Book of Abstracts

VIII Multidyscyplinarna Konferencja Nauki o Leku

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Abstrakt

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Welcome

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Programme

Tuesday, 29 May

Kolacja
Tuesday evening, 29 May, 19:00

Wednesday, 30 May

Śniadanie
Wednesday morning, 30 May, 7:00

Sesja wykładowa I
Wednesday morning, 30 May, 9:00
Chair: A. Kutner, R. Ostaszewski

9:00 Invited oral

Pharmaceutical technologies – why do they have to be innovative

Janusz Obukowicz[1], Wiesław Szelejewski, Andrzej Kutner[2]

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An overview will be given of the syntheses of pharmaceutical substances, using representative examples selected from the syntheses completed at the Institute, over the last two decades. The discussion will start from a one step convergent synthesis of antihypertensive dihydropyridin and methods of their isolation and purification, used in middle eighties to get a pharmaceutical substance that meets the purity requirements of that time. Convergent synthesis of a PDE-5 inhibitor will be presented to prove an innovative synthesis that might be competitive with the innovator's process. Linear synthesis of a class IB anti-arrythmic will demonstrate an innovative one step reductive amination, leading efficiently to target molecule. The same linear synthesis was used to construct a molecule of tyrosine kinase inhibitor and obtain the final product in a patent non-infringing polymorphic form. A contribution of the Institute to the technology of anticancer oxazaphosphorinane will also be discussed. New phase-transfer catalytic process was used efficiently to synthesize a selective aromatase inhibitor. Recent convergent strategies for the preparation of vitamin D active metabolites and analogs and also of anti-glaucoma analogs of prostaglandin F_{2a} will be discussed in some more details. The retro-synthetic analysis of prostaglandins from this group resulted in a design of the new advanced intermediate that was lately proved to be useful in a synthesis of not only of a lead analog from this group but also of other two analogs used in a therapy. The concept will be discussed how to approach a problem of diastereomeric purity for a synthetic multi-chiral pharmaceuticals to comply with current very strict regulations in this matter. The advantage of using chiral pool of natural products as raw materials for syntheses will be demonstrated on examples of side-chains of prostaglandin analogs.

All the syntheses discussed were developed in a way required for pharmaceutical substances and this is why they could have been implemented at the Institute and manufactured on an industrial scale for the European pharmaceutical market. Most of the inventive steps have been protected by national or international patents and the technologies were awarded with distinctive national and international prizes. Past and present challenges faced in syntheses of pharmaceuticals will be outlines and successful approaches will be presented that were developed over the years at the Institute.

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3. I. Dams, M. Chodyński, A. Kutner, M. Krupa, A. Pietraszek, P. Cmoch, M. Zezula, Process for the preparation of 13,14-en-15-ol analogs of prostaglandin F_{2a}, IF 932, 2012.

9:45 Invited oral

Pyrimidine nucleoside analogues with appended heterocylic ring and their antiviral properties

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Modification of heterocyclic base moiety of naturally occurring nucleosides by additional ring often results in new interesting physicochemical (e.g. enhanced lipophilicity, fluorescence) or biological properties. It has been shown that transformation of pyrimidine nucleosides into their 6-alkyl-2,3-dihydrofurano[2,3-*d*]pyrimidin-2(1H)-one or 6-alkyl-2,3-dihydropyrrolo[2,3-*d*]pyrimidin-2(3*H,7H*)-one derivatives leads to new bicyclic nucleoside analogues which exhibit antiviral activity. The type of activity, its potency and selectivity, depend on structural factors: (i) structure of the sugar or pseudosugar moiety, and (ii) the length of lipophilic alkyl substituent in the 6-position. In our search for new biologically active nucleosides we have obtained a series of furano- and pyrrolo[2,3-*d*]pyrimidine compounds having as a sugar part 2'-C-β-methyl-β-d-ribofuranosyl substituent, which often increases activity of nucleosides against hepatitis virus type C (HCV). In a related project, we have elaborated transformation of the 6-alkyl-2,3-dihydrofurano[2,3-*d*]pyrimidin-2(1H)-one system into a new class of heterocycles, 6-(β-d-ribofuranosyl)-5,6,7,8-tetrahydropyrimido[4,5-*c*]pyridazine derivatives, in the reaction with hydrazine. The series of new compounds of this type included nucleosides of β-d-ribofuranose, 2'-deoxy-β-d-ribofuranose, 2'-C-β-methyl-β-d-ribofuranose, and acylonucleosides of the Acyclovir type, which were additionally modified by alkyl substituents of different length in the 3-position.

All new nucleoside analogues were tested *in vitro* against a variety

of RNA- and DNA-viruses at Rega Institute for Medical Research, Katholieke Universiteit Leuven, Belgium, and the results will presented.

Ackowledgements: This work was partly supported by Aminoscience Laboratories, Ajinomoto Co. Inc., Kawasaki-ku, Kawasaki, Japan.

Przerwa na kawę

Wednesday morning, 30 May, 10:30

Sesja wykładowa II

Wednesday morning, 30 May, 10:50

Chair: Ł. Kaczmarek, T. Brodniewicz

10:50 Invited oral

Nanotechnolgy in pharmacy and medicine – contemporary status and future perspectives

Aleksander P. Mazurek

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Even though that atomistic theory emerged from the discussions between Democritus and his teacher Leukippos, and was sounded by Dalton, it is a fact that Richard Feynman was the first scientist who suggested that devices and materials could someday be fabricated to atomic specifications, saying that principles of physics, as far as he can see, do not speak against the possibility of maneuvering things atom by atom. Later the term nanotechnology was coined. Today nanotechnology is reshaping technology.

Contemporary nanotechnology was derived from carbon compounds. So far a few allotropes of carbon were discovered with various chemical interest and extent of industrial use. Starting from diamond, through graphite, amorphous carbon, fullerenes, carbon nanotubes, aggregated diamond nanorods, glassy carbon, carbon nanofoam, lonsdaleite and chaoite, graphens those various structural forms were discovered or designed based on classical approach to possible existence of carbon compounds in linear or four, five, six and seven membered rings forms stable as isolated molecules.

Continuous progress in nantechnology creates promising field of new applications in pharmacy and in medicine. Surgical and diagnostic tools will be elegant and cheap. Research and diagnosis will become fairly more efficient. Small medical devices can be probably implanted permanently. Diagnosis and treatment may be semi-automated. Health will improve and life spans increase. Genetic therapy will be facilitated. Some organs will be replaceable. The molecular manufacturing will be a significant breakthrough, comparable perhaps to the Industrial Revolution

Nanotechnology can provide solutions to many current problems in pharmacy and medicine by means of smaller, lighter, faster, and better-performing materials, components, and systems. Nanomaterials are being used for current and future medical developments. The major application fields and fast developing areas are: drug delivery systems, diagnostics aids and regenerative medicine materials. Within the drug delivery systems polymer systems and polymeric micelles (amphiphilic block copolymers), liposomes, dendrimers and transdermal delivery (e.g. nanoneedles etc.) are very promising tools. The diagnostics like device for use in identifying early-stage disease "cellular endoscope', gene 'ignition switch' for potential use in aiding cancer detection and treatment, polymer-caged nanobins, nanocrystals (quantum dots), magnetic nanoparticles (e.g. iron oxide for MRI), nano-scale determination of biomarkers acting as a predictor of deterioration in heart failure became reality. Future of respirocytes, vasculocytes, vasculoid and biologically useful membranes is now emerging. Nanotechnology tackles also borderline products zone.

11:35 Oral

Synthesis and anticancer activity of acetylenic derivatives of betulin

Ewa B. Bębenek[1], Katarzyna Kempińska[2], Joanna Wietrzyk[2], Maria Jastrzębska[3], Joachim Kusz[3], Stanisław Boryczka[1]

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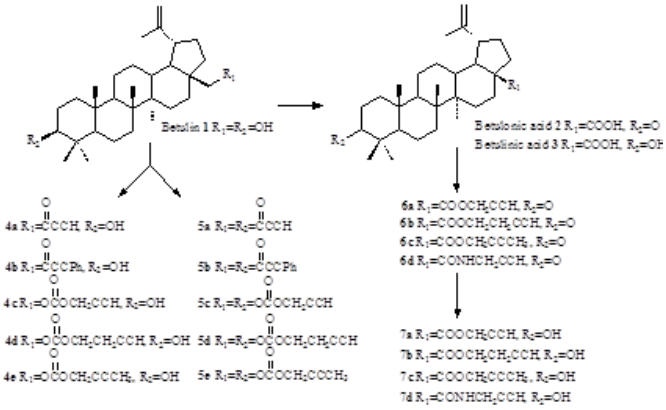
Betulin [lup-20(29)-ene-3β,28-diol, $C_{30}H_{50}O_2$] (**1**), also known as betulinic alcohol, is a pentacyclic triterpene of the lupane type which was one of the first natural products identified and isolated from plants as a pure chemical substance in 1788 by Lowitz [1]. The still growing interest in betulin (**1**) and its derivatives results from their wide spectrum of biological activities such as anticancer, antiviral, anti-inflammatory, antibacterial and hepatoprotective properties [1-3]. 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-20. 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. 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 [1,4]. Despite the fact that the carbon-carbon triple bond is one of the most important functional groups in medicinal and organic chemistry, only a few reports of acetylenic derivatives of betulin have been described.

In this paper we present the synthesis of new derivatives of betulin bearing one or two an acetylenic function at the C-3 and/or C-28 positions. This interest has resulted from the recognition of the value of such compounds in a wide range of biological and chemical synthetic aspects. The starting material (**1**) was isolated from the bark of birch by extraction with dichloromethane. Synthesis of acetylenic derivatives of betulin (**4-7**) has been described in scheme presented below.



The structure of all new compounds (**4-7**) were determined on the basis of their ^{1}H, ^{13}C NMR, IR and MS spectra, together with elemental analyses and for compound **4a** was also confirmed by X-ray crystal analysis. 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. Most of the obtained compound exhibited antiproliferative activity with ID^{50} values ranging from 0.4 to 3.8 μg/ml, comparable to that of cis-platin used as reference compounds.

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[3] T.G. Tolstikova, I.V. Sorokina, G.A. Tolstikov, A.G. Tolstikov, O.B. Flekhter, *Russ J Bioorg Chem***32** (2006) 291-307

[4] S. Alakurtti, T. Heiska, A. Kiriazis, *Bioorg Med Chem***18** (2010) 1573-1582

11:55 Oral

The influence of crenotherapy on glutathione level in human body.

Ewelina M. Błońska-Sikora, Zygfryd Witkiewicz, Jerzy Oszczudłowski

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Glutathione (γ-glutamyl-cysteinylglycine) occurs in several forms in human body. The most important forms are reduced (GSH) and oxidized (GSSG) glutathione. It plays a lot of useful functions in human body and it is one of most important antioxidants.

Characteristic element of glutathione structure is thiol group (-SH) which is responsible for biological functions of this compound. GSH protects organism against reactive forms of oxygen (hydrogen peroxide, organic peroxides), egzogenous endogenous electrophilic substances and also oxidized forms of other antioxidants for example vit. E or C. Glutathione is also presented in specific selenoenzym-glutathione peroxidase protecting organism from hydrogen peroxide and lipid peroxides. GSH antioxidative activity appears also in reduction of thiol group of different amino acids and protect them from oxidation to sulfonic or sulfinic acids and losing their activities.

GSH presents also immunomodulatory effect. This compound participates in cytokine production and humoral immune response and cell-mediated response by antigen presentation to T lymphocytes. It also has an influence on proliferation of lymphocytes and endothelial cells, increases cytotoxic lymphocytes T, granulocytes and natural killers cells activity. Even a small aberration in glutathione content may lead to lymphocyte T cells dysfunction.

Huge meaning of this compound highlights the fact, that it is synthesized in all body cells as necessary to cover high demand for this compound. If not the glutathione, the body would not be able to protect itself against infections and cancer development, the liver would not have any ability to detoxify heavy metals, toxins, xenobiotics and cells would be subject of continuous destruction. Thus measuring both forms of this peptide seems to be interesting in investigating oxidative/antioxidative status in various physiological and pathophysiological conditions.Despite the fact, the impact of glutathione in various physiological processes is well known, there are still no specific reports concerning the purposefulness and effectiveness of supplementation this substance.

The most popular modulators of glutathione content are: glutathione esters, amino acids (cysteine, glutamine), their analogues: S-adenosylmethionine, N-acetylcysteine and a lot of other substances such as: vitaminum C and E, silymarin, α-lipoic acid, melatonine, whey protein.

The objective of the study was to agree whether crenotherapy (drinking therapy) with sulfide/hydrogen sulfide (SHS) waters from "Zuzanna" spring located in the area of Busko-Zdrój leads to increasing of glutathione (GSH and GSSG) content in human blood. Crenotherapy is a branch of balneotherapy – the method generally applied to everything relating to spa treatment, including the drinking of waters and the use of hot baths and natural vapor baths, as well as of the various kinds of gases or peloids such as: mud, sand used for hot applications.

SHS water in distinct from mineral water is characterised by specific pharmacokinetic, invariable content and natural microbiological purity. SHS waters contain at least 1 g of total sulfur per kilogram of water and a treatment effect also depends on other bioelements.

A lot of earlier experiments confirmed positive influence of H_2S on antioxidative properties of organism. The fact, that H_2S takes part in many important processes in human body is obvious, because even the one of the first organisms on the earth-cyanobacteria use it in anaerobic photosytntesis.

In vivo H_2S is synthetized by the pyridoxal phosphate dependent enzymes cystathionine-γ-lyase, cystathionine b-synthase and 3-mercaptopyruvate sulfurtransferase utilizing the amino acids l-cysteine,l-homocysteine and l-cystathionine. SHS water is used in rheumatology to treat: rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis, gouty arthritis and other disorders of muscles and bandapparat, in post-traumatic and post-operative disorders of locomotor.

In this study GSH and GSSG ratio were analyzed in whole blood. The group of 40 volunteers consisted of both women and men, in different age range. Volunteers were either non-responsive, intolerant, or had a contraindication to crenotherapy.

The therapy with SHS waters lasted 2 weeks. We recently

demonstrated that the administration of H_2S in SHS waters increases GSH concentration in blood, and therefore crenotherapy could be used in therapeutics. Increase of GSH concentration is a parameter of improvement of health in patients which take part in experiment and it has an influence on immune system and antioxidative activity. The subjective wellbeing of patients was also observed.

The method employing capillary electrophoresis with UV detector for the analysis of glutathione in human blood was developed.

12:15 Oral

Plant phenolics as new drug leads. What's missing?

Teresa M. Brodniewicz[1], Grzegorz Grynkiewicz[2]

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Secondary metabolites which are phenolic in chemical character, belong to the most widespread constituents of higher plants and their presence is believed to be largely responsible for beneficial influence on human health of various vegetal foods, herbs and spices. Modern pharmacognosy, backed up by sophisticated analytical techniques, allows to trace biological activity of individual secondary metabolites and consequently many of them became registered as drugs directly or after minor structural modifications. This review will concentrate on low molecular weight phenolics from such secondary metabolite categories as: phenolic acids, stilbenes, quinones, coumarins, anthocyanes, flavones, flavonolignans, isoflavones, catechines, curcuminoids and lignans. All these compounds share certain structural features, to which some general physicochemical characteristics is assigned – e.g. all of them are recognized as active antioxidants – a property desirable in food constituents, but hardly an asset in drug candidate. Remarkably, some of them also exhibit selective pharmacological activities, which make them widely accepted drug leads, with very convincing results in molecular pharmacology tests. However, although plant phenolics are generally biocompatible, their ADME properties are usually sub-optimal. We will review exemplary phenolics from natural sources and discuss their potential as future medicinal products, considering outcomes of their numerous clinical trials.

12:35 Oral

SEL-120-small molecule CDK8/9 kinase inhibitors as potential targeted therapy in colorectal cancer treatment.

Tomasz Rzymski, Adrian Zarębski, Agnieszka Dreas, Wiesław M. Chołody, Agnieszka Wardęga, Karolina Krawczyńska, Katarzyna Prymula, Mariusz Milik, Renata Windak, Karolina Osowska, Katarzyna Kucwaj, Małgorzata Żurawska, Nicolas Beuzen, Krzysztof Zając, Ewa Trębacz, Agnieszka Szamborska-Gbur, Krzysztof Brzózka

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Recent advances in molecular biology of tumors led to novel innovative therapies. Targeted therapies block the development of cancer by interfering with specific molecules involved in carcinogenesis and have shown a great promise in preclinical and clinical settings. Application of anti-EGFR antibodies for the treatment of colorectal cancer (CRC) is a good example of such an approach. EGFR receptor is commonly amplified and promotes tumorigenesis and tumor progression of CRC. The presence of activating KRAS mutations has been identified as a potent predictor of resistance to EGFR-directed antibodies. These agents should therefore be applied only in tumors with a wild-type status of the KRAS.

There are very few therapeutic options for patients resistant to anti-EGFR antibodies with activating mutations in KRAS. SEL120 is a series of small molecule inhibitors of cyclin-dependent kinases CDK8 and CDK9, with high and selective cytotoxicity towards CRC cells with mutated KRAS. Both CDK8 and CDK9 phosphorylate RNA polymerase II (Pol II) and regulate gene expression programs during oncogenic transformation and progression of CRC. CDK8 is a colon cancer oncogene necessary for b-catenin activity which is deregulated in over 90% of CRC cases. Moreover it was shown that expression of CDK8 significantly increased colon cancer-specific mortality what correlated with CDK8 copy number gain and high activity of b-catenin. CDK9 is frequently induced during oncogenic transformation and is activated by RAS/RAF/MEK pathway and stress. Inhibition of CDK9 plays a crucial role in compounds originally developed as CDK2/4/6-specific cell cycle inhibitors.

Here we report development of selective, ATP- competitive inhibitors of CDK8/9. Compounds from the SEL120 series have binding affinities towards CDK8/9 kinases in the low nM range. Results from the kinome screen (299 kinases) indicated that selectivity of SEL120 compounds was comparable with some of the most selective clinical kinase inhibitors. In addition, several compounds in the series exerted also significant activity on the mitotic kinase Haspin, what could be potentially beneficial for lowering proliferation rate and stabilization of aggressive forms of CRC.

SEL120 compounds inhibited proliferation and clonogenic survival of a number of CRC tumor cell lines, with particularly good activity in CRC cell lines with G13D mutation in KRAS. Slightly lower sensitivity was observed for cells with mutated P53 and other mutations in KRAS/BRAF pathway. Treatment with SEL120 compounds repressed also phosphorylation of Pol II and reduced levels of anti-apoptotic protein Mcl-1 and survival of cancer cells, due to inhibition of CDK8/9 kinases. Furthermore, co-administration of SEL120 compounds with Oxaliplatin, the standard of care in CRC, resulted in strong synergistic cytostatic effects. Oral administration of SEL120 revealed favorable pharmacokinetics profile and strong, dose dependent potency in the HCT116 colon cancer mouse xenograft model, with observed tumor growth inhibition around 80%. Notably, even high doses were safe as tested by blood biochemistry, blood morphology, limited body weight loss and lack of visible histopathological changes in major organs. Presented data validate inhibition of CDK8 and CDK9 as a promising strategy for anticancer treatment, particularly for solid tumors such as CRC resistant to current therapies.

Przerwa obiadowa

Wednesday afternoon, 30 May, 12:55

Sesja wykładowa III

Wednesday afternoon, 30 May, 14:30
Chair: S. Chłopicki, M. Fedoryński

14:30 Invited oral

Molecular medicine - Facts and myths

Jerzy Ostrowski

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The diagnosis and classification of a disease are limited by the diagnostic methods. Current medicine defines symptoms mostly as disturbances related to the state of classical physiology and employs diagnostics which is mostly based on anatomic/microscopic imaging and basic biochemical tests. However, individual susceptibility to disease is based on genetic variance, which determines the patient's defense and adaptive mechanisms against environmental factors, particularly on the molecular level. Consequently, diseases arise from the sum of the cell-specific, developmental-stage-specific, and metabolism-related changes in gene expression, leading to alterations of cellular signaling and regulatory pathways. Thus, understanding the molecular mechanisms of diseases requires the introduction of molecular diagnostics into medical practice.

Implementation of highly efficient molecular imaging methods on a genomic scale (such as next-generation sequencing, functional genomics, transcriptomics and metabolomics) to medical practice will allow the creation of molecular medicine, so called because of the research methodology and methods of disease imaging. In a consequence, molecular medicine might personalize disease prevention, diagnosis, and treatment that, in turn, would increase therapeutic efficacy and lower costs of the health care system. However, although with the introduction of high-throughput nanotechnologies, molecular biology came to the point of nearly unlimited resources on cell status, characteristics of data acquisition still resemble the taking of a single photo as opposed to a fast evolving movie. Current medicine is limited to elements of molecular diagnostics, usually on the scale of individual genes. In a consequence, the ready availability of current molecular methods enable molecular diagnostics and genetic counseling rather for monogenic disorders. On the other hand, molecular diagnostic of complex diseases is a much greater challenge, largely due to the limited number of established guidelines or procedures that determine the impact of a cumulative result of these "weak" genetic alterations and a set of environmental expositions on humans over the span of a lifetime.

Although many years have passed from the announcement of the first assembly of the human genome sequence, current clinical practice is hardly affected by the knowledge of the sequence of the human genome itself. The lecture will present the possibilities and limitations of modern molecular diagnostics in relation to expectations and doubts raised by the concept of the molecular medicine.

15:15 Oral

Impurity profiling using 2D-HPLC-IT-TOF

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The specification test of APIs at quality control has usually employed non-volatile buffer. The requirements of impurity identification in quality management has been recently increasing due to the regulatory issues and the globalization of supply chain. Needs are rising to shorten the turn around time. The mobile phase conversion from non-volatile to volatile buffer is labor intensive task and involves multiple risks. The Shimadzu prepared special 2D-dimentional system which lets the scientists to work with nonvolatile buffers in combination with MS system. This solution lets to avoid the mistake in identification and do not require the need of the new analytical method development.

Combination with IT-TOF with is a powerful tool for impurities identification gives some extra advantages.

15:35 Oral

Genotoxic impurities in pharmaceuticals

Katarzyna E. Filip

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Residual impurities resulting from manufacturing, formulation or degradation of the active pharmaceutical ingredient (API) and excipients, may be present in pharmaceutical products. Some of these impurities may show a potential for genotoxicity and therefore pose an additional safety concern. Since the turn of the millennium, the subject of genotoxic impurities (GIs) has been one of the most emotive issues facing the industry. GIs have now equal significance in terms of both, safety and quality, and are of increasing interest to regulatory agencies and industry scientists. Although some guidelines were pubished, there are still concerns and uncertainty due to the complexity of the issue. A proactive, multidisciplinary approach is needed to assess potential for GIs to affect the quality of API.

In this presentation the most important aspects relating to GIs will be described, like: interpretation of regulatory guidelines, determination of acceptable limits for such impurities in active substances, test methods used to qualify potential GIs, using of structure-activity relationship (SAR) and alerting structure concept in assessment of potential GIs. Examples of compounds known to be GIs as well as analytical methods used for their determination will be also covered.

15:55 Oral

The analysis of plant-based raw materials of the unknown origin

Marek Wasek[1,2], Piotr Wroczyński[1], Natalia Lal

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Some chosen aspects of the safety of use of several herbs received from the National Medicines Institute, which came from smuggling, have been examined. The analysis has been conducted in three different aspects:

(1) Possibilities of contamination of plant-based raw materials by metals of the heavy elements (As, Cd, Cu, Cr, Pb, Hg)

(2) Conscious smuggling of intoxicating preparation or narcotics in plant-based raw materials

(3) Radioactive contamination originating mostly from 137Cs isotope.

To solve the problem, analytical methods of GFAAS and ICP-MS, X-ray diffraction and high-distributive spectrometry of gamma radiation have been applied.

Determined concentration of arsenic in all analysed samples and the concentration of lead in one sample exceeded allowable concentration recommended by WHO. In the analyzed materials, no presence of narcotics or radioactive contamination of 137Cs isotope has been detected.

16:15 Oral

Iron chelators- the novel perspective in cancer treatment

Maciej Serda[1], Robert Musiol[1], Anna Mrozek-Wilczkiewicz[1,2], Jaroslaw Polanski[1], Des R. Richardson[3]

1. *University of Silesia, Institute of Chemistry (UŚ), Szkolna 9, Katowice 40-006, Poland* **2.** *University of Silesia, August Chełkowski Institute of Physics, Department of Solid State Physics, Uniwersytecka 4, Katowice 40-007, Poland* **3.** *University of Sydney, Sydney 2006, Australia*

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Iron, due to its unique biochemical and biophysical properties is present in the most important processes within human cells. The transportation of oxygen and its presence in complex proteins, such as transferrin and ferritin, can be used to highlight the role of iron. It is proven that most of cancer cells have a higher requirement for iron than normal cells as they rapidly proliferate. Hence, iron metabolism is altered within these cells. This fact is reflected by higher number of Tf receptors on their cell surface, mediating a high rate of iron uptake.

All compounds were synthesized in microwave reactor (CEM-DISCOVERY®) and the purity of final products was determinated by HPLC. The stereochemistry and structures of final compounds were confirmed by NMR spectroscopy and HRMS spectroscopy.

To sum up, novel iron chelators based on thiosemicarbazone moiety have been synthesized and tested for antiproliferative activity. Theywere found to be active against **HCT116** p53+/+and p53 -/- and **SK-N-MC** cancer cells (nanomolar cytotoxicity).Moreover the ability to induce cellular iron release and inhibit iron uptake from the iron-binding protein, transferrin, was at the same level that most active iron chelator Dp44mT [1,2]

References:

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Acknowledgments:

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Przerwa na Kawę

Wednesday afternoon, 30 May, 16:35

Sesja Posterowa I

Wednesday afternoon, 30 May, 17:00

17:00 Poster 1

Novel dual target ligands derivatives of isoindoline-1,3-dione as potential treatment for Alzheimer's disease

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Alzheimer's disease (AD) is a complex and still not completely understood disorder. There are a number of processes involved in the neuropathological changes leading to the neuronal death, which is the main cause of the disease. Thus, a large number of therapeutic targets have been identified and many strategies are being pursued in search for potential anti-AD drugs. The multiple ligand approach has been applied to design and synthesize a wide variety of potential dual- and multi-acting anti-AD drugs [1]. As a continuation of our studies [2], we designed compounds with inhibitory activity against cholinesterases and β-amyloid (Aβ) aggregation as dual target ligands against AD. Several series of hybrid molecules bearing alkylamino- or arylalkylamino- groups linked by alkyl chain with heterocyclic isoindolino-1,2-dione (phthalimide) were obtained. The activity of the synthesized compounds against acetylcholinesterase (AChE from electric eels) and butyrylcholinesterase (BuChE from horse serum) was evaluated in spectrophotometric Ellman's method. The influence of the compounds on the formation of Aβ plaques was examined in the modified Thioflavine T test.

Within the obtained compounds, series of 2-(diethyaminoalkyl)-isoindoline-1,3-dione derivatives showed selective AChE inhibition with IC_{50} values ranging from 0.9 to 19.5

μM and weak Aβ anti-aggregation activity (micromolar concentrations). Within the arylalkylamino- series, fluorobenzylamine derivatives displayed inhibitory activities against AChE in nanomolar ranges and activity against Aβ plaque formation similar to the above series. These results support the outcome of docking studies with the compounds targeting both the catalytic and the peripheral anionic sites of cholinesterases.

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Acknowledgements
Presented work is financially supported by the Polish Ministry of Science and Higher Education (Grant no N N405 16339).

17:00	Poster	2

Physicochemical characterization of sunitinib and its impurities

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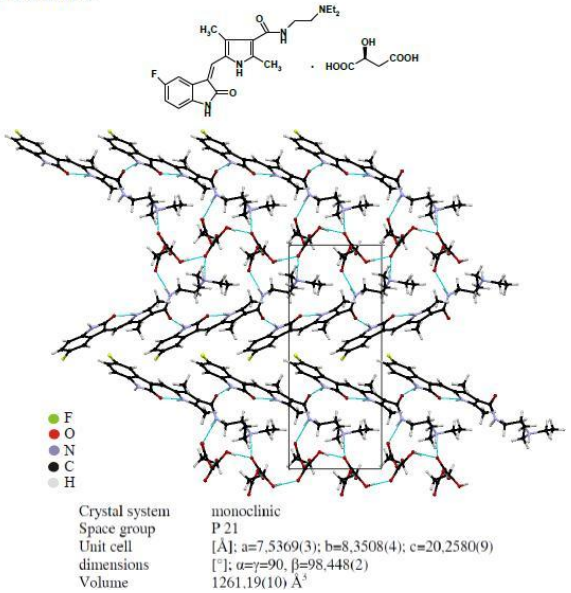
Sunitinib is an oral small-molecule tyrosine kinase inhibitor (TKI) that targets and blocks the signaling pathways of multiple selected receptor tyrosine kinases (RTKs). Sunitinib was approved by the FDA for the treatment of renal cell carcinoma (RCC) and imatinib-resistant gastrointestinal stromal tumor (GITS) on January 26, 2006.

During the investigation on the synthesis of pharmaceutically pure sunitinib *L*-malate based on the procedure described in EP 1255752B1 (Sugen Inc. and Pharmacia & Upjohn Co.) the physicochemical studies of sunitinib as well as its potential degradation products and process-related impurities were performed. The structure elucidation of the compounds was accomplished by means of the following techniques: NMR, IR and MS. Furthermore, X-ray single-cristal studies of sunitinib unambiguously proved the structure. A selective HPLC method for the determination of chemical purity of sunitinib was applied.

Chemical name: (N-[2-(diethylamino)ethyl]-5-[(Z)-(5-fluoro-1,2-dihydro-2-oxo -3H-indol-3-ylidene)methyl]-2 ,4-dimethyl-1H-pyrrole-3-carboxamide · *L*-malate)
Empirical formula: $C_{22}H_{27}FN_4O_2 \cdot C_4H_6O_5$
Structural formula:



Research Project is supported by European Union, Project no UDA-POIG.01.03.01-14-069/08-00, 26.02.2009„Innovative technologies of oncological medicines of special therapeutic and social importance".

17:00	Poster	3

New cyclic dermorphin analogues containing a carbonyl bridge

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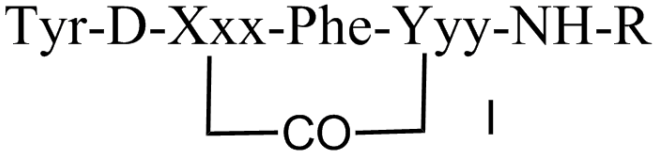
The cyclic dermorphin analogues, containing a carbonyl bridge have already been published [1-3]; all of them were N-unsubstituted amides.

The object of this study was the synthesis of N-substituted amides of dermorphin cyclic analogues containing an ureido group (Formula I) in the hope of preparing more proteolytical stable and analgesic active peptides.



For the synthesis of N-substituted amides both approaches were used: solid phase peptide synthesis (SPPS) and classical method in solution. SPPS was carried out on functionalized MBH-resin [4]

whereas classical metod was found to be more convenient to prepare a series of differently substituted amides starting from a common synton(s). All analogues were purified by semi-preparative HPLC metod.

The resistence of all new peptides to proteolysis was demonstrated by high resolution mass spectroscopy (HR-MS) method which showed full stability (no degradation products) during 24-hours incubation with chymotrypsin and pepsin. New analogues showed also remarkable antinociceptic effect.

