarkable activity against 23 of human tumor cell lines representing leukemia, lung, colon, CNS, melanoma, ovarian, renal and breast at low micromolar GI_{50} level in the range of 13.8 – 19.9 μM.

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17:25 Poster 100

Role of gene polymorphism in the optimalisation of the treatment with vitamin K antagonist.

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Oral anticoagulant treatment such as warfarin, acenocoumarol or warfarin, is universally used for treatment and prevention of thromboembolic events. Their clinical use is complicated by a the narrow therapeutic concentration range and high variability in dose requirements among

individuals. Cytochrome P450 (CYP) 2C9 is involved in coumarin metabolizm and two allelic variations are most relevant for dose-response variability: CYP2C9 430C®T (CYP2C9*2) and CYP2C9 1075A®C (CYP2C9*3). The aim of presented study was to genotype these allelic variants with the use allele-specific amplification ASA-PCR assay among 104 patients with long QT syndrome. Genomic DNA was prepared from peripheral blood leukocytes and ASA-PCR with internal and external pair of primers was performer to detect wild type or mutant allele according the number and the lenght of PCR products. The study showed the presense of 17 heterozygous allelic wariant and 2 homozygous for CYP2C9*2 and 9 heterozygous allelic wariant and 1 homozygous for CYP2C9*3. Presented method is significantly faster and less expensive than sequencing and very useful for pharmacogenetic information.

17:25 Poster 101

Detection of the poor metabolizer-associated CYP2D6 gene by long- and tetra-primer PCR technology

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Cytochrome P450 (CYP) 2D6 is one of the most important enzymes involved in the metabolism of drugs. Genotyping of CYP2D6 could be applied in individualization of drug therapy to improve therapeutic efficacy and decrease adverse effects enable to avoid the therapeutic failure following drug treatment. Several mutations have been described in the CYP2D6 gene that abolish CYP2D6 activity, however, four mutations accounts for the majority of the poor metabolizers. Genomic DNA was etracted from a total of 109 EDTA-supplemented blood samples of patients with dilated cardiomyopathy.

Multiplex long PCR was performed to genotype the CYP2D6*5 allele and three tetra-primer PCR assays were developed to detect the mutations in the CYP2D6*3, CYP2D6*4, CYP2D6*6 alleles. Analysis of 109 alleles showed the CYP2D6*4 polymorphism to occur at the allelic frequency of 22.93%, whereas CYP2D6*3, CYP2D6*5 and CYP2D6*6 at 1.83%, 1.83% and 0.91% frequency, respectively. The method allows rapid and cheap genotyping of the majority of poor metabolizers of CYP2D6.

17:25 Poster 102

The application of HPLC method for investigating the stability of Vitamin C

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Vitamin C (ascorbic acid, 2-(1,2-dihydroxyethyl)-4,5-dihydroxyfuran-3-one, AA) is a water-soluble organic compound essential in many biological process e.g., it protects the immune system, enhances iron bioavailability, reduces cholesterol levels and is necessary in the formation and maintenance of collagen. Moreover, ascorbic acid is strong antioxidant and it is extensively used for the prevention and treatment of the common cold, some mental illnesses, and cancer [1-3]. The main disadvantage of ascorbic acid is relatively low stability of water solution.

The aim of our research was investigation of ascorbic acid stability depending on time, temperature, concentration. and type of stabilizer. The analysis was performed by HPLC-DAD method in reversed phase C-18 system using water with addition of trifluoric acid at concentration of 0.025% as a mobile phase.

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17:25 Poster 103

The effect of strontium ions fertilization on isoflavones content in Glycine max (L.) Merr.

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Soy (*Glycine max* (L.) Merr.) is an annual plant cultivated worldwide mostly for high-quality food. Moreover, due to its pharmacological properties it is widely used in pharmacy for alleviating the symptoms of osteoporosis. Numerous literature data suggest that isoflavones, the major phenolic compounds present in soy, protect against post-menopausal osteoporosis associated with endogenous estrogen deficiency [1]. On the other hand, strontium salts have shown unique pharmacological effects on bone resorption and formation [2, 3].

The aim of our paper was investigation the influence of strontium ions fertilization on isoflavones content in Glycine max. Our research can be useful to obtain herbal preparations containing both phytoestrogens and strontium to prevent postmenopausal osteoporosis.

The analysis of isoflavones was performed by HPLC-DAD method; the content of strontium in plant depending on its concentration in growth media was determined by flame atomic absorption spectrometry (High-Resolution Continuum Source atomic absorption spectrometer ContrAA 700).

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17:25 Poster 104

The biological activity of quinoline derivatives

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Compounds containing quinoline moiety are well known due to their broad biological activities. A number of them have been widely investigated and clinically used as antifungal or antibacterial agents [1, 2]. Quinoline derivatives have gained strong attention recently due to their activity as perspective HIV integrase inhibitors [3]. Recently, some quinoline-based compounds have been synthesized and reported as potent antitumor agents by our research team [4]. Anti-proliferative activity of the synthesized compounds was tested by the MTS assay against the human colon adenocarcinoma cell lines with normal expression of p53 protein (Hct116 p53+/+) and mutants with disabled TP53 gene (Hct116 p53-/-). The compounds were also tested for their cytotoxicity against mouse melanoma cell line B16-F10 and nontumor cell line NHDF. Anti-proliferative activity of quinoline derivatives was determined. Compound (Figure 1) demonstrated the highest anti-proliferative activity (**IC_{50}=1,40µM**). The most active compound makes it promising for further development.

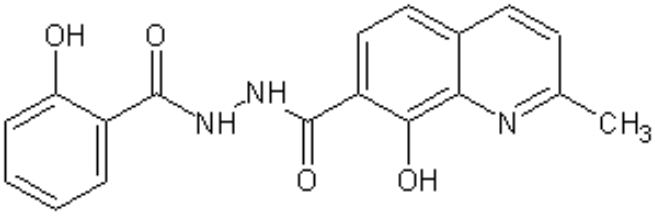

Figure 1. Chemical structure 8-hydroxy-*N*'-(2-hydroxybenzoyl)-2-methylquinoline-7-carbohydrazide.

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17:25 Poster 105

Rational design and synthesis of new 5-HT$_6$R ligands with the use of bioisosteric strategies. Crystal structures, biological evaluation and molecular modeling studies.

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The 5-HT$_6$ receptor, which is localized practically only in the brain [1] is a promising target for different new psychotropic drugs. 5-HT$_6$ receptors are supposed to be responsible mainly for motor control, memory and learning and its antagonists can be used to improve cognitive and memory functions in cognitive impairments [2, 3, 4] and also as an antiobesity drugs [5, 6]. Up to date several thousands of structurally diverse ligands have been synthesized but their binding mode has not been fully identified.

During virtual screening campaign for the search of novel 5-HT$_6$R

ligands, a 3-fold less active bioisostere of literature compound [7] was found. A series of its isosteres was synthesized in an attempt to increase affinity. One of this compounds proved to be 10-times more potent than the parent one. Crystal structures and molecular modeling studies justified structure-activity relationship.

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17:25 Poster 106

Development and validation of the GC method for quantitative determination of semi-volatile solvents in pharmaceutical substance Bosentan

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Bosentan is an active substance in the orphan drugs used to treat pulmonary artery hypertension (PAH). It is a dual endothelin receptor antagonist (ERA) with the affinity for both receptors of the A and B: endothelin-A (ETA) and endothelin-B (ETB). Under normal conditions, ETA or ETB receptors cause constriction of the pulmonary blood vessels. By blocking this interaction, bosentan decreases pulmonary vascular resistance [1].
The new gas chromatography method with direct-injection for quantitative determination of residual semi-volatile solvents such as acetic acid, dimethyl sulfoxide (DMSO) and ethylene glycol in bosentan - the pharmaceutical active substance has been developed and validated. The optimization of the method consisted in the selection of experimental conditions that allowed to meet the requirements for this procedure, including:

- The appropriate level of detection limit (LOD) of analytes, in particular for ethylene glycol,
- The stability of determination - decomposition peak of Bosentan were observed depending on the temperature of the injector,
- The appropriate specificity of the method between all solvents used in the synthesis of Bosentan
- The appropriate range of the determination which includes 10 - 120 % of specified limits of residual solvents. According to the Guideline Q3C (R5) 11 [2] acceptable limits (maximum allowable limit) of ethylene glycol is 620 ppm, acetic acid 5000 ppm and DMSO 5000 ppm in respect to sample preparation.

The developed method was validated according to the requirement of ICH (International Conference of Harmonization) validation guidelines Q2R1 [3]. Specificity, precision, accuracy, linearity, limits of detection and quantitation and robustness were determined and excellent results were obtained.

Acknowledgements:

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17:25 Poster 107

Synthesis of new piroxicam derivatives and their influence on lipid bilayers

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Modification of the surface properties of membranes by any ligand could lead to several phenomena like aggregation, leakage of trapped contents or permeabilization, fusion, etc. Such modulation of surface properties is a fundamental requirement for many biological processes [1]. In this work, we present the synthesis and interaction of new piroxicam derivatives with model lipid bilayers.

The starting material for the synthesis of the above mentioned compounds was 1,1-dioxo-1,2-benzothiazol-3-one (saccharin). It was condensed with 2-bromo-4’-fluoroacetophenone in dimethylformamide (DMF) in the presence of triethylamine giving 1,1-dioxo-2-(4-fluorophenyl)acetyl-1,2-benzothiazol-3-one, which was then rearranged to the corresponding 1,2-benzothiazine ring. The final compounds were prepared by alkylation of corresponding 1,2-benzothiazine with 4-aryl/heteroaryl-1-(2-chloroacetyl/3-chloropropyl)piperazine giving four new structures (PD 28-31). The separated products were purified by the crystallization from ethanol. The structures of the compounds obtained were confirmed by elemental and spectral (IR, H^1NMR) analyses.

In spectroscopic experiments we assessed the influence of the examined new piroxicam derivatives on laurdan and prodan fluorescence in liposomes made of lecithin from egg yolk (EYPC) and dipalmitoylphosphatidylcholine (DPPC). Laurdan and prodan both possess the same fluorophore connected to an alkyl chain of different length (three carbon atoms in prodan and twelve in laurdan). Therefore prodan molecules locate closer to the hydrophilic surface of a bilayer than laurdan whose fluorophore is positioned close to phospholipid glycerol groups [2, 3].

PD 28-31 quenched the fluorescence of both laurdan and prodan. Additionally, the influence of PD 29 and PD 31 at 25 μM/l concentration on main DPPC phase transition was monitored by the use of laurdan generalized polarization.

In our present work we have shown that new piroxicam derivatives PD 28-31 interact with the model membranes under consideration.

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17:25 Poster 108

The effect of ionic strength on the aggregation of bacteriophage T4

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The therapeutic use of lytic bacteriophages has a potential as a viable solution to the worldwide problem of increasing number of drug-resistant bacteria (1). In recent years, there has been a great emphasis towards the modification of bacteriophage particles for their use as a nanomaterial for diagnostic and therapeutic applications, vaccines or for systems employed in bacteria detection.

This study concerns physicochemical properties of bacteriophage T4, purified using the extraction method (2) and membrane filtration. The purification process allowed removal of endotoxins, culture medium components and residues generated after disintegration of bacterial cells. The titer of bacteriophage T4 in purified preparation was $4{,}2 \times 10^{13}$ pfu/ml and endotoxin level, determined by Limulus Amebocyte Assay, was 76 EU/ml. The effect of ionic strength on stability of bacteriophage suspension was studied using atomic force microscopy (AFM) and dynamic light scattering (DLS) measurement. On the basis of size measurements it was found that critical level of sodium bicarbonate concentration, below which the process of size increase starts, is about 0.03 M. The AFM imaging indicates that increase in particle sizes can be linked to the process of aggregation of bacteriophage particles emerging in the alkaline environment of lower ionic strength.

Presented results prove that physicochemical properties of bacteriophage T4 depend on the medium composition, in particular on the ionic strength and pH.

This project was supported by the National Centre for Research and Development, Poland (Grant No 13-0089-06).

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17:25 Poster 109

Elaboration of travoprost eye drops formulation.

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Prostaglandin analogs are an important therapeutic agents used in treating glaucoma. This increasingly common disease manifests itself *inter alia* by increased intraocular pressure. Consequently, it causes irreversible degenerative changes in the visual organ, hence importance of efficacious pharmacological treatment. Research towards development of a formulation process for generic prostaglandin eye drops will be presented on the example of travoprost. Typical hurdles of the process development involving prostaglandin are: low dose of the active substance, difficulties connected with dissolving it in the target formulation and obtaining a homogenous solution. The result of experiments demonstrating significance of various factors used in the process elaboration to select the most effective way of transferring the active pharmaceutical ingredient to the final form of preparation, while avoiding the content loss and creation of impurities, will be presented. As a result of conducted research, product specification fulfilling the requirements imposed by pharmacopoeia, and documenting suitable stability, was established.

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17:25 Poster 110

Fluorinated carbanucleosides and acyclic nucleosides

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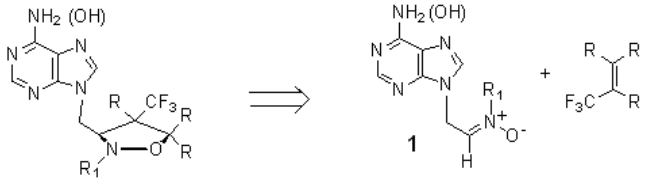
Fluorinated analogs of nucleosides find use as antiviral and anticancer agents. This is possible due to their ability to act as inhibitors of nucleotide metabolism- they are indistinguishable by active site of enzymes. Properties of fluorine atom are very important here (similar size to the hydrogen atom, large electronegativity, the possibility of replacing a hydroxyl group).

Interesting examples of nucleoside derivatives with bioactive properties are acyclic nucleosides and carbanucleosides, reffered to in our work.

In recent years, a number of studies describing isoxazolidinyl analogs of nucleosides with isoxazolidinyl ring instead of sugar moiety has appeared. These compounds, which have N-O bond in the five-membered ring, are prone to breaking in appropriate conditions [1].

Products of opening isoxazolidinyl ring can be classified both as a 1,3-aminoalcohols as well as acyclic nucleosides.



Our synthetic task was to obtain presented above nitrone (1) from the respective purine bases. These compounds would be subjected to the 1,3-dipolar cycloaddition with fluorinated olefins. Next step would be reaction of opening isoxazolidinyl ring.

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17:25 Poster 111

Study on the model of risperidone release from poly(L-lactide-co-glycolide) (85:15) matrices

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The drug formulations providing prolonged releasing are preferred in the treatment of mental diseases. Biodegradable matrices based on copolymers of lactide and glycolide have been proposed for risperidone (RSP) targeting. They degrade releasing substance. In principle, drug release is usually controlled by diffusion, degradation or their combination. There are two widely used diffusion models for the explanation of diffusion-controlled release from matrices, i.e. Higuchi model and Korsmeyer-Peppas model. The aim of this study was to estimate the release rate of RSP from poly(L-lactide-co-glycolide) (L-PLGA) (85:15) matrices.

The matrices were obtained by solution casting method from L-PLGA (100000 Da) (85:15). Matrices (5 wt-% of RSP, with the diameter of 10 mm, thickness of 0.6273 mm ± 0.0196, n=3) were degraded in phosphate buffered saline (pH 7.4) at 37ºC under constant agitation.

The release rate was determined by high-performance liquid chromatography (VWR Hitachi, Merck). The theoretical profiles according to the Higuchi, Korsmeyer-Peppas equation were used. The data were processed by means of software package Excel 2013 and Visual basic 5.0.

RSP was released for 215 days and the cumulative amount was 2009.74 µg ± 24.49 (n=3). The fraction of initial release was 1.53 % ± 0.24. The loss of dry weight of matrices followed exponentially with a constant k = 0.0104 1/day. The total fraction of released RSP was the sum of initial release, relaxation-induced drug dissolution release and diffusion controlled release. The correlation coefficient of the Higuchi equation R2 = 0.89 ± 0.002 and the values of release exponent of the Korsmeyer-Peppas equation n > 0.89 indicate that drug release occurs by both swelling and erosion. The proposed models are adequate for the interpretation of RSP release form L-PLGA (85:15) matrices.

This work was financially supported by the National Centre for Research and Development, grant RYSPCONT no. PBS1/A7/2/201.

17:25 Poster 112

Preliminary studies on application of library of artificial receptors for differentiation of metabolites in urine of healthy and cancer mice

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Metabolites are small molecules necessary for the maintenance, growth and normal function of a cell. The term metabolome refers to the complete set of metabolites in an organism. Metabolomics and metabonomics cover all techniques to identify and quantify all metabolites in a biological system, as well as the monitoring of changes in the metabolome of a biofluid, cell culture or tissue [1]. Metabolomics carries a huge potential for early diagnosis, therapy monitoring and for understanding the pathogenesis of many diseases [2]. Despite of the extensive progress in this field, there is still a great need for development of new non-invasive diagnostic methods. The microarrays have emerged as one of the most prominent and revolutionary technologies currently available for multiplexed detection. Peptide microarrays, usually synthesized by SPOT technology, are gaining prominence as a vital tool for high-throughput screening. This techniques are most commonly applied in epitope-mapping, substrate profiling and probing peptide-ligand interactions [3].