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17:00 Poster 4

New 4,8-dihydroxy-1-oxo-6-phenyl-1,2-dihydro-2,7-naphthyridine-3-carboxylate derivatives with potential anticancer activity

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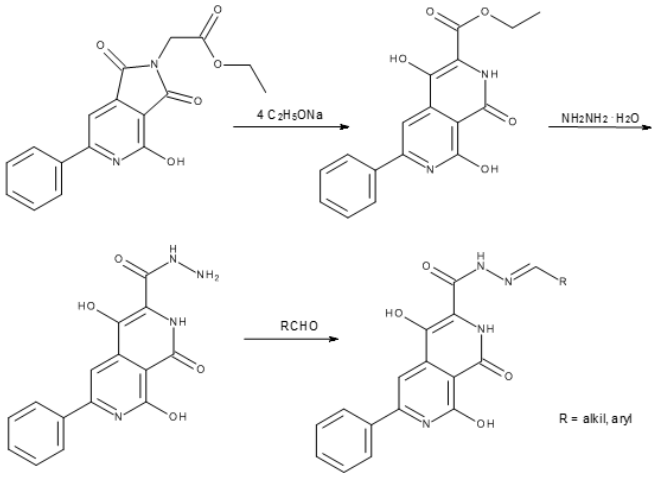
2,7-Naphthyridine is one of the six structural isomers of pyridopyridine, which have a broad spectrum of biological activity such as antimicrobial, antifungal, antimalarial, analgesic, and antiphlogistic [1]. Many of the natural alkaloids containing the 2,7-naphthyridine scaffold show anticancer activity [1]. The various biological properties encourage the synthesis of new 2,7-naphthyridine derivatives. Recently we presented the methods of synthesis and *in vitro* antitumor screening of novel 1-oxo-6-phenyl-2,7-naphthyridine-3-carboxylate derivatives [2]. In our studies we have found that the most active were the 2,7-naphthyridine hydrazide derivatives. As a continuation of our research we decided to synthesize a new derivatives by modification of the substituent in position 8 of 2,7-naphthyridine ring. New compounds have been obtained according to the methods described by us before [3]. Heating the ethyl 2-(4-hydroxy-1,3- dioxo-6-phenyl-pyrrolo[3,4-*c*]pyridin-2-yl)acetate **1** in a fourfold excess of sodium ethoxide resulted in the rearrangement to the ethyl 4,8-dihydroxy-1-oxo-6-phenyl-2H - 2,7-naphthyridine-3-carboxylate **2**. Ester **2** with hydrazine monohydrate gave appropriate hydrazide **3**. The series of Schiff's bases **4** were synthesized by treating the novel 4,8-dihydroxy-1-oxo-6-phenyl-1,2-dihydro-2,7-naphthyridine-3-carboxylic acid hydrazide **3** with appropriate aldehydes. Most of newly obtained compounds were qualified by the National Cancer Institute (Bethesda, USA) for *in vitro* antiproliferative screening against the 60 different human tumor cell lines.



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17:00 Poster 5

Synthesis and in vitro anticancer evaluation of the new thiazolo[4,5-d]pyrimidines.

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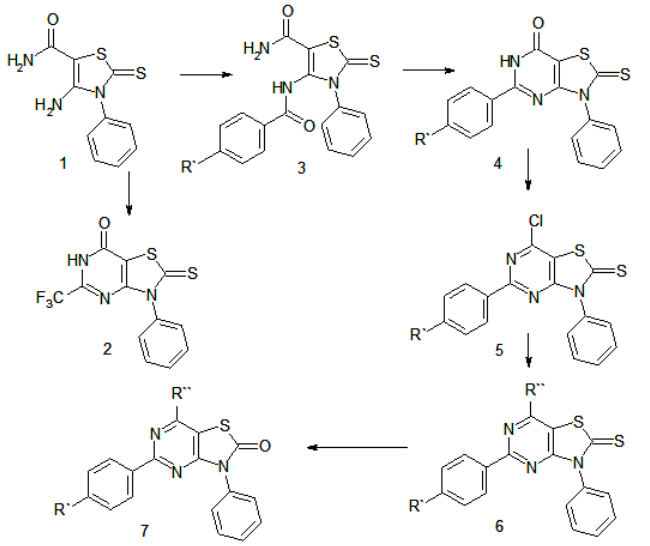
Intensive efforts in anticanver drug discovery are still needed to develop more effective antitumor agents. Thiazolo[4,5-d]pyrimidines are important class of fused heterocycles. Variety of biological activities have been reported earlier. In our previous research, we found that they possess cytotoxic activity [1]. As a 7-thio guanine analogues, thiazolo[4,5-d]pyrimide derivatives may interfere with the synthesis of guanine. Recently we have synthesized and evaluated *in vitro* a series of the new derivatives of thiazolo[4,5-d]pyrimidines, which exhibited anticancer activity (Scheme). Treatment of a starting 4-amino-5-carbamoyl-3-phenyl-2-thioxo-2,3-dihydrothiazole **1** with triflouroacetic anhydride gave directly 5-trifluoromethyl-3-phenyl-2-thioxo-thiazolo[4,5-d]pyrimidine-7-on **2**, while with benzoyl chloride acylation of the 4-amino group was occurred. Intramolecular cyclization of intermediate diamides **3** was affected by base and underwent in the presence of sodium ethoxylate. The subsequent chlorination of obtained compound **4** with phosphorus oxychloride afforded the 7-chloro derivatives **5** in good

yield . Nucleophilic substitution of the chlorine atom by reaction of **5** with the appropriate amine in boiling 1-propanol gave the target 7-substituted amino-thiazolo- [4,5-d]pyrimidines **6**. Thiazolo[4,5-d]pyrimidin-2-ones **7** were obtained by the replacement of the 2-thioxo group with 2-oxo. To evaluate the cytotoxic effect, newly synthesized compounds were submitted for testing at the National Cancer Institute (Bethesda, USA), against 60 human tumor cell lines. Most effective 7-(4-chlorobenzyl)-3,5-diphenyl-thiazolo-[4,5-d]pyrimidin-2-on **7** turned out to be active against 40 cell lines (highest activity parameter for U0-31 renal cancer, GI_{50} = -7.37). Interesting was the fact that, although in varying degrees, all active compounds inhibited the growth of ovarian carcinoma cell line IGROV1, even if it was the only line across the 60 cell panel (in the case of 7-4-methylpiperazi-1-yl **6** derivative). IGROV1cell line expresses high-affinity IL-4 and IL-13 receptors shared by both cytokines that regulate proliferation and apoptosis. Inhibition of the interaction between interleukin and receptor, by inhibiting the synthesis or by blocking the receptor is a way to influence on biological processes. It is important to search for small synthetic molecules that maintain a high binding affinity for the receptor [2,3].

Scheme



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17:00 Poster 6

1-(2-Mercaptobenzenesulfonyl)-3-hydroxyguanidines – synthesis of novel potent in vitro antiproliferatives

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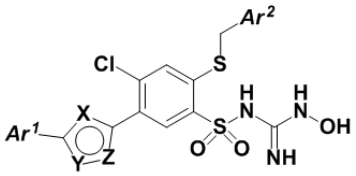
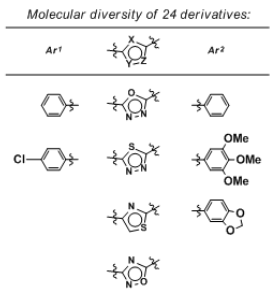
Our systematic study on 2-mercaptobenzenesulfonamide derivatives led us to discover novel anticancer,[1–5] anti-HIV[6,7] and antibacterial[8] agents. Our ongoing research program aimed at the synthesis and biological evaluations of novel 2-mercaptobenzenesulfonamides (MBSA) with five-membered heterocycles located at 5 position of MBSA scaffold[4,5] led us for finding the unconventional group of compounds bearing *N-2*-unsubstituted 1-benzenesulfonyl-3-hydroxyguanidine moiety, as potent *in vitro* cytostatic agents against numerous of NCI-60 cell lines of human leukemia, melanoma, non-small cell lung cancer, colon, CNS, renal, prostate, ovarian and breast cancers.





Nineteen of twenty four examined compounds at US, NCI (Bethesda), showed significant and broad cytostatic effect at low micromolar GI_{50} level (mean value in the range of 1.62–18.62 μM), and mean TGI ranged from 3.72–75.86 μM, over most of 60 tested cell lines.

QSAR studies were conducted to better understand structure-activity relationship (SAR) against human cancer cells, involving CoMSIA field descriptors. Additionally, COMPARE analyses were performed for representative 11 compounds. For at least 6 compounds NCI-60 cell lines panel inhibition profiles were correlated (Pearson correlation coefficient >0.5) with dactinomycin, bruceantin, chromomycin A3 and echinomycin – nucleic acid interfering agents.

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17:00 Poster 7

Anitiviral activity of chalcones

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We have prepared a new class of chalcones characterized by strong cytotoxic activity. Looking for other possible application of the compounds, preliminary tests of antiviral activity of selected derivatives were performed. Six randomly selected compounds was screened against classical swine fever virus (CSFV) which is the causative agent of a highly contagious, economically damaging disease of swine and wild boars.

The inhibition of the in vitro propagation of CSFV by tested compounds was evaluated following a procedure reported previously [1]. In brief, Swine kidney cells (SK6) were infected with CSFV and incubated with each compound at various concentrations from 0.1 - 3 μg/ml for up to 3 days. Due to the lack of cytopathic effect caused by CSFV, accurate virus propagation measurement was based on the visualization of the foci caused by CSFV (pseudoplaques) using immunoperoxidase monolayer assay (IPMA) which detects the areas of maximum concentration of glycoproteins of the viral outer layer. The effective doses for 50% virus reduction (IC_{50}) and cytotoxic concentrations at 50% cell death (CC_{50}) of the above compounds determined in CellTiter 96 AQ_{ueous} non-radioactive cell proliferation assay (MTS) are summarized in the Table. Both CC_{50} and IC_{50} values were used to calculate the in vitro selectivity index (CC_{50}/IC_{50}).

Compound	CC_{50} (μg/ml)[a]	IC_{50} (μg/ml)[b]	SI[c]
AMG-161	1.95	0.26	7.5
AMG-164	1.78	0.18	9.9
AMG-167	> 2	0.28	7.1
AMG-168	2.12	0.53	4
AMG-169	ND	1.2	NA
AMG-170	ND	1.8	NA

[a] Compound concentration required to reduce cell viability by 50%.

[b] Compound concentration required to reduce virus plaque formation by 50%.

[c] In vitro selectivity index (CC50/IC50).

ND: not determined.

NA: not available.

The results of the preliminary studies demonstrated that all tested compounds exhibited antiviral properties against CSFV in a dose-dependent manner evidenced by the reduction in the number and size of pseudoplaques, suggesting that the drugs inhibited proliferation and spread of the virus. Among the tested compounds, AMG-164 showed the most potent inhibitory activity on CSFV with an IC_{50} value of approximately 0.18 mg/ml. All the other compounds displayed a lower inhibitory potency, with IC_{50} values ranging from 0.26 mg/ml for AMG-161 to 1.8 mg/ml for AMG-170. The difference in antiviral activity of tested compounds could be due to the differences in cellular uptake and intracellular distribution.

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17:00 Poster 8

Comparison of bisoprolol pharmacokinetics after 10 mg oral administration of Bisocard and reference formulation to healthy subjects

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Bisoprolol, 1-(propan-2-ylamino)-3-[4-(2-propan-2-yloxyethoxymethyl)phenoxy]- propan-2-ol is a synthetic a highly selective beta1-adrenoceptor antagonist. Bisoprolol reduces the heart rate and is useful in treating abnormally rapid heart rhythms. It also reduces the force of contraction of the heart and lowers blood pressure.

The aim of the study was to compare pharmacokinetics of bisoprolol observed in two separate bioequivalence studies. The test products were Bisocard 10 mg and Bisocard 2.5 mg film-coated tablets, both manufactured by ICN Polfa Rzeszów S.A. The reference products were Concor 10® and Concor COR 2.5 film-coated tablets, both manufactured by Merck KGaA, Beerse, Belgium. Bisoprolol concentration in plasma samples was determined using LC-MS/MS method at the Good Laboratory Practice certified laboratory of the Pharmacology Department, Pharmaceutical Research Institute.

The results indicated that generic and branded formulations were bioequivalent at both doses. In both studies the 90% CI for geometric mean test/reference-ratios for the primary pharmacokinetic parameters ($AUC_{(0-t)}$, $AUC_{(0-\infty)}$) and C_{max}) were within acceptance limits of 80.00 – 125.00%. The secondary parameters (t_{max}, $t_{1/2}$, MRT) recorded in both studies were comparable.

The study was supported by ICN Polfa Rzeszów.

17:00 Poster 9

Effect of valproic acid and cisplatin on the proliferation of tumor cell lines A375 and C32

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An early diagnosis, in the initial phase of development of *melanoma malignum,* increases the chance of complete recovery, whereas the advanced stage of melanoma is almost fully resistant to all available therapies. Surgical removal of the lesion is the primary method of melanoma regimen, whereas radio-, chemo- and immunotherapy are a supplementary treatment. From among platinum – based chemotherapeutic drugs, cisplatin (*cis*-diamminedichloroplatinum, CPT) was the first used in treatment of melanoma. However, CPT has a low efficacy in therapy of melanoma, which is associated with cell resistance to this drug, and high toxicity. For this reason, novel therapeutic strategies are sought (analogues of cisplatin). A new promising group of compounds used in the therapy of melanoma are histone deacetylase (HDAC) inhibitors. One of them is valproic acid (2-propylpentanoic acid, VPA) which undergoes phase I and II clinical trials.

The aim of our study was to evaluate the influence of valproic acid and cisplatin on morphology and growth rate in human melanoma cell lines: A375 (melanotic) and C32 (amelanotic). Cell proliferation was measured using sulforhodamine B (*In Vitro Toxicology Assay Kit, Sigma-Aldrich)*, a dye binding to cellular proteins.

Our results suggest that CPT and VPA exerted synergistic inhibitory effect on the growth of both studied cell lines. Cisplatin alone inhibited the cell growth at concentrations ≥0.3 μM. Inhibitory effect of valproic acid was seen at concentrations ≥1 mM. However, the combination of both compounds displayed the strongest cytotoxic effect.

17:00 Poster 10

Examination of free radicals in melanins isolated from A-375 cells exposed on valproic acid and cisplatin by EPR spectroscopy

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Treatment of malignant melanoma (*melanoma malignum*) is still challenging because of its aggressiveness, rapid development and resistance to therapy. *Melanoma malignum* is a tumor originated from the melanocytes, pigment cells differentiated from neural crest. Mammalian melanocytes synthesize two types of the melanin, black to brown eumelanin and yellow to red-brown pheomelanin, that gives color to skin and hair. Synthesis and accumulation of the melanin in malignant melanoma cells is a crucial factor that influences the effectiveness of therapy. It is believed that melanin hinders the radiotherapy and photodynamic therapy of melanoma. Moreover, melanin, binding with cytostatics , decreases the efficacy of melanoma chemotherapy. Therefore, it's worth to consider the influence of melanin to the melanoma resistance to the treatment, and to investigate the melanin as a possible target of the chemotherapy.

o-Semiquinone free radicals with spin S = ½ [1-3] and biradicals with spin S = 1 [2] exist in melanin. Paramagnetic centers of melanin take a part in complex formation of this polymer with drugs [2-3].

In this work the effect of valproid acid (VPA) and cisplatin (CPT) on free radical properties of melanin isolated from A-375 cells was studied by the use of electron paramagnetic resonance (EPR) spectroscopy.

The measurements were performed by an X band (9.3 GHz) EPR spectrometer of Radiopan Firm (Poznań, Poland) and the Rapid Scan Unit of Jagmar Firm (Kraków, Poland). The first-derivative EPR spectra of melanin isolated from A-375 cells treated with VPA, CPT, and both VPA and CPT, were analysed. Amplitudes (A), integral intensities (I), linewidths (ΔB_{pp}), and g-factors were compared. Free radicals concentrations in the melanin samples were determined. Changes of the EPR parameters with increasing of microwave power in the range of 0.7-70 mW were examined. Spin-lattice and spin-spin interactions in the melanin samples were compared. It was shown that CPT and VPA change the parameters of the EPR spectra of melanin and they influence the free radicals concentration in melanin. It was shown that the EPR method may be used in the tests for clinical applications of the antitumor drugs. The performed spectroscopic studies broaden the knowledge about paramagnetic centers in melanin-drug complexes and they role in drug binding to melanin in human *melanoma malignum* tumor cells.

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17:00 Poster 11

Crystal structures of betulin and its derivatives

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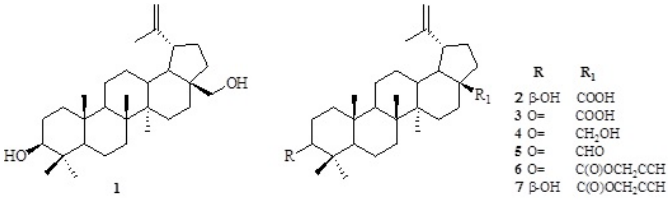
Betulin [lup-20(29)-ene-3β,28-diol, $C_{30}H_{50}O_2$] (**1**), also known as betulinic alcohol, is a pentacyclic triterpene of the lupane type which was one of the first natural products indentified and isolated from plants as a pure chemical substance in 1788 by Lowitz [1]. The high content of betulin (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.

The still growing interest in betulin (**1**) and its derivatives results from their wide spectrum of biological activities such as: anticancer, antiviral, antibacterial or hepatoprotective properties [1-3]. The structure of **1** is based on a 30-carbon skeleton comprising of four 6-membered rings and one 5-membered ring. Betulin (**1**) has three available sites for simple chemical modification, namely: secondary hydroxyl group at position C-3, primary hydroxyl group C-28 and isopropenyl side chain at position C-20. These group and their positions, mutual distance and orientation with respect to the rings can influence hydrogen bonding and the interctions of betulin derivatives with active sites of surrounding species. Despite the fact that betulin has been known for over 200 years, the X-ray structure of this compound was investigated for first time by Drebushchak and Boryczka as betulin-EtOH and betulin-DMSO solvates, respectively [4,5]. Various solvent used in the crystallization process and different melting points reported may indicate the existence of several crystal polymorphic forms of betulin. It is well know, that the large numbers of natural molecules are capable of exhibiting polymorphism or solvatomorphism [6]. More importantly, different polymorphic forms of pharmaceutical compounds display varying physicochemical properties, such as: solubility, stability, density as well as bioavailability, particularly when the drug substance is poorly soluble.

In the present work, we describe the synthesis and X-ray crystal structures of the betulinic acid (**2**), betulonic acid (**3**), betulone (**4**), betulonal (**5**) and new acetylenic derivatives of betulin (**6**,**7**) in order to gain better understanding of the structure-activity relationship of these important molecules.



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17:00 Poster 12

Methods for methotrexate determination in macromolecular conjugates drug-carrier

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Methotrexate (MTX) is one of the oldest and currently used drugs. It is an antimetabolite of folic acid, and a specific inhibitor of dihydrofolate reductase (DHFR), having an indirect and direct effect on other molecular targets, influencing on DNA replication and cell proliferation. MTX has a wide use in the treatment of tumor and autoimmunological diseases. This drug at the same time demonstrates a range of disadvantages, characteristic for low molecular compounds such as fast metabolism and fast excretion from an organism, as well as adverse biodystribution and a low selectivity of therapeutic use. Solving these problems involves the binding process of MTX with macromolecular carriers resulting in enhancement of the delivery, selectivity and improvement of pharmacological properties of MTX. Research on MTX conjugates with natural and synthetic polymers such as dextrans, albumin, fibrinogen, polyethylene glycol and other is in progress. During a study on potential carrier for MTX a fast and precise method is needed to determine the total amount of the drug bound and free in investigated preparation. In this paper, two simple, cheap and fast methods regarding quantification of methotrexate in the conjugated preparations are presented. In both methods sodium bicarbonate solution was used as a solvent. For both methods basic parameters such as linearity, range of the method, limit of detection (LOD) and limit of quantification (LOQ), reproducibility and recovery were validated. The method for analysis of a total MTX in preparations was based on absorption spectrophotometry. Validation was performed by measuring absorbance at 372 nm of the sodium bicarbonate solution. Curve describing drug concentration against absorption had a linear character in the range of 0,60µg/ml-20µg/ml. The method for free methotrexate determination was based on size exclusion chromatography and UV-VIS detection at the wavelength of 302 nm. Superdex® Peptide column (150 x 4.6 mm) and a mobile phase 0,1M sodium bicarbonate flow rate of 400µl/min was used. In a free drug determination method a curve had a linear character in the range of 1,00-100µg/ml. LOD of the method was 0,15µg/ml, and LOQ was 0,50µg/ml.

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17:00 Poster 13

Efficient preparation of a key intermediate in the exemestane synthesis

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One-third of breast cancers is hormone-dependent and uses estrogen to proliferate. The major source of estrogens in post-menopausal women is enzymatic conversion of circulating androgens. Therefore, the inhibition of enzyme activity causes tumor regression. One of the Type I aromatase inhibitors is exemestane (6-methyleneandrosta-1,4-dione), the only orally active irreversible steroidal aromatase inhibitor, effectively used in postmenopausal women with advanced breast cancer.

In general, there are two different routes to obtain exemestane. The first exploits 6-methylation of androstenedione or boldenone followed by oxidation of the appropriate intermediate. The above mentioned processes are unfavorable because of toxic and environmentally unsafe reagents as well as harsh reaction conditions. The second method proposed by a German group starts from androsta-1,4-diene-3,17-dione (ADD) and appears to be an advantageous alternative. The crucial point of synthesis is the formation of 1α,3-di(1-pyrrolidino)androsta-3,5-diene-17-one (DPA) followed by transformation into 6-(hydroxymethyl)androsta-1,4-dien-3,17-dione.

In order to develop the technology of exemestane in a pilot scale the both reaction were examined. The sampling of reaction parameter space such as reaction time, temperature, the amount of acid catalyst and some details of the work-up procedure in the course of several experiments led us to formulation of a reasonable conditions for ADD transformation to DPA. The use of the adequate amount of the acid catalyst at room temperature and the NMR monitoring of the reaction mixture is essential for this process. The developed method produces a high yield intermediate on a level of purity which is suitable for the last step of exemestane synthesis. Taking into consideration our observation about the instability of DPA during the studies on various work-up procedures, a hypothetical transformation routes of ADD to DPA were analyzed. The suggested reaction pathways were calculated with the theoretical B3LYP/6-31G(d,p)quantum mechanical method. The theoretical molecular structures were verified with ^{1}H and ^{13}C NMR spectra.

17:00 Poster 14

Investigation of unknown impurities in cyclic dermorphin analogue by HPLC-MS

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Dermorphin (Tyr-D-Ala-Phe-Gly-Tyr-Pro-Ser-NH2) is highly selective, μ-opioid receptor ligand. The synthesis, biological activity and conformations of several side-chain to side-chain cyclized dermorphin analogues, containing a carbonyl bridge, has been previously described [1, 2, 3]. The object of this study, ureidopeptide UP-1, also belongs to the mentioned group, however - in comparison with the described series of analogues - posses some new modification of the *N*-terminal segment.

Developed synthetic process led to peptide UP-1 having a good HPLC purity (above 96%, after purification by semi-preparative HPLC). Unfortunately, the overall yield of the synthesis was wery low. One of the possible reason is forming some unknown by-products. In crude ureidopeptide several impurities were detected by HPLC-UV and attempts to make structural assignment of these compounds by HPLC-MS-MS were the main goal of this work.

Two impurities were isolated by semi-preparative reversed-phase high performance liquid chromatography. Based on mass spectrometric data and synthetic specifics the structure of one impurity was confirmed. Founded on HPLC-MS-MS analysis the potential structures of the others process-related by-products were also proposed.

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17:00 Poster 15

Vitamin D analogs enhance the activity of imatinib mesylate in human non-small cell lung cancer model

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Background

Recent ideas related to the anticancer treatment are mostly focused on the connection of different signaling pathway modulators in the cell. Imatinib (STI571, Glivec),a cell cycle inhibitor is one of them. Its anticancer activity relays mainly on the specifical inhibition of a number of tyrosine kinases. Imatinib can suppress the phosphorylation and decrease the activity of c-kit tyrosine kinase and also restrains cell proliferation and tumor growth in human NSCLC model A549 acting through the inhibition of PDGFR-α phosphorylation and decreasing the VEGFR expression. The hormonally active form of vitamin D, calcitriol, is known to reveal a therapeutic effect aga-

inst many type of cancers, including lung cancer, mainly by regulation of many signaling pathways in the cell. Calcitriol influences the activity of protein kinase C, ras, MAPK, prostaglandins, cyclic AMP and many others, what can lead in consequence to cell proliferation inhibition, differentiation or apoptosis. Low-calcemic vitamin D3 analogs; PRI-2191 and PRI-2205 were previously tested for their antiproliferative activity against different cancer cell lines. In our latest studies, the influence of vitamin D analogs PRI-2191 and PRI-2205 on the activity of imatinib mesylate against NSCLC has been evaluated.

Materials and methods

Cell line: The human lung cancer A549 cell line was purchased from American Type Culture Collection (ATCC, USA).

Compounds: The vitamin D analogs: PRI-2191 (tacalcitol) and PRI-2205 (5,6-trans- calcipotriol), and imanitib mesylate are certified synthetic materials provided by the Pharmaceutical Research Institute, Warsaw, Poland.

In vivo studies

Two in vivo experiments were performed. NOD/SCID female mice were subcutaneously (s.c.) inoculated in the right flank of the abdomen with 5x106 viable A549 tumor cells per mouse in 0.2 ml of Hanks buffer (day 0) and then randomly divided into groups receiving different treatment agents used alone or in combination. In both experiments the treatment was started from day 7 after tumor cells inoculation (when tumors were palpable). Imatinib was administered intraperitoneally (i.p.) in the dose of 50 or 75 mg/kg/day. In the first experiment the vitamin D analogs PRI-2191 and PRI-2205 were administered subcutaneously (s.c.) in the doses 1 or 10 mg/kg/day respectively, 3 times a week. The second experiment was performed to evaluate the correlation of the administration route and the activity of compound PRI-2191 used alone or combined with imatinib. Analog PRI-2191 was administered in the dose of 2 mg/kg/day subcutaneously or orally, 3 times a week.

Evaluation of the therapeutic effect. Tumor volume was calculated using the formula (a2 x b)/2, where a = shorter tumor diameter in mm and b = longer tumor diameter in mm. Inhibition of tumor growth is calculated from the following formula: TGI [%] (tumor growth inhibition) = (WT/WC) x 100 – 100%, where WT is the median tumor weight of treated mice and WC – that of untreated control animals. The minimal expected inhibition used to estimate the effect of combination of two compounds is calculated using the formula: %H=100-[(100-TGI for cytostatic) x (100-TGI for vitamin D analog) /100]. Statistical analysis using the Kruskal-Wallis was performed. Animal weight and tumor volume were measured three times weekly.

Results: In the first experiment mice receiving imatinib (GV) in combination with vitamin D analogs had lower tumor volume than animals from the control group or from the group treated with imatinib alone. Tumor growth inhibition (TGI) at 26th day of the experiment was 30% for GV administered alone, 69 and 55% for GV administered in combination with PRI-2191 and PRI-2205 respectively. This difference for analog PRI-2191, as compared to GV alone, was statistically significant. Moreover, in almost all days of experiment, the tumor volume of mice treated with GV combined with PRI-2191 or PRI-2205 was statistically significant as compared to control mice. The second experiment showed, that the antitumor effect of PRI-2191 used alone or in combined treatment with imatinib depends on the route of administration. In the case of oral administration, PRI-2191 used alone exhibits better effect than after s.c. injections. However, in combined treatment with GV, synergistic interaction was observed when PRI-2191 was administered subcutaneously. Comparing two days of experiment: 14 and 21, when both schedules of treatment lead to statistically significant tumor growth inhibition as compared to control or imatinib administered alone, analysis of interactions showed synergistic effect only in the case of s.c. administration. At day 14th the tumor growth inhibition index TGI for groups receiving combined treatment achieved 51 and 38% for GV+PRI-2191 s.c. and GV+PRI-2191 p.o. respectively. Imatinib administered alone in the dose and schedule used (75mg/kg/day) didn't affect tumor growth.

Conclusions

These findings suggest that therapeutic agents that can augment the activity of imatinib without additional toxicity, like vitamin D analogs, may be of potential use in anticancer therapy - to improve the activity and overcome resistance and relapse.

Patent application no P-397662, 30.12.2011, Combined therapy in non-small cell lung cancer therapy; J. Wietrzyk, B. Filip-Psurska, A. Kutner, W. Szelejewski, M. Chodyński, 30.12.2011 This work was supported by Ministry of Science and Higher Education Grant No PBZ-MNiI-1/1/2005,"New drugs with specific therapeutic and social values" Task: "Vitamin D Analog (PRI-2191) in combination with anticancer agents. In vitro and in vivo" period of 09.11.2006-08.11.2009.

17:00	Poster	16

Validated HPLC method for the determination of temozolomide in human plasma

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Temozolomide (TMZ) is one of the most often used drugs in the treatment of malignant primary brain tumors.
The aim of the study was to develop the bioanalytical method for the determination of TMZ in human plasma. A high-performance liquid chromatography method with UV detection (HPLC-UV) is based on the method described by Kim et al. [1]. The method was validated according to European Medicines Agency (EMA) [2] and Food and Drug Administration (FDA) [3] guidelines, in compliance with the principles of Good Laboratory Practice (GLP). Plasma concentration of TMZ was determined on C18 column after liquid-liquid extraction. Isocratic elution was applied with mixture of aqueous acetic acid and methanol. Theophylline was used as an internal standard. To prevent chemical degradation of TMZ to an active metabolite at physiological pH, plasma samples were acidified to pH < 3.
All of the validation parameters met acceptance criteria. Calibration curve, prepared using freshly spiked plasma samples, was linear within the range of 0.1-20.0 µg/mL. The method was found to be sufficiently accurate and precise over the studied range of concentrations. TMZ was stable in acidified plasma samples for at least 50 days.

The method recovery of TMZ from human plasma was consistent and ranged 37.1-41.1%.

The study was supported by the European Union (European Regional Development Fund) under the Innovative Economy Operational Programme 2007–2013 (Project No. UDA-PO-IG.01.03.01-14-069/08).

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17:00 Poster 18

Synthesis of potential impurities of loteprednol etabonate and methods for chemical purity determination

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Loteprednol etabonate (LE, chloromethyl 17α-ethoxycarbonyloxy-11β-hydroxy-3-oxoandrosta-1,4-diene-17β-carboxylate, Fig. 1), a soft corticosteroid antiinflammatory drug was developed for topical use. It is derived from the inactive metabolite of prednisolone, i.e. 11β,17α-dihydroxy-3-oxoandrosta-1,4-diene-17β-carboxylic acid, by introduction of a chloromethyl ester to the 17β-position and ethylcarbonate ester to the 17α-position.

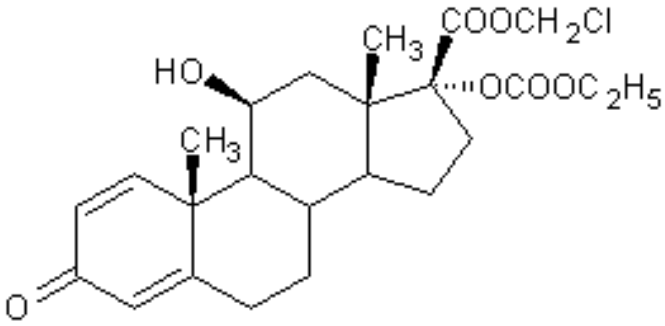

Fig. 1. Loteprednol etabonate.

Depending on the reaction sequences, reagents, reaction conditions as well as a substrate used in the synthesis of LE the final product (active pharmaceutical ingredient) is accompanied by different contaminants. Therefore, to evaluate purity of LE, we have synthesized eight compounds being potential process impurities or degradation products. Moreover, two additional process-impurities were isolated from the laboratory batches of LE by chromatographic methods. All these compounds were fully characterized by FT-IR, 1H and 13C NMR techniques.

Finally, UPLC analytical methods have been developed to assess the quality and safety of the final LE product. These methods have been also initially evaluated regarding their suitability for the intended purpose.

17:00 Poster 19

Synthesis and tuberculostatic activity of novel 2-(2-cyclohexylethyl)-1H-benzo[d]imidazoles

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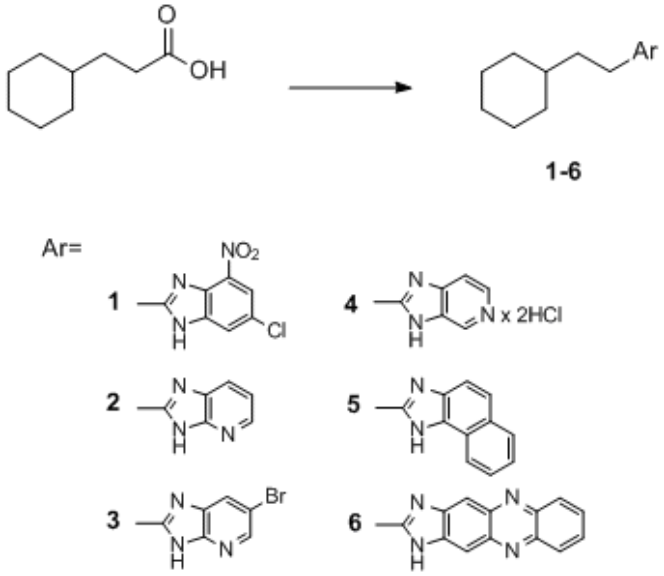
Previously we described a significant tuberculostatic activity of 2-phenylalkyl- and 2-cyclohexylalkylbenzimidazoles [1]. The designated minimum concentration inhibiting the growth of *M. tuberculosis* strains (MIC) *in vitro* was at the level appropriate for applied chemotherapeutics. We found that tested compounds were more active against resistant than sensitive strains and the presence of cyclohexylethyl substituent at position C-2 of the benzimidazole system was important for their activity.