In our studies, we tried to apply the new type of microarrays formed by self-organization of N-lipided peptides immobilized on the cellulose for systematic searching the differences in the composition of the body fluids of healthy and malignant tumor bearing mice. The arrays of N-lipidated peptides are prone to formation of monolayer of highly organized structures able to molecular recognition. These structures very efficiently recognize the shape, size, polarity of ligands [4]. As the recognition of ligands depends on the structure of immobilized peptides fit is assumed that appropriately designed peptidic fragments allow selective binding of cancer markers from body fluids. In this preliminary studies urine was used and the different profile of binding was observed for healthy and malignant tumor bearing mice.

Acknowledgement:

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17:25 Poster 113

Attempts to application of libraries of N-lipidated amino acids and peptides immobilized on cellulose as a stereosectors

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Differentiation of enantiomers remains one of the most attractive and important research areas due to its impact on pharmaceutical, chemical, biotechnology, and food industries. The development of fast and reliable chiral sensors remains a challenge to achieve on-line analysis of enantiomers [1].

In our studies we found that structures formed by self organization of *N*-lipidated amino acids [2] and peptides [3] immobilized in a regular pattern on a cellulose surface *via* triazine linker are able to recognize the shape and properties of ligands, and then selectively bind guest molecules matching the requirements of the binding pocket. Our previous studies documented that the process of binding guest molecules is reversible and competitive.

Due to the conformational flexibility of both interacting partners, the relative direction of the functional groups of a ligand as well as that of the binding pockets could be readjusted to the most energetically favored orientation of both counterparts. Therefore, it has been expected that ligand binding profile to the receptor pocket formed from chiral *N*-lipidated peptides immobilized on chiral solid support (cellulose) will be depended on the configuration of the ligand. To check the versatility of artificial receptors formed by self-organization of *N*-lipidated amino acids and peptides immobilized on cellulose as a stereosectors it has been compared binding profile for both enantiomers of Aib-Ala. It has been found that binding pockets of artificial receptors are able to differentiate configuration of docked molecules.

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17:25 Poster 114

Serotonin transporter inhibitors inducing hypothermia at mice

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During our work concerning new antidepressive drugs we obtained several serotonin transporter inhibitors of nanomolal affinity [1]. During behavioural experiments on mice some of the compounds exhibited moderate activity at the force swim test and induced hypothermia. Similar behavioural profile exhibit citalopram, clinically used serotonin selective reuptake inhibitor (SSRI, it is assumed that hypothermia as a part of serotonergic syndrome is conveyed by the 5-HT_{1A} and 5-HT_{1B} receptors) [2]. One of our compounds (AZ-07) although did not evoke hypothermia, amplified substantially 8-OHDPAT (the referenced 5-HT_{1A} receptor agonist) induced hypothermia. It should be noted that another SSRI, fluoxetine, reversed 8-OHDPAT induced hypothermia [3]. Because our compounds did not exhibit any substantial 5-HT_{1A} receptor affinity one could assumed off-target of allosteric effects.

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17:25 Poster 115

The measurement of the radioactivity of ^{137}Cs in materials of plant origin with potential radioactive contamination

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The appearance of radioactive isotopes in the natural environment is very dangerous phenomenon leading to radioactive contamination. The main causes of radioactive contamination include: improper storage of radioactive materials, radioactive leakage from nuclear installations, emissions of radioactive gases as a result of the explosion at the nuclear power plant and falling of radioactive substances from a cloud of radioactive nuclear explosion after a bomb explosion. To acquire raw materials from such environment and using them in various industries can have a disastrous impact on human life. One such example is the acquisition of plants and herbs for the preparation of medicinal products. This situation may occur when the plant material is derived from radioactively contaminated areas. This applies mainly to the isotope ^{137}Cs. Half-life of this isotope is long enough to be deposited in the soil and plants for many years. Trial nuclear explosions in the atmosphere have caused widespread presence of fission and activation products in the environment. It is estimated that about 9,6 x10^{17} Bq of ^{137}Cs has been put into the atmosphere, with 76 % of it deposited in the northern hemisphere .

The spectrum of gamma radiation and radioactivity of ^{137}Cs in plant medicines available in pharmacies and plant products of unknown origin were measured. The aim of our interest comprised plant materials such as dried bilberry fruit, red blueberry and swamp cranberry. In addition, the dose of radioactivity of dried mushrooms obtained from south-east Poland (Bieszczady Mountains, Solska forest) was estimated as well.

17:25 Poster 116

The measurement of antioxidant capacity and polyphenol content in selected food supplements

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Oxidative stress (OS), defined as a disturbance in the balance between the production of reactive oxygen species (ROS) and antioxidant defenses, can result in development of many serious diseases like diabetes or cancer. Moreover, the role of oxidative stress

in the acceleration of aging process is also confirmed. ROS are constantly produced in the natural biochemical processes, mainly during cellular respiration. Their enhanced production may be the result of e.g. inappropriate diet high in saturated fats, low in fiber, fruits and vegetables, insufficient physical activity or smoking. To prevent oxidative stress, besides life style changing, the additional supplementation of antioxidants is proposed. On a Polish market the number of food supplements with declared antioxidant activity still increases. However, their antioxidant properties are rarely confirmed experimentally.

The aim of our study was to determine the antioxidant potential of selected dietary supplements available on the market and recommended in chronic fatigue syndrome. The antioxidant potential was measured using four methods: FRAP, ORAC, HORAC, EPR/DPPH. Moreover, the content of polyphenols in the dietary supplements was also determined.

17:25 Poster 117

Antiproliferative activity of inositol hexaphosphate against human skin melanoma cells

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Human malignant melanoma is a highly metastatic tumor with poor prognosis. The majority of metastatic melanomas are resistant to diverse chemotherapeutic agents. Consequently, the search for novel antimelanoma agents continues. In recent years, the interest in medicinal plants and their biologically active constituents as a source of novel potential drugs significantly increased. Inositol hexaphosphate (IP6) is a naturally occurring compound that has been shown to inhibit the growth of a wide variety of tumor cells in multiple experimental model systems.

The aim of this study was to evaluate the antiproliferative and cytotoxic influence of inositol hexaphosphate on melanotic melanoma cells *in vitro*. The A2058 cells used as a model of human skin melanoma malignum were exposed to various concentrations of IP6 (0,1-5 mM) for a various period of time and their growth was determined by sulforhodamine B assay after 24, 48 and 72 h. The cytotoxicity of IP6 was measured at 24 and 72 h by XTT assay.

IP6 has been found to cause dose-dependent growth suppression of A2058 melanoma cells. At low concentrations (0,1 and 0,5 mM) it did not exert any influence on the proliferation of cells as compared to control cultures. Higher concentrations of IP6 (from 1 to 5mM) had a statistically significant, suppressive effect on cell proliferation after 24 hour incubation. When the experimental time period was increased up to 72 h, statistically significant inhibition of cell proliferation was monitored at all IP6 concentrations used. Data obtained from XTT assay indicated that IP6 had dose- and time-dependent cytotoxic effect on melanoma cells.

The results demonstrated the potent antiproliferative and cytotoxic properties of inositol hexaphosphate in a wide range of concentrations on human A2058 melanoma cells. Hence, it can be suggested that IP6 could have a potential therapeutic significance in treating cancer.

17:25 Poster 118

The antiproliferative effect of pterostilbene on colon cancer cells in vitro

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Colon cancer remains the second leading cause of cancer mortality in Poland in the last years. Epidemiological studies showed that dietary phytochemicals may exert chemopreventive and therapeutic effect against colorectal cancer. There is a growing interest in identifying new biologically active agents from dietary sources in this respect. Pterostilbene (trans-3,5-dimethoxy-4-hydroxystilbene) is a naturally occurring stilbene, that has been found to have antioxidant, anti-inflammatory and antiproliferative properties. The greater bioavailability of pterostilbene than other stilbenes indicated that it could be potentially developed for clinical applications. Recent studies showed that pterostilbene exhibits the hallmark characteristics of an effective anticancer agent based on its antineoplastic properties in several common cancers.

The aim of this study was to analyze antiproliferative and cytotoxic effects of pterostilbene on human colon cancer cells Caco-2. They were cultured using standard techniques and exposed to increasing doses of pterostilbene (5-100 mM) for 48 and 72 h. Cell proliferation was determined by sulforhodamine B assay. The growth of treated cells was expressed as a percentage of untreated control cells. Pterostilbene decreased proliferation rate of Caco-2 cells in a dose- and time-dependent manner. Its concentrations $\leq$ 25 mM did not affect cell growth for 48 hours treatment. Significant growth inhibition was observed in cell cultures incubated with higher concentrations of pterostilbene (40-100 mM). The prolongation of cells exposure to stilbenoid at all concentrations used (5-100 mM) up to 72 h resulted in their considerable growth inhibition. The maximum growth reduction was observed at 100 mM pterostilbene (73-78% of control).

The findings of this study showed significant antiproliferative effects of pterostilbene against human colon cancer cells *in vitro*.

17:25 Poster 119

The dissolution model and comparison of the analytical methods used for the assay of sunitinib malate released from capsules

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The purpose of this study was to optimize the dissolution model of sunitinib malate released from gelatine capsules and then to quantify the released amount of the active substance by the appropriate

HPLC and spectrophotometric analytical methods.

The study was carried out on the reference capsules Sutent 12,5 mg, 25 mg and 50 mg manufactured by Pfizer Ltd.

The dissolution was performed using the USP apparatus 1, with baskets rotating at 100 rpm and a 900 ml of the dissolution medium.

The dissolution conditions were optimized through the appropriate choice of a dissolution medium. It was not possible to achieve the sufficient dissolution level using hydrochloric acid (0,1 mol/l) as the dissolution medium, although sunitinib malate is not difficult to dissolve in water. The addition of a surfactant to the dissolution medium was required. The final choice of different surfactant amounts was made by comparing the dissolution profiles. The dissolution profiles were developed in the range from the time point of 5 minutes to the point when over 85% of the active substance was released and later compared in relation to the results obtained by the HPLC and spectrophotometric methods.

Two analytical methods - the selective and not highly time-consuming reversed-phased HPLC and the spectrophotometric one were developed for the quantitation of the amount of the active substance released from the capsules. Both methods were validated in accordance with the International Conference on Harmonization (ICH) requirements.

Both methods can be used to assay the amount of sunitinib malate released from capsules.

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17:25 Poster 120

The comparison of the stability indicating two HPLC methods and their application for the determination of bosentan in coated tablets

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Nowadays, there is an increasing need for fast HPLC separations characterized by high efficiency and good resolution. The ultra-high pressure liquid chromatography (UHPLC) has been considered suitable to meet this challenge, but it was found that this method also has serious disadvantages. Currently, the range of applications of the UHPLC in the analysis of pharmaceutical substances and dosage forms is discussed in the literature [1- 2]. In this study we investigated the consequences of shortening the analysis time. Bosentan, a non-peptide antagonist of human endothelin receptors, was chosen as an example due to its therapeutic importance and lack of analytical methods described. Two high-performance liquid chromatography methods were compared, both in the reversed phase, with UV detection at 220 nm by performing the validation of the methods and comparing the resulting performance characteristics. The first separation (method A) has been achieved on Kinetex column, 2.6m C18 100A, 150 x 4.60 mm, the second – fast (method B) employed Kinetex column, 1.7m XB-C18 100A 50 x 3.0 mm. Both methods were performed with a buffered mobile phase containing 0.1% of triethylamine (TEA) in water brought to the pH 2.5 with phosphoric acid and methanol as the solvent A and acetonitrile as the solvent B. Gradient program was used and flow rate 0.8 ml/min and 0.4 ml, for the methods A and B respectively. The methods were validated according to the ICH guidelines for specificity, precision on the specified and LOQ limits, intermediate precision, accuracy, linearity (correlation coefficient=0.999) and robustness. The robustness was confirmed using four factors: the mobile phase pH, the flow rate of the mobile phase, column temperature and the other batch of the column. The limits of detection and quantification were established. Both validated methods fulfilled the acceptance criteria. The method B was 3.5 times faster than the method A, but the method A showed much better sensitivity (LOQ 0.0132 and 0.1505 mg/mL for method A and B, respectively) and resolution (R_s between compound B and bosentan 3.39 and 1.75 for method A and B, respectively). This lowered sensitivity limits the utility of the method B, especially in the analyses of low levels of active substances (e.g. bioanalysis, validation of the cleaning procedures).

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17:25 Poster 121

Characterization of dutasteride polymorphic forms

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It is well recognized that the active pharmaceutical ingredient (API) can exist in different crystalline forms, and therefore differ in their bioavailability, stability and physical properties. Characterization of polymorphism of drug substances is required by the ICH Q6A guideline [1].

Dutasteride is a 5α-reductase inhibitor. It is mainly used in treatment of benign prostatic hyperplasia (BPH) and prostate cancer [2]. Dutasteride is also used in treating the male pattern hair loss [3].

The patent and scientific literature reveal two polymorphic forms of dutasteride known as form I [4,5] and form II [5,6], form III called hemihydrate [7,8] as well as the amorphous dutasteride [5].

We present physicochemical characterization of dutasteride polymorphs, involving analytical techniques such as X-ray powder diffraction (XRPD), IR and Raman spectroscopy as well as thermal analysis (differential scanning calorimetry and thermogravimetry). These studies proved that form II is a pseudopolymorph. Additional XRPD temperature measurements showed phase transitions in the dutasteride forms II and III.

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17:25 Poster 122

Quantitative analysis of triterpenic acids in Viscum album from various hosts

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Viscum album L. (*Viscaceae*) known as mistletoe is evergreen semi-parasitic plant which grows on various hosts. Mistletoe preparations have multiple applications, e.g. as antiatherosclerotic, sedative, anticancer and analgestic remedies. Moreover, ethanol extract is used as a hypotensive drug. The anti-inflammatory effect was also reported in literature [1,2].

Pentacyclic triterpenes belonging to common secondary plant metabolites have strong anti-inflammatory activity and can influence on biological properties of *V.album* [3].

In our paper, the content of two triterpenes: olanolic (OA) and betulinic (BA) acid in *V.album* of different origin was determined by HPLC-DAD method. The separation was achieved on RP 18 column at 1ml/min^{-1} flow rate and at temperature of 10°C. Acetonitrile, water and phosphoric acid (80:20:0.5 v/v/v) were used as a mobile phase. The established equation of calibration curve (y = 62898x+17711 for BA and y = 55925x+126773 for OA) and the other validation parameters: correlation coefficient (r □ 0.9999) and precision (RSD values ranged from 0.01% to 2.2%) were found to be satisfactory for the proposed method. As showed our investigation, the amount of both acids differed significantly depending on host. It ranged from 0,36 ng/g (host: *Sorbus aucuparia, Malus John Downie*) to 1.57 ng/g (*Tilia cordata*) for BA and from 3,11 ng/g (*Malus John Downie*) to 9,56 ng/g (*Malus Jonathan*) for OA.

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17:25 Poster 123

The phenolic acids content and antioxidant activity of Cimicifuga racemosa

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Cimicifuga racemosa (L.) Nutt. (black cohosh) is a perennial herb with a diverse and long history of medicinal use. The roots and rhizomes are traditionally employed to treat a variety of disorders, e.g. malaria, rheumatism, sore throat, menstrual irregularities and for the relief of menopause-related symptoms [1,2]. The two groups of secondary metabolites: triterpene glycosides and phenolics compounds are the main bioactive constituents of black cohosh [3]

In the present investigation, the content of phenolic acids in two varieties: *C. racemosa (L.) Nutt. var. racemosa* and *C. racemosa (L.) Nutt. var. cordifolia (Pursh) Gray* was determined by HPLC method in reversed-phase system. Analysis was performed on C18 column using mixture of acetonitrile, water and trifluoroacetic acid as a mobile phase

Moreover, the antioxidant activity of metanolic extracts from roots and rhizomes of *C. racemosa* was investigated. DPPH test with ascorbic acid as a references compound was used for the assay.

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17:25 Poster 124

Synergistic antiproliferative effect of valproic acid and 5,7-dimethoxycoumarin against A2058 human melanoma cells

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Melanoma is one of the most malignant tumours of a dangerous high incidence and high metastatic potential. It grows quickly and in an advanced stage is resistant to radio-, chemo- and immunotherapy, which makes it difficult to cure. Therefore, research efforts are focused on the development of new therapeutics or chemopreventive strategies [1]. One of the histone deacetylase inhibitors - valproic acid (VPA) – appears to be very promising new anticancer agent with pro-apoptotic, antiproliferative, and differentiation-stimulating properties. The natural compound, 5,7-dimethoxycoumarin (DMC), also has chemopreventive potential due to its pro-differentiation activity. Probably the differentiation activity of these compounds is associated with activation of the neoplastic cell differentiation inducing genes and the interference with the mitogen-activated protein kinase signalling pathway [2, 3].

The aim of the study was to investigate whether the VPA and DMC has a synergistic antiproliferative activity against A2058 human melanoma cell line.

A2058 cells were cultured on Minimal Essential Medium in standard conditions. Cells were cultured in 96-well plates (initial density 10^3 cells/well) for 24 h. Subsequently, the cells were treated with VPA (concentration range: 0.1–10 mM), DMC (10–500 μM), and a combination of 1 mM VPA with 10, 50, 100 or 150 μM DMC. The effect of VPA and DMC on the cell proliferation was measured using the sulforhodamine B based *In Vitro* Toxicology Assay Kit (Sigma–Aldrich) after 72 hours of incubation.

Pronounced antiproliferative effect was observed when using 1 mM of VPA or 50 μM of DMC. Slightly synergistic effect was observed in the case of combined use of 1 mM VPA and lower concentrations of DMC (10 and 50 μM).