These findings prompted us to extend our studies on the development of novel tuberculostatic agents, here we disclose the synthesis of novel 2-(2-cyclohexylethyl)-1*H*-benzo[*d*]imidazoles. We synthesized structures in which the cyclohexylethyl group is connected to the benzimidazole system or systems of benzimidazole type structure. Target compounds were obtained by the heating of 3-cyclohexylpropanoic acid with respective diamines according to the method described by Algul and co-workers [2] and based on the use of polyphosphoric acid (PPA) as a solvent with strong acidic properties (method A).

Promising results of biological studies encouraged us to undertake the synthesis of other benzimidazole type compounds **2-6**. Imidazopyridines **2** and **4** were obtained by the heating of 3-cyclohexylpropanoic acid and appropriate diamine in a diglyme (di(2-dimethoxyethyl) ether) solution (method B). Due to the liquid form of 2-(2-cyclohexylethyl)-3*H*-imidazo[4,5-*c*]pyridine the compound **4** was synthesized as dihydrochloride.



All the newly synthesized compounds were characterized by IR and ^{1}H NMR spectra as well as the elemental analysis. They have been also screened for their tuberculostatic, antibacterial and cytotoxic activities.

This study is supported by the National Science Centre, Cracow (grant no. 2011/01/B/NZ4/01187).

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17:00 Poster 20

The role of a sugar moiety in new genitein derivatives affecting proliferation and apoptosis of cancer cells

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Genistein, one of the major soy isoflavones is a promising agent for cancer chemoprevention. There are many experimental evidence showing that the inhibition of human cancer cell growth by genistein is mediated via the modulation of genes related to the control of the cell cycle and apoptosis.[1] Several glycoconjugates of genistein appeared more active than parent compound in preliminary screening for inhibition of cancer cell proliferation. The most potent compound was Ram-3, in which genistein is linked with a 2,3-unsaturated sugar moiety through an alkyl chain containing three carbon atoms.[2]

In the present study we report the synthesis of new glycoconjugates of genistein. We also describe the results of preliminary in vitro screening of antiproliferative and proapoptotic activity of several representatives of these glycoconjugates in cancer cells. Inhibition of proliferation was assessed in MTT assay, cell cycle was studied with flow cytometer. Mitotic and apoptotic indices and cell senescence were determined by microscopical observation.

Our results indicate the role of the sugar moiety is essential for activity of compounds. It is necessary to maintain the half-chair conformation of sugar moiety as an important structural factor. In particular the 2,3-unsaturated sugars and 2,3-anhydro derivatives (oxiranes) are the objects of interest. Moreover, polyfunctional sugar moieties offer ample opportunities for almost continuous changes in shape, electron density and polarity. Change of the protecting groups led us to derivatives with wide range of lipophilicity, which significantly differ in anti-cancer activity.

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

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17:00 Poster 21

Synthesis and anti-proliferative activity of selected aromatic bis(aminomethylidenebisphosphonic) acids

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Bisphosphonates are a class of drugs which show a broad spectrum of biological activity. The main clinical application of bisphosphonates is connected with diseases characterized by calcium metabolism disorder, like osteoporosis, Paget`s disease or hypercalcemia. Bisphosphonates are also used to prevent skeletal fractures and as auxiliary therapy in case of bone metastases of some cancer.

In literature, about 10.000 bisphosphonic acids are described and most of them are hydroxybisphosphonic acids. From all bisphosphonates present on the drug list only one is a aminomethylidenebisphosphonic acid - *Incadronate* (cycloheptylamino- methylidenebis-phosphonic acid). The most often used method for preparation of this group of bisphosphonic acids relays on the reaction of trialkyl orthoformate, primary or secondary amine and dialkyl phosphonate. The crude reaction mixture is hydrolyzed and the free acid is separated. This reaction has a few disadvantages: reaction yield that rarely exceeds 30-50%, harsh reaction conditions and formation a number of a sideproducts which hinder separation of the main product.

We recently developed a new method of synthesis of the aminomethylidenebisphosphonic acids. It is based on the reaction of isocyanide with trialkyl phosphite in the presence of stoichiometric amount

of hydrochloride. This reaction occur at mild conditions and gives a high yield. As a result, a series of aromatic bis(aminomethylidenebisphosphonic) acids were obtained. Their anti-proliferative activity towards MCF-7 human breast cancer cells, *HL-60* human promyelocytic leukemia cells and J774E mouse macrophages were evaluated. Some of them shown promising activity comparable with commercially available zoledronic acid.

17:00 Poster 22

Anticancer activity of the conjugates: modified fibrinogen – methotrexate.

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The majority of currently used anticancer drugs belong to the low molecular weight compounds. These medications demonstrate a range of disadvantages, such as fast metabolism and excretion from an organism, as well as adverse biodistribution and a low selectivity of therapeutic use. 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 and pharmacological properties of both conventional and innovative drugs.

In our laboratory antifoliates conjugated with carriers such as dextrans, albumin, fibrinogen and glycated proteins, were examined. Particularly promising investigations pertained the use of fibrinogen as a carrier of methotrexate (MTX). Many prerequisites such as accumulation in tumors determined this type of protein to be selected. Fibrinogen-MTX conjugates were characterized by considerably higher anticancer efficacy in comparison to free drug. One of the proposed mechanisms regarding this phenomenon assumes retention of fibrinogen/fibrin and its conjugates in peritoneum of animals with ascites tumors. It also shows a gradual enzymatic degradation connected to the drug release.

In this paper we show examination of anticancer properties of the MTX-fibrinogen subjected to limited hydrolysis conjugates *in vivo* and previously described generation of these conjugates with native fibrinogen. Leukemia mouse model P388 was used in this study, the cancer cells were engrafed intraperitoneally. High anticancer efficacy of the examined conjugate was observed by prolonging mice lifespan at 145% in comparison to lifespan of the control mice. Accordingly free methotrexate prolonged the lifespan of mice at 36% in comparison to the control mice. Toxicity in the dosage pattern of the preparation used was not observed. Data described above emerges a therapeutic potential in drug binding with macromolecular carriers, as well as the potential use of the derived fibrinogen in this system.

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

17:00 Poster 23

The immunochemical studies of the glycinated glycoconjugates based on synthetic thioglycosides as mimic factors of bacterial endotoxins using in the polysaccharide vaccines preparation

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The biological importance of lipopolysaccharides (LPS), component of bacterial cell wall has not been explained sufficiently. The glycine epitope present in these structures could play an important role in the immunological response after bacterial infections, during sepsis and septic shock. Modified thioglycosides conjugated with glycine residue could be used for broadly reactive antibodies production which would be able to neutralize endotoxin biological activity.

Previously, in our studies we obtained synthetic and stable substituted N-acetylated glycine 1-thioglycosides as amine functional group derivatives of monosaccharides, e.g., D-glucose or D-galactose as well as disaccharides, e.g., melibiose, lactose or maltose. The conditions of aminoacylation rections were validated and specific products were separeted by using chromatography methods. Their structures were confirmed by NMR.

Next, these model aminoacylated sugar structures were conjugated with two different carrier proteins e.g. bovine serum albumin (BSA) and horse myoglobin (MYO) to obtain reactive antibodies after experimental animals immunization. In order to obtain carrier proteins capable to bind the sugar ligand- amine containing group, BSA and MYO were modified by using glycidol to introduce aldehyde functional groups. In these experiments we used monomeric BSA as a result of purification of commercial material. Treatment of the stable protein-diol intermediates after glycidol application with sodium periodate resulted in the generation of reactive aldehyde functionalities through the oxidation of the glycol moieties.

Modified and formylated carrier proteins were conjugated with aminoacylated thioglicosides in the presence of sodium cyanoborohydride in order to complete reduce of the labile Schiff base intermediate to a chemically stable bond between aldehyde and amine functional groups. Subsequently, obtained products were separated by using HW-55S size-exclusion chromatography and analyzed in SDS-PAGE.

The presence of aminoacylated glycoconjugates fractions were expected in protein immunoblot with specific anti-aminoacylated antibodies obtained after rabbit immunization with *Escherichia coli* K12 C600 core oligosaccharide glycine-containing glyconjugate. Received glycine-acylated glycoconjugates as one of the non-sugar substituents localized in various bacterial LPS as an inherent compo-

nent, can mimic common epitopes in different bacterial endotoxins responsible for sepsis development and propagation. These synthetic compounds could be used as glycoconjugate vaccines in protection against serious bacterial infections.

Research studies are part-financed by Ministry of Science and Higher Education of Poland (Grant No. N N209 186338) as well as European Union (POIG.01.01.02-02-003/08-00) and (POKL.08.02.02-02-001/10).

17:00	Poster	24

Synthesis of galactothiophosphoesters of uridine and preliminary tests to evaluate their activity against selected glycosyltransferases

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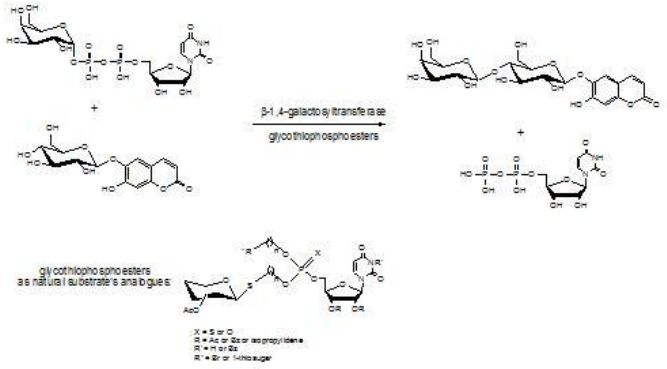
Due to the functions in energy metabolism and storage, as components of the genetic material as well as of structural elements carbohydrates contribute to life in many ways, and their connection with proteins or lipids play important roles in cellular communication during cell differentiation and development [1]. The main classes of enzymes responsible for the addition or removal of sugar residues are glycosyltransferases (GTs). They catalyze the transfer of sugar moiety from an activated donor (e.g. UDP-glucose) onto sugar or nonsugar acceptor [2]. Donor's or acceptor's analogues as GTs inhibitors may find applications as novel therapeutics for a wide range of diseases.

In our previously report we described glycoconiugates – analogues of UDP-glucose, in which variously protected 5'-uridine derivatives were connected with 1-thioglucose with thiophosphoesters fragment [3]. This compounds were synthesized in sequence of reactions: phosphitylation, secondary oxidation with sulphur presence and finally condensation reaction of obtained products with 1-thiosugars.

Now we presented new scale of conections, in which we replaced thioglucose fragment with 2,3,4,6-tetra-O-acetyl-1-thio-b-D-galactose. To estimate activity of these compounds as potential GTs inhibitors we tried to check if they will be competitive with natural sugar donor – UDP-galactose – in glicosylation of esculine catalized by β-1,4-galactosyltransferase (scheme 1). To track the progress of the reaction we have chosen HPLC method with UV and fluorescence detectors.



Scheme 1

Now we try to evaluate the conditions of separation of substrates and products reaction, so that on the basis of their concentrations to determine the impact of glycoconjugates on the activity of GTs. After optimalizing the separation conditions we plan to test the influence of whole range of glycoconiugates.

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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17:00	Poster	25

Polyunsaturated fatty acids inhibit melanoma cell growth in vitro

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Human malignant melanoma is a highly aggressive and incurable cancer due to intrinsic resistance to apoptosis and reprogramming proliferation and survival pathways during progression. Numerous studies, including our own, linked arachidonic acid (AA, 20:4 n-6), eicosapentaenoic acid (EPA, 20:5 n-3) and docosahexaenoic acid (DHA, 22:6 n-3) supplementation to induction of apoptosis and decreased proliferation of various cancer cells. The cytotoxic effects result from lipid peroxidation and formation of reactive oxygen species (ROS), which modify proteins, and nucleic acids. DNA damage by ROS causes mutations, and genomic instability leading to uncontrolled proliferation or cell death.

In the present work four human melanoma cell lines differing in origin, doubling time, metastatic potential, and melanin content: A375, A2058, G361, C32 were exposed to AA, EPA or DHA added into culture media in the concentrations ranging from 0 (control) to 100 μM. After 24 h incubation cytotoxicity of the analyzed acids was determined with TOX-2 (In Vitro Toxicology Assay Kit XTT Based, TOX-2, Sigma) test. At the some time oxidative protein modifications were measured using Aldehyde Site (DNA and Protein) Detec-

tion Kit (Cayman).
All the acids tested showed marked inhibition of cell proliferation. The observed effects were statistically significant and depended on the concentration. Decrease of proliferation, associated by oxidative protein and DNA damage (measured as aldehyde sites in cells), was observed for EPA and DHA (50 μM and 100 μM) in A375, A2058, and G361 cells. In case of C32 cell line, which is amelanotic melanoma, EPA and DHA inhibited cell proliferation at 100 μM only. The effect of DHA was more pronounced. AA did not show its antiproliferative action in this cell line.

The obtained results suggest that antiproliferative effects of the fatty acids in cultured human melanoma cells depend on the type of acid, its concentration and may be diverse when different melanoma cell lines are used

This work was supported by SUM grant KNW-1-002/P/1/0.
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17:00 Poster 26

Effect of Gly-Gly-His, Gly-His-Lys and their copper complexes on the TNF-α – dependent IL-6 secretion in normal human dermal fibroblasts

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Cosmeceuticals represent a marriage between cosmetics and pharmaceuticals [1] There are numerous cosmeceutically active products which can be broadly classified into the following categories: antioxidants, oligopeptides, growth factors and pigment lightning agents [2]. Much attention has been focused on the tripeptides such as Gly-His-Lys (GHK) and Gly-Gly-His (GGH) and their copper complexes, which have a high activity and good skin tolerance. Recent data suggested their physiological role in process of wound healing, tissue repair and skin inflammation [3]. The mechanism of anti-inflammatory properties of these peptides is not clear. The aim of the study was evaluation of influence of two peptides GGH, GHK and their copper complexes and saccharomyces/copper ferment (Oligolides ® Copper) on secretion of pro-inflammatory IL-6 in normal human dermal fibroblasts NHDF cell line. IL-6 was evaluated using the ELISA kit. GGH, GHK, $CuCl_2$ and their copper complexes decreased TNF-α - dependent IL-6 secretion in fibroblasts. IL-6 is crucial for normal wound healing, skin inflammation and UVB-induced erythema [4]. Because of the anti-inflammatory properties the copper-peptides could be used on the skin surface instead of corticosteroids or non-steroidal anti-inflammatory drugs, which have more side effects. Our observations provide some new information about the role of these tripeptides in skin inflammation.
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17:00 Poster 27

Molecular properties of antifungal agents – Impact on bioavailability – The Biopharmaceutics Classification System

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The main target of our investigations was to identify molecular determinants that have an impact on bioavailability of 14 azole antifungal agents.

Solubility and permeability are two properties that have an effect on compounds bioavailability, which is a crucial for biological activity of drugs in humans. These two parameters are used to create the BCS – the Biopharmaceutics Classification System. It classifies active substances into four classes based on their aqueous solubility and intestinal permeability. Experimental determination of those parameters is cumbersome and frequently impossible

The parameters which describe both solubility and the tendency to cross biological membranes were calculated: the free enthalpy of solvation (ΔG_{solv}) in water and organic solvents (e.g. chloroform or chlorobenzene) and electrostatic potential range give valuable information on solubility, polarity, lipophilicity of compounds and explain solute-solvent interaction phenomena. The experimental and theoretical log P values which, as first approximation, describe lipophilicity and permeability of pharmacological substances were determined and compared to calculated factors. All these parameters define drugs properties clearly and precisely.

The results also serve as description of properties relevant to the Biopharmaceutics Classification System (BCS) and seem to be promising tool for fast and clear classification of chemical substances within BCS. Characteristics, like solubility and permeability are considered when active substances are classified into group I-IV. We managed to propose BCS categorization for 14 antifungal drugs, as a step to improve the BCS system at the international level.

17:00 Poster 28

Preparation of protoaescigenin from escin.

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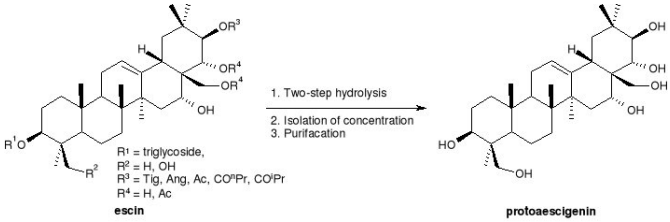
β-Escin (β-Aescin) is an active ingredient of popular OTC drugs (Escin, Reparil, Venitan) with anti-inflammatory, vasoprotective and vasoconstrictor effects. It is used in treatment of chronic venous insufficiency (CVI) and also in cosmetics. The substance obtained from the seed of the horse chestnut (*Aesculushippocastanum*) is a mixture of over a dozen saponins, which comprise polyhydroxylated triterpene aglycone of olean-12-ene type, acidic oligosaccharide, and some short chain fatty acid residues. Preparative separation of native mixture of escins would be a daunting task since even analytical HPLC is difficult and inconclusive until supported by a suitable collection of standard compounds for positive identification.

In connection with a project aimed at new, semisynthetic, vasoactive compounds, it has been decided to obtain escin sapogenins by partial degradation of native saponins. Preparation of escin aglycons was achieved in two-step hydrolysis, in which glycosidic and ester substituents were removed sequentially. It has been confirmed that the main terpenoid constituent of escin mixture is protoaescigenin (approximately 50% of the saponin hydrolyzate), but its isolation in state of reasonable purity by scalable process posed considerable difficulty.

A method of preparation of protoaescigenin of purity >98% from β-escin without using chromatography was developed. The method consists of three steps (Scheme 1): i) successive acidic and alkaline hydrolyses, ii) isolation of the protoaescigenin concentrate, iii) purification of the concentrate by crystallization and obtaining pure protoaescigenin. The method was successfully scaled up to a kilogram scale, allowing to obtain certified protoaescigenin as a prospective fine chemical and/or pharmaceutical intermediate.



Scheme 1

Preliminary polymorphism studies indicated that obtained crystals of protoaescigenin represent a sapogenin monohydrate which has not been reported in patent or published scientific literature.

17:00 Poster 29

Experience of the Office for Registration of Medicinal Products, Medical Devices and Biocidal Products in assessment of quality of medicinal products

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Prior to its authorization every medicinal product undergoes an appropriate regulatory process. The Member States of the European Union possess institutions responsible for conducting the registration and granting relevant decisions. In Poland this institute is called the Office for Registration of Medicinal Products, Medical Devices and Biocidal Products. The Polish Office of Registration participates actively in all types of procedures.

The registration process is multistage and consists of formal validation of the application form submitted by the applicant (Department of Registration of Medicinal Products) and dossier assessment (Department of Documentation Assessment). The final stage is granting a marketing authorization or rejection of an application. For medicinal products authorized via centralized procedure these decisions are granted by the European Commission. For the medicinal product authorized via national procedure, mutual recognition procedure and decentralized procedure the decisions are granted by national competent authorities. In Poland the decisions are granted by the President of the Office for Registration of Medicinal Products, Medical Devices and Biocidal Products.

Assessment of chemical, pharmaceutical and biological documentation submitted in the registration process is performed with regards to its compliance with requirements of pharmaceutical law, actual editions of Pharmacopoeia and actual CHMP ,CVMP, ICH guidelines. Assessment of quality of a medicinal product results in assessment report issued by an expert. During the assessment the applicant is obliged to update the documentation according to comments arising from the assessment. According to the legislation analytical tests of a medicinal product during the registration process are allowed in case the doubts of the experts may be solved only on experimental basis. Before the product is tested the President of the Office informs the Marketing Authorization Holder and provides a rationale of the testing.

17:00 Poster 30

Enzymatic synthesis of beta-2-deoxy-D-galactopyranosides from glycals

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Glycoside hydrolases, a widely distributed group of enzymes, cleave glycosidic bonds in glycosides, glycan and glycoconiugates, and

they can play roles in the development of biofuels and in desease research. Glycosidases also participate in a broad range of biological processes including the virulence of pneumococci, which cause a number of serious diseases that are responsible for millions of deaths annually [1]. Although these enzymes belong to the class of hydrolases, they can also synthesize glycosidic bond through the transglycosylation or reverse hydrolysis reaction.

The enzyme β-galactosidase (EC 3.2.1.23), which is usually hydrolyzing lactose to the monosaccharides like D-glucose and D-galactose, may also catalyse the formation of galacto-oligosaccharides [2,3]. In the synthesis of biologically active glycosides via enzymatic systems are still rare in literature. Indeed, the enzyme systems, the frequently observed low yields, the stringent specificities or the lack of an available catalyst still often favour chemical systems. In view of the often long-winded protection stages required for most chemical glycosylations, hovewer, the use of enzyme-catalysed one step systems is clearly an attractive proposition. Furthermore, enzymatic methods of glycosylation seem to be promising as a means of synthesizing unstable glycosides, because they can be carried out under mild conditions with no accompanying decomposition of the starting materials or products [4]. The synthesis of novel 2-deoxygalactosides is interesting because of their important role in several biological processes [5]. Therefore, to study biological activity, it is essential to elucidate the structure of the products.Glycosidases are capable of catalyzing glycosidic linkages with absolute stereoselectivity of the anomeric center.

We report an efficient synthesis of alkyl and aryl β-D-2-deoxygalactopyranosides derivatives in presence of β-galactosidases .

Acknowledgement

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17:00 Poster 31

Manufacturing of modified release tablets

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Modified release tablets nowadays are very popular form of introduction of active substances for medicinal treatment. The main advantage of these forms is much simpler dosing scheme with only one tablet per a day instead of 2-4 in case of normal drug product. The benefits of modified release drug product requires more complex multistep manufacturing process and moreover an understanding of functionality of human digestion system and changes of conditions during digestion process.The fundamental differences of modified and normal release of active substances are presented with visualization of both variants on graphs.

This article shows the mechanism for modifying release of active substance and present common technical procedure for manufacturing of modified release drug product with regards to excipients used and manufacturing process. All information are supplemented with graphs and pictures which helps to understand the principle of modified release drug product.

17:00 Poster 32

Glycosyl thiocarbamates in orthogonal synthesis of oligosaccharides

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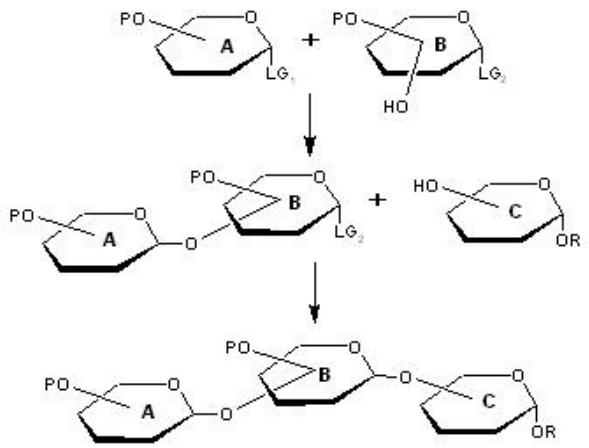
In orthogonal glycosidation a range of glycosyl donors that bear different leaving groups (**LG_1** / **LG_2**) and that can be selectively activated in the presence of each other, are utilized. A highly reactive donor is required for the first glycosidation in the orthogonal sequence[1].

The O-glycosyl-N-methyl thiocarbamates (**A**), readily obtained from anomerically-unprotected sugars[2], are very reactive glycosyl donors. Thiocarbamates can be easily activated with bromine. On the other hand the S-ethyl glycosides (**B**), obtained from S-glycosyl-N-methyl thiocarbamates, can be used as glycosyl acceptors in the first step of orthogonal glycosylation sequence. The second step requires activation of disaccharide baring thioethyl group. This activation can be done with thiophilic reagents, thus with the addition of the second glycosyl acceptor (**C**) the orthogonal sequence can be completed. We will present the application of this method in the synthesis of trisaccharides. This methodology will be used in the preparation of biologically active glycoconjugates.



LG$_1$: OC(S)NHMe; **LG**$_2$: SEt; **P**: Bn; **R**: isopropylidene

Acknowledgement

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17:00 Poster 33

On tautomerism of the pyridodiazepines. A DFT study.

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The benzodiazepine derivatives are known for their wide applications in medicinal chemistry since they are used as anticonvulsant, anti-anxiety, analgesic, hypnotic, sedative, antidepressant, and anti-inflammatory agents.

Since few years we have been studying tautomerism [1-5]. Lately, we have been focused on substituent influence on the tautomeric equilibria in benzodiazoles [4] and their aza analogues [5].

The aim of the present study has been to investigate tautomerism of the aza analogues of benzodiazepines: the pyridodiazepines. The geometry and energetic differences between the pyridodiazepine tautomers have been studied based on the Gibbs free energies calculated in standard conditions at the DFT/B3LYP/cc-pVTZ level. The calculations were performed for both the gas-phase and the water surrounding simulated by the polarizable continuum model (IEF-PCM).

The results indicate that for the pyrido[1,2]- and pyrido[1,4]-diazepines the N_1-H tautomer is more stable than the other two tautomers. However, for the pyrido[1,3]diazepine, the N_3-H tautomer is more stable if the N atom is placed in the position 7 and 8 of the pyrido ring, whereas the N_1-H tautomer is more stable when it is placed in the position 6. For the pyrido[1,5]-diazepine where N1 and N5 positions are somehow equivalent by symmetry, the N_1-H tautomer is more stable when the N atom is placed in the position 7 (or 9) of the pyrido ring whereas the N_5-H tautomer when it is placed in the position 6 (or 8). The aromaticity of the studied structures is studied in details by means of the HOMA aromaticity index. It is shown that aromaticity of the pyridodiazepine tautomer is the main factor for its stability.

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17:00 Poster 34

An improved preparation of 3,5-bis(2-cyanoisopropyl)toluene - key intermediate in Anastrozole synthesis

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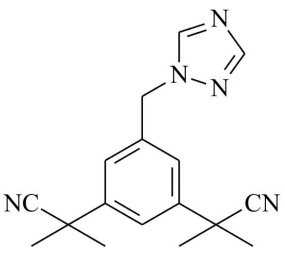
Anastrozole **1** is a highly selective aromatase inhibitor, used in advanced breast cancer treatment of post menopausal women.



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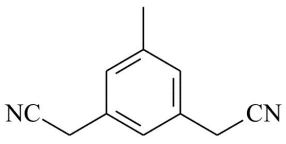


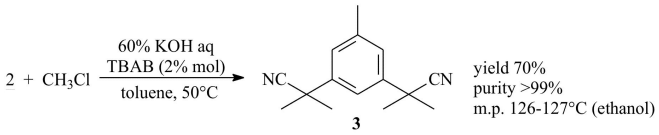
2

A key step in synthesis of **1** is exhaustive methylation of 3,5-bis(cyanomethyl)toluene **2**, synthesized from commercially available 5-methylisophthalic acid [1]. Methylation of **2** was performed using sodium hydride in DMF as a base and methyl iodide [2] or methyl *p*□toluenesulphonate [1] as alkylating agents. Application of

phase transfer catalysis (PTC) for this purpose was patented [3] (methyl bromide or chloride, 50% aq NaOH in the presence of a catalyst, benzyltriethylammonium chloride), however purity of product obtained precluded its use for synthesis of **1** [4]. It is well known, that introduction of the second alkyl group to the 2-arylalkanenitriles under standard PTC conditions proceeds with difficulty. However, it was found recently, that replacement of the typical 50% aq NaOH by 60-75% aq KOH results in substantial improvements in the overall yields and purity of products of alkylation, and especially bis alkylation of arylacetonitriles [5, 6].

Application of such system to the methylation of **2** (methyl bromide, 60% aq KOH, tetrabutylammonium bromide, TBAB as a catalyst, toluene) resulted in formation of tetramethylated product **3** in high yield and purity [7]. Methyl bromide, however, is much more expensive then methyl chloride, commonly used in alkylation reactions.

Now, we have found, that the use of methyl chloride for alkylation of **2**, carried out under PTC conditions, results in the formation of **3** in high yield and after one crystallization, high purity. Optimization experiments were carried out using model 1,3-bis(cyanomethyl)benzene.



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17:00 Poster 35

Application of silica-bonded ovomucoin protein chiral stationary phase for the determination of paroxetine, clinically used psychotropic drug

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Paroxetine
)-3-[(1,3-benzodioxol-5-yloxy)methyl]-4-(4-fluorophenyl
{(-)-(*3S*,*4R*)piperidine]} is an SSRI antidepressant. Marketing of the drug began in 1992 by the pharmaceutical company SmithKlineBeecham. The effectiveness of paroxetine in major depressive disorder has been proven by six placebo-controlled clinical trials [1, 2]. Since in medicinal products beside (-)-*trans*-paroxetine its probably inactive enantiomer [3] may be present, appropriate analytical procedures are necessary to determine a content of the second enantiomer (which in best case could be devoid of any pharmacological activity but very often may be responsible for serious adverse effects).

Here we report validated analytical procedure enabling determination of (+/-)-*trans*-paroxetine in medicinal products containing (+)-*trans*-paroxetine as a main active ingredient with the aid silica-bonded ovomucoin protein chiral stationary phase. Validation included: specificity, linearity, limit of detection (LOD), limit of quantification (LOQ), assay, precision and recovery.

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17:00 Poster 36

Preliminary studies on a new way of nucleic acids extraction using hydrogel microsphere

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In many areas of modern medicine and molecular biology the isolation of a nucleic acid from the biological material is a key process. It is a preliminary step for many procedures without which the study on DNA or RNA would not be possible [1]. Hence there is a need to develop better methods to perform these procedures. The most serious problem with the techniques used today is the use of toxic solvents are often harmful to the environment and the laboratory personnel performing isolation. This work presents the results of the studies on the use of polymeric hydrogels based on fully biocompatible and biodegradable biomaterials for this purpose.

The process of binding nucleic acids is based on the attraction between the negatively charged DNA and positively charged surface of the hydrogel. A similar effect was observed for heparin [2]. Dextran used as a starting material was crosslinked to obtain hydrogel and functionalized with positively charged ammonium groups in the reaction with ammonium epoxide and diepoxide. In order to obtained hydrogel microspheres with dimensions of several microns the

whole process was carried out in an inverted emulsion. The resulting material was investigated using optical microscopy and atomic force microscopy.

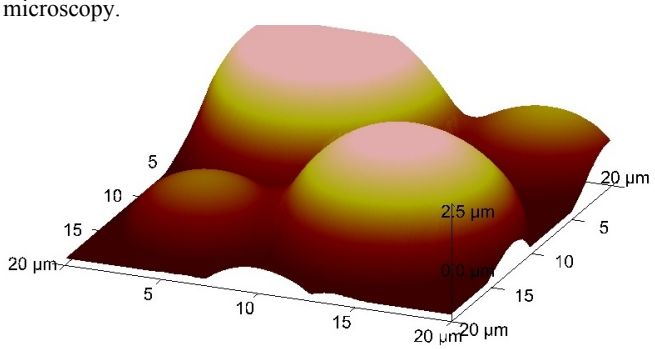

Figure 1. Atomic force microscope picture of the studied microspheres

The average size of the obtained structures, and size distribution was determined. The ability of microspheres to bind DNA and other anionic biopolymers was studied. Also, the the possibility of the interaction of the microspheres with bovine serum albumin was examined.

A similar experiment was carried out for silica gel augmented with NH_2 groups and quaternary ammonium groups. None of these silicone materials was able to decrease concentration of DNA or other polymers tested. This may suggest that in addition to the positive charge also the chemical structure of the adsorbing material used for DNA or RNA extraction is important.