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17:25 Poster 125

The effect of sulphasalazine and 5-aminosalicylic acid on the human colon myofibroblasts

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Sulfasalazine (SAS) is a drug commonly used in the treatment of inflammatory intestinal diseases. In addition to its bacteriostatic, anti-inflammatory and immunosuppressive action, it greatly reduces the risk of neoplastic lesions of the colon and rectum in patients suffering from ulcerative colitis. Sulfasalazine therapeutic effect results from pharmacological activity of 5-aminosalicylic acid (ASA), the metabolite of SAS formed in the reaction catalysed by bacterial azoreductases. The number of myofibroblasts, the cells involved in organogenesis, growth and differentiation of the intestinal epithelial cells, mucosa regeneration as well as in fibrosis and carcinogenesis process, significantly increases in the course of inflammatory bowel diseases.

The aim of the study was to evaluate the effect of SAS and its active metabolite (ASA) on the viability of colon subepithelial myofibroblasts.

The tests were performed on normal human myofibroblasts CCD-18Co derived from the American Type Culture Collection (ATCC, Manassas, VA). Cells were cultured in Minimal Essential Medium supplemented with 10% fetal bovine serum (FBS), 100 IU/ml penicillin G and 100 mg/ml streptomycin (Gibco) and 10 mM HEPES. Cell were cultured at 37°C in a 5% CO_2 atmosphere. Cytotoxicity of SAS and ASA against myofibroblasts was evaluated with XTT (Toxicology *In Vitro* Test Kit, XTT based, TOX-2, Sigma). Cells were seeded on 96-well plate at density of 3000 cells/well. Subsequently, they were cultured in the presence of 0.5 mM, 1 mM, 2.5 mM, 5 mM and 10 mM ASA, and 0.05 mM, 0.1 mM, 0.25 mM, 0.5 mM and 1 mM SAS for 72 hours at standard conditions. Myofibroblasts were incubated with XTT solution for 4 hours and absorbance was measured at 450 nm (reference 690 nm) using a plate reader.

It was found that 1 mM SAS and 5 mM ASA decreased the number of myofibroblasts as compared to the control, which could be explained by the cytotoxic action of these compounds against investigated cells.

17:25 Poster 126

LC-MS/MS method's development for detection of selected β-adrenolytic drugs metabolites in surface water

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Consumption of pharmaceuticals in Poland is fairly high in comparison to other European countries. The most often prescribed are cardiovascular drugs. In recent years high concentrations of cardiovascular drugs were detected in municipal wastewaters and surface waters. Due to quite high hydrophilic properties of these drugs, apparently they are not completely eliminated during wastewater treatment processes. In a consequence they reach surface waters and tap waters, making a real threat to aquatic organisms and humans, respectively. Consequences of chronic and constant exposure to low concentration of active compounds with similar mode of action remains unknown. Unlikely to parent compounds, the presence of cardiovascular metabolites is rarely investigated in surface waters. The main reason for that is their low commercial availability or high prices of standards.

The goal of this paper was to develop the method for detection of metabolites of selected
β-blockers: metoprolol, bisoprolol and propranolol. These metabolites were obtained by incubation of parents compounds with S9 rat's liver fraction. The fraction contains cellular cytosol as well as endoplasmic reticulum, where metabolically active enzymes are located. The obtained metabolites were used for development and optimization of the LC-MS/MS method utilized for detection of selected metabolites in surface waters. Solid phase extraction (SPE) was selected to extract metabolites from water matrix. For this purpose Oasis HLB cartridges (Waters) were used which allowed 500-fold samples concentration.

Project was founded by National Science Centre, Poland, grant no DEC-2011/03/B/NZ7/00751.

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17:25 Poster 127

Optimization of cloud-point extraction method for determination of bisoprolol in human plasma by LC-MS/MS

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Beta-blockers are a group of pharmaceuticals frequently used in cardiology, in the treatment of arterial hypertension, ischemic heart rhythm disorders and heart failure. One of the most common prescribed and the safest, especially for patients with bronchial diseases and/or diabetes, and peripheral circulatory disorders is bisoprolol.

There are numerous methods of its extraction from serum or plasma, which were used for sample preparation during pharmacokinetic studies. The most popular is solvent extraction method (LLE), because is fast, inexpensive and require only minimal equipment investment. The alternative method possessing all the advantages is an environmental friendly extraction technique - cloud point extraction (CPE). CPE is based on fact that low surfactant concentrations above the critical micelle concentration can exist as homogeneous isotropic liquid phase which separates into two isotropic phases, both of which contain surfactant but which differ in total surfactant concentration. In the surfactant micellar-rich phase any hydrophobic organic components originally present in the sample will be concentrated and separated to other, more hydrophilic under the given conditions compounds. The aim of this work was to create a fast, simple, accurate and environmental friendly method for determination of bisoprolol in plasma using CPE.

CPE was performed using Trition X-114 as a surfactant and metoprolol as internal standard. The optimization of the extraction included testing of various NaOH concentration, temperature and Trition X-114 concentration. The CPE method was compared to previously reported liquid-liquid extraction. The concentration of extracted analyte was determined using the liquid chromatography-electrospray ionisation-tandem mass spectrometer (LC-MS/MS) method operated under the multiple reaction monitoring mode (MRM).

The experimental results suggest that CPE can be an innovative sample preparation method applied for bisoprolol determination in human plasma.

17:25 Poster 128

Determination of organic volatile impurities in Nepafenac by GC method

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The active substance Nepafenac is used to prevent and treat the pain and inflammation that can occur after an operation to remove a cataract from the eye and also to reduce the risk of macular oedema that can occur after cataract surgery. Nepafenac, is a 'pro-drug' of amfenac (a non-steroidal anti-inflammatory drug). It works by blocking an enzyme called cyclo-oxygenase, by reducing the production of prostaglandins in the eye. Nepafenac can reduce complications caused by eye surgery, such as inflammation, pain and swelling [1].

Several methods to control of residue solvents and reagent in active substance and the starting material purity were developed by using gas chromatography (very important and popular instrumental method in analytical chemistry). Our methods were validated according to the requirement of ICH (International Conference of Harmonization) validation guidelines Q2R1 [2] and guideline for residual solvents Q3C [3].

In our original synthetic route 2-(methylthio)acetamide (NF1A) was used as starting material. Thus, the gas chromatography method with direct injection has been applied to control the quality of this material. The validation of this method (normalization method procedure) includes tests of specificity, system precision, detection limit and range at the area normalization.

Moreover it has been confirmed, that the gas chromatography methods with direct injection is effective technique to control of triethylamine - reagent and NF1A in nepafenac by using limit test procedure. The validation of these methods includes the examination of specificity, system precision and detection limit.
It has been demonstrated, that the gas chromatography methods with headspace injection may be develop to control of residual solvents from the first to the last synthetic step.
Residual acetone and 2-propanol (i.e. solvents from the final step of synthesis) - have been examined by using quantitative test procedure, and the validation of this method includes the examination of specificity and selectivity, system precision, repeatability, intermediate precision, accuracy, linearity, robustness as well as quantitation and detection limit.
Residual solvents from first step of synthesis and the staring material: methanol, toluene, dichloromethane and benzene - potential contaminant of acetone have been examined by using limit test procedure and the validation of these methods includes the examination of specificity, system precision and detection limit.

Acknowledgements:

The study was supported by European Union under European Regional Development Fund No. UDA-POIG.01.03.01-14-068/08 "Innovative technologies of ophthalmic medicines of special therapeutic and social importance".

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[3] ICH Harmonised Tripartite Guideline Impurities: Guideline for Residual Solvents Q3C(R5)

17:25 Poster 129

Influence of cyclosporin A on expression pattern of genes associated with DNA repair in human dermal fibroblasts

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Cyclosporin A (CsA) is a cyclic nonribosomal peptide with immunosuppressive activity. Chronic immunosuppressive medication is associated with time distant side effects and is the cause of the different secondary diseases, including cancers (especially skin cancers). Anomalies in the functioning of DNA repair mechanisms are closely related to the processes of neoplastic transformation [1-3].

The object of this study was to assess the impact of CsA exposure (8 hours, early cell response) on expression of genes associated with DNA repair in human dermal fibroblasts (NHDF).

NHDF cells were obtained from Clonetics (San Diego, CA) and routinely maintained in FBM medium (Lonza, Basel, Switzerland). Using oligonucleotide microarray technique HG–U133A 2.0 (Affymetrix) we compared transcriptional activity of genes associated with DNA repair in NHDF after 8 hours of cells exposition to CsA (c=100 ng/ml) in relation to control cells.

GeneSpring GX fluorescence signals analysis of 1514 probes, which represented the expression of 875 genes selected from the NetAffx Analysis Center database for "DNA repair" query, demonstrated the inhibited expression of 38 probes (p-value < 0.05; Fold Change > 2.0), including: *BRCA1*, *RAD51*, *TOP2A*, *EXO1*, *RRM2*, *CDK1,* and *FEN1*.