Acknowledgement: The project was operated within the Fundation for Polish Science Team Programme (TEAM/2008-2/6) and Ventures Programme (Ventures/2009-4/4) cofinanced by EU European Regional Development Fund

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17:00	Poster	37

Design, synthesis and anticonvulsant activity of new N-Mannich bases derived from 3-phenylpyrrolidine-2,5-diones

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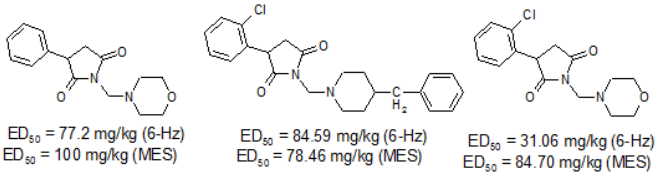
In aim to search of new anticonvulsants our attention was focused on a group of pyrrolidine-2,5-diones differently substituted at position-1 and -3 of imide ring. Among these compounds, the most active were N-Mannich bases with the spiro-β-tetralone moiety at position-3 of pyrrolidine-2,5-dione ring and 4-phenyl-piperazines with electron-withdrawing atoms as basic fragment. The obtained ED_{50} values were comparable to the that of known antiepileptic drugs [1].

Taking into consideration the above, in aim to obtain more potent compounds, in the present studies we designed and synthesized new series of derivatives in which we replaced the spiro-β-tetralone nucleus in position-3 into the phenyl ring without or with chloro atom at position-2, -3 or -4. At the imide nitrogen atom differently substituted piperazines, morpholine or 4-benzylpiperidine have been introduced. The results of pharmacological investigations showed, that introduction of mentioned aromatic rings in place of spiro fragment as well as exchange of phenyl moiety at position-4 of piperazines into other substituents increased anticonvulsant activity especially in MES and 6-Hz tests. The structures of the most active molecules (data in mice, *i.p.*) are presented in **Fig**. **1**.

Fig. 1.



All the compounds were tested for their anticonvulsant activity within the Antiepileptic Drug Development (ADD) program (Epilepsy Branch, Neurological Disorders Program, National Institute of the Neurological and Communicative Disorders and Stroke (NINCDS), Rockville, USA), by using of the procedures described elsewhere [2].

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17:00	Poster	38

The effect of inositol hexaphosphate on the transcriptional activity of genes encoding metalloproteinases and their tissue inhibitors in colon cancer cellsstimulated with phorbol-12-myristate 13-acetate

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Inositol hexaphosphate (IP6) is a naturally occurring phytochemical, found in abundance in cereals, legumes and other high-fiber-content diets. IP6 has shown promising efficacy against a wide range of cancers. Its anti-cancer activity involves anti-proliferative, pro-apoptotic and anti-metastatic effects. However, molecular mechanisms of its action have yet to be established. IP6 has been found to block phosphatidylinositol-3 kinase (PI3K), activating protein-1 (AP-1), protein kinase C (PKC) and mitogen-activated protein kinases (MAPK). Both matrix metalloproteinases (MMPs), a family of endopeptidases capable of degrading extracellular matrix proteins and their tissue inhibitors (TIMPs), are implicated in tumor growth, metastasis, and

angiogenesis. Phorbol-12-myristate 13-acetate (PMA) is a well-known inflammatory stimulator and tumor promoter that activates PKC and increases the invasiveness of various types of cancer cells by activating MMPs.

The aim of the present study was to examine the influence of IP6 on the expression of selected MMPs, i.e., MMP-1, -2, -3, -9, 10, -13 and their TIMP-1 and -2 in unstimulated and PMA-stimulated colon cancer cell line Caco-2. Quantification of genes expression in Caco-2 cells treated with 100 ng/ml of PMA, 2.5 mM of IP6 and both for 6 and 12h was carried out using real time QRT-PCR technique. Stimulation of cells with PMA only resulted in an up-expression of MMP-3, MMP-9, MMP-10, MMP-13 and TIMP-1 mRNAs at 6 and 12h. Caco-2 revealed an increase in MMP-2 and decrease in MMP-1 genes expression after 12h of PMA treatment. The quantity of TIMP-2 transcript was reduced by PMA compared to control cells at both 6 and 12 h. A significant decrease in MMP-2, MMP-3, MMP-10, MMP-13, and TIMP-1 expression in response to 2.5 mM IP6 was observed. IP6 down-regulated MMP-9 transcription induced by PMA in 6h lasting culture and the level of both MMP-2 and MMP-3 mRNAs was increased by PMA at 12h. Caco-2 treated with both PMA and IP6 at 6 – 12h showed a significant decrease in MMP-1 expression in comparison to PMA-stimulated cells. The results of this study show that PMA, protein kinase C activator, can modulate MMP and TIMP genes transcription in colon cancer cells Caco-2. 2.5 mM IP6 exerts an influence of basal mRNA expression of some MMPs and their tissue inhibitors and down-regulates MMP-1, MMP-2, MMP-3 and MMP-9 in cells treated with PMA.

17:00 Poster 39

Modulation of the expression of genes encoding TGF-β isoforms and their receptors by inositol hexaphosphate in human colon cancer cells

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Transforming growth factors-β (TGF-β) are multifunctional cytokines involved in the regulation of cell development, differentiation, survival and apoptosis. They are also potent anticancer agents that inhibit the uncontrolled proliferation of cells. Incorrect TGF-b regulation have been implicated in the pathogenesis of many diseases including inflammation and cancer. In humans, the TGF-β family consists of three members (TGF-β1, 2, 3) that show high similarity and homology. TGF-bs exert their biological activities on various cell types including neoplastic cells via their specific receptors. In recent years, there has been a growing interest of functional foods which, besides nutrient components, contain foodstuffs that improve overall health and reduce the risk of disease. Inositol hexaphosphate (phytic acid, IP6), a phytochemical present in large amounts in legumes, cereals, oilseeds and nuts, has been reported to possess various health benefits. IP6 selectively inhibits cancer cells without affecting the normal and acts synergistically with standard therapeutics.

The aim of this study was to examine the effect of IP6 on the expression of genes encoding TGF-b1, TGF-b2, TGF-b3 isoforms and their receptors TbRI, TbRII, TbRIII in human colorectal cancer cell line Caco-2. The cells were treated with 0.5, 1 and 2.5 mM IP6 for 3, 6 and 12h. The untreated Caco-2 cells were used as the control. Quantification of genes expression was performed by real time QRT-PCR technique with a SYBR Green I chemistry. The experimental data revealed that the TGF-b1 mRNA was the predominant isoform in human Caco-2 cells and that IP6 enhanced transcriptional activity of genes of all three TGF-b isoforms and their receptors TβRI, TβRII TβRIII in these cells. At concentrations up to 1 mM, IP6 over-expressed the genes in 6 h lasting cultures, however, its higher dose (2.5 mM) caused successively increased transcript level of TGF-b isoforms and receptors with the duration of experiment up to 12 h. The findings of this study show that IP6 enhanced the genes expression of TGF-b isoforms and those of their receptors in colon cancer cells at the transcriptional level in a way dependent on its concentration and time of interaction.

17:00 Poster 40

Bioequivalence study of 500 mg cefuroxime film-coated tablets in healthy volunteers

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Cefuroxime is a semisynthetic, broadspectrum cephalosporin antibiotic.

The aim of the study was to investigate the bioavailability of a generic formulation of 500-mg cefuroxime film-coated tablets (test) as compared to that of a branded formulation (reference) at the same strength to 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 formulation was followed by 7-day wash-out period. Cefuroxime concentration was determined by validated HPLC-UV method [1] in compliance with the principles of Good Laboratory Practice. The formulations were considered bioequivalent if the 90% CI of the geometric mean ratios (test/reference) for $AUC_{(0-t)}$, $AUC_{(0-\infty)}$ and C_{max} were within the range 80.00 – 125.00% [2,3].

24 healthy male and female volunteers completed the study. There were no significant differences in pharmacokinetic parameters between formulations. The results of the study indicate that the 500 mg film-coated tablets of Tarsime manufactured by Tarchomińskie Zakłady Farmaceutyczne "Polfa" (test formulation) are bioequivalent to those of Zinnat manufactured by GlaxoSmithKline Export Ltd (reference formulation). Both formulations were well tolerated.

The study was supported by Tarchomińskie Zakłady Farmaceutycz-

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17:00 Poster 41

Interaction of endotoxin and polymyxin B in B16 mouse melanoma model of metatstasis

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Lipopolysacharides (LPS) has been recognized as an efficient immune stimulator as well as multicytokine inducer and it is believed to have a potent antitumor and antimetastatic activity through a host-defense mechanism. So far it has been demonstrated that LPS could regress tumor growth in animal cancer models, which occurs to be promising even for cancer immunotherapy in humans. However, its use in human cancer therapy has been limited only to one trial, since is toxic causing sepsis.

Numerous peptides have been designed to bind and neutralize LPS. One of them is a natural peptide polymyxin B (PMB) which prevents noxious LPS effects occurrence during LPS-mediated endotoxin shock in animal models. The Polymyxin B mechanism of action depends on its interactions with bacterial cell membrane phospholipids what results in disruption of membrane structure.

In order to obtain the anticancer effect of LPS avoiding its toxicity we investigated the influence of LPS complexes with polymyxin and LPS complexes with anti-LPS antibody on lung metastases formation in the mice bearing B16 melanoma. In mice treated with LPS and polymyxin the number of metastatic foci was approximately 50-70% lower in comparison to control group of mice. We didn’t observed antimetastatic effect in mice treated with LPS alone. Treatment with polymyxin alone showed stimulation of metastatic foci formation.

This work was partly supported by Polish Ministry of Science and Higher Education -**The National Centre for Research and Development** (NCBiR) NR 13 0089 06 “Products of cell lysis of Gram negative bacteria”

17:00 Poster 42

Search for Huntington's disease biomarkers - amino acid profile analysis using HPLC method

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Huntington’s disease (chorea, HD) is a hereditary disease, which causes death of neurons in certain parts of the brain. Since 1993 its genetic basis has been known and it is associated with IT15 gene mutation on chromosome 4 encoding the huntingtin protein. The disease symptoms are involuntary movements and dementia. While HD progresses, the patient loses the ability to control the body movements, emotions and thinking. The patients with HD have a poor prognosis: the disease inevitably leads to death within 10 to 20 years since the occurrence of the first symptoms.

The pathogenetic factors responsible for the development of the disease include transcriptional deregulation and impaired energy metabolism. The metabolic defect is potentially associated, among other things, with decreased levels of branched-chain amino acids (valine, isoleucine and leucine), which are indirectly involved in the energy-giving Krebs cycle and the respiratory chain.

The purpose of this work was to determine the plasma amino acid profiles in a control group and in HD patients and to identify the amino acids which in time could prove to be HD biomarkers. Defining biomarkers of this neurodegenerative disease could help to early diagnose HD, to monitor the progression of changes and to assess the administered neuroprotective therapy.

For this purpose, blood samples were collected from 30 patients with HD and 29 healthy controls (approval of the Bioethics Committee at the Poznań University of Medical Sciences No. 770/09).

Plasma samples deproteinized by ultrafiltration and a model mixture of amino acids were submitted to the derivatization reaction with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC). The obtained stable derivatives were tested by the gradient HPLC method using a fluorescence detector (E_X = 250 nm, E_M = 396 nm). As the quantitative assessment of amino acids in biological material requires a highly selective method, an ion-pairing mechanism was used, due to using a triethylamine buffer with additional N,N-dimethyloctylamine in the mobile phase. The separation conditions were optimized by selecting the appropriate pH value, column temperature, counterion concentration and acetonitrile gradient. The presented chromatographic conditions allowed separation and quantitative determination of 23 amino acids.

The statistical analysis of the obtained results was performed using the SYSTAT software for Windows, version 13.00.05 (SYSTAT Inc., USA). The use of the non-parametric Mann-Whitney test helped to demonstrate that 5 amino acids (asparagine, histidine, leucine, serine, threonine) have significantly lower plasma levels in patients with HD as compared to healthy controls ($p<0.05$, $\alpha=0.05$).

17:00 Poster 43

Synthesis of marker compounds for detailed explanation the mechanism of anticancer activity of peptidomimetics with β-acyloxymethacrylic fragment

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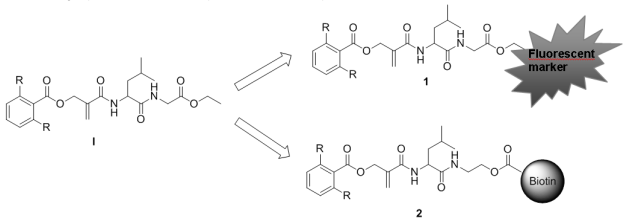
During recent studies on development new inhibitor of thioredoxin system inhibitors, the new class of β-acyloxyamides **I** has been found. Studied peptidomimetics exhibited interesting biological properties and were very potent anticancer agents either in vitro (in cellular assays) or in vivo (mice model). [1,2]



Detailed mechanistic studies indicated, however, that compound **I** activates apoptotic pathways not only by inhibiting the thioredoxin system. To establish mechanism of action of new compounds in a cell, we developed marker compounds containing fluorescent fragments as well as biotinylated compounds **1** and **2**. Here, we will present the proposed methodology as well as the results of studies on the synthesis of compounds **1** and **2.**

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[2]: Ostaszewski, R., Klossowski, S., Szokalska, A., Swiech, M., Ziuzia I., Golab, J., Patent App. PK/1081/AG

17:00 Poster 44

Application of different α-1-thioglycosides preparation methods in synthesis of 5-nitro-pyridyl 1-thioglycosides - substrates in construction of conjugates with uridine moiety

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Glycosyltransferases (GTs) are enzymes involved in the synthesis and modification of the multitude of glycoconjugates that exist in the biosphere. They typically act by adding monosaccharides one at a time to specific positions on specific precursors. The biosynthesis of glycans is primarily determined by these sequentially acting enzymes, which assemble monosaccharides into linear and branched sugar chains [1]. In general GTs catalyze transglycosylation reactions where the monosaccharide component of a high-energy nucleotide sugar donor (e.g., GDP-Fuc or CMP-Sia) is transferred to a precursor – acceptor, forming glycosidic bonds. The result of glycosyl transfer can be a glycoside, oligo- or polysaccharide. Glycosyl transfer can also occur to protein residues, may also use lipids as an acceptor, forming glycolipids, or even lipid-linked sugar phosphate donors [2,3]. GTs donor type natural substrates generally consist of three different moieties that can be distinguished: carbohydrate part, diphosphate linkage and nucleoside moiety (mostly uridine).

Inhibition of glycosyltransferases have an enormous significance in controlling of synthesis of cell-surface glycoconjugates. It leads to the modulation of oligosaccharides biosynthesis and enables recognition of their biological functions. Therefore some inhibitors of GTs may be interesting from therapeutic point of view.

Our previous research on glycosyltransferases inhibitors revealed biological activity of these connections against classical swine fever virus (CSFV) [4,5]. Our recent research led us to obtain glycoconjugates derivatives of D-glucose, D-galactose, 2-deoxy-D-glucose and 2-deoxy-D-galactose including α-1-thioglycosidic part connected to selectively protected uridine through the amide bond. Improvement of the synthesis and investigation of the biological activity are in progress.

Research studies part-financed by the European Union within the Structural Funds in Poland (POIG.01.01.02-14-102/09). Roman Komor was supported by European Funds (POKL.04.01.01-00-114/09-01).

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17:00 Poster 45

The biological activity of sugar derivatives based on quinoline scaffold

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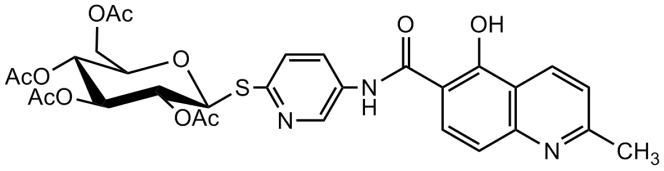
The quinoline moiety can be considered as privileged structure (1).

It is present in many synthetic drugs and natural products with spectrum of activity covering antifungal, anticancer and antiviral effects(2-3). Nevertheless bioeffectors designed on the core of quinoline moiety still suffers from poor bioavailability/membrane transport. This prompted us to incorporate biologically relevant sugar scaffold into some quinoline related biomolecules.



The example of synthesized compound

Sugar part could be mono- or oligosaccharide connected to quinoline derivatives by amide, ester, thioester or glycosidic bond. Choice of sugar part structure was caused by earlier performed researches (4-5).

Obtained structures and their expected and examined biological activity will be presented. Moreover all synthesized compounds are part of the novel **Polish Patent Application** (**2012**).

Research studies are part-financed by Ministry of Science and Higher Education of Poland (Grant No. N N209 186338). Maciej Serda was supported by a TWING fellowship and NCN grant DEC-2011/01/N/NZ4/01166.

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17:00	Poster	46

Synthesis of new class of cytotoxic chalcone derivatives

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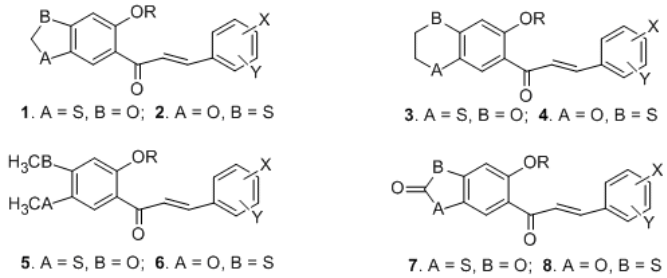
Chalcones are widely explored as potential lead compounds for new drugs, including antitumor medicaments [1-10]. In vitro cytotoxicity of most of chalcone derivatives is in 1 – 50 micromolar range. Now we found that chalcones bearing a fused oxathiole ring (**1** or **2**) display cytotoxic activity even in a nanomolar range, e.g. for compound **AMG-304** (**5**, R = CH_3, X = 4-OCH_3, Y = 3-OH), IC_{50} = 10 nM (A549) and 5 nM (HeLa).

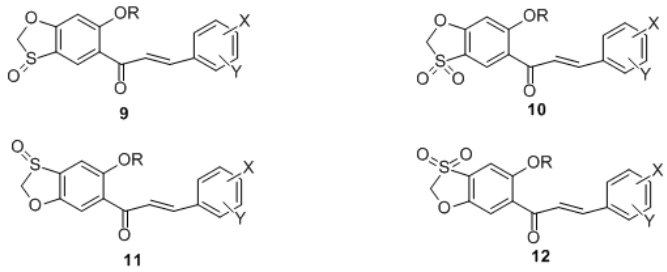
Change of the ring size from five to a six membered one (**3**, **4**) does not influence much the activity, while opening of the heterocyclic ring (**5**, **6**) or its substitution in the position 2 with oxygen (**7**, **8**) diminish the activity (Figure 1).

Figure 1.



Oxidation of isomer **1** to sulfoxide (**9**) or sulfone (**10**) result in decrease in cytotoxicity, while analogous oxidation of isomer **2** lead to derivatives **11** and **12** with increased cytotoxic activity (Figure 2).

Figure 2.



The results suggest, that either the relatively small modifications result in change of the mechanism of the cytotoxicity, or structure of the "left hand part" of the molecules is extremely important for interaction of the compounds with target.

Activity of the compounds was also influenced by radical R in the OR alkoxy group, and by substituents X and Y. No simple relationship exist between the influence of the listed structural factors on the cytotoxic activity in vitro of different chalcones.

Cytotoxic activity of chalcones is often associated with their effect on polymerization of tubulin. For this reason inhibition of tubulin polymerization and inhibition of ^{3}H-colchicine binding to tubulin by selected AMG chalcones were studied. Next, the obtained values were correlated with cytotoxicity (Table 1).

Table 1.

Compound	Colchicine site binding IC_{50} [μM]	Tubulin polymerization IC_{50} [μM]	Cytotoxicity A549 IC_{50} [μM]
colchicine	4.260	nd	
combretastatin A4	0.023	1.73	0.008

Compound	Colchicine site binding IC_{50} [μM]	Tubulin polymerization IC_{50} [μM]	Cytotoxicity A549 IC_{50} [μM]
AMG-175 (structure **1**)	0.489	5.80	0.352
AMG-190 (structure **2**)	0.108	4.15	0.369
AMG-202 (structure **3**)	0.770	4.45	0.224
AMG-221 (structure **11**)	0.195	4.55	0.033
AMG-228 (structure **12**)	0.164	7.35	0.293

nd – not determined

The comparison revealed that there is no correlation between cytotoxicity and inhibition of tubulin polymerization, or colchicine binding.

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17:00 Poster 47

Proapoptotic effects of new pentabromobenzylisothioureas and CK2 inhibitor in prostate adenocarcinoma cell line

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Prostate cancer is one of the most common cancers in elderly men in the Western world. Its growth is dependent on androgens and androgen receptor signaling. Androgen ablation by orchidectomy and/or treatment with LHRH-analogs or anti-androgens are the most common therapy strategies. Chemical castration often leads to a selection of hormone-refractory cells, which are mostly characterized by activation of anti-apoptotic signaling pathways. The agents capable of inducing apoptosis in prostate cancer cells seems a promising approach to treatment of this malignancy. In prostate cancer cells seems a promising approach to treatment of this malignancy. In this study we describe the synthesis of several new modified S-2,3,4,5,6-pentabromobenzylisothiouronium bromides and their activity against human prostate adenocarcinoma PC-3 cell line. All the tested compounds induced apoptosis and cytostatic effect in the tested cancer line. Apoptosis induced by the most effective compounds ZKK-3, ZKK-9 and ZKK-13 (20 μM) was 41,5%, 46,3% and 65,7% respectively, after 48h incubation time. We also determined synergetic effects of combination of two selected S-2,3,4,5,6-pentabromobenzylisothiouronium bromides (ZKK-3, ZKK-9) with new casein kinase II inhibitor 2-(4-methylpiperazin-1-yl)-4,5,6,7-tetrabromo-1H-benzimidazole (TBIPIP) in prostate cancer PC-3 cell line. Flow cytometry analysis were run on a FACSCaliur Flow Cytometer (Becton Dickinson Co., San Jose, CA, USA).

Acknowledgment

This study was supported by the Ministry of Science and Higher Education (Poland), grant No. N N209 371439.

17:00 Poster 48

HPLC method for determination of the enantiomeric purity of a new ω chain aldehyde synthon used in the synthesis of travoprost

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A new optically pure synthon, precursor of the ω chain, was applied for synthesis of Travoprost (Fig.1), which is a topical medication used for controlling the progression of glaucoma or ocular hyperten-

sion, by reducing intraocular pressure. It is a prostaglandin analogue that works by increasing the outflow of aqueous fluid from the eyes. [1]

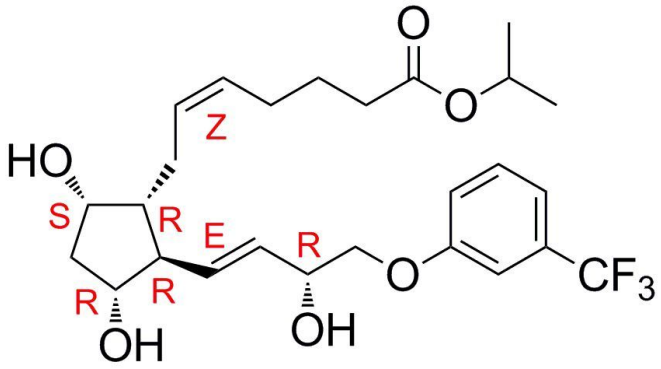

Fig 1. Travoprost, namely izopropyl (Z)-7-{(1R,2R,3R,5S)-3,5-Dihydroxy-2-[(R,E)-3-hydroxy-4-(3-trifluoromethylphenoxy)but-1-enyl]cyclopentyl}hept-5-enoate

Synthesis of the new ω chain synthon consisted of seven optimized synthetic steps. Identity and enantiomeric purity of the synthesized compounds were determined.

Determining the enantiomeric purity of chiral therapeutic agents is important in the development of active pharmaceutical ingredients (API). Analytical method used for this purpose must ensure fast and effective separation of isomers. The common and widely applied method which meets these requirements is high performance liquid chromatography (HPLC).

The HPLC methods for determination of investigated compounds were developed. The individual compounds were separated using columns containing polysaccharide chiral stationary phase. The mobile phase consisted of hexane with addition of an alcohol (methanol, ethanol or isopropanol). UV detection with detector wavelength was 220 nm was applied to determine the investigated compounds. In course of extensive research it was proven, that the developed methods are selective and sensitive for all separated compounds. Elaborated method fulfills the criteria of European Pharmacopoeia for API analysis and can be used to control the synthesis process. [2][3]

[1] leki-nformacje.pl

[2] www.usp.org

[3] European Directorate for the Quality of Medicines & HealthCare, European Pharmacopoeia 7.0, Chapter: 2.2.46 *Chromatographic separation techniques* (2010)

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nr UDA-POIG.01.03.01-14-068/08-00 „Opracowanie innowacyjnych technologii leków oftalmicznych o szczególnym znaczeniu terapeutycznym i społecznym”

17:00 Poster 49

ESI-MS/MS techniques for structural analysis of boron clusters

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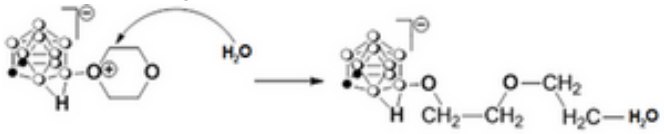
Boron-containing compounds are actually widely used as insecticides, bactericides, reducing reagents (organic chemistry), catalysts, materials for the production of some types of ceramics, the liquid crystal components, temperature-resistant polymers and many others. As potential drugs, they were met with great interest in cancer therapy using technique called “Boron Neutron Capture Therapy” (BNCT). The idea of this therapy is based on the fact that we target molecules containing boron isotope ^{10}B into the tumor cells or in their direct surrounding. Tumor tissue is subjected to irradiation in a stream of slow neutrons. Boron atoms are able to capture neutron and as a result, they disintegrate with emittion of alpha particles. Nowadays, scientists test a big variety of boron carriers including: amino acids, sugars, lipids, porphyrins, DNA-oligonucleotides and their components-nucleosides. Carboranes are boron cage systems in which one or more carbon atoms are bound as an integral part of electron-delocalized borane framework. They are characterized by high boron content, remarkable thermal and chemical stability, spherical geometry and high hydrophobicity. Hydrophobicity is the main reason why particularly important are reactions of boron clusters with macromolecules in aqueous or aqueous-organic solutions.

Techniques of mass spectrometry (ESI-MS/MS) are not common for analysis of compounds containing boron. This is due to the generation of complex spectra related to the natural content of boron isotopes ^{10}B and ^{11}B. However, these techniques have the high possibilities of throughput for sample analysis.

Analyzed compounds were carborane and carborane metal complexes adducts of dioxane that were generated in order to react with biomolecules. We proved that in an aqueous-organic solution undergo structural modifications in the structure of the dioxane ring of cluster boron adduct. Mechanism of structure modification was determined as binding of the water molecule that is shown below:



Observed modification in structure of boron cluster dioxane adduct had a direct impact on further ability to react with macromolecules.

17:00 Poster 50

Vaccines for swine authorized on Polish market in 2008 and 2009-change of trends after harmonization according to EU pharmaceutical codex

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According to pharmaceutical codex authorization of immunological veterinary medicines products (ivmp, vaccines) on European Union market bases on mutual recognition agreement. Polish market, after harmonization these rules in 2009, has got full admittance to the EU veterinary medicines products (vmp) wholesaler system. In 2008 there were 1303 vmps, 348 ivmps included: 70 vaccines for swine, authorized on national market. While in 2009 these figures accounted: 1184; 331; 63**,** respectively. Statistics of doses within the ivmp batches revealed 24,57 million for swine, authorized in 2008, since after harmonization the dose numbers increased to 32,35 million. Analysis of particular ivmp batches according to prophylaxis destination revealed substantial quantity differences of dose scores between 2008 and 2009. It concerned both the vaccines against bacterial and viral diseases. The most pronounced increase, were recorded for circoviral type 2 ivmp in swine. Thus, the harmonization considerably influenced the country market within the number of products, because of not standing for registration procedure of national "old" ivmp. On the other hand, marked quantity changes within some vaccines butches being authorized imply prophylactic programmes reorientation. The reason for particular vaccines should be analysed out by species specialists looking for rationale answer for it.

17:00 Poster 51

Karl Fisher determination of a residual moisture in veterinary vaccines - practical implementation in market monitoring

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Residual moisture content plays a significant role in assessing the stability of veterinary vaccines. Analysis of water amount is often critical parameter, which determines the quality of product, its appearance as well as the expiration date. The aim of the study was to validate a coulometric Karl Fisher method for practical use in the monitoring of veterinary vaccines national market. Immunological veterinary medicinal product (ivmp) for three different animal species – cats, dogs and rabbits - were used. Automated coulometric analysis in chamber without diaphragma was used, as well as a solution for titration, which was a mixture of diethanolamine, imidazole, methanol and sulfur dioxide. The weight of a single sample was 15 – 100 mg. The most important concern was optimisation of the way of transferring a vaccine sample into titration cell, so that atmospheric moisture would not affect baseline drift and repeatability of the results. Humidity level in lyophilised biopharmaceuticals was validated in accordance with the guidelines. Method was linear in the range of one to five percent of water content with R^2=0,9998. Repeatability for different sample types was found to be not higher than CV% = 5,9. The method was used for vaccines market monitoring in 2010 and 2011. Thirteen vaccines from the market were tested and all were found to be compliant with official EU guidelines.

17:00 Poster 52

Examination of antimicrobial activity of selected non-antibiotic products

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A variety of pharmaceutical preparations, which are applied in the management of non-infectious diseases, have shown *in vitro* some antimicrobial activity. These drugs are called "non-antibiotics". So far, a lot of attention has been focused on phenothiazines, thioxanthenes and other agents with affinities to cellular transport systems or agents showing other inhibition mechanism. Several authors confirmed that some non-antibiotics are "helper compounds", which enhance the *in vitro* activity of certain antibiotics against specific bacteria (ex. omeprazole and nizatidine enhance the effect of metronidazole on *Helicobacter pylori*). The aim of this study was to detect and characterise the antimicrobial activity of non-antibiotic drugs, selected from the pharmaceutical products analysed during the state control performed in National Medicines Institute. Over 90 pharmaceutical preparations were randomly chosen from different groups of drugs. The surveillance study was performed on standard ATCC microbial strains used for drug control: *S. aureus*, *E. coli*, *P. aeruginosa* and *C. albicans*. It was shown that the drugs listed below inhibited growth of at least one of the examined strains: Arketis 20 mg tab. (paroxetine), Buvasodil 150 mg tab. (buflomedile), Halidor 100 mg tab. (bencyclane), Hydroxyzinum espefa 25 mg tab. (hydroxyzine), Norifaz 35 mg tab. (risedronate), Strattera 60 mg cap. (atomoxetine), Tamiflu 75 mg tab. (oseltamivire), Valpro-ratiopharm Chrono 300 mg tab. (valproate), Vetminth oral paste (24+3) g/100 ml (niclozamide, oxybendazole). Atomoxetine and bencyclane showed broad activity spectrum. They inhibited growth of all examined strains (MIC of atomoxetine: 2.6 - 13 mg/ml; MIC of bencyclane: 12.5 - 31 mg/ml). The highest activity against Gram-negative bacteria was found for risendronate (MIC 1.4 mg/ml). *S. aureus* was susceptible to the mixture of niclozamide and oxybendazole in concentration 0.24 and 0.03 mg/ml, resp. Paroxetine was active against *S. aureus* and *E. coli* (MIC 2.5 mg/ml and 5 mg/ml, resp.). For twenty-fold diluted solution of Inhalol consisting of aromatherapy oils, antiseptic activity was found against: *S. aureus*, *E. coli* and *C. albicans*. The antimicrobial activity of the pharmaceutical products necessitates neutralisation of this activity during pharmacopoeial microbial purity assays.

17:00 Poster 53

Examination of optical isomerism - compounds of antihypertensive effects.

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Chemical compounds containing chiral centers in their molecules show optical isomerism. Angiotensin converting enzyme inhibitors (ACE inhibitors) used in arterial hypertension treatment is a large group of medical substances containing chiral centers.

One of them - benazepril hydrochloride contains two stereogenic centers, but is currently available as single enantiomer (S,S configuration) for the treatment of hypertension. Its enantiomer (R,R configuration) and the diastereoisomeric pair (R,S and S,R) can be regarded as impurities.

The aim of this study was to estimate stereochemical stability of S,S isomer of benazepril hydrochloride as well as examining their potential susceptibility to conversion in the active substance and in Lisonid tablets.

At the first stage of the study the chromatographic methods of separation of examined enantiomers were explored applying TLC and HPLC methods.

The best separation with the use of the TLC method was obtained the following system: chromatographic plates Chiralplate and a mobile phase methanol – acetonitrile - 1mM copper (II) acetate (4:2:4) with saturation of glacial acetic acid for 1 hour.