The obtained results suggest that CsA can have a silencing effect on DNA repair genes. The risk of skin cancer development during CsA therapy can result not only from immunosuppressive effects of the drug, but is also likely to arise from inhibition of DNA repair pathways.

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17:25 Poster 130

Polyunsaturated fatty acids potentate cytotoxicity of cisplatin in A549 cells

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In normal and tumor cells, polyunsaturated fatty acids (PUFAs) act as intracellular second messengers, which play a role in signaling, proliferation and cell death. PUFAs have selective tumoricidal action and may alter sensitivity of tumor cells to cisplatin (CDDP), a commonly used anticancer agent [1-3].

The aim of this study was to evaluate the influence of arachidonic acid (AA, 20:4 n-6), eicosapentaenoic acid (EPA, 20:5 n-3), docosahexaenoic acid (DHA, 22:6 n-3) and CDDP on autophagy and apoptosis in A549 human lung adenocarcinoma cells.

Viability of A549 cellstreated with CDDP and PUFAs was measured using the XTT tetrazolium salt based assay. Caspase-3/7 activity was estimated using ApoTox-Glo kit (Promega). Autophagic vacuoles were detected by Cyto-ID Autophagy Detection Kit (Enzo). The results were compared to control cultures maintained in the absence of CDDP and PUFAs.

PUFAs, in particular EPA and DHA, added to the cultivation medium, increased the antitumour activity of CDDP in A549 cells in a concentration dependent manner. In case of AA this effect was observed at the highest of the concentrations tested only (100mM). Both, EPA and DHA, but not AA significantly increased the amount of autophagic vacuoles and induced caspase-3/7 activity.

The obtained results suggest that the antiproliferative effect of CDDP in A549 cells can be enhanced by AA and in particular by EPA and DHA through their influence on autophagic and apoptotic cell death. It is likely that EPA and DHA incorporated to the tumour cells may improve outcomes in lung cancer patients.

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17:25 Poster 131

Effect of Cd(II) on free radicals in DOPA-melanin tested by EPR spectroscopy

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Melanins are the large organic compounds, which belong to natural pigments [1]. Melanin biopolymers bind major chemical compounds, especially metal ions, polycyclic aromatic hydrocarbons and drugs [1]. The binding of drugs by melanin biopolymers depends on the presence of free radicals and metal ions in their structure [1-3].
In this work effect of Cd(II) on free radicals in the model eumelanin as DOPA-melanin was examined. Electron paramagnetic resonance (EPR) spectroscopy was used as the experimental method. EPR spectra of DOPA-melanin, and DOPA-melanin-Cd(II) complexes were measured by an X-band (9.3 GHz) EPR spectrometer of Radiopan Firm (Poznań, Poland) and the Rapid Scan Unit of Jagmar Firm (Kraków, Poland). Synthetic DOPA-melanin was formed by the autooxidative polymerization of 3,4-dihydroxyphenylalanine (DOPA). The metal ions/DOPA molar ratios in the reaction mixtures were 2:1, 1:1 or 1:2. Free radical concentration in DOPA-melanin was high. Formation of melanin complexes with Cd(II) increased free radical concentration in DOPA-melanin. For the samples DOPA-melanin-Cd(II) (2:1) and DOPA-melanin-Cd(II) (1:1) free radical concentration increased with the increasing of Cd(II) in melanin. Free radical concentration in DOPA-melanin-Cd(II) (1:2) complexes were lower than for DOPA-melanin-Cd(II) (1:1) complexes. This effect was the result of free radical recombination during formation of these samples. g-Values characteristic for o-semiquinone free radicals were obtained. Broad EPR lines were measured. Linewidths increased after Cd(II) binding to DOPA-melanin. Changes of amplitudes and linewidths with microwave power shown the homogeneous broadening of EPR lines of DOPA-melanin complexes with Cd(II), independently on the metal ion concentration. Slow spin-lattice relaxation processes existed in all the tested samples, their EPR lines saturated at low microwave powers. Cd(II) caused fastening of spin-lattice relaxation processes in DOPA-melanin. The results are very important for evaluation of drugs binding to eumelanin in the presence of Cd(II).

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17:25 Poster 132

EPR studies of free radicals in A-2058 human melanoma cells treated by valproic acid and 5,7-dimethoxycoumarin

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Electron paramagnetic resonance (EPR) examination pointed out the strong effect of valproic acid (VPA) and 5,7-dimethoxycoumarin (DMC) on free radicals in A-375 and G-361 human *melanoma malignum* cells [1].

In this work changes in free radical concentrations in tumor A-2058 cells after treatment by VPA and DMC cells were tested, and compared to those for A-375 and G-361 cells. Human *melanoma malignum* A-2058 cells were exposed to interactions with VPA, DMC, and both VPA and DMC. The malignant melanoma A-2058 cell line were purchased from LGC Prochem (Lomianki, Poland), and they were grown in the standard conditions: at temperature 37 ^{o}C and in an atmosphere containing 95% air and 5% CO_2, in the Minimum Essential Medium Eagle (MEM, Sigma-Aldrich). The cells were incubated with VPA (1 mM) and DMC (10 μM) for 7 days. Free radicals in A-2058 cells were studied by an X-band (9.3 GHz) EPR spectroscopy. The first derivative EPR spectra were measured for the original A-2058 cells, and the cells cultured with VPA, DMC or VPA and DMC together. The following parameters of the EPR spectra: amplitude, integral intensity, linewidths, and g-factor, were analysed. The effect of microwave power in the range of 2.2-70 mW on the EPR spectra of the cells was evaluated. Free radical concentrations were determined via double integration of the spectra, as the values proportional to the area under the absorption lines. o-Semiquinone free radicals of melanin biopolymer are mainly responsible for the EPR lines of *melanoma malignum*

A-2058 cells. The changes in free radical system in A-2058 cells caused by the tested substances (VPA, DMC) and their application as the antitumor agents were discussed. The usefulness of electron paramagnetic resonance spectroscopy in the study of free radicals in tumor cells treated with drugs was confirmed.

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17:25 Poster 133

Synthesis of a new series of N-acylbenzenesulfonamide derivatives with potential anticancer activity

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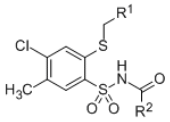
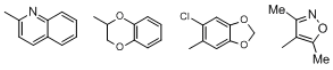
N-Acylation of sulfonamides is an important transformation used for lead optimization as well as lead generation. *N*-Acylsulfonamides have diverse pharmacological activities including antiproliferative activity as cyclooxygenase [1], tubulin [2] and cyclin-dependent kinases [3] inhibitors. We have recently reported that some *N*-acylsulfonamides exhibit antiproliferative activity against broad spectrum of cancer cell lines [4].

The aim of presented work was the synthesis of a series of new *N*-acylbenzenesulfonamides with potential anticancer activity. New compounds were obtained in the one-step reaction of *N*-[4-chloro-5-methyl-2-(R^1-methylthio)benzenesulfonyl]cyanamide potassium salts with appropriate carboxylic acids (acetic, propionic, isobutyric, cycloheksylopropionic acids and solution of trans-cinnamic or benzoic acids in water) at reflux. The structure of compounds was confirmed by IR, ^{1}H NMR, ^{13}C NMR spectroscopy and by elemental and X-ray structure analyses.



R^1 = CH=CH$_2$, C≡CH, Ph, 3-CF$_3$Ph, 4-CF$_3$Ph, 4-ClPh, 4-MeOPh, 4-pirydyl, 1-naphthyl, 2-naphthyl,



R^2 = Me, Et, iPr, Ph, PhCH=CH 

Anticancer *in vitro* screening was performed at the Department of Biotechnology, UG – MUG on three cell lines of breast (MCF-7), colon (HCT-116) and cervix (HeLa) cancer, and at the National Cancer Institute (Bethesda, USA) on panel of 60 cell lines derived from 9 types of human tumors. The prominent compounds *N*-(4-chloro-5-methyl-2-naphthalen-1-ylmethylthiophenylsulfonyl)acetamide and *N*-(4-chloro-5-methyl-2-naphthalen-1-ylmethylthiophenylsulfonyl)cinnamideshowed the highest antiproliferative activity toward MCF-7, HCT-116, HeLa cell lines with IC_{50} in the range of 6-96 μM.