For the separation with the HPLC method numerous chiral columns as well as mobile phases were tested. The system, described in Ph.Eur., proved to be the best one: Chiral AGP column (150mm x 4,0mm x 5µm) and a mobile phase: phosphate buffer pH=6,0 – methanol (80:20), column temperature: 30°C, flow rate: 0,9 ml/min, wavelength λ=240 nm.

The system was subsequently used for examination of stereochemical stability of benazepril hydrochloride in the substance and Lisonid – coated tablets 20 mg.

The active substance - benazepril hydrochloride and coated tablets Lisonid 20 mg were subjected to the impact of different stress factors: temperature of 40°C, 1 N sodium hydroxide solution and temperature of 40°C, 0,05 N sodium hydroxide solution and temperature of 40 °C, 1 N hydrochloric acid solution and temperature of 40°C, 6% hydrogen peroxide solution and temperature of 40°C and 1000 watt UV light, 3 times each 10 minutes.

Tests were carried out after 1 and 6 weeks.

It was found that none of the applied stress factors caused the transformation of SS enantiomer of benazepril hydrochloride in the substance and tablets to other identified stereoisomers - only the compound decomposition has occurred.

17:00 Poster 54

Actualities of the European Pharmacopoeia in the Polish Pharmacopoeia

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Elaboration and publication of the Polish Pharmacopoeia are essential parts of the mission of the Office for Registration of Medicinal Products, Medical Devices and Biocidal Products, i.e. “Our main priority is to ensure the proper quality, efficiency and safety of medicinal products, medical devices and biocidal products in benefit of the society’s wellbeing”.

The new, IXth edition of the Polish Pharmacopoeia, which is in full accord with the European Pharmacopoeia (Ph. Eur.), was published in November 2011. It is the first edition of the Polish Pharmacopoeia which contains Polish version of full texts of Ph. Eur. published up to now systematically in the VII and VIII editions of Polish Pharmacopoeia.

Since December 2006 Polish representatives have been taking part in the activities of the European Pharmacopoeia Commission and its groups of experts in Strasbourg. Proposals concerning the introduction or revision of monographs Ph. Eur. have been made by the Polish party.

The IXth Edition of the Polish Pharmacopoeia contains also systematically extended section of national monographs for galena products, a register of doses and A, B, N registers of active substances described in monographs.

The Act of 18 March 2011 on the Office for Registration of Medicinal Products, Medical Devices and Biocidal Products introduced also organization changes in the activity of the Polish Pharmacopoeia Commission. In November 2011 a new Commission composed of 7 members has been established. In February 2012 eleven expert groups have been called up. Their activity is coordinated by the Pharmacopoeia Department.

The publication system of the European Pharmacopoeia requires constant updating of the Polish Pharmacopoeia. Therefore, in 2012, the Supplement 2012 based on the Supplements 7.3 – 7.5 Ph. Eur. will be published. It will also contain successive national monographs.

17:00 Poster 55

An application of accelerator mass spectrometry (AMS) in pediatric clinical studies. Paracetamol, midazolam and spironolactone radiosynthesis and certification.

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Combined application of linear accelerator and high resolution mass spectrometer (AMS) for detection of organic compounds containing suitable label, allows to achieve levels of sensitivity which are not attainable for other analytical methods. For active pharmaceutical substances, which contain molecules labeled with carbon 14, femtomolar concentrations can be determined, which allows to study pharmacokinetic parameters (PK) and metabolic fate of a drug, from single sub-pharmacological dose (e.g. 100 micrograms). This novel way of collecting the PK data, customary obtained during Phase I clinical trials, is described as “microdosing” (or Phase 0 clinical trial).

In case of isotopically labeled compound designated for microdosing study with AMS detection, design of synthetic route requires taking into consideration all known methods of preparation, and commercially available or potentially labeled synthons, securing reasonably late entry of radioactive material into the process. Additionally, metabolic transformations have to be considered, in order to place the label in a position which is not susceptible to biotransformation. Labeled compounds require slightly different certification then parent drug compounds and they may need elaboration of a new method of qualitative analysis, as well as for quantification.

Pharmaceutical Research Institute (PRI) currently participates in second EU project which uses microdosing/AMS technique, this time for benefits of pediatric patients. The origin of this studies is the problem that to this date there is no data about PK or metabolism routes for children in a different age because clinical studies on children were (and still are) generally considered as unethical. Because of very small quantities of labeled drug which are given to patients and using very sensitive AMS technique as an analytical tool it should be finally possible to find a proper way for medical treatment of children.

In the ERA-NET PrioMedChild program which is planned for three years, the role of PRI is to provide three [14]C-labeled active pharmaceutical ingredients: Paracetamol, Spironolactone and Midazolam. These API have to have exact quality, they should also be certified and permitted as a suitable for using as a medicine for children. On the poster presentation selected synthetic issues connected with mentioned above [14]C-drugs will be shown. We also would like to outline a development of analytical methods, analysis and certification procedures for labeled compounds that are done by PRI. Moreover further aspects about clinical studies and AMS utility in this project will be briefly disclosed.

17:00 Poster 56

An improved process for the preparation of 2-amino-N-tert-butyl-2-cyanoamide hydrochlorie.

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Non commercially available 2-amino-N-tert-butyl-2-cyanoamide hydrochloride is the one of the two reagents used in the synthesis of 5-amino-N-tert-butyl-1-methylcarbamoyl imidazole-4-carboxamide. This compound is a key starting material in the straightforward synthesis of temozolomide, an oral alkylating agent which can be used for the treatment of an aggressive brain tumor or some forms of skin cancer [Figure 1].

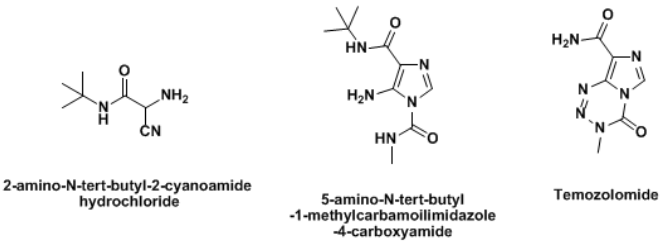

Figure 1

Known from the literature two-step synthesis of 2-amino-N-tert-butyl-2-cyanoamide hydrochloride is based on a reaction of (diphenylmethylene)-aminoacetonitrile with tert-butyl isocyanate in the presence of potassium tert-butoxide and THF as a solvent followed by purification of the product and further acid hydrolysis which removes diphenylmethylene protecting group from the nitrogen. Unfortunately there are many drawbacks for using procedure described above, like non environmental friendly solvents. This process is also time and energy consuming. Furthermore the yield and purity of such obtained product are not satisfying.

Our invention provides an improved route for the preparation of 2-amino-N-tert-butyl-2-cyanoamide hydrochloride with high yield (72-80%) and very high purity (>99.5%, HPLC). Instead of a solution of the base, solid potassium tert-butoxide is used in the reaction with tert-butyl isocyanate in dichloromethane as a solvent. Moreover further hydrolysis in the presence of hydrogen chloride is carried out without the purification of the intermediate and both reactions with tert-butyl isocyanate and hydrolysis are done in the same solvent. Simultaneously the idea of the presented innovation is also to simplify technological operations by reducing the amount of toxic solvents, wastes, time and energy which is crucial for multi-scale synthesis.

17:00 Poster 57

Application of amylose tris(3,5dimethylphenyl- carbamate) chiral stationary phase for the determination of paroxetine, clinically used psychotropic drug

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Paroxetine
)-3-[(1,3-benzodioxol-5-yloxy)methyl]-4-(4-fluorophenyl {(-)-(*3S*,*4R*)piperidine]} is a potent, selective serotonin reuptke inhibitor exhibiting minor affinity to muscarinic and cholinergic receptors. In clinics it is mainly used in major depression, anxiety and obsessive-compulsive disorders. In pharmaceutical preparations - beside (-)-*trans*-paroxetine (**I**) - (+)-*trans*-paroxetine, its probably inactive enantiomer [1], may be present.

Here we report validated analytical procedure enabling determination of (+/-)-*trans*-paroxetine in medicinal products containing (+)-*trans*-paroxetine as a main active ingredient with the aid of amylose tris(3,5dimethylphenylcarbamate) chiral stationary phase. Validation included: specificity, linearity, limit of detection (LOD), limit of quantification (LOQ), assay, precision and recovery.

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of paroxetine, *Bioorg. Chem. 31, 248-258 (2003)*.

17:00 Poster 58

Assessment of selected synthesis of bosentan towards elimination of known impurities.

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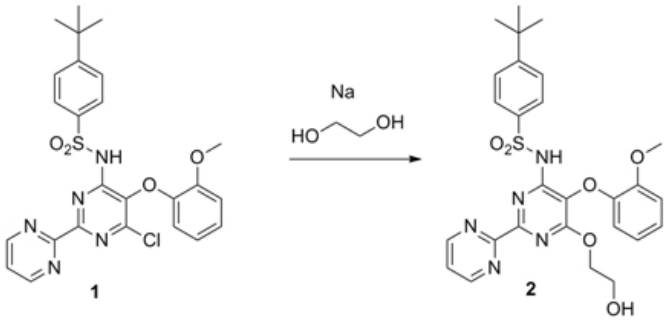
Bosentan is first oral dual endothelin A and B receptor (ETA/ETB) antagonist. The drug is indicated for the treatment of pulmonary arterial hypertension (PAH). In patients with systemic sclerosis and ongoing digital ulcer disease it is used for the reduction of new digital ulcers.



In selected synthesis of bosentan (2) the reaction of halogenoderivative (1) with sodium glycolate is carried out in the excess of ethylene glycol [1] (scheme 1). Reaction is accompanied by formation of two main impurities known as “deshydroxyethylbosentan” and” dimer”. First of them appears to be a product of bosentan molecule degradation process, while the second is formed as a result of substitution of both hydroxyl groups of ethylene glycol with two molecules of the starting compound 1.

Modern quality requirements for active pharmaceutical ingredients permit the amount of a single, known impurity below 0,15 %. Available literature data indicate that raw bosentan obtained that way should be purified by means of three subsequent crystallizations but level of impurities in such purified material still exceed acceptable limit [2, 3].

Considering implementation of this synthetic method for manufacturing of bosentan critical selection of particular reaction condition is required to achieve reduction of impurities level at the stage of synthesis before any purification method is applied. We investigated dependence between impurities level and selected process parameters such as temperature, reaction time and amount of sodium glycolate used in the reaction in order to propose most favourable conditions for evaluated process. In process control as well as impurities level monitoring was performed, by means of HPLC analysis of raw reaction mixtures.

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[3]
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17:00 Poster 59

Searchıng for new ısoxazole derıvatıves wıth potentıal ımmunorestorıng actıvıty

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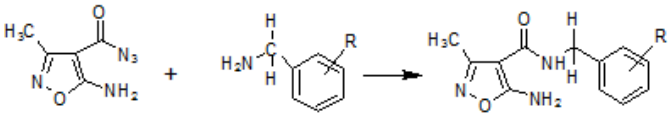
Our investigations of biological activity of isoxazole derivatives showed very interesting immunological properties. Searching for new active immunomodulators we synthesized a few groups of isoxazole derivatives, which showed significant immunological activity in several *in vitro* and *in vivo* assays in mice and humans [1-8]. A new series of substituted benzylamides of 5-amino-3-methyl-4-isoxazolecarboxylic acid (I) was synthesized in reaction of substituted benzylamines with 5-amino-3-methyl-4-isoxazolecarboxylic acid azide [9]. Each of these new structures opens possibility of a variety of structural modification. Pure products were obtained in average yields, therefore the optimal conditions were determined. The change of parameters of the reactions (temperature, equivalent of reagents, solvents etc.) lead

to different products and of course, may influence on efficiency and/ or purity of the obtained products. Described benzylamides of 5-amino-3-methyl-4-isoxazolecarboxylic acid (I) will be used as a starting material for the synthesis a new isoxazole derivatives. Structures of new compounds (I) were proven with elemental analysis and IR, NMR spectroscopy.



(I)

The influence on the PHA-induced proliferation of human mononuclear blood cells and toxicity to the PHA-induced proliferation of human mononuclear blood cells of derivatives (I) were tested. Preliminary assays showed very interesting immunosupresory and anti-inflammatory activities with low toxicity. Computational study, molecular modeling and structure/activity relationship was performed.

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

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17:00	Poster	60

Application of HPLC for studying purity, content and release profile of temozolomide capsules

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The aim of the study was to develop analytical methods for the determination of temozolomide, an anti-cancer drug from the imidazotetrazine group [1]. Temodal 5 mg, 20 mg and 100 mg capsules manufactured by SP Labo N. V. (Belgium) and the drug obtained in the Pharmaceutical Research Institute (Poland) were used.

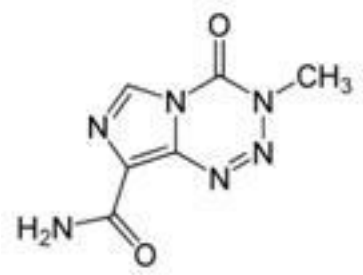

Fig. 1. Temozolomide

The analytical method for the purity study was characterized by good HPLC parameters:

- High separation
- Short time of analysis
- Favorable peak's symmetry
- A lot of theoretical plates

No unidentified impurities were detected both in the reference product and the capsules manufactured in the Pharmaceutical Research Institute. The only identified impurity AIC (5-amino-imidazole-4-carboxamide) met tight acceptance criteria. The developed methods are in accordance with the Pharmacopea's [2] requirements regarding HPLC methods.

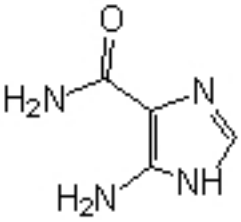

Fig. 2. 5-amino-imidazole-4-carboxamide (AIC)

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2. Ph.Eu. VIII

17:00	Poster	61

The influence of fluphenazine and its newly synthesized derivatives on the properties of liposomal membranes

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Fluphenazine, from the group of phenothiazines, is mainly known as

a very potent antipsychotic drug [1]. Phenothiazines also belong to one of the eldest classes of modulators of multidrug resistance (MDR) in cancer cells. MDR is a development of the mechanism of active, outward drug transport, which is a common strategy used by cancer cells to defeat themselves against chemotherapeutic agents [2]. To obtain the accumulation of anticancer drugs in resistant cells either the permeability of the cell membrane must be increased or the efficiency of efflux pumps must be reduced. The direct relationship between membrane permeability and physical state of lipids is obvious, but also the activity of transporters should be modulated by the lipid composition [3] or biophysical properties (e.g. fluidity) of the membrane [4].

To optimize and modulate the biological effects of fluphenazine some efforts are made to synthesize the derivatives of this phenothiazine. In the present work we studied the influence of fluphenazine itself and its new derivatives, synthesized in our department – SM1, SM8, SM10, SM12 – on lipid bilayers. Phenothiazine effects were assessed by spectrofluorimetry, using unilamellar liposomes labeled with DPH.

The studied phenothiazines caused concentration-dependent increase of DPH fluorescence polarization anisotropy. The effect was observed for bilayers in the liquid-crystalline phase, because liposomes were made of lecithin from egg yolk (EYPC), which is liquid at room temperature. The results presented allow the conclusion that studied phenothiazines interact with the lipid bilayers and decrease their fluidity in the liquid-crystalline phase. All of the studied compounds increased DPH fluorescence polarization anisotropy in EYPC liposomes, which can be interpreted as a rigidifying effect exerted by this compound on the hydrocarbon chains regions of the lipid bilayer.

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[3] Y. Romsicki, F.J. Sharom, The membrane lipid environment modulates drug interactions with the P-glycoprotein multidrug transporter, Biochemistry 38 (1999) 6887–6896,
[4] R.M. Wadkins, P.J. Houghton, The role of drug–lipid interactions in the biological activity of modulators of multi-drug resistance, Biochim. Biophys. Acta 1153 (1993) 225–236.

17:00 Poster 62

On Substituent Effect in the on the Benzodiazepinone System

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Tautomerism, aromaticity, and electron density in the ring critical points were analyzed for the 1,3-dihydro-benzo[e][1,4]diazepin-2-one (BDA) molecule substituted at the position C7 of the benzo ring at the B3LYP/ 6-31G** level. We found that the N1H tautomer is much more stable (a matter of at leat 8 kcal/mol) than the N4H in the gas phase and in water. It is shown that the grater is the π-electron donation effect the more stable is the N1H tautomer. The benzo ring is aromatic and the diazepinone is antiaromatic in N1H tautomers while in the N4H tautomers the former is practically not aromatic and the latter is much more antiaromatic. This is the main reason why the N4H tautomers are so much less stable than N1H ones. The AIM analysis of the electron density or its Laplacian in ring critical point of the seven-membered ring correlate well with the pEDA descriptor of the substituent effect on π-electron system, while for the benzo ring the analogous correlations are much weaker. This indicates that the susbstitution at the benzo ring modifies significantly the electron dencity of the diazepinone ring.

17:00 Poster 63

Colon cancer treatment with the use of vitamin D analogs and irinotecan

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Colorectal cancer is the second most common cause of mortality due to cancer among men and women [1]. Epidemiological studies strongly suggest a protective effect of calcitriol (1,25-dihydroxyvitamin D3) against this type of cancer. Moreover, the experimental research reveals its anticancer properties but only in hyper-physiological doses, which can lead to hypercalcemia. For this reason the synthesis of vitamin D analogs has been started in order to obtain compounds with better therapeutic activity. On the basis of previous studies we selected two analogs coded: PRI-2191 (tacalcitol, 1,24-dihydroxyvitamin D_3) and PRI-2205 (5,6-trans-isomer of calcipotriol), which reveal higher antitumor and lower calcemic activity as well as lower toxicity than calcitriol [2, 3].

One of the anticancer research directions is the development of novel combined treatment strategies. The benefit from such an approach is a possibility of enhancing the therapeutic effect of a drug, which is the basis of a standard therapy [4]. In the current work, it is presented the influence of vitamin D analogs on antitumor activity of irinotecan in mice bearing transplantable murine colon cancer MC38. The antitumor effect of combined treatment was evaluated as tumor growth inhibition and increase in life span of treated mice over control. The monitored parameters were body weight and tumor volume, which was calculated using the formula $(a^2 \times b)/2$, where a = shorter tumor diameter in mm and b = longer tumor diameter in mm.

It was observed that both vitamin D analogs improve antitumor activity of irinotecan in murine MC38 colon cancer model. Tumor growth inhibition by irinotecan and PRI-2191 or PRI-2205 was ob-

served starting from the eight day of the experiment till the last day (in comparison to the group treated with cytostatic alone). At the first stage of experiment the additive effect was observed which than changed to synergy. Moreover, the combined application of irinotecan and PRI-2191 caused the increase of life span of mice in 47% in relation to control group, whereas cytostatic applied alone only in 5%. In case of combined treatment with PRI-2205 - 11% increase of life span was observed. Both analogs in combination with irinotecan indicated synergistic interactions in the increase of life span of mice [5].

Obtained data suggest that vitamin D analogs combined with irinotecan could be promising agents supporting the standard treatment against colon cancer.

This work was supported by Ministry of Science and Higher Education Grants: No. N N401 014535, „Supporting anticancer therapy of colon cancer by using new vitamin D analogs", period of 2008–2011.

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17:00 Poster 64

Sulforaphane modulates MRP expression and activity in Caco-2 cells

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Sulforaphane (SFN) is a chemopreventive isothiocyanate (ITC) found in vegetables from the *Brassica* family. SFN has been already shown to be inducer not only of metabolism phase 2 detoxification enzymes but also of phase 3 detoxification enzymes, which include, among others transport proteins like MRP (multidrug resistance-associated protein). The accumulation of products of the metabolism phase 2 enzymes may lead to reduced activity of detoxification system. Therefore, the metabolism phase 3 detoxification enzymes involved in carcinogen transport across the cell membrane finally eliminate the xenobiotics from the cell. Thus, the metabolism both phases (2 and 3) contribute to the protection the cells against hazardous agents.

In this study we evaluated the impact of SFN on MRP expression and activity. The study was carried out in human intestinal Caco-2 cells. The dose and time–dependent changes in MRP activity and expression after incubation witch SFN has been studied. The expression of MRP isoform MRP1 was determined by quantitative real-time PCR (QRT-PCR). The total MRP activity was estimated by Calcein-AM Assay and visualized by confocal microscopy.

Our results have shown that SFN stronger affects the activity of MRP than its expression in Caco-2 cells.

17:00 Poster 65

The combined effect of 5-fluorouracil and sulforaphane in prostate cancer cell line

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One of the most commonly investigated anticancer drug is 5-fluorouracil. It is employed clinically to treat solid tumors, e.g. colorectal, breast head and neck and several others. 5-fluorouracil is an antimetabolite which incorporates into DNA or RNA in place of thymine or uracil. It blocks the cell cycle and it is a proapoptotic agent. 5-fluorouracil has some adverse effects: stomatitis, bone marrow depression, diarrhea, nausea, vomiting, angina and cerebellar ataxia.

As it was described , chemopreventive agents may increase anticancer activity of chemotherapeutic agents and decrease their toxicity. Sulforaphane is an isothiocyanate naturally occurring in plants from the family *Crucifera e.g.broccoli.* It is widely known as a chemopreventive agent whose properties were proved in many cancer cell lines. Sulfaraphane induces the phase II enzymes and apoptosis, blocks the cell cycle and inhibits the phase I enzymes. Its multi-path activity provokes a question about possible interactions with anticancer drugs, among others 5-fluorouracil.

The aim of the study was to determine the type of interaction between 5-fluorouracil and sulforaphane in prostate cancer cell line PC-3. Antiproliferative effects of the substances were tested by the MTT assay. The type of interaction was established by the Chou and Talay method after sequential and co-administrative treatments. The mechanism of action was investigated with the flow cytometry assay: cytotoxic effect was examined with fluorescein diacetate/ propidum iodide staining and cytostatic effect was determined using the cell cycle distribution test. Additionally, the cell number after alone treatments and combination treatment was estimated.

The type of interaction depended on the cytotoxicity level. The antagonism was observed at the cytotoxicity level 0.75 after co-administrative treatment and at the cytotoxicity level 0.25 and 0.5 after sequential treatment. The synergic effect was shown at the cytotoxicity level 0.25 after co-administrative treatment. At the other cytotoxicity levels the additive effects were observed. Mechanism of antagonism was explained by the influence on different phases of a

cell cycle by 5-fluorouracil and sulforaphane.The synergetic effect did not depend on cytotoxicity of the tested compounds or their cytostatic activity.

17:00	Poster	66

Biological activities of new amidrazone derivative and its copper (II) complex

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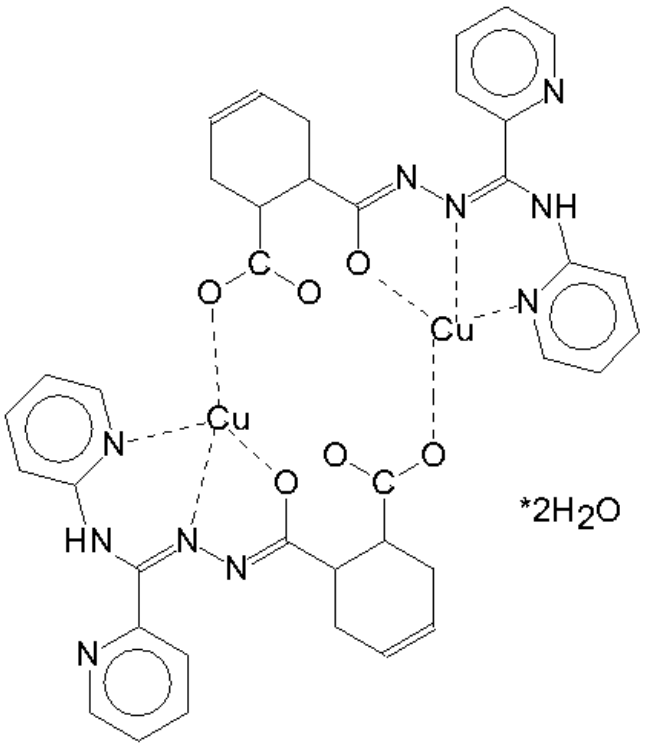
Present-day antitumor drugs act by killing cells that divide rapidly, one of the main properties of most cancer cells. This means that they are also toxic to cells that divide rapidly under normal circumstances and cause severe side effects. The aim of researches on new substances is to find a derivative with similar antitumor activity to applied drugs but with better selectivity.

A novel derivative was obtained in reaction of N-(pyridin-2-yl)picolinoamidrazone with cis-1,2,3,6-tetrahydrophthalic anhydride and its Cu(II) complex which was created in reaction with copper (II) acetate. New structures were confirmed with IR and NMR spectroscopy, elemental and X-ray diffraction analyses.



The compounds were evaluated *in vitro* against Gram-negative and Gram-positive bacterial strains to assess their antimicrobial activity. The MIC_{50} values (the minimal inhibitory concentration required to inhibit the growth of 50% of organisms) ranged from 90 to 400μg/mL for the free ligand and from 50 to 250μg//mL for copper complex. Both tested substances had a relatively low antibacterial activity, although the potency of the complex with Cu was better than the activity of the free ligand.

In cytotoxicity research the ligand shown anti-inflammatory effect by inhibition of IL-6 synthesis. Cu (II) complex in concentration 100m/ml displayed strong antiproliferative activity against tumor lines SW 948, CX-1 and A-431 comparable to cisplatin used as a reference drug. Morover copper complex posses low cell toxicity and shown no TNF-α inhibition which makes it interesting substance for potential antitumor drug.

17:00	Poster	125

Development of new multitarget GPCRs ligands (opioid agonist- tachykinin antagonist).

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Our group proposed to develop new chimeric analgesics in which opioid pharmacophores are covalently hybridized with other types of pharmacophores that positively modulate effects of the opioid part. Synergistic enhancement of opioid analgesia and/or decrease of unwanted side-effects should result from such hybridization.

It is generally accepted, that opioids and tachykinins are classified as functional antagonists. Therefore, hybridization of opioid agonist with tachykinin antagonist should result with very effective analgesics. Melanoma cancer cells overexpress tachykinin receptors. It has been already documented that tachykinin antagonists express antiproliferative properties of melanoma cells. Therefore chimeras of opioid agonists and tachykinin antagonists should express both analgesic and anticancer properties that make them ideal for cancer pain treatment.

Series of new opioid agonist-tachykinin antagonists conjugates have been developed, synthesized and tested. The affinities to opioid receptors mu and delta has been evaluated. The antiproliferative properties of new compounds has been evaluated in vitro in comparison to substance P antagonist aprepitant.

The new compounds express high affinity to opioid receptors as well as antiproliferative properties of melanoma cancer cells *in vitro* and they are good candidates for further studies as analgesics for cancer pain treatment.

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17:00 Poster 126

Methods of pharmaceutical availability enhancement

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Introduction

The major challenge in pharmaceutical pre-formulation studies are APIs, which exhibit poor water solubility and low bioavailability. Several techniques are known to enhance the solubility and bioavailability of drugs i.e. supercritical fluid technology, grinding method, solid liquid systems.

Materials & Methods

Ibuprofen (Shasun Chemicals and Drugs, India) and furosemide (IPCA, India) were chosen as model drugs insoluble in water. Ibuprofen represents the API from BCS class II, whereas furosemide belongs to BCS class IV. To improve their solubility Neusilin US2 (Fuji Chemical Industry), PEG 400, PEG 4000 (Merck) or Labrasol (Gattefosse, France) were used. In case of furosemide supercritical fluid technique was applied to prepare solid dispersion of the API in PEG 4000 (1:11). The substances were treated by carbon dioxide in supercritical conditions using a high pressure reactor Roth. Furosemide liquisolid formulations were prepared using PEG 400 as liquid vehicle with two different drug concentations 10% and 20%. Microcrystalline cellulose, anhydrous dibasic calcium phosphate - Fujicalin®, and Neusilin US2 were used as s solid excipients. After adsorption of furosemide liquid formulation on solid carriers, liquisolid formulation were compacted directly on Korch EK0 tabletting machine. In case of ibuprofen, the API was dissolved in Labrasol and then the solution was adsorbed on Neusilin US2 by simple blending. Dissolution test was performed to examine the influence of both the process parameters and carriers on the API solubility.

Results

All the results showed that the carrier as well as process parameters had influence on the API dissolution. In case of formulations obtained after carbon dioxide treatment of furosemide in supercritical conditions, a higher solubility and dissolution rate was obtained, when the drug was present in the form of solid dispersion. Afert 1 h the amount of furosemide dissolved was approximately 30 times grater than drug alone. With regard to liquisolid formulations the best results were found for formulations containing 10% furosemide solution and Fujicalin® as a carrier material. After 2 h the amount of released drug was over 2-fold higher as compared to commercial tablets. The results showed that the release rate of furosemide increased markedly when present in liquisolid tablets. Similar results were found in case of ibuprofen liquid formulation adsorbed on Neusilins US2. After 1 h the amount of the drug dissolved increased from 36% to 76%.

17:00 Poster 127

Structure-activity relationship studies of 1-diphenylacetyl-4-aryl/alkyl thiosemicarbazides.

Edyta Kuśmierz[1], Monika Wujec[1], Agata Siwek[1], Urszula Kosikowska[2], Anna Malm[2], Joanna Stefańska[3], Katarzyna Dzitko[4]

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Thiosemicarbazide derivatives are important class of biologically active compounds that exhibit an impressive array of promising biological properties including antiviral, anticonvulsant, antituberculosis, analgesic, antibacterial, antifungal, and anticancer activities. Among them, their antimicrobial properties have been extensively described and systematically studied in our research group.

Twenty-two 1-diphenylacethyl-4-aryl/alkyl-thiosemicarbazides were synthesized and their *in vitro* antibacterial potency was evaluated. Among studied compounds, 4-(4-chlorophenyl)-1-diphenylacethyl thiosemicarbazide showed activity comparable to control antibacterial ampicillin at non-toxic concentration. Thus, it potency and low toxicity makes it valid lead for synthesizing new compounds with better bioactivity.

Fianlly, some structural and electronic parameters have been determined in hope to get insight into different biological activity of close related isomers. Based on computational studies some SAR trends can be summarized as follows: (i) electron-withdrawing substitution seems to be better than electron-donating, (ii) *para*-position tends to be better than *ortho* or *meta*, (iii) antibacterial response is correlated rather with electronic than structural parameters.

17:00 Poster 128

Anticancer properties of hair proteins digestive peptides

Joanna Matalińska[1], Andrzej W. Lipkowski[1,3], Sergiusz Markowicz[2], Katarzyna Kurzepa[3], Marta Bochyńska[1], Marzena Biernacka[2], Anna Samluk[2], Dorota Dudek[2], Henryk Skurzak[2]

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ANTICANCER PROPERTIES OF HAIR PROTEINS DIGESTIVE PEPTIDES

Joanna Matalinska[1], Andrzej W. Lipkowski[1], Sergiusz Markowicz[2], Katarzyna Kurzepa[3], Marta Bochynska[1], Marzena Biernacka[2], Anna Samluk[2], Dorota Dudek[2], Henryk Skurzak[2]

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Mechanical and thermal protection of the body is believed to be the major function of hair and fur that cover mammalian skin. Proteins that construct hair are characterized by extremely high resistance for degradation. However, it should be taken into account that even such environmentally resistant material may be also slowly broken down by mechanical wiping or biodegradation due to skin endogenous enzymes or hosting of numerous microbes. Similar processes may take place not only in skin, but also in stomach due to well known phenomena of fur skein swallowing by animals. We hypothesized, that biodegradation products of hair or fur may possess bioprotective properties which are supplementary to the physical hair protective features. The evolutionary process resulted in a reduced hair-covered space on human body, but it may be assumed that bioprotective effects of hair degradation products persist. We applied the process of partial enzymatic digestion of hair and fur with pepsin. Enzymatic digestive water soluble lysate consists of mixture of peptides, including fragments of keratins and keratin associated proteins. Human skin is exposed for various environmental cancerogenic factors. Therefore, we hypothesized that bioprotective mechanisms of hair lysate, if any, could affect proliferation of melanoma cells. Indeed, we found that mixtures of soluble peptides originated from human hair inhibited proliferation of human melanoma cells *in vitro*. The inhibitory effect of such peptide mixtures on B lymphoma cells and urinary bladder cancer cells was also observed. Normal human cells populations varied in their susceptibility to lysate effects. Hair-originated peptide mixtures inhibited proliferation of normal human fibroblasts, but did not affect proliferationof human mesenchymal cells. Identification of active components of hair and mechanisms of their action may delineate a new avenue for anticancer drug development.

BREAK
Wednesday evening, 30 May, 18:30

Kolacja konferencyjna

Oprawa muzyczna Kwartet smyczkowy AQuartet Gwiazda wieczoru Michał Czachowski - gitara flamenko z Zespołem Viva Flamenco
Wednesday evening, 30 May, 19:30
Restauracja Kryształowa

Thursday, 31 May

Śniadanie
Thursday morning, 31 May, 7:00

Sesja wykładowa IV
Thursday morning, 31 May, 9:00
Chair: Z. Leśnikowski, R. Jachowicz

9:00 Invited oral

The link between CpG methylation and depletion and a new CpG specific methyltransferase

Marek Wojciechowski[1], Honorata Czapinska[1], Matthias Bochtler[1,2]

1. *International Institute of Molecular and Cell Biology, Ks. Trojdena 4 Warszawa, Warszawa 02-109, Poland* **2.** *Polish Academy of Sciences, Institute of Biochemistry and Biophysics (IBB/PAS), Pawińskiego 5A, Warszawa 02-106, Poland*

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In vertebrates, CpG methylation plays a key role in controlling the epigenetic state of DNA. However, there is a price to pay. Cytosine methylation promotes deamination, and hence leads to the depletion of CpG sequences in the genome (which are currently about 5-fold underrepresented with respect to statistical expectation in mammals). Is this link between cytosine methylation and deamination also applicable to prokaryotic genomes? We have systematically looked for new CpG methyltransferases by screening genomes for the expected collateral damage. In my talk, I will describe the discovery of a new bacterial CpG methyltransferase and its possible role, present the structure of the enzyme in a catalytically relevant complex with target DNA, and discuss some unexpected findings on the generality of the link between CpG methylation and depletion in prokaryotic genomes.

Przerwa na kawę
Thursday morning, 31 May, 10:30

Sesja wykładowa V
Thursday morning, 31 May, 10:50
Chair: J. Pałka, Z. Lipkowska

10:50 Invited oral

New trends in search for bioactive compounds: EU-OPENSCREEN and boron clusters.

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The end of XX century witnessed both, a fast growth of number of new drugs available on the market as well as reversal of the growth trend around the year 1997. There are several reasons for that phenomenon, they include exponential growth in cost of drug development, meeting of medical needs in some areas (“the low hanging fruit has been picked”), increased demands to document efficacy and safety (larger and more costly clinical trials), and several others. Nevertheless, large number of unmet needs still exists and awaits better therapy: tumor disease, type 2 diabetes, atherosclerotic cardiovascular disease, asthma, neurodegenerative disease, and many others. Therefore pharmaceutical industry as well as academia is strongly interested in overcoming the present difficulties.

Ways to respond to the challenges include better screening technologies and strategies, better chemistry and increasing of pools of innovative molecules, and better academia-industry interactions, among others. These efforts are supported by the state and financed both by the government and pharmaceutical industry. Herein two different examples of such undertakings will be briefly presented. The first is EU-OPENSCREEN, an international programme financed within the 7FP under the auspices of European Commission; the second is a new concept in drug design based on use of boron clusters as pharmacophores and modulators of biomolecules. The EU-OPENSCREEN project is aimed at keeping Europe at the forefront of the biological and medical sciences and at stimulation industrial research and commercial exploitation of chemical biology potentials. As a major objective, the project aims at bioprofiling and identifying small-molecule modulators for individual functions of proteins and nucleic acids, and forms a base for increasing the availability of new bioactive compounds.

Application of boron clusters in drug design is based on unique properties of polyhedral heteroboranes and makes it possible to design boron-containing molecules with new biological characteristics. This offer medicinal chemists and pharmacologists a rare opportunity to explore and pioneer new areas of molecular design and medicinal applications.

11:35 Oral

Development and validation of HPLC method. Review of selected cases.

Maria Puchalska, Łukasz Jedynak, Magdalena Kossykowska, Marta Zezula, Elżbieta Lipiec-Abramska, Marta Kościuch, Katarzyna E. Filip, Joanna Zagrodzka

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Quality assurance of analytical results is one of the most crucial points during the development of analytical methods and their implementation to routine analytical control. The aim of validation of analytical procedure is to prove that the developed procedure fulfils the requirements concerning the assumed purpose. Validation is based on statistically documented results. Statistical tests applied during validation of analytical methods may be employed for optimization and verification of the method as early as at the stage of method development.

HPLC is one of the most often used analytical techniques during development and production of drug substances. Range of application, accuracy, precision and linearity of chromatographic method are dependent on several factors such as interactions between chromatographed analyte and both mobile and stationary phase, absorption of UV-VIS radiation (if spectrophotometric detection is used) or stability of the sample.

Application of statistical tools during the development of chromatographic methods applied for determination of chemical purity and assay of drug substances is presented using a few chosen examples. Use of the statistical tests for determination of such parameters as linearity, range, accuracy, precision and limit of quantification is also demonstrated.

11:55 Oral

CPL-200-075, a novel potent renal sodium-glucose co-transporter 2 (SGLT2) inhibitor

Monika Lamparska-Przybysz, Piotr Guzenda, Joanna Hucz-Kalitowska, Mateusz Mach, Michał Mroczkiewicz, Katarzyna Bazydło, Krzysztof Dubiel, Maciej Wieczorek

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Background and aims:

Renal sodium-glucose co-transporter 2 (SGLT2) inhibition is a promising new approach in therapy of type 2 diabetes. Currently, there are several clinical trials ongoing, with almost ten SGLT2 inhibitors in Phase III CPL-200-075 is a novel, selective and potent SGLT2 inhibitor that induce urinary glucose excretion with better pharmacokinetics than most advanced in development other SGLT2 inhibitors. In this studies we focused on pharmacodynamics of CPL-200-075.

Materials and methods:

Drug potency was evaluated in intraperitoneal glucose tolerance test (IGTT) and in mouse streptozoocine-induced diabetic model. In IGTT C57BL/6 males fasted for 12 h were given single oral dose of compound or placebo followed by i.p. administration of glucose (1 g/kg). Blood glucose levels were measured in several time points up to 120 min post glucose bolus. Mice with streptozotocin induced diabetes were fasted 3 h before dosing and were given a single oral dose of compound or vehicle. Food was given again to animals 4 h post dose. Blood glucose levels were measured in several time points up to 24h. In both studies, glucose levels were measured in a sample of tail vein blood using standard glucometer. Pharmacokinetic study was performed in normal Wistar rats. Overnight fasted animals were given compound in single dose of 1 or 5 mg/kg. Blood samples were collected via tail vein and compound's serum concentration of was analyzed by LC-MS/MS.

Results:

CPL-200-075 displayed very good pharmacokinetic parameters. In rats after administration of single oral dose, we observed Cmax reaching 1563 ng/ml and 4233 ng/ml for 1 and 5 mg/kg, respectively. In the higher dose we observed that Cmax was stable from 2 to 8h post dosing. In IGTT CPL-200-075 decreased blood glucose levels by almost 50% when given in dose 5 mg/kg. The compound lowered blood glucose in dose-depended manner by 40, 60 and 80% when administrated in doses 5, 20 and 50 mg/kg, respectively. In streptozoocine-induced animal model of diabetes CPL-200-075 reduced blood glucose by over 100 mg/dl with little shift in minimum peak when compared to other SLGT2 inhibitors that currently are in clinical development. The effect on blood glucose after refeeding of STZ animals was comparable with SLGT2 inhibitors in clinical development used as control. Beneficial effect was stable up to 24h after dosing as indicated by glucose levels below that for vehicle group.

Conclusions:

Administration of CPL-200-075 results in a dose-depended decrease in blood glucose levels in healthy and diabetic animals. This new compound is characterized by good pharmacokinetics and lover risk of hypoglycaemia.

12:15 Oral

Dextran-based materials reducing anticoagulant activity of heparin in animal models in vitro and in vivo

Kamil K. Kamiński[1], Krzysztof Szczubiałka[1], Maria Nowakowska[1], Bartłomiej Kalaska[2], Emilia Sokołowska[2], Andrzej Mogielnicki[2], Włodzimierz Buczko[2]

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Heparin is a naturally occurring polysaccharide used in many medical applications, especially for treating cardiovascular diseases [1]. This polymer has a natural ability to prevent blood clot formation in mammals both in vitro and in vivo. Anticoagulative effect of heparin is often difficult to predict and in the case of an emergency (e.g. haemorrhage due to the heparin overdose) it has to be reduced. The only substance currently used in such situations is protamine sulphate (PS)[1]. This protein, however, can be a cause of many adverse effects, e.g. life threatening allergic response [2] or hypotension. Therefore, there is a necessity of developing a safer alternative which could replace it.

Polysaccharide derivatives with positively charged molecules seem to be promising candidates that could be the answer for this issue [3]. Here we are reporting the studies on the application of the cationic derivatives of dextran (DeX-GTMAC) for heparin deactivation [4]. Studies on the influence of different doses of DeX-GTMAC on the heparin activity in the rat blood using various coagulation tests were performed and compared with those for (PS)First, the *in vitro* studies were performed to estimate the effective dose of the obtained polymer. The effective dose of to reverse the effects of heparin in rats in vitro was 5 mg/120 U of heparin (Figure 1).

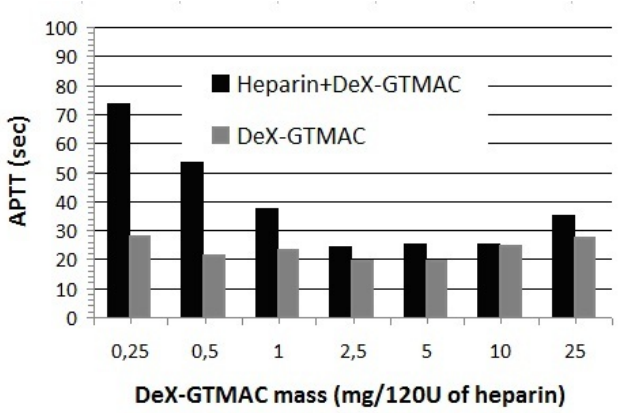

Figure 1. Dependence of activated thromboplastin time (aPTT) on the applied dose of the polymer

The reversal of heparin action by DeX-GTMAC was clearly seen in the mice developing chemically induced venous thrombosis and was even more potent than the effect of PS. Both DeX-GTMAC and PS reversed the prolongation of bleeding time and aPTT by heparin administered in rats and mice. Moreover, other routinely measured blood parameters are significantly affected. DeX-GTMAC, in contrast to the PS, significantly increase red blood cell counts, hemoglobin level and haematocrit value. The data obtained show that the cationically-modified dextran may be potentially used to reverse anticoagulative heparin activity.

Acknowledgement: The project was operated within the Fundation for Polish Science Team Programme (TEAM/2008-2/6) and Ventures Programme (Ventures/2009-4/4) cofinanced by EU European Regional Development Fund

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12:35 Oral

Quinoline based antifungals

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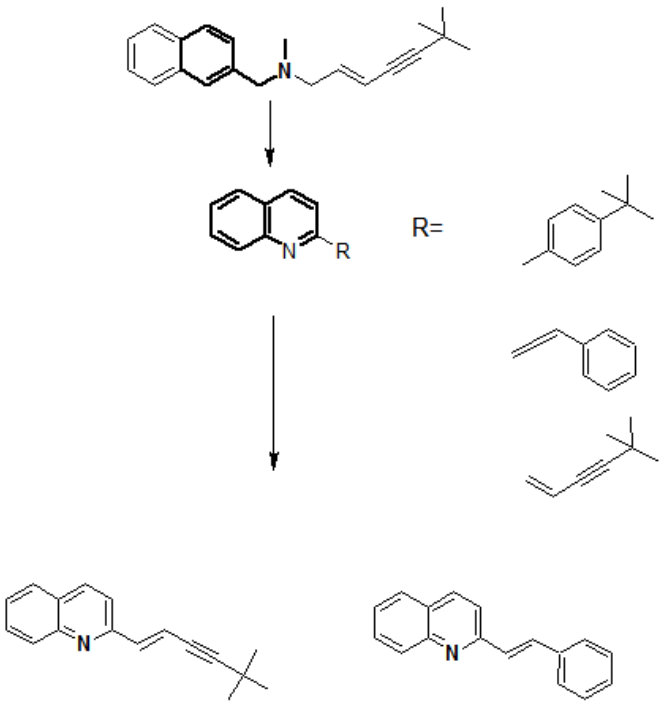
Modern antifungal therapy have to deal with number of issues associated with broad but inadequate arsenal of drugs. The most commonly used agents have serious drawbacks as toxicity and resistance. On the other hand new drugs and drug candidates under clinical trials do not guarantee better overall performance. This is not the only reason of strong need for new antifungals. The other is – observed during last four decades – steadily growth of the fungal infections. Especially in patients with compromised immune system [1]. Morbidity and mortality of some endemic and opportunistic mycoses is still very high[2]. Thus we strongly need new drugs acting through new mechanisms on new targets.

Lack of expected success form established methodology like combinatorial chemistry and high throughput screening we turn on fragment based design and search for privileged structures. Quinoline moiety by its abundant presence in various natural and bioactive compounds may be considered as such structure [3-6]. We wish to introduce here the fragment based approach to quinolines mimicking known antifungal allylamines.



This approach lead to well known for their biological activity styrylquinolines as naftifine analogues. Styrylquinolines were broadly studied for their anticancer and antiviral activity and FZ-41 is an example of HIV integrase inhibitor under clinical trials. We explored the styrylquinolines finding some highly active antifungals. Further modification of the quinoline scaffold led us to even more active compounds. Namely we used dicholroquinoline as this moiety has established position between antimicrobial agents. Chloroxine is used for long time as antibiotic for shampoos or topical ointments. Combine chloroxine scaffold and styryl moiety afforded us with compounds active against fungi and bacteria. The most active compounds were up to ten times more active than bacitracin against bacteria including metilicin resistant strains of Staphylococcus aureus.

Literature

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Przerwa obiadowa

Thursday afternoon, 31 May, 12:55

Sesja wykładowa VI

Thursday afternoon, 31 May, 14:30

Chair: W. Szeja, W. Szczepek

14:30 Invited oral

Experimental pharmacology of endothelium: MNA/ COX-2/PGI_2 pathway

Stefan Chłopicki

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Healthy endothelium is essential for undisturbed functioning of the cardiovascular system, while endothelial dysfunction - characterized by the deficiency of vasoprotective mediators such as NO, PGI_2 and EDHF and robust activation of pro-inflammatory and pro-thrombotic phenotype of endothelium - leads to cardiovascular oathologies including *atherothrombosis* and diabetes. COX-2/PGI_2 pathway represent an important defensive mechanisms of vascular wall. We described that this vasoprotective pathway is stimulated by 1-methylnicotinamide (MNA), a major metabolite of nicotinamide (vitamin PP, vitamin B_3) and MNA afford anti-thrombotic, anti-inflammatory and vasoprotective activity *in vitro* [1,2] including the ability of MNA to reverse endothelial dysfunction in hypertriglicerydemic and diabetic rats [3] as well as anti-diabetic action [4]. Furthermore, endogenous MNA, produced in the liver from nicotinamide by NNMT (N-nicotinamide-methyl-transferase), as an endogenous activator of COX2/PGI_2 pathway and may play an important regulatory role in the cardiovascular system for example as a compensatory response in atherosclerosis [5] or liver failure [6].

In brief exogenous MNA, displays a unique profile of vasoprotective activity mediated by COX-2/PGI_2 pathway. On the other hand, endogenous MNA, produced in the liver by NNMT, appears to be an endogenous activator of COX2/PGI_2 pathway that may play an important role in the homeostatis of cardiovascular system. Finally, treatment with nicotinic acid provide robust activation of MNA pathway and MNA-dependent mechanisms that may explain some of the pharmacological effects of nicotiinic acid. In conclusion, we propose novel mechanism of vasoprotective activity of nicotinic acid dependent on MNA, and suggest MNA as a prototype stimulator of COX-2/PGI_2 vasoprotective pathway.

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15:15 Oral

Supramolecular assembly and properties of dendrimeric peptide mimics

Zofia Lipkowska[1], Marta K. Sowińska[1], Paulina Zielińska[1], Magdalena Zimnicka[1], Jolanta Solecka[2,3], Aleksandra M. Rajnisz, Tanja Lind[4], Marité Cárdenas[4]

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Intermolecular interactions at various levels are driving forces of biological, chemical and physical processes, Therefore, their knowledge is essential in medicinal chemistry, particularly in the designing of a new therapeutic substances.

During last 20 years, dendrimeric compounds gained a lot of recognition as prospective carriers of poorly soluble substances in therapy of numerous diseases and as diagnostic tools. Although structural variability and globular structure of dendrimeric compounds were recognized early, *de novo* design of new original compounds for technology and medicine is still not very popular. Particularly in the case of dendrimeric peptides, intrinsically non-active amino acid residues after assembling on dendrimeric tree achieve new biological dimensions

Here we present rationale behind synthetic design of various types of biologically active peptide dendrimers and studies on their conformation and molecular assembly in various media. In particular, interactions with biologically relevant monovalent (Na^+ and K^+) and divalent (Mg^{2+} and Ca^{2+}) cations, and impact of high concentrations (i.e. physiological conditions) on biological activity will be presented. Molecular assembly of the selected dendrimers with model zwitterionic and anionic phospholipids, studied in the gas phase by electrospray ionization mass spectrometry along with application of quartz crystal microbalance technique, revealed details of dendrimer-membrane interactions on molecular level.

Studies on influence of high salt conditions on antimicrobial potency and interactions with phospholipids might be important for knowledge of bactericidal mechanism and establishing similarities between mechanistic behavior of the natural linear antimicrobial peptides and the title branched peptidomimetic molecules.

Financial support from the National Center for Research and Development, grant NR13-0153-10/2010 and in part grant N204 239436 from the Ministry of Sciences and Higher Education, are acknowledged.

15:35 Oral

The molecularly imprinted polymers as selective sorbents to pharmaceutical analyses

Dorota Maciejewska, Piotr Luliński, Teresa Żołek, Mariusz Dana, Monika Pawłowska

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Molecular imprinting is a tool by which selective recognition sites can be created in a polymer during the synthetic procedure. The monomers and the cross-linkers form a three-dimensional matrix around chosen molecule named a template. After removal of the template molecules, the cavities created in polymer matrix are complementary both in terms of shape and functionality to the template. New molecularly imprinted polymers (MIP) are being developed for solid phase extraction (SPE), chromatographic separation, organic syntheses as protecting groups and catalysts. Many parameters are important for production of MIPs with good recognition ability. For example, crucial role plays properties of: the monomers, the porogens, the cross-linkers, and also the polymerization procedures.

The aim of this presentation is to show achievements in synthesis, physicochemical analysis, and theoretical studies on molecularly imprinted materials. Our laboratory is engaged in searching for selective materials which could be used in analysis of 2-(3,4-dihydroxyphenyl)ethylamine (dopamine) and its metabolites (1-3). Synthesis - non-covalent approach was applied to form MIPs by the radical bulk polymerization with the different functional monomers, the cross-linkers, and the porogens. First evaluation – the imprinting factors (IF) were calculated as the relationship between the binding capacity of MIP and its non-imprinted counterpart. Analysis of adsorption parameters - the best imprinted resins were analyzed using Scatchard equation, scanning electron microscopy, spectroscopic methods. Analysis of recognition mode - the compounds of similar structures were tested in non-competitive binding experiments. Theoretical analysis - it is not easy task to model real polymer system, but it is possible to work up step by step procedure for evaluation of the polymer matrix. Steps - the most promising monomer was chosen on the basis of the energies of prepolymerization complexes; the structure of the most stable complex was used to simulate the cavity in the polymer matrix. In the computations, solvent effects were approximated by the distance dependent dielectric constants εr_{ij}.

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15:55 Oral

Synthesis and biological evaluation of new amino acid and dipeptide derivatives of neocryptolepine as anticancer agents.

Katarzyna Sidoryk[1], Marta Świtalska[2], Joanna Wietrzyk[2], Anna Jaromin[3], Magdalena Piętka-Ottlik[4], Piotr Cmoch[1,5], Joanna Zagrodzka[1], Wojciech J. Szczepek[1], Łukasz S. Kaczmarek[1], Wanda Peczyńska-Czoch[4]

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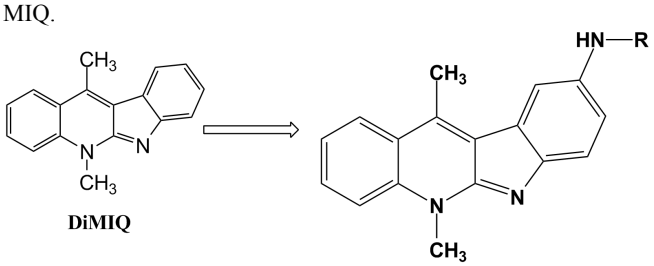
The syntheses of neocryptolepine derivatives containing an amino acid or a dipeptide at the C-9 position, their evaluation for antitumor activity in vitro and in vivo were reported. To establish the influence of the amino acid or the peptide on the physicochemical properties of 5H-indolo[2,3-b]quinoline (DiMIQ), lipophilic and hemolytic properties were investigated. Most of the compounds displayed a high anticancer activity in vitro, and strongly inhibited growth of tumor in mice compared to cyclophosphamide. The attachment of the hydrophilic amino acid or peptide to the hydrophobic DiMIQ increased its hydrophilic properties, and decreased its hemotylic activity.

The glycylglycine conjugate (**7a**) was the most promising derivative. It strongly inhibited the growth of the tumor in mice (at dose 50mg/kg/day it inhibited the tumor growth by 46-63% on days 11th-16th, and by 29-43% on day 18th-23th), and significantly decreased hemolytic activity and lowered the in vivo toxicity compared to DiMIQ.

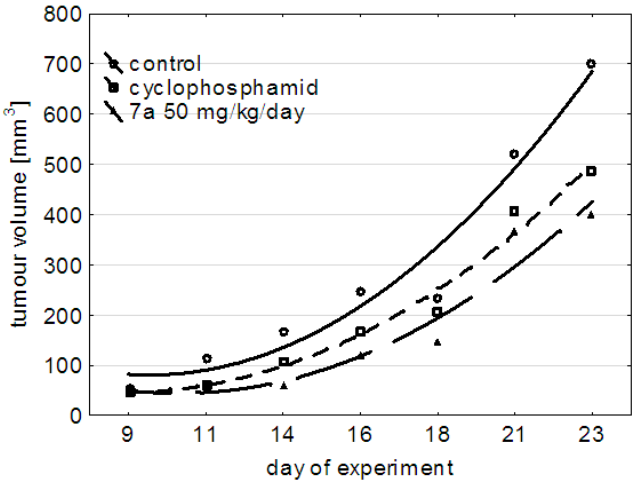


DiMIQ

2a R = Gly
3a R = *L*-Pro
4a R = *D*-Pro
5a R = *L*-His
6a R = *D*-His

7a R = Gly-Gly
8a R = *L*-His-Gly
9a R = *L*-Pro-Gly
10a R = *L*-Pro-*L*-Pro
11a R = Gly-*L*-Pro

In vitro and in vivo anticancer activity pKa, logP and hemolytic activity were investigated



16:15 Oral

Design and development of CPL-407-22 – novel, potent and selective JAK2 protein kinase inhibitor.

Filip Stefaniak[1], Karolina Dzwonek[1], Michał Mroczkiewicz[1], Joanna Paradowska[1], Bartosz Stypik[1], Paweł Gunerka[1,2], Daria Zdżalik[1], Barbara Dymek[1], Paulina Grygielewicz[1], Monika Lamparska-Przybysz[1], Maciej Wieczorek[1]

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Small molecule kinase inhibitors comprise a growing class of drugs used in cancer treatment for over decade. As opposed to traditional cancer therapies which interfere with DNA synthesis and repair system, kinase inhibitors are directly aimed in specific molecular targets involved in oncogenesis. Due to their selective activity they entail significantly different toxicity profile from traditional cancer drugs.

One of the recently investigated potential targeted therapies are JAK2 kinase inhibitors which constitute promising treatment strategy in several mieloproliferative disorders. Considerable number of patients suffering from primary mielofibrosis, polycythemia vera or essential thrombocytopenia carry a point mutation in JAK2 gene [V617F] which is assumed to be an oncogenic driver in these malignancies. There are several JAK2 inhibitors under development, however, most of them, including Ruxolitinib, are accompanied with severe hematological side effects. Therefore there is still a strong need for designing more selective compounds with less off-target activity. To fulfill these requirements, *in silico* methods are employed at early stages of drug development process. Among those are virtual libraries screening and *de novo* ligand design. Further steps include improving compound activity by QSAR and machine learning analysis, selectivity profiling and ADMET prediction.

Using this approach we have designed and developed a novel JAK2 kinase inhibitor with high activity and excellent selectivity comparing to leading compounds in this class. We present subsequent stages of that process, from idea, through virtual screening to hit profiling and lead molecule.

Przerwa na kawę

Thursday afternoon, 31 May, 16:35

Sesja posterowa II

Thursday afternoon, 31 May, 17:00

17:00 Poster 67

Application of Corona detector for analysis of 4-cyclohexyl-(S)-proline – key intermediate in Fosinopril synthesis

Barbara Kąkol[1], Magdalena Jezierska-Zięba[1], Bożenna Obukowicz[1], Mirosława Szpakiewicz[1], Michał Fedoryński[2], Jacek Cybulski1

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The aim of our work was elaboratating an analytical method to control the last step of the synthesis of *trans* 4-cyclohexyl-*S*-proline, which is semi-product in the synthesis of fosinopril.

Fosinopril is an antihypertensive medicine which belongs to the group of ACE inhibitors. It posses a long-acting formulation (for 24 h) and a high degree of assimilation.

ACE inhibitors block the conversion of angiotensin I to angiotensin II. They, therefore, decrease the arterial resistance and increase the venous capacity.

The most often applicable drugs of these group are: enalapril, lisinopril and captopril.

This synthesis is based on simple, well-known reactions like aldol condensation, Michael addition and hydrogen reduction. The last step of this work is the hydrolysis of ethyl ester of *trans* 4-cyclohexyl-*S*-proline.

The analytical control of this process is difficult because of nonvolatile product and lack of chromophore group in a molecule enabling detection. The product of reduction with using a UV detector in background impurities present in the mixture is practically invisible.

The crude product of reduction, having of small amounts of substrate and other compounds with high absorbance in the UV wavelength range, was hydrolysed. The analytical control of hydrolysis of ethyl trans-4-cyclohexyl-S-proline to the corresponding acid is possible by using the Corona CAD detector. Detector operating principle is based on corona discharges and the signal does not depend on the structure of the analyzed compound.

Corona CAD detector allows work in gradient, which we cannot be used in work with RI detector. In our case it is necessary due to the presence of compounds with very different polarity. Conditions of the control of hydrolysis of the ethyl ester of *trans* 4-cyclohexyl-*S*-proline were developed. Indications were performed using standard HPLC systems.

*This work was financially supported by Ministry of Science and Higher Education grant No***N N209237336**.

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17:00 Poster 68

HPLC methods for in–process control and chemical purity determination of olopatadine

Karolina Kłos, Ewelina Czerniec-Michalik, Joanna Zagrodzka, Katarzyna Badowska-Rosłonek

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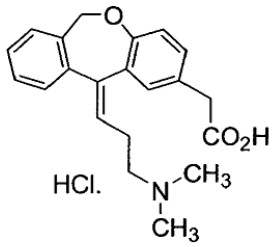
Olopatadine-HCl

([(*Z*)-3-(Dimethylamino)propylidene]-6-11-dihydrodibenz[b,e] oxepin-2-acetic acid hydrochloride) is a relatively selective H1-receptor antagonist that is used for the treatment of ocular symptoms of seasonal allergic conjunctivitis. The compound represented by formula (I), commonly known as Olopatadine, has been used as an active constituent drug, in form of its hydrochloric salt and may be administered as an ophthalmic solution.



(I)

During the synthesis of (*Z*)-olopatadine hydrochloride a number of impurities are formed. The (*E*) olopatadine isomer is the one of the most significant impurities.

The quality control of the starting materials, by-products identification and the purity of active substance are essential in the manufacturing process of drugs. The aim of this control is based on the requirements of ICH and EMA and described in scientific guidelines [1]. Critical points defined during the development are gathered in indoor specifications, taking into account selected parameters important from the synthetic point of view. The main analytical technique used for quantitative determination of impurities in the olopatadine**,** as well as the in-process control of manufacturing process is HPLC with UV detection.

Two HPLC methods were developed to establish purity of drug substance, control synthetic route and the isomerization process of crude final product. The following potential impurities were determined: starting material – 6,11-Di-hydro-11-oxodibenz[b,e]oxepin-2-acetic acid (Isoxepac), p-toluenesulfonic acid, metabolite the (Z)-olopatadine and the (E)-olopatadine isomer. Only one of the compounds mentioned above (isomer E) was considered in the HPLC method recommended by USP [2]. The constant evolution of olopatadine synthesis route de-

mand the adjustment of quality control analytical tools. The HPLC method described in USP is dedicated to samples demonstrating the high degree of purification, published methodology is not suitable for reaction control.

Satisfying separation of the API from impurities formed during the production process was achieved on C-8 and C-18 columns using trifluoroacetic acid solution, phosphorous buffer, methanol and acetonitrile as the mobile phases in gradient mode. The flow rate was 1.0 mL min^{-1} and the detection wavelength was 225 nm. The efficiency of the procedure was verified by its application to standards of potential impurities found during the synthesis development. Presented methods confirmed the acceptance criteria given by Eur. Phar [3].

1. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. ICH Harmonised Tripartite Guideline: *Impurities In New Drug Substances* Q3A(R2). ICH Secretariat. Geneva. Switzerland (2006*)*

2. USP Olopatadine Hydrochloride, USP34, p. 3711

3. European Directorate for the Quality of Medicines & HealthCare. European Pharmacopoeia 7.0. Chapter: 2.2.46 *Chromatographic separation techniques* (2010)

17:00 Poster 69

Anti-inflammatory activity of novel hydrazide derivatives

Bożena Modzelewska-Banachiewicz[1], Renata Paprocka[1], Andrzej Eljaszewicz[2], Małgorzata Wiese[2], Jacek Michałkiewicz[2]

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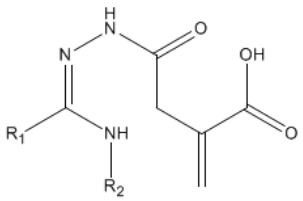
Amidrazones and their derivatives are known for a wide spectrum of biological effects: antifungal, antibacterial, anti-inflammatory, antithrombotic and antitumor activity. The aim of the studies was to obtain new biologically active substances.

Three hydrazide derivatives (**1-3**) were synthesized in reaction of N^3-substituted amidrazones with itaconik anhydride in anhydrous ether. Their structures were determined by nuclear magnetic resonance spectroscopy. Potential biological effects of novel compounds were predicted with PASS (Prediction Activity Spectra for Substances) software. According to PASS calculations hydrazides (**1-3)** could develop anti-inflammatory activity.



1) R_1=2-pyridyl R_2=phenyl
2) R_1=2-pyridyl R_2=4-nitrophenyl
3) R_1=4-pyridyl R_2=phenyl

Hydrazides (**1-3**) were studied for their ability to affect cytokines (IL-6 and TNF-α) production in human whole blood cultures to determine their anti-inflammatory effect. Both chosen cytokines are important inflammatory mediators. Compounds were used in concentrations 0,1µg/mL, 1µg/mL and 10µg/mL. The cell cultures were stimulated with lipopolisaccharide (LPS) to produce IL-6 and TNF-α. The activities of cytokines were measured in the supernatants using bioassays.

The results indicate that TNF-α production was slightly reduced (about 10%) by compounds **1** and **3** in concentration 1µg/mL. However the same compounds in concentration 10µg/mL caused stronger inhibition: 57% and 23%, respectively. Derivative **1** did not affect production of IL-6, while hydrazide 3 increased this cytokine production. Compound **2** showed no TNF-α or IL-6 inhibition.

17:00 Poster 70

Development and validation of a liquid chromatography / single quadrupole mass spectrometry method for the determination of aripiprazole in human plasma

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Aripiprazole is an atypical antipsychotic that has serotonin 5-HT1A-receptor partial agonist and 5-HT2A-receptor antagonist properties as well as being a partial agonist at dopamine D2 receptors. It is used in the management of schizophrenia and in acute manic or mixed episodes associated with bipolar disorder in adults over 18 years of age [1].