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17:25 Poster 134

Inhibition of cell proliferation by novel pyridine platinium(II) complexes on the Ishikawa endometrial cancer cell lines

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The aim of this study was to compare the effect of cisplatin and novel dinuclear platinum(II) complexes with berenil and pyridine ligands on the Ishikawa endometrial cancer cell line. The chemical formula is $[Pt_2L_4(berenil)_2]Cl_4$, where L is 3-ethylpyridine (Pt10), 3-(n-butyl)pyridine (Pt11), 4-ethylpyridine (Pt12) and 4-(t-butyl)pyridine (Pt13). The results showed higher cytotoxicity of Pt10-Pt13 compounds in comparison with cisplatin on the Ishikawa endometrial cancer cell line. In relation to human skin fibroblasts, Pt10-Pt13 compounds were characterized with lower cytotoxicity than in case of examined neoplastic cells. Moreover, it was shown that all examined compounds Pt10-Pt13 inhibit DNA biosynthesis and collagen biosynthesis in the Ishikawa endometrial cancer cell line stronger than in human skin fibroblasts. The influence of berenil complexes of Pt10-Pt13 on apoptosis induction in the cancer cell lines was evaluated with the use of the flow cytometry and the fluorescent microscope. The Pt10-Pt13 compounds showed higher ability to induce apoptosis in the Ischikawa endometrial carcinoma cells in comparison with cisplatin. The results suggest that apoptosis of cells in the presence of Pt10-Pt13 follows the mitochondrial pathway, with the decrease in mitochondrial membrane potential and activation of caspase 9, as well as by the external pathway with the significant increase in FADD protein expression and caspase 8. The determination of caspase 3 activity with the flow cytometry using the antibody recognizing the active form of this protein in the process of apoptosis showed that caspases cascade was started, particularly effector caspase 3, engaged in the executive phase of apoptosis. The obtained results in the present study demonstrated the cytotoxic activity of new berenil complexes of platinum(II) can be connected with their ability to impair of DNA biosynthesis and induction of apoptosis.

17:25 Poster 135

Synthesis of 1'-(saccharide)substituted 28-O-(1'H-[1',2',3']triazolyl-4'-methyl) derivatives of protoescigenin

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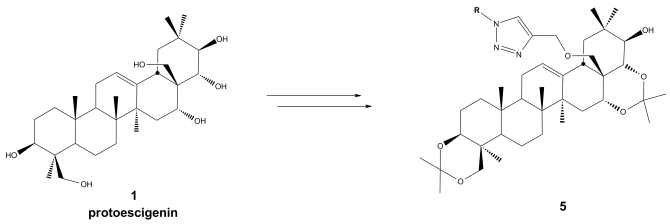
Selective functionalization of protoescigenin [1,2]: 3,16,21,22,24,28-hexahydroxy-olean-12-ene **1**, either directly, or by application of protective group strategy, proved unexpectedly difficult. Since various approaches to glycosylation of **1** or its partially protected derivatives have failed [3,4,6], we have decided to use chemical ligation with sugar containing synthons, based on click chemistry (CC) protocols which involve alkyne – azide 1,3-dipolar cycloaddition reaction [5,7]. Triazoles obtained in such reactions feature characteristics, which are considered favorable from drug design point of view: chemical and metabolic stability, polarizability, hydrogen bond accepting properties, and predictable influence on logP and pK values. Protoescigenine diacetonide 28-O-propargyl ether, obtained by modified classical Williamson method was used as an alkyne component, while azides varied from substituted benzoic acid to mono and disaccharides, with azido substituent placed either in the pyranose ring or in an alkyl aglycone part. Various conditions for CC reaction were tested, including influence of Cu(I) and Cu(II) salts promotion. Obtained triazoles were purified by SiO_2 column chromatography and characterized by standard spectroscopic methods.



Acknowledgement:

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Biesiada grillowa
Tuesday evening, 13 May, 19:00

Wednesday, 14 May

Śniadanie
Wednesday morning, 14 May, 7:00

Sesja wykładowa VIII
Wednesday morning, 14 May, 8:30
Sala konferencyjna
Chair: K.Woźniak, A.Lipkowski

8:30 Invited oral

Biologistics in bacteria: protein motion and gene regulation

Robert Hołyst

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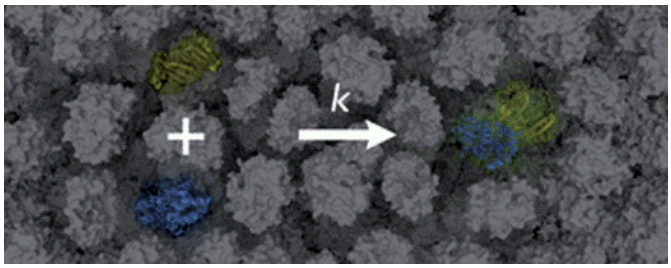

Biologistics and biochemistry in a crowded environment are two emerging interdisciplinary fields of science. They provide quantitative analysis of mobility of proteins and their interactions involved in gene expression and regulation. I will discuss the mobility of small ligands, proteins and plasmids in cytoplasm of E.coli HeLa and Swiss 3T3 cells [1, 4] and in complex liquids [1-3]. I will explain why proteins move so fast in seemingly highly crowded environments (e.g. cell nucleus, mitochondria or cytoplasm of bacteria). Most proteins (of sizes below 5 nm) have the same mobility in the cytoplasm of eukaryotic cells as in water [1]. Therefore their association rate does not differ much from the one measured in vitro (in a buffer) [5, 6]. For the first time such association of two freely diffusing proteins in a cytoplasm of HeLa cell was measured in 2012 [6]. I will also provide specific-site searching time for 180 known transcription factors in E.coli and discuss the results from perspective of facilitated target location and mobility in living cells [4, 7, 8, 9, 10]. Finally I will give a simple prescription for quantitative analysis of interactions between proteins and macrostructures inside living cells based on their mobility data [10].

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9:15 Invited oral

Rare diseases in diagnosis, medical practice and therapy

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The term «rare diseases» was introduced in the seventies of the last century, especially in the field of hereditary metabolic diseases or inborn error of metabolism. The European Union defines rare diseases as those with a prevalence of less than 5 per 10,000 inhabitants. According to that definition, an estimated 30 million people in the EU-27 are affected.

It is estimated that about 90% of rare diseases are genetic, mainly monogenetic.

Despite having quite low prevalence separately, together they represent a fairly significant group. Enormous progress in diagnostic possibilities made rare diseases an important issue in medicine.

Diversity is an intrinsic characteristic in a group of rare diseases. The nature of the pathological processes varies from diseases that affect a single organ system to multisystem diseases. The people who suffer from these diseases have, in majority serious, chronic, progressive disorders that can appear at early age as well as in adulthood. Therapeutic options for rare diseases are scarce and not very effective. That care must be considered in a context of global management, involving pediatrics and the specialties that understand the specific clinical problems, nursing and physiotherapy, social services and psychological support.
Rare diseases become health, scientific, research and social interest problem.

10:00 Oral

Keratin Associeted Proteins as a New Type of Bandage for Wound Healing

Marek Konop, Piotr Kosson, Dorota Sulejczak, Marta Bochyńska-Czyż, Aleksandra M. Szczucińska, Andrzej W. Lipkowski

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Skin protein structure reconstruction is one of the major factors of skin healing when injured by accidents, suffered by infection or disease degeneration (e.g. diabetes). Unfortunately, the healing processes are very slow. To enhance the healing processes we developed KAP's preparations, which added to healing space, could be easily colonized by migrated epithelial cells. We predicted that KAP's structures should be highly complementary to proliferated cells in the wound and to their keratins. To test this approach we adopted diabetic rodent model for studies of wound healing processes. In this model we tested histology of wound healing processes with or without keratin preparation applied. Mouse fur or human hairs were primary sources of keratin preparations. Developed keratin preparations were characterized by lack of immunogenicity that is the independent from the original source. Keratin dressing has been adsorbed in regenerating tissue during healing process, which eliminates painful and risky stages of dressing replacement. The preliminary animal in vivo results confirm that obtained keratin powders could be used as a part of wound dressing that will be adsorbed in regenerating skin during healing process.

Acknowledgement:

This work was supported by the Polish National Science Center (NCN), 2011/01/B/ST5/07818 as well as Mazowian Peptide Cluster.

Przerwa kawowa

Wednesday morning, 14 May, 10:20

Sesja wykładowa VIII

Wednesday morning, 14 May, 10:40
Sala konferencyjna
Chair: L.Kozerski, J. Wietrzyk

10:40 Invited oral

The molecular basis for therapeutic effectiveness of β-escin

Oliwia Zegrocka-Stendel[1], Dominik Domański[2], Magdalena Kowalewska[1], Dorota Maciejko[1], Anna Perzanowska[2], Katarzyna A. Koziak[1]

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β -escin, which is a mixture of triterpene saponins isolated from of the horse chestnut seeds (*Aesculushippocastanum)* has been traditionally attributed with the anti-edematous, anti-inflammatory and venotonic properties. As such, β-escin is an active ingredient of popular vascular anti-inflammatory and anti-edematous drug formulations (Escin®, Reparil®, Venitan®). However, despite the widespread clinical use, the pharmacological mechanisms of β -escin action remains largely unknown. We therefore aimed to determine the molecular basis for therapeutic effectiveness of β-escin using i) human gene expression microarrays covering all the transcriptional activity of endothelial cells, ii) global proteomic analysis providing mechanistic information on β-escin action and iii) broad panel of cellular responses including migration, proliferation, permeability and apoptosis. We identified several novel pathways responsible for the protective effects of β-escin on the vascular endothelium under inflammatory conditions.