A bioanalytical method was developed and validated according to Food and Drug Administration (FDA) [2] and European Medicines Agency (EMA) [3] guidelines with the OECD Principles of Good Laboratory Practice (GLP), to study pharmacokinetics of aripiprazole. The method was based on liquid-liquid extraction of aripiprazole with n hexane/izopropanol mixture, followed by reversed-phase liquid chromatography. Positive electrospray ionization mass spectrometry in single ion monitoring mode was applied as the detector. Isotope labelled aripiprazole was used as internal standard. No matrix effects and no back-conversion of dehydro-aripiprazole to aripiprazole were observed. Calibration curve, fitted to 1/x2 weighted linear regression model, was linear in the range of 1.0 – 80.0 ng/mL. Extraction recoveries for both aripiprazole and internal standard were approximately 75–80%. The method was successfully validated and applied in pharmacokinetic study in humans after a oral administration of aripiprazole.

[1] T. Swainston Harrison, C. M. Perry, Aripiprazole - A Review of its Use in Schizophrenia and Schizoaffective Disorder, Drugs, 64, 2004, 1715-1736

[2] Guidance for Industry. Bioanalytical Method Validation. U.S. Department of Health and Human Services. Food and Drug Administration. Center for Drug Evaluation and Research (CDER). Center for Veterinary Medicine (CVM). May 2001.

[3] Guideline on bioanalytical method validation. European Medicines Agency (EMEA/CHMP/EWP/192217/2009). London, 21 July 2011.

17:00 Poster 71

Biological activity of naphthyl-1,5-diamino-bis (methylidenebisphosphonic) acid – a new bisphosphonate

Anna Nasulewicz-Goldeman[1], Waldemar Goldeman[2]

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Bisphosphonates are a class of drugs commonly used to prevent the loss of bone mass due to osteoporosis and other diseases related to the disorder of calcium homeostasis. Bisphosphonates have also been shown to possess anti-tumour activity when applied alone or in combination with known cytostatics. In clinics, bisphosphonates are usually applied in complementary treatment of cancer patients since they are very potent modifiers of a progression of skeletal metastasis in several forms of cancer, especially breast cancer. It is postulated that bisphosphonates decrease the rate of bone resorption not only due to their direct effect on osteoclasts, but also through the impact on macrophages present in microenvironment of tumour metastases.

In these studies, we evaluate the anti-proliferative activity of a new bisphosphonate (naphthyl-1,5-diaminobis(methylidenebisphosphonic) acid) on MCF-7 human breast cancer cells and J774E mouse macrophages. Naphthyl-1,5-diaminobis(methylidenebisphosphonic) acid exerted a potent, anti-proliferative effect on J774E cells with IC_{50} values over 10 times lower as compared to zoledronic acid – a control bisphosphonate. For MCF-7 cells IC_{50} values were respectively twice higher than for zoledronic acid. Moreover, it showed an additive or even synergistic anti-proliferative effect when applied in combination with cisplatin or doxorubicin. Interestingly, the effect was manifested the most distinctly when MCF-7 or J774E cells were treated with naphthyl-1,5-diaminobis(methylidenebisphosphonic) acid and cytostatics simultaneously or were pre-treated with cytostatics.

We also evaluated the influence of naphthyl-1,5-diaminobis(methylidenebisphosphonic) acid on the adhesive and migratory properties of MCF-7 or J774E cells. An inhibitory effect of naphthyl-1,5-diaminobis(methylidenebisphosphonic) acid on cell adhesion to fibrinogen and migration through matrigel was observed.

In summary, naphthyl-1,5-diaminobis(methylidenebisphosphonic) acid seems to be of particular interest for further studies concerning the influence of bisphosphonates on cells involved in tumour development and progression.

17:00 Poster 72

Molecular complexes of topo I and topo II inhibitors from camptothecin and flavonoid families with model DNA oligomers. Search from sequence specific binding. Verification of biological assay on atomic coordinates level.

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1. *Instytut Chemii Organicznej Polskiej Akademii Nauk (ICHOPAN), Kasprzaka 44/52, Warszawa 01-224, Poland* **2.** *Narodowy Instytut Lekow (NIL), Chełmska 30/34, Warszawa 00-725, Poland*

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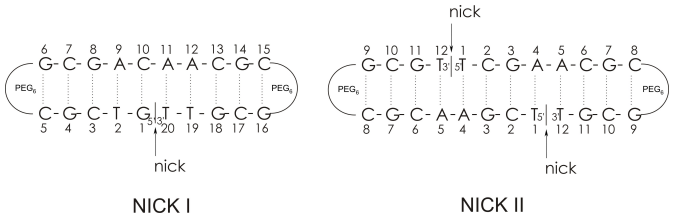
The role of topoisomerases I and II inhibitors involves the ternary complexes composed of enzyme/DNA/Inhibitor. Topoisomerases are essentially enzymes which, *inter alia*, relax the torsional strain in supercoiled DNA generated in replication process. The enzyme cleaves one strand, creating a nick, and thus enables the rotation around the uncleaved strand followed by ligation of the broken strand and restoring DNA duplex.

Topotecan is in clinacl use as an antitumor agent Hycamtin ™. It act by binding to the covalent complex formed between nicked DNA and topoisomerase I and acting as a poison.

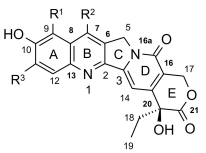
Human topo I involves Tyr 723 , of which OH group attacks phosphate in the main chain forming 3'-phosphotyrosine. After relaxation, strain free DNA duplex is restored in nucleophilic attack of free 5'-OH group on phosphorous atom in 3'-phosphotyrosine. Dumbbell DNA (NICK I) mimics nicked DNA in a ternary complex and was designed by us as a general model of DNA duplex for screening its interactions with topo I inhibitors from camtothecin group.

Similarly to topo I , topo II generates nicks separated by four base pairs in both strands forming doubly broken duplex covalently linked to topo II through 5'-phosphotyrosines. Dumbbell DNA (NICK II) mimics doubly nicked DNA and was designed by us as an adequate general model of DNA duplex for screening its interactions with topo II inhibitors from flavonoid group of derivatives.

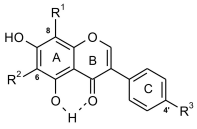


The essential role of inhibitor in a ternary complex is specific binding in a nick, either covalently or noncovalently, and preventing its ligation and further proliferation of cytotoxic DNA.

The chemical basis of selected compounds from both classes of inhibitors is shown below.



R^1= H, CH_2OH, CH_2OMe,CH_2NMe_2,$CH_2N^+HMe_2$ X^-,
R^2 = H, C_2H_5
R^3 = H
X^- = Cl^-, $CF_3SO_3^-$



R^1 = H, alkylamino
R^2 = H ,alkylamino
R^3 = OH

In a poster will be presented characteristics of molecular complexe of NICK I and parent TPT inhibitor from camptothecin family and also design of NICK II model dumbbell DNA and molecular complexes of parent genistein and its derivatives.

17:00	Poster	73

Synthesis of new 2-aminobenzimidazole derivatives. Reaction of 2-arylideneaminobenzimidazole with selected nitriles containing active methylene group.

Anna Nowicka[1], Wanda P. Nawrocka[1], Hanna Liszkiewicz[1], Andrzej Dryś[2]

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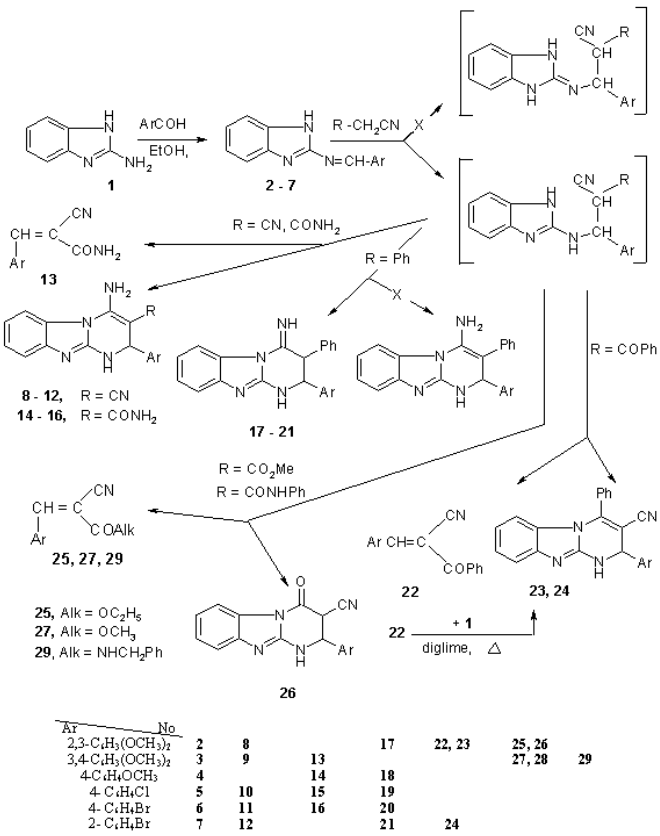
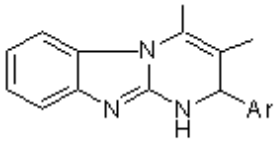
Schiff bases **2-7** obtained from 2-aminobenzimidazole (**1**) with 2,3-dimethoxy-, 3,4-dimethoxy-, 4-methoxy-, 4-chloro-, 4-bromo- and 2-bromobenzaldehyde. A series of pyrimido[1,2-*a*]- benzimidazole and α-cyanocinnamic derivatives have been synthezied in the reactions of Schiff bases **2-7** with selected nitriles α-containing active methylene group: malononitrile **8-12**, cyanoacetamide **13-16**, benzoylacetonitrile **17-21**, benzylonitrile **22-24**, cyanoaceticmethyl ester **25-28** and cyanoacetic benzylamide **29** (Scheme 1). The structure **8-29** were confirmed by the results of elemental analysis and their IR, 1H-, 13C-NMR and MS spectra.

Scheme 1

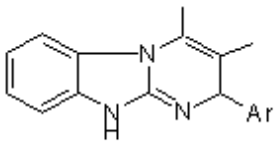


Ar \ No						
2,3-$C_4H_3(OCH_3)_2$	2	8	17	22, 23	25, 26	
3,4-$C_4H_3(OCH_3)_2$	3	9	13		27, 28	29
4-$C_4H_4OCH_3$	4		14	18		
4- C_4H_4Cl	5	10	15	19		
4- C_4H_4Br	6	11	16	20		
2- C_4H_4Br	7	12		21	24	

Ab initio geometry optimization calculations for three pairs 1H and 10H of possible products **10** and **10a**, **17** and **17a**, **24** and **24a** was performed within the density functional theory (DFT). The computational study was performed using Gaussian 98 program. calculated energies for optimized structures are presented in Table 1. Energy differences ΔE suggest that **10**, **17** and **24** are more propable products than corresponding structures **10a**, **17a** and **24a** respectively.



1 H



10 H

Table 1 Comparison of calculated energies for selected compounds.

Comp.	Total energy /Hartree/	ΔE / Hartree /	ΔE / kJ/mol /
10	-1388.9829027		
10a	-1388.9748138	-0.008089	-21.24
17	-1182.7152963		
17a	-1182.7040707	-0.011226	-29.47
24	-3676.1827237		
24a	-3676.1747843	-0.007939	-20.84

The products **8-29** of various chemical structure were obtained, which are interest for biological studies or which can be substrates for

further syntheses.

17:00 Poster 74

Synthesis and anticonvulsant activity of new 5-(cyclo)alkyl-5-phenyl-imidazolidine-2,4-diones derivatives with arylpiperazinylpropyl moiety

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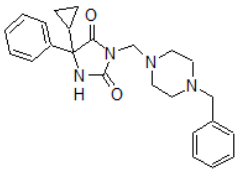
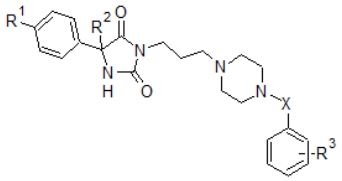
Epilepsy is a disease of different cerebral disorders of central nervous system, and it is characterized by paroxysmal, excessive and synchronous discharges of large numbers of neurons. In fact, epilepsy is the third most frequent neurological disorder and affects patients of different age groups and sex. [1].

Searching for the new safer and more effective antiepileptic drug, we modify the structure of available one – phenytoin, which has already known to possess a useful mechanism of action. As a continuation of our work involving five-member heterocyclic anticonvulsants [2], we have designed and synthesized new 5-(cyclo)alkyl-5-phenylimidazolidine-2,4-dione ring with arylpiperazinylpropyl moiety. The major modifications of newly synthesized compounds were change of substituent at position 5 of hydantoin and elongation of linker between heterocyclic ring and arylpiperazine fragment, to three methylene units.



ED_{50}MES = 13,11 mg/kg

ED_{50}scMet = 17,58 mg/kg



R^1 = H, Cl; R^2 = C_3H_5, $CH(CH_3)_2$

R^3 = H, 2-F, 4-F, 3-Cl, 3-CF_3, 2-OCH_3 X = -, CH_2

The investigated compounds were prepared in three step process, in which cyclization of hydantoin ring followed by alkylation with 1-bromo-3-chloropropan and condensation with appropriate arylpiperazine moiety.

The compounds were evaluated for their anticonvulsant properties within the Antiepileptic Drug Development Program, by testing procedures, which have been described earlier [3].

This work was supported by the Polish MNiSW grant No N N405 298536

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17:00 Poster 75

Synthesis, anticonvulsant activity and 5-HT_{1A}/5-HT_7 receptors affinity of new 1-[(4-substituted-piperazin-1-yl)-alkyl]- 3-methyl- 3-(2-methylpropyl)-pyrrolidine-2,5-diones

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The incomplete information on the pathogenesis of human epilepsy and the complex mechanism of action of majority antiepileptic drugs makes it difficult to use rational methodologies of discovery that are based on three-dimensional structure of biological target. Thus the most useful for the design of new anticonvulsants are ligand-based approaches that rely on the use of different pharmacophores established through the analysis of structural characteristics of clinically effective antiepileptic drugs (AEDs) as well as other anticonvulsant active compounds [1]. The two past decades have demonstrated many attempts to identify the structural features of compounds crucial for anticonvulsant activity. As a result it was proved that one of the important core fragments is defined by nitrogen heteroatomic system, usually imide or lactam, at least of one carbonyl group and phenyl or alkyl groups attached to the heterocyclic system [2,3].

The previous studies from our laboratory have demonstrated potent anticonvulsant activity in groups of 1-[(4-phenyl- piperazin-1-yl)-alkyl]-3-phenyl-pyrrolidine-2,5-diones [4,5]. These molecules were effective mainly in the maximal electroshock test (MES) which an animal model of human generalized tonic-clonic epilepsy. As a continuation of our systematic SAR analysis in the present studies we have obtained a series of 1-[(4-substituted-piperazin-1-yl)-alkyl]-3-methyl-3-(2-methylpropyl)pyrrolidine-2,5-diones. These molecules have been designed as analogues of respective, active 3-phenyl-pyrrolidine-2,5-diones. The structures of compounds synthesized are shown in **Fig. 1**.

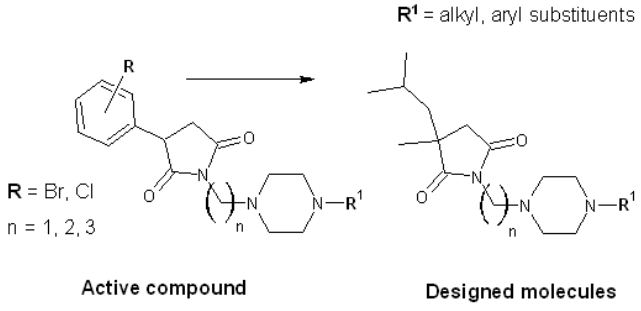

Fig. 1.

All the compounds were evaluated for their anticonvulsant activity and neurotoxic properties within the Antiepileptic Drug Development (ADD) Program (Epilepsy Branch, Neurological Disorders Program, National Institute of the Neurological and Communicative Disorders and Stroke (NINCDS), Rockville, USA) [6]. Because se-

veral molecules can be regarded as long-chain arylpiperazines (LCAPs) in the current studies the affinities for 5-HT_{1A} and 5-HT_7 receptor subtypes have been assessed.

We are pleased to acknowledge the generous financial support of this work by the grant of the Minister Committee for Scientific Research, Poland (Grant NoN405 298536).

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17:00 Poster 76

Microwave-assisted synthesis and antimicrobial activity of O-alkylamino derivatives of benzofurancarboxylic acids

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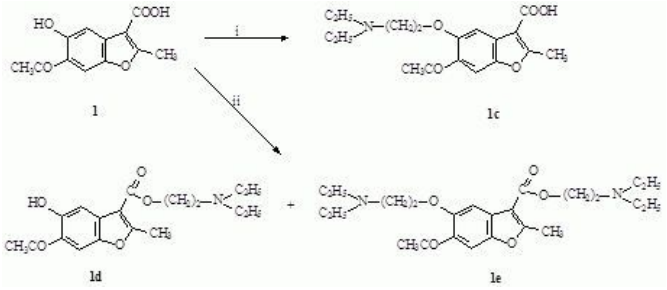
It is widely known that numerous compounds containing the benzo[b]furan system, both synthetic and isolated from natural sources show antimicrobial activity [1-2]. As a continuation of our research [3] we have synthesized a series of benzofurancarboxylic acids bearing *O*-aminoethyl substituents with 2-chloroethyl-*N,N*-diethylamine, and have tested their antimicrobial activity. We have used microwave-assisted syntheses to obtain *O*-alkylamino derivatives of methyl esters benzofurancarboxylic acids (7-acetyl-6-hydroxy-3-methyl-2-benzofurancarboxylic acid, 2-methyl-5-hydroxy-6-acetylbenzofuran-3-carboxylic acid, 7-acetyl-6-hydroxy-5-methoxy-3-methyl-2-benzo[b]furancarboxylic acid, 6-hydroxy-7-(*p*-methoxycinnamoyl)-3-methyl-2-benzo-[b]furancarboxylic acid, 7-acetyl-6-methoxy-3-methyl-2-benzo[b]furancarboxylic acid, 5-bromo-7-hydroxy-6-methoxy-2-benzo- [b]furancarboxylic acid and 7-methoxy-2-benzo[b]-furancarboxylic acid) by *O*-alkylation of appropriate esters. Benzofurancarboxylic acids reacted with 2-chloroethyl-*N,N*-diethylamine under similar conditions. Microwave-assisted alkylations yielded the mixture of two products. Observed data for antibacterial activity indicated strong activity of 5-bromo-7-hydroxy-6-methoxy-2-benzo[b]furancarboxylic acid against fifteen tested strains of bacteria.



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17:00 Poster 77

Comparison of biological activity and stability of selected C-glycosidic and O-glycosidic genistein derivatives

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Intensive research on genistein biomedical properties prompted many researchers to synthesize its derivatives with intention to improve their stability, activity and bioavailability. Our previous study revealed very promising properties of unsaturated sugar substituted genistein derivatives. The purpose of this study was to synthesize and screen in vitro the library of their analogues for antiproliferative properties and stability in water solutions and in culture media. We have compared the activity and stability of genistein derivatives which contain a substituent at C-7 or C-4' position, connected with a molecule of genistein by *O*-glycosidic bond. We have also compared the stability of C-7 substituted analogues differing with the type of the glycosidic bond: *O*-glycosides vs. *C*-glycosides.

Cytotoxicity of the analogues of genistein was assessed by MTT assay after 72-hour incubation of HCT116 and DU 145 cell lines with the tested compounds. The influence of selected compounds on the cell cycle was determined using flow cytometer. Comet assay was used to check whether the derivatives cause DNA damage. LC / MS^n was used to determine the stability of the compounds in after-culture medium. Isoflavones in the medium were separated using isocratic methods and detected by positive ionization mode and using electrospray ionization interface (ESI).

On the base of the obtained results the influence of the position of a

substitution of genistein on the antiproliferative activity and inhibition of cell cycle was determined. Genistein analogues which contained a sugar substituent at C-7 position showed the ability to inhibit the cell cycle in G2 and M phase. Compounds in which the sugar was attached at C-4' position caused the inhibition of the cell cycle in G1 phase. Compounds of Ram-X ' series and Gen-5' did not cause either single or double strand brakes in DNA structure. In contrast, derivatives of Ram-C series increased the level of DNA damage in relation to the untreated control.

The stability of compounds depended on the type of glycosidic bond connecting the substituent with a molecule of genistein. Derivatives which contained *C*-glycosidic bond had higher stability in comparison to compounds containing *O*-glycosidic bond. The type of a glycosidic bond did not influence the biological activity of compounds. Glycoconjugates of Ram-C series showed similar ability to inhibit the cell proliferation as their O-glycosidic analogues.

Acknowledgements

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17:00	Poster	78

Impact of celecoxib on soluble intercellular adhesion molecule-1 (sICAM-1) and soluble E-cadherin (sE-cadherin) concentrations in human colon cancer cell line cultures exposed to phytic acid (IP6) and TNF-α (tumor necrosis factor-alpha)

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Information about discovery of antineoplastic activity of non-oncological drugs spreads very quickly. One of them seems to be a class of non-steroidal anti-inflammatory drugs (NSAIDs), coxibs. Celecoxib, a NSAIDs representant selectively inhibit cyclooxygenase-2 (COX-2), an enzyme that activate synthesis of prostaglandines, pro-inflammatory molecules, that promote inflammation. Analysis of COX-2 gene expression in colonic epithelium showed that it's level was significantly elevated in colon cancer and familial adenomatous polyposis. There are scientific reports that celecoxib, besides it's analgesic, anti-inflammatory, and antipyretic activity is able to inhibit development of colon cancer and reduce risk of liver colon cancer metastasis. It is well known that these molecules play a key role in carcinogenesis. The measurement of their concentrations in serum and in other patient's body fluids may be helpful for diagnostics, advancement evaluation, and therapy response monitoring in patients with different types of cancer. The additional factors, e.g. dietary components in colon cancer, may influence therapeutic effect of drugs, such as cytokines. TNF-α (tumor necrosis factor - alpha) is a cytokine, which concentration significantly increases in serum of patients with inflammatory and cancer diseases. Last studies demonstrate, that phytic acid (IP6), a phosphorylated carbohydrate, abundantly present in high-fiber diets could substantially reduce colon cancer incidence.

The aim of the present study was to estimate the influence of celecoxib on sICAM-1 and sE-cadherin concentrations in transformed epithelial colon cell cultures simultaneously exposed to IP6 and TNF-α. Human intestinal HT-29 and Caco-2 cell lines derived from colon adenocarcinoma were used in our studies. The cells were cultured in the presence of 50 ng/ml celecoxib (f.c.) 1.0 mM IP6, and 100 ng/ml TNF-α and their combination: TNF-α plus IP6, TNF-α plus celecoxib, IP6 plus celecoxib, and TNF-α with celecoxib plus IP6, for 96 hours. Nonexposed cell line cultures served as controls. Concentrations of sICAM-1 and sE-cadherin were measured in the culture medium by enzyme-linked immunosorbent assay (ELISA) using Quantikine – Human sICAM-1/CD54 Immunoassay (R&D Systems) and Quantikine–Human sE-Cadherin Immunoassay (R&D Systems). All the results obtained were expressed as ng per ml. The statistical analysis was performed with the use of Statistica 10.0 Software.

The results of this studies showed that mean sICAM-1 concentration in HT-29 cell line control culture was 2.26 ng/ml (+/- 0.13), while in the control Caco-2 cell line culture amounted 5.56 ng/ml (+/-0.58). Celecoxib (50 ng/ml) alone as well as in the presence of 1 mM IP6 or TNF-α did not cause a significant change in this adhesion molecule concentrations in Caco-2 and HT-29 cell lines either. In both cell line types exposed to a combination of celecoxib, IP6 and TNF-α a statistically significant decrease in sICAM-1 level was observed compared with the cell cultures exposed to a combination of IP6 plus TNF-α. In HT-29 cell line culture sICAM-1 concentration was statistically significantly higher, than in the control culture because of high concentration of the molecule deriving from TNF-α exposed cells, but the effect was not observed in Caco-2 cell line cultures. Mean sE-cadherin concentrations in HT-29 and Caco-2 cell line cultures were 17.34 ng/ml (+/- 1.2) and 16.23 ng/ml (+/-0.78), respectively. There was no significant difference in sE-cadherin concentration in HT-29 cell line culture exposed to celecoxib compared with the control one, but in Caco-2 cell line culture it was significantly lower. The addition of celecoxib to the HT-29 cell line culture containing TNF-α did not cause changes in sE-cadherin concentration compared with cell culture containing the cytokine exclusively, while in Caco-2 cell line culture it was significantly lower than in culture containing only the cytokine. In both, HT-29 and Caco-2 cell lines, low sE-cadherin concentrations were observed in the presence of celecoxib introduced along with 1 mM IP6. Co-treatment with celecoxid, IP6 and TNF-α caused a significant decrease in sE-cadherin concentrations in culture medium deriving from HT-29 and Caco-2 cell lines down to 0.5 ng/ml (+/-0.4) and 2.1 ng/ml (+/-1.1), respectively.

17:00 Poster 79

The influence of TNF-α (tumor necrosis factor - alpha) on concentration of soluble adhesion molecules in cultures of HT-29 cells exposed to inositol hexaphosphate (IP6)

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Continuous improvement in colon cancer therapy is one of the most difficult challenges of contemporary medicine, especially with regard to a high tumor metastatic potential, which is the main cause of treatment failure. The latest studies suggest that adhesion molecules are involved in the arising of malignant changes and in distant metastasis induction. The changes in their expression, and consequently, in the concentration of their soluble forms in serum, could be modulated by many different factors affecting cancer cells. In the case of colon cancer one of the factors is a high-fiber diet, containing an anti-cancer chemical, inositol hexaphosphate (IP6). It is known, however, that in both inflammatory diseases and the malignant tumor microenvironment the concentration of pro-inflammatory cytokines increases, including TNF-α. This cytokine can modify the adhesive properties of cells and influence the concentration of soluble adhesion molecules deriving from malignant colon cells. Nevertheless, there is no literature data on the synergistic effect of TNF-α and IP6 in the cancer cells microenvironment.

The goal of this study was to evaluate the influence of TNF-α on the concentration of soluble intercellular adhesive molecule-1 (sICAM-1) and soluble E-cadherin (sE-cadherin) in the microenvironment of HT-29 malignant cells stimulated with IP6. The elevated concentration of these soluble adhesive molecules suggests the intensification of the migration ability of cancer cells, and the concurrent increase in cancer cells invasiveness and metastasis.

HT-29 cell line derived from human colorectal cancer was used to evaluate sICAM-1 and sE-cadherin concentrations in their microenvironment. The cells were stimulated with 0.5 mM, 1.0 mM and 2.0 mM IP6, 10 ng/ml and 100 ng/ml TNF-α and TNF-α plus IP6 for 96 hours. Nonexposed cell line cultures as well as a culture medium without colon cancer cells served as controls. sICAM-1 and sE-cadherin concentrations were measured in the culture medium by immunoenzymatic method ELISA using Quantikine – Human sICAM-1/CD54 Immunoassay (R&D Systems) and Quantikine–Human sE-Cadherin Immunoassay (R&D Systems). All the results obtained were expressed as ng per ml.

The experiments showed the presence of both soluble ICAM-1 and soluble E-cadherin in HT-29 cell cultures exposed and unexposed to TNF-α and IP6. The control medium without the cells was negative for these adhesion molecules. Therefore, sICAM-1 and sE-cadherin were shed from HT-29 cells. After TNF-α treatment at concentration of 10 ng/ml of HT-29 cells, the values of sICAM-1 in cell culture supernatants did not change, but 100 ng/ml TNF-α caused a six-sevenfold increase of these soluble molecules compared with control culture. Interestingly, TNF-α at that concentration caused the increase in the sICAM-1 level, but to a lesser degree in the presence of higher concentrations of IP6. Similarly, as in the case of sICAM-1, TNF-α and IP6 caused dysregulation of sE-cadherin molecules in the intestinal epithelial of HT-29 cells. In our studies TNF-α caused an increase in sE-cadherin level in cultures exposed to 100 ng/ml of the cytokine, but 10ng/ml TNF-α did not influence their concentration in HT-29 cells cultures. A decrease in sE-cadherin in cultures supernatant under the influence of 2 mM IP6 was observed. The addition of TNF-α at a concentration of 10 ng/ml to the cultures containing 0.5, 1.0, and 2.0 mM IP6 did not cause any increase in the sE-cadherin level. Taking into account the concentrations of these molecules in the control culture, TNF-α at concentration of 100ng/ml did not significantly increase the sE-cadherin level in the presence of various IP6 concentrations either. Their level was most likely associated with IP6 activity. The higher concentration of IP6 and the lower sE-cadherin level in the presence of TNF-α was observed.

17:00 Poster 80

Synthesis and evaluation of inhibitory activity of novel C-5 substituted uridine derivatives

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The biosynthesis of polysaccharides and glycoconjugates mediated by glycosyltransferases (GTs) has attracted increasing interest. Modulation of GTs activity by selective inhibitors has become a tool for investiagation of biochemical pathways and also a new field for a novel therapeutics discovery.

The natural donor substrates for the majority of glycosyltransferases are UDP-sugars. They are bound to the active site of enzyme by coordination to divalent metal ion through diphosphate group. The aim of this work was to synthesize potential β-1,4-galactosyltransefrase inhibitors that are analogues of natural donor substrate (UDP-Gal). A variety of pyrophosphate analogues that can mimic pyrophosphate-metal interactions have been developed[1-6]. We decided to replace the diphosphate unit with β-ketoenol group which is known to coordinate to divalent metal ion. The designed compounds were composed of uridine unit connected to substituted aromatic ring through different linkages. Structures of all final products were confirmed by ^{1}H and ^{13}C NMR spectroscopy.

All synthesized compounds were tested as potential inhibitors in a competition assay against bovine milk β-1,4-galactosyltransferase. We have applied the fluorescence assay developed by Praly and coworkers to measure GTs activity[4]. This method utilizes fluorescent-labeled acceptor β-GlcNAc-O-$(CH_2)_6$-dansyl instead of the natural substrate. The samples were analyzed using reversed-phase HPLC with fluorescent detector. Synthesis of target compounds and evaluation of their activity will be presented.

Acknowledgement

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17:00 Poster 81

Comparative permeation studies of tacalcitol through the human skin from brand product versus generic product

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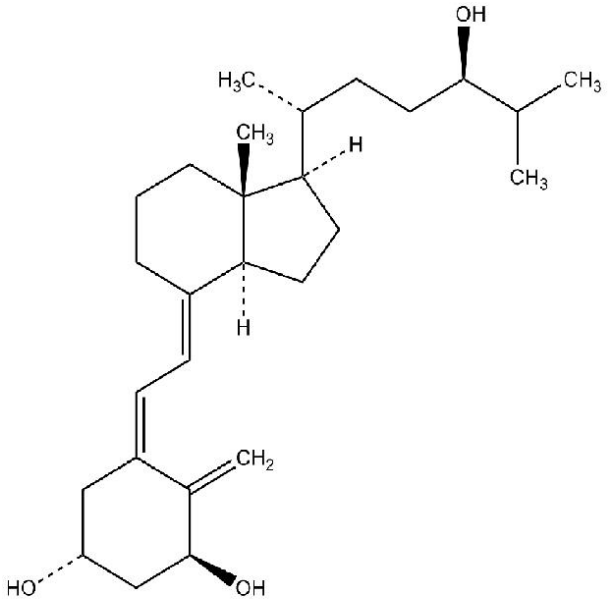
Tacalcitol is one of the analogues of vitamin D_3 used to treat plaque psoriasis. Its chemical structure is as follows:



with the chemical name: (1S, 3R, 5Z, 7E, 24R) – 9,10 – secocholesta – 5,7,10 – triene – 1,3,24 – triol. The clinical efficacy of the keratolytic product is difficult to assess taking into account the influence of others ingredients. The comparative studies of in vitro release testing and skin permeation provide a lot of details in the whole development of the manufacturing process.

The objective of this study was to compare the permeation of tacalcitol through human breast skin in the brand product versus a generic cutaneous lotion manufactured by Pharmaceutical Institute. Ex vivo permeation was investigated using a Franz diffusion cell. The method of the extraction was developed and the recovery was examined in the range from 5 ng to 50 ng of the active substance. The skin extracts were analysed using the HPLC method with normal phase at the flow rate 0.6 mL/min. and a UV detector. The concentration of tacalcitol was analysed in the *stratum corneum* and in both layers- *epidermis/dermis*. It was observed that the permeation of tacalcitol from both formulations tested was comparable.