11:25 Oral

Hexenoses in design of glycoconjugates – from chemistry to function

Piotr Krzeczyński[1], Grzegorz Grynkiewicz[1], Wiesław Szeja[2]

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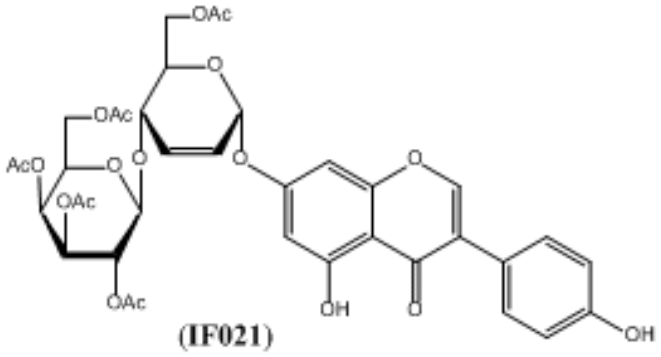
Isoflavones have received a great deal of attention over the last decades as possible drug leads with demonstrated activity against multiple targets. However, their poor bioavailability and quick metabolism result in low efficacy and call for well designed synthetic modifications before potential medicinal application. Our lasting interest in unsaturated pyranosides prompted syntheses of various isoflavone glycosides, exemplified by genistein derivative IF021, containing hex–2–enopyranose moiety.



(IF021)

Although IF021 was designed as genistein pro-drug, and similar effects in cell tests were predicted, it turned out that it has distinctly different biological activity profile than the parent isoflavone [1; 2] with unexpected cytotoxicity related to interference with the mitotic spindle dynamics. Despite our efforts, laboratory synthesis of IF021 proved poorly stereoselective, offering no hope for elaboration of efficient manufacturing process. Considering hex-2-enopyranosides as the new type of saccharide scaffold which may be useful in medicinal chemistry, we have decided to examine synthetic methods of its linkage to selected polyphenolic substrates by several strategies involving Ferrier rearrangement and transition metals promoted glycosylations. Apart from straight isoflavone – glycon bonding, variety of linkers were tried for obtaining

O-linked or *C*-linked glycoconjugates. Generally, all types of unsaturated isoflavone glycoconjugates exhibited specific influence on cell cycle, which warrants more specific pharmacological research.

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Acknowledgement:

This work was supported by the Statutory Fund of Pharmaceutical Research Institute.

11:45 Oral

EPR studies of thermally sterilized Vaselinum Album

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Sterilization process in pharmacy is preformed to kill microorganisms in vaselinum album and as the result to rise the safety of the pharmacotherapy [1]. The popular method of sterilization is the thermal sterilization with hot air circulation in the vaselinum album samples [1]. The parameters of thermally sterilization is determined by pharmaceutical norms[2-5]. Thermal sterilization should not produce the high amount of free radicals in the vaselinum album and their chemical structure should not be destroyed. Free radicals in the sterilized vaselinum album be studied by electron paramagnetic resonance (EPR) spectroscopy. Free radicals and chemical structure of thermally sterilized vaselinum album were examined in this work. The aim of this study is to find optimal conditions of thermal sterilization of the analysed vaselinum album.

Sterilization was performed at temperatures: 160^{o} C (120 minutes), 170^{o} C (60 minutes), and 180^{o} C (30 minutes). The electron paramagnetic resonance spectra were measured at room temperature by the use of an X-band (9.3 GHz) EPR spectrometer of RADIOPAN Firm (Poznań) and the system of numerical acquisition of JAGMAR Firm (Kraków). g-Factors, amplitudes, integral intensities, and line widths of the EPR spectra were analysed. Free radicals concentrations were determined. The effect of microwave power on the line shape and parameters of the EPR spectra was presented.

The original vaselinum album sample was diamagnetic. EPR spectra were obtained for the thermally sterilized vaselinum album. The parameters of the EPR spectra, microwave saturation of the lines, and free radicals concentrations in the heated samples depend on the conditions of sterilization. It was shown that EPR method may be used to optimize the thermal sterilization process of vaselinum album and conditions of its storage.

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12:05 Oral

Influence of structural and surface properties on the release of risperidone from poly(D,L-lactide-co-glycolide) (70:30) matrices

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Only one medical product with a prolonged release of risperidone (RSP) is available at present, i.e. a long-acting injection including encapsulated microspheres of poly(D,L-lactide-co-glycolide) (D,L-PLGA) (75:25). The administration of aqueous suspension may be painful and microspheres cannot be removed upon complications. The aim of this study was to develop an alternative solutions based on implantable formulation composed of D,L-PLGA (70:30).

Matrices containing 10 wt-% (n =10, 10 mm) were prepared by solution casting method from D,L-PLGA (70:30) (460000 Da). They were incubated in phosphate buffered saline (pH 7.4) at 37ºC under constant agitation.

The tests performed included: determination of RSP concentration

by high-performance liquid chromatography (VWR Hitachi, Merck); composition and chain structure by nuclear magnetic resonance spectroscopy (AVANCE II Ultra Shield Plus Bruker 600 MHz spectrometer); thermal properties by means of differential scanning calorimetry (TA DSC 2010 apparatus, New Castle, DE); morphological study by a scanning electron microscope (Quanta 250, FEI Quanta FEG) and atomic force microscopy using MultiMode 3 (di-Veeco, CA).
RSP was detected within the period of 51 days. The cumulative amount was 4498 mg ± 682 (n= 10). The loss of dry weight followed exponentially with a constant k = 0.047 1/day. The incubation led to the decrease of the amount of glycolidyl units and increase in the amount of lactidyl units; the reduction of the average length of glycolidyl and lactidyl blocks; the decrease of glass transition temperature. The copolymer became more random. The morphological study shows a greater morphological differentiation of the incubated samples. The properties of D,L-PLGA (70:30) matrices afford the possibility of obtaining an implantable drug formulation for a prolonged release of RSP.
This work was financially supportedby the National Centre for Research and Development, grant RYSPCONT no. PBS1/A7/2/201.

12:25 Oral

Starch derivatives as vehicles for drug delivery in anticancer therapy

Tomasz M. Goszczyński, Beata Filip-Psurska, Joanna Wietrzyk, Janusz Boratyński

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The search for new, innovative methods of cancer treatment is an ongoing challenge undertaken in the scientific community. A major limitation to the most conventional low-molecular weight anticancer chemotherapeutics is their unprofitable uptake by healthy tissue, fast metabolism and lack of tumor cell selectivity. Solving these problems involves the binding process of drugs with macromolecular carriers. Such structures were designed with the aim to enhance delivery and to improve the selectivity of both conventional and innovative drugs. Research into drug binding with macromolecular carrier systems has joined the search for new therapeutic strategies.

In the present contribution we discusses the potential application of starch derivatives as effective, high molecular, carriers for anticancer, therapeutic substances (e.g. methotrexate, MTX). Nanoparticles composed of a hydroxyethyl starch with a covalently bound MTX(ester bond) were obtained. Physicochemical properties (hydrodynamic size, zeta potential and drug release kinetics) of the obtained conjugates wereanalyzed, characterizing alterations in relation to the starting carrier and analyzing biological implications. *In vitro* biological characteristics were determined using different cancer cell lines. The antitumor effect *in vivo* was tested on NOD/SCID mice subcutaneously inoculated with MV4-11 human leukemia cells.

Obtained conjugates demonstrate differences in physicochemical properties when compared to initial polymer and exhibit high efficacy for the treatment of experimental tumors. Conjugates reduced the volume of MV4-11 tumors (10-fold increase in therapeutic effectiveness) to a significant degree ($P< 0.05$) when compared to the control group and MTX-treated group.

The present observation opens the way for the application of modified starch as a drug carrier, especially for anticancer treatments.

This project was supported by the National Science Centre, Poland (N N302 098434)

Czas wolny
Wednesday afternoon, 14 May, 12:45

Przerwa obiadowa
Wednesday afternoon, 14 May, 13:30

Dyskusja panelowa

Przyszłość MKNOL wśród nowych wyzwań; Ogłoszenie wyników konkursu na najlepsze postery
Wednesday afternoon, 14 May, 14:45

Prezentacje nagrodzonych posterów
Wednesday afternoon, 14 May, 16:00

Zamknięcie konferencji
Wednesday afternoon, 14 May, 17:15

Czas wolny
Wednesday afternoon, 14 May, 17:20

Kolacja pożegnalna
Wednesday evening, 14 May, 19:00

Thursday, 15 May

Sniadanie
Thursday morning, 15 May, 7:00

Odjazd autokarów do Kielc
Thursday morning, 15 May, 9:00

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Index

I

J

K

L

M

N

O

P

R

S

T

W

Z