17:00 Poster 82

Determination of Sunitinib in human plasma using LC/MS

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Sunitinib is an inhibitor of several receptor tyrosine kinases and is used for treatment of gastrointestinal stromal tumors as well as advanced renal cell carcinoma. Considering the need for low-cost cancer treatments, it is essential to develop reliable and sensitive bioanalytical methods of sunitinib determination in human plasma for conducting pharmacokinetic studies of new generic drug products.

Currently available bioanalytical methods are fast and sensitive, predominantly based on protein precipitation and MS/MS detection, providing LLOQs (lower limit of quantification) at the level of 0.1 ng/mL [1]. Presented work describes validation of a method developed for sunitinib determination using LC/MS instrument, conducted according to respective FDA (U.S. Food and Drug Administration) [2] and EMA (European Medicines Agency) [3] guidelines. In order to achieve required LLOQ, liquid-liquid extraction of 500 µL plasma sample with hexane/isopropanol (90/10 v/v) mixture was used. Chromatographic analysis was carried out using Zorbax SB-C18 150 x 3 mm, 3.5 µm column and isocratic elution with 55/45 (v/v) mixture of 0,1% HCOOH and ACN/MeOH (80/20 v/v). The detector was a single quadrupole setup with ESI (+) probe, operating in Single Ion Monitoring (SIM) mode.

Applied sample preparation procedure as well as instrumental conditions allowed recoveries of approximately 70 % for both sunitinib and sunitinib-d10 (internal standard) and linear detection range of 0.1-150.0 ng/mL.

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17:00	Poster	83

Y308 is a key residue of the β_2-adrenoceptor to distinguish the G_s selective derivatives of fenoterol

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β_2 adrenergic receptors (β_2-ARs) belong to the large family of the G protein-coupled receptors (GPCRs). With almost 50–60% of drugs in the market targeting GPCRs, they are the most important family of receptors in the drug discovery field.

We studied stereoisomers of fenoterol (Fig. 1) and its derivatives which are a selective β_2-AR agonists [1,2].

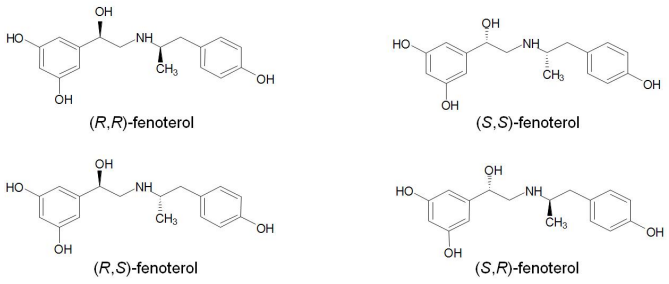

Fig. 1. Stereoisomers of fenoterol.

Our studies show the association of molecular structure fenoterol analogs with significant differences in activation patterns of the β_2-AR. The results of the *pertussis toxin* -controlled (PTx-controlled) myocyte contraction analysis indicate that subtle changes in a chemical constitution of fenoterol derivatives play a decisive role in inducing G_s selective or G_s/G_i (G stimulatory/G inhibitory) patterns of receptor coupling [3]. While (*R*,*R*)-isomers of fenoterol, 4-methoxy-fenoterol and 4-amino-fenoterol activate β_2-AR to a form which couples selectively to G_s protein (group A, G_s selective compounds), the receptor activated by e.g. 1-naphtyl-fenoterol or 4-methoxy-1-naphtyl-fenoterol is able to couple uniformly both G_s and G_i proteins (group B, non G_s selective).

Molecular modeling studies show that hydrogen bond (HB) formation between N-alkyl moiety of a ligand with $Y308^{7.35}$ is a key interaction distinguishing G_s selective derivatives from others, non selective derivatives. This hypothesis has been positively validated using Y308A mutant of the β_2-AR: G_s selectivity of the mutant is lost in the experiments with (*R*,*R*)-fenoterol and (*R*,*R*)-4-amino-fenoterol. The Y308A mutant shows significantly reduced affinities for compounds of group A as compared with the wild type-related data (WT β_2-AR). When compounds of group B were tested on the mutant, their binding affinity (K_i) were not affected by Y308A mutation and were very close to those determined for WT β_2-AR.

In addition, docking studies indicate two alternative binding modes of studied compounds interacting with β_2-AR in its binding site. The binding pattern depends on the structure of the N-alkyl part of these derivatives. The molecules containing benzyl ring substituted in the 4' position with oxygen or nitrogen atom are able to form HB with $Y308^{7.35}$ (group A) independently from interactions with the aromatic cleft. These derivatives while in the (*R*,*R*)-stereoconfiguration, induce PTx insensitive contractile response. If a derivative contains the naphtyl moiety (group B), the Π-Π interactions with residues forming the aromatic cleft of the receptor seem to be dominated.

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17:00	Poster	84

Naturally occurring hydroxyalkyl isothiocyanates and isothiocyanatoalkyl benzoates - structure-activity and structure-mechanism of action relationship studies.

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Isothiocyanates are among most extensively studied natural compounds as a potential anticancer drugs present in a cruciferous vegetables in high amounts and variety. Numerous studies showed that benzyl isothiocyanate or sulphoraphane are capable to induce cell cycle arrest and apoptosis in a variety of cancer cell lines *in vitro* and *in vivo*. However, only a few most abundant representatives of this large and diversified group were extensively studied so far.Herein, we describes studies over the structure-activity relationship for previously never tested representatives of natural isothiocyanates - hydroxyalkyl isothiocyanates and isothiocyanatoalkyl benzoates. Both groups showed high antiproliferative activity against several cancer cell lines including lung, breast, drug-sensitive and drug-resistant colon cancer. This activity was comparable to the activity of extensively studied benzyl, phenylethyl isothiocyanate and suforaphane, e.g. IC_{50} values measured for drug-sensitive colon can-

cer cell line (LoVo) were in range 3 – 13 μM and only slight activity decrease was observed when drug-resistant subline (LoVoDX) was used (IC_{50} in range 6 – 18 μM). The length of the alkyl chain connecting the hydroxyl moiety with isothiocyanate moiety had a direct impact on this activity – the most active compounds were those with the longest alkyl chain - 6-isothicyanohexan-1-ol and 6-isothiocyanatohexyl benzoate.

Isothiocyanates mechanism of action is based on fast accumulation in cells via passive diffusion followed by the reaction with glutathione reduced and protein's thiocarbamoylation which leads to a number of changes in cell's metabolism and signaling routes. At least 30 proteins were recognized as isothiocyanates molecular targets so far, thus, studies over the structure-mechanism of action relationship are difficult. However, several parental molecular targets can be set and the most important target for almost all isothiocyanates appears to be glutathione, main redox status guardian present in high amounts within cells. Because mitochondria of most cancer cells do not functioning properly, they produce high amounts of reactive oxygen species and to counteract this phenomenon the biosynthesis of glutathione route is highly active and overexpressed. Based on this observations we evaluated the structure-mechanism of action relationship for tested isothiocyanates using glutathione level, ROS level, caspase-3 activity and cell cycle distribution as markers to asses this correlation. Generally, we have found that hydrophobic representatives like 6-isothicyanohexan-1-ol caused high glutathione depletion and caspase-3 activation correlated with high ROS production level. Hydrophilic compounds like 3-isothicyanopropan-1-ol caused only a moderate glutathione depletion without significant caspase-3 activation, but a cell cycle arrest was observed in G_2/M or in G_0/G_1 phase.

17:00 Poster 85

Novel bifunctional synthetic isothiocyanates with high antiproliferative activity

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Herein, we describe novel class of biologically active compounds containing two distinct chemical moieties - isothiocyano-aralkylphosphonate diphenyl esters. The isothiocyanate moiety is responsible for a fast compound's accumulation in the cells via passive diffusion and high reactivity against reduced glutathione which leads to its depletion correlated with several proteins thiocarbamoylation, cell cycle arrest and apoptosis. Numerous studies performed with use of natural isothiocyanates such as benzyl isothiocyanate or sulforaphane strongly suggest that even such simple compounds can be a good candidate for an anticancer drug. Moreover, despite of the high chemical reactivity of the isothiocyanate moiety (this property is often used in organic chemistry), they show remarkable selectivity towards cancer cells. Second moiety – phosphonate diphenyl ester is the transition state analogue for the peptide bond hydrolysis carried out by serine proteases, thus, its responsible for inhibitory activity towards this class of enzymes. Because of the proteases substrate high specificity, both carbon α substitution and phenyl ring substitution allows us to obtain highly active and specific inhibitors. In this presentation, chymotrypsin and elastase-directed compounds will be presented. Both enzymes are an important enzymes in carcinogenesis and metastasis. Chymotrypsin-like proteasome activity, as a part of a large proteasome multicatalytic proteinase complex, plays an important role in several critical cellular functions including the processing of proteins involved in cell cycle progression and gene expression. It is responsible for a fast degradation of p53, p27 and cyclin B, and for the activation of the transcription factor NF-κB through the degradation of its regulatory subunit IκBα. Elastase is an enzyme important during the process of metastasis, because of its capability to cleave almost every protein contained within the extracellular matrix (ECM) including, but not limited to, elastin, collagen, fibronectin, laminin, and proteoglycans. Uncontrolled activity and overexpression of elastase allows cancer cells within a tumor to develop and metastasize directly through degradation of ECM.

In this presentation, we present synthetic routes used in order to obtain over 20 compounds, phosphonate analogues of amino acids with unsubstituted phenyl ring. For all of them, the antiproliferative activity against drug-sensitive and drug resistant colon cancer cell line (LoVo and LoVoDX) and the chymotrypsin and elastase enzymes inhibition potency was evaluated. The IC_{50} for the most active compounds was in range 7-15 μM (determined with SRB method, 72 hour drug treatment) for both cancer cell lines. Simultaneously, the most active chymotrypsin inhibitors demonstrated k^2/K_i second order rate constant in range 1 100 - 1 320 [$M^{-1}s^{-1}$] and elastase inhibitors in range 400 - 505 [$M^{-1}s^{-1}$]. For the most promising compounds, the molecular mechanism was additionally evaluated. The glutathione reduced depletion (up to 70% depletion after 2 hours of treatment) was observed followed by caspase-3 activation (up to 2,5-fold change compared to control after 24h treatment) and chymotrypsin-like proteasome inhibition (up to 1,7-fold change compared to control). These results are a good starting point for further development of biologically active compounds based on both isothiocyanates and phosphonate diphenyl esters.

17:00 Poster 86

Tiocarbamates derivatives of p-nitrobenzyl alcohol as donors in the synthesis of glycoconjugates

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Formation of carbon – oxygen single bonds is one of the most fundamental and most frequently used important reaction in synthetic chemistry of carbohydrates. The choice of efficient protective group in the synthesis of a complex molecules is often main stage.[1,2] The p-nitrobenzyl protecting group has seen use in the protection of alcohols, thiols, amines and carboxylic acids.[3] p-Nitrobenzyl glycosides are stable building blocks used in synthesis of oligosaccharides or complex glycoconjugates which exhibiting various biological activity. p-Nitrobenzyl groups can be reduced to the amino group which

can be used as spacer in the synthesis of glycoconjugates.

In this communication we report the novel method protection of hydroxyl functionality group in the sugars and isoflavonoids using p-nitrobenzyl thiocarbamates as donors. These compounds are readily obtained from p-nitrobenzyl alcohol in the reaction with commercially available N-allyl isothiocyanate[4], N-benzyl isothiocyanate and N-phenyl isothiocyanate. We obtained p-nitrobenzylglycoside with high α-stereoselectivity and p-nitrobenzyl derivative of isoflavonoid with good yield. In summary both this products can be used in the synthesis of glycoconjugates.

Acknowledgement

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17:00 Poster 87

Synthesis and cytotoxic activity of new Pt (II) complexes with nitropyrazoles in aerobic and hypoxic conditions

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Hypoxic conditions are usually the common feature of solid tumors resulting from their inefficient microvascular systems due to the rapid tumor growth [1]. This behavior distinguished solid tumor cells from normal cells causing their resistance to the chemo- and radiotherapy but, on the other hand, also giving new opportunities for selective cancer treatment. In our approaches to the design and synthesis of targeted anticancer prodrugs for tumor site-specific activation we paid our attention to Pt(II) complexes with nitrodiazoles. This class of compounds potentially should exhibit dual mode of action, binding to DNA and undergo the bioreductive activation process.

On presented poster, the synthesis of new platinum complexes with nitropyrazoles and their cytotoxic activity against tumor line cells in aerobic and hypoxic conditions [2] is going to be shown. Revealed activity of tested complexes shows strong cytotoxic activity of new platinum complexes against tumor lines, in a few cases, stronger than reference drug cisplatin.

Research was granted by KBN financial support, grant nr N N401 052938

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17:00 Poster 88

New method of obtaining of 2-cyano- 3-alkoxy-2-butenoates

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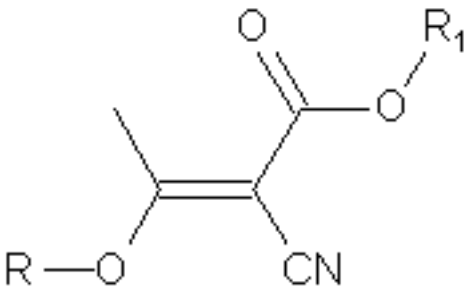
New method of obtaining of known (*E*)-2-cyano-3-alkoxy-2-butenoates **1** are going to be presented. These esters are very useful to synthesis of isoxazole and pyrazole derivatives [2], from which there are many biologically active compounds [1]. So, they are very essentially valuable compounds in organic synthesis but so far methods of their obtaining were not yielded (about 40%) [3,4] or very hazardous diazomethane was used to synthesize them [5].



1

The way of the synthesis of 2-cyano-3-alkoxy-2-butenoates **1**,developed by us, as a result of reaction of appropriate cyanoacetates with orthoacetates in the tertiary amine presence, is the new efficient method giving practically quantitative yield of these valuable semi-products [6].

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17:00 Poster 89

Development and validation of the HPLC-UV method for impurities determination in duloxetine hydrochloride

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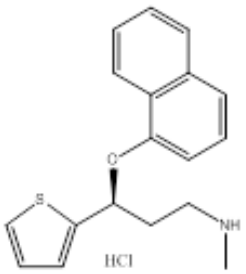
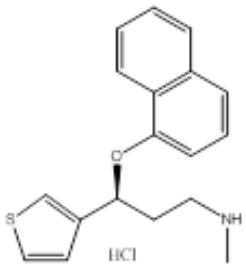
Duloxetine hydrochloride (DX) [(+)-(S)-N-Methyl-3-(naphthalen-1-yloxy)-3-(thiophen-2-yl)] propan-1-amine hydrochloride] is an antidepressive drug effective for mood disorders and also the stress urinary incontinence. Duloxetine is a serotonin-norepinephrine reuptake inhibitor (SNRI). The serotonin and norepinephrine are neurotransmitters responsible for connection between nerve cells. Besides effectiveness in treatment of mental disorders these hormones are involved in process of pain reduction. Duloxetine increases the level of neurotransmitters in the brain, which affect course of anxiety, diabetic neuropathic pain and attention deficit hyperactivity disorder (ADHD).



duloxetine (DX)



DX isomer

Reversed phase liquid chromatography (RP - HPLC) as an analytical technique was used to control the synthesis route and for the determination of impurities of duloxetine hydrochloride. Resolution between potential contaminants and by-products of DX is based on the difference in polarity. One of potential impurities determined in duloxetine samples is DX isomer described in pharmacopoeia monograph [1]. During the method development it was found that the separation between DX and its isomer is the critical point of the HPLC method. The value of resolution (RS) should be higher than 1.5.
The final HPLC method does not require ion-pair and buffer use in contrast to methodologies described in available analytical literature [1,2].

The chromatographic separation was achieved on a C-18 column using gradient elution and the mixture of water, methanol, tetrahydrofuran with the addition of trifluoroacetic acid as the mobile phase. Analysis were performed at 230 nm [2].

The validation of the HPLC method included: specificity and selectivity studies, determination of linearity, limits of detection (LOD) and quantification (LOQ), accuracy, repeatability and robustness [3,4].

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17:00 Poster 90

Role of estradiol in chromium-induced oxidative stress

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Chromium is widely present in the environment. Chromium toxicity is directly dependent on its valency. Chromium III (CrIII) is an essential factor of glucose metabolism as a constituent of the glucose tolerance factor (GTF) and chromium VI (CrVI) is considered carcinogenic in humans and is listed on the International Agency for Research on Cancer list of carcinogens. People exposed to chromium compounds express a number of disorders of variable background in some cases due to chromium-induced oxidative stress. Estrogens are involved in the anti- and pro- oxidative transformations as inducers or scavengers of reactive oxygen species.The aim of this study was to investigate the role of 17-β-estradiol in chromium-generated oxidative stress in order to determine whether it has a detoxifying activity or increases the toxic effects of chromium compounds.

Analyses described in this study were performed on an *in vitro* model of whole human blood, purified erythrocytes or mitochondria isolated from human placenta upon exposure to varying concentrations of CrIII, CrVI, and estradiol. Reduced glutathione (GSH) levels, membrane lipid peroxidation (levels of malondialdehyde - MDA), glutathione peroxidase (GPx), and superoxide dismutase

(SOD) activities were measured in blood. Isolated mitochondria were used to investigate the MDA levels and hydroxyl radical (˚OH) generation. The results show varying influence of estradiol on the chromium-induced oxidative stress.

We have shown previously (Dlugosz *et al*, 2012) that CrIII does not influence the levels of erythrocytal GSH, while CrVI at concentrations of 5 and 10 µg/mL reduces the level of GSH in human red blood cells *in vitro*. In this work we noticed, that 17-β-estradiol shows a positive effect when erythrocytes are exposed to moderate concentrations CrVI and increases the levels of erythrocytal GSH. Estradiol does not show any interactions with chromium on the antioxidative enzymes (SOD in erythrocytes and GPx in whole blood) activity measurements. Additionally, we show that estradiol plays a positive role in the chromium-induced lipid peroxidation in erythrocytes, reducing the levels of MDA produced under exposition of erythrocytes to both forms of chromium. Unexpectedly, the interaction of estradiol with chromium can be seen on the MDA formation in human mitochondria, where estradiol increases MDA levels induced by both forms of chromium. Estradiol increased the ˚OH generation triggered with CrVI when compared to control mitochondria exposed to CrVI alone. It appears that estradiol acts protectively on lipid peroxidation caused by chromium in erythrocytes but gives an interaction with Cr in mitochondria, which partially correlates with hydroxyl radical formation in this organelle.

17:00 Poster 91

New form of coenzyme Q10 (PureSorb-Q^{TM}40) inhibits free radicals processes caused by cisplatin.

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Reducing the side effects of cancer chemotherapy is an important task for clinicians and researchers. The role of free radical processes in carcinogenesis is established and studies of free radical scavengers are intensive. In our study on the activity of KP972 (derivative of pyridopyrazolopyrimidine i.e. 4-phenyl-2-(4'-trifluoromethyl-beta-styryl)pyrido (2',3':3,4)pyrazolo(1,5-a)pyrimidine in comparison with cisplatin, we found that cisplatin toxicity is associated significantly with the generation of free radicals [1,2]. This is also confirmed by the clinical observations (an increase of MDA-malondialdehyde in the urine of patients) [3]. There is information about beneficial effects of antioxidants (vitamins C and E, N-acetylcysteine) in the treatment of cisplatin [4] Our *in vitro* study demonstrated that oxidative stress caused by xenobiotics can be in some of cases effectively inhibited by coenzyme Q10 (CoQ10) [5].

Currently presented research concerns the assessment of the effectiveness of a new soluble in water form of coenzymeQ10-P40 (PureSorb-Q ^{TM}40) in relation to cisplatin. The study was performed on an *in vitro* model of human erythrocyte. The degree of lipid peroxidation in the erythrocytes was measured as the TBARS (thiobarbituric active reagent species)level according to Stocke's method. The hydroxyl radical (OH·) concentration was determined by deoxyribose degradation, while the activity of antioxidant enzymes : SOD (superoxide dismutase) and GPx (glutathione peroxidase), using Randox kits. Final verification of the suitability of a new form P40 as an antioxidant has been leading as a series of the same experience with fat-soluble CoQ10.

In our observations new form - P40 and lipophilic CoQ10 decreased TBARS level in red blood cell. Furthermore, it was observed that both forms of antioxidant acted protectively in concentrations: 1,0 - 120 µg/ml in oxidative stress induced by cisplatin. Inhibition of lipid peroxidation, elevated after exposure to cisplatin, was more effective in the higher doses (20 -120 µg/ml) of P40 or CoQ10. The influence of P40 on antioxidant enzymes activity was varied, causing both an increase and decrease of their activity. During our *in vitro* studies we didn't obtained significant differences between the effects of the two forms of CoQ10 on lipid peroxidation. It can be assumed that the solubility in water of P40 is important rather at the stage of absorption *in vivo* but not during action in cells.

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17:00 Poster 92

5H-indolo-[2,3-b]quinolines loaded polymeric micelles

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The purpose of this study was to optimize and characterize novel po-

lymeric mixed micelles loaded with 2,5,9,11-tetramethyl-5*H*-indolo-[2,3-*b*]quinoline, a compound bearing the highest antitumor activity in 5*H* series derivatives of indoloquinolines and 5,11-dimethyl-5*H*-indolo-[2,3-*b*]quinoline which displayed a strong antibacterial, antimycotic and cytotoxic activity *in vitro* against leukemia P388 and L1210 and melanoma B16.

Mixed polymeric micelles were prepared by thin-film hydration method in several proportions in order to confirm optimal value of variables i.e. polymers mass fraction and concentration, amount of derivative of a indoloquinoline, amount of water and level of hydration temperature. Mixed polymeric micelles proved higher sizes and lower polydispersity than micelles prepared from only one of these Pluronics. The determined drug-loading coefficients (DL%), encapsulation ratios (ER%) and Zeta potential values allow to assume that the particles have proper size and density to remain in suspension which leads to repulse. Such combination ensures that the solution or dispersion resists aggregation.

This observation gives a strong ground to claim that mixed polymeric micelles may be considered as an effective nanocarrying system for 2,5,9,11-tetramethyl-5*H*-indolo-[2,3-*b*]quinoline and 5,11-dimethyl-5*H*-indolo-[2,3-*b*]quinoline.

17:00 Poster 93

Fungal N-myristoyltransferase as potential target for thiosemicarbazide-based compounds

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During the past two decades, the frequency of invasive and systemic fungal infections has increased dramatically due mainly to *Candida* species among which *Candida albicans* is the most dreadful human pathogen.(???) Although several antifungal agents such as amphotericin B and the azoles are available, the limitations of current antifungal drugs, increased incidence of systemic fungal infections, and rapid development of drug resistance have highlighted the need for the discovery of new antifungals, preferably with novel modes of action.(???)

In the course of our search for prototype antifungal agents from class of thiosemicarbazide derivatives, we identified two isoquinoline-thiosemicarbazides (see Fig.) as promising leads for design of novel small-molecule antifungal agents. The compounds showed inhibitory activity against opportunistic pathogens *C. albicans* with MIC range of 25-50 µg/mL. More importantly, *ortho*-methyl derivative displayed antifungal activity at non-cytotoxic concentrations in mammalian cells. Based on docking and molecular modeling studies it was proposed that possible target for antifungal thiosemicarbazide-based compounds is *N*-myristoyltransferase and ligand recognition process is connected with both structural and electronic parameters such as dipole moment and the highest occupied molecular orbital energy.

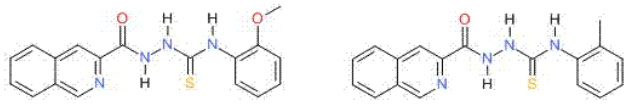

Fig. Structure of isoquinoline-thiosemicarbazides with antifungal activity

??? Giraud F, Guillon R, Logé C, Pagniez F, Picot C, Le Borgne M, Le Pape P. Bioorg Med Chem Letters 19 (2009) 301-304.

??? Babu KS, Li X-C, Jacob MR, Zhang O, Khan SI, Ferreira D, Clark AM. J Med Chem, 49 (2006) 7877-7886 and references therein

17:00 Poster 94

Synthesis and antitumor activity of novel 2 alkylthio-N-(4-R-quinazolin-2-yl)benzenesulfonamides

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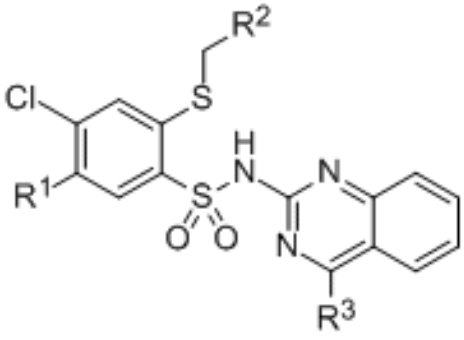
Our recent studies on 2-benzylthiobenzenesulfonamides revealed that a number of their *N*-substituted derivatives possess substantial *in vitro* antitumor activity [1-5]. This prompted us to develop a facile method for the synthesis a new series of sulfonamides bearing heterocyclic rings attached to the sulfonamide nitrogen atom. The synthesis of the target 2-alkylthio-4-chloro-*N*-(4-R^3-quinazolin-2-yl)benzenesulfonamides were achieved by reacting of the corresponding *N*-(2-alkylthiobenzenesulfonyl)cyanamide potassium salts with suitable *ortho*-substituted anilines, such as *o*-aminoacetophenone or *o*-aminobenzophenone derivatives in glacial acetic acid.



R^1 = Me, PhNHCO, 4-substituted PhNHCO
R^2 = Ph, 6-chloropiperonyl, $CONH_2$
R^3 = alkyl, aryl

Fourteen of compounds obtained were screened at the NCI (Bethesda MD, USA) for their *in vitro* activities against NCI-60 panel of human cancer cell lines. Many of these compounds exhibited

a pronounced activity against numerous cancer cell lines. They noticed that, the susceptibility of individual subpanels to the tested compounds was being arranged in the following order: leukemia, non-small cell lung cancer, melanoma, colon, prostate and renal cancer, while the number of leukemia and lung cancer cell lines was largest.

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[2]. Sławiński J., Gdaniec M. *Eur. J. Med. Chem*. **40**, 377 (2005).

[3]. Sławiński J., Brzozowski Z., *Eur. J. Med. Chem*. **41**, 1180 (2006).

[4]. Sławiński J., Brożewicz K., Fruziński A., Główka M.L. *Heterocycles*, **83**, 1093 (2011).

[5]. Brożewicz K., Sławiński J., *Monatsh. Chem*. doi:10.1007/s00706-012-0732-6 (2012).

17:00 Poster 95

Synthesis of novel 4-substituted pyridine-3-sulfonamide derivatives with potential anticancer activity

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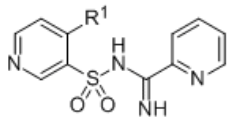
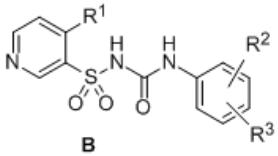
Previously we reported a broad spectrum of biological properties and chemical behaviours of 4-substituted pyridine-3-sulfonamide [1-4]. Our current investigations are focused on the synthesis and biological evaluations of novel pyridine-3-sulfonamide derivatives containing *N*-[imino(pyridin-2-yl)methyl] or *N*-(phenylcarbamoyl) moieties, and diversified with substituent at 4 position of pyridine ring. Thus, starting 4-chloropyridine-3-sulfonamide were converted to the novel 4-(4-arylpiperazin-1-yl)-, 4-(1*H*-pyrazol-1-yl)- or 4-(aryl/alkylthio)pyridine-3-sulfonamides, which were subjected to the reactions with either methyl picolinimidate or the corresponding phenyl isocyanates, to afford the desired *N*-(4-R^1-pyridine-3-ylsulfonyl)picolinamidine (**A**) or *N*-(4-R^1-pyridine-3-ylsulfonyl)-*N*′-phenylurea (**B**) derivatives, respectively.



A



B

R^1 = 4-arylpiperazin-1-yl; 3,5-dialkyl-1*H*-pyrazol-1-yl; aryl/alkylthio
R^2, R^3 = H, 4-Cl or 3,4-diCl

Several compounds obtained were screened at the National Cancer Institute (Bethesda MD, USA) for their *in vitro* activities against NCI-60 panel of human cancer cell lines. Many of them exhibited moderate to weak ability of growth inhibition a number of cancer cell lines. Among them, *N*-[imino(pyridin-2-yl)methyl]-[4-(4-phenylpiperazin-1-yl)]pyridine-3-sulfonamide (**A**) with *para*-halo substituted phenyl ring showed moderate activity against many of leukemia cells, while *N*-(4-R^1-pyridine-3-ylsulfonyl)-*N*′-phenylurea derivatives of thpe **B** possessed activity against colon, melanoma and renal cancer cell lines.

References:

[1] Brzozowski Z., Sączewski F., Sławiński J., *J. Heterocyclic Chem*., 45 (2008) 1407.

[2] Brzozowski Z., Sławiński J., Kędzia A., Kwapisz E., Gdaniec M., *J. Heterocyclic Chem.*, 46 (2009) 1396.

[3] Brzozowski Z., Sławiński J., Innocenti A., Supuran C.T. *Eur. J. Med. Chem*. 45 (2010) 3656.

[4] Brzozowski Z., Sławiński J., Gdaniec M., Innocenti A., Supouran C.T. *Eur. J. Med. Chem.* 46 (2011) 4403.

17:00 Poster 96

Interaction of an potential copper(I) radiopharmaceuticals with human serum albumin

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The coordination chemistry of the aqua-soluble copper(I)-PTA complexes has received an increased interest in recent years. This type complexes may play an important role in the mechanism of cytotoxicity and antitumor activity. They may also be utility in positron emission tomography and targeted radiotherapy. Plasma proteins binding have profound effect on drug distribution and pharmacokinetics. Human serum albumin is the most abundant plasma protein and is responsible for the majority of known drug-protein binding interactions in blood.

In this work, our objective is to study the effect of potential copper(I) radiopharmaceutical on the albumin structure. The binding studies have been performed using spectroscopic methods – fluorescence and circular dichroism. CD spectra indicate that the metal complex-HSA interaction cause conformational changes with the loss of helical stability of protein. Fluorescence data revealed that the fluorescence quenching of HSA by copper complex was the result of the formed complex of HSA-Cu-PTA. The binding constants (Ka), number of binding sites and Stern-Volmer constants have been established.

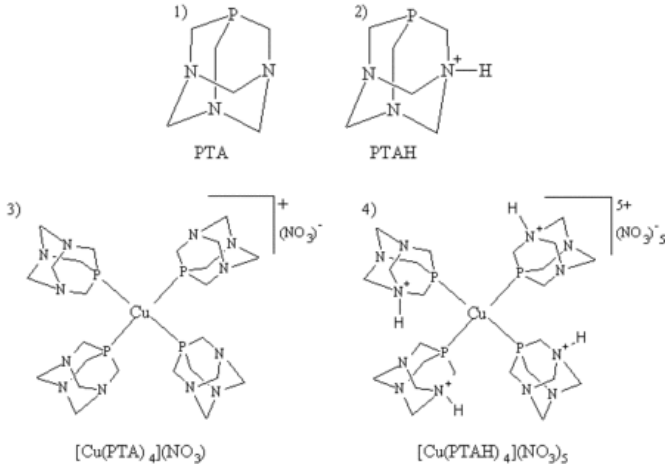

Figure 1. Structure of the investigated copper(I) complexes and PTA ligands

Keywords: 1,3,5-triaza-7-phosphaadamantane (PTA), copper(I)-PTA complexes, human serum albumin (HSA), radiopharmaceuticals, positron emission tomography (PET)

17:00 Poster 97

Novel amphiphilic dendrimers as antibacterial and antitumoral agents.

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The continuing rise in microbial drug resistance has led to widespread problems in the treatment of bacterial infections. It is therefore necessary, to find new compounds able to overcome this problem. Such a promising new class of antibacterial drugs are dendrimers, which are structurally well-defined artificial macromolecules with branched structure and polyvalent nature. There are innumerable ways of designing dendrimers, as various types of initiator cores and building blocks can be used. Length of the dendritic arms, shape, character of the surface, and display of terminal functional groups can all be customized to fit a specific purpose.

Here, we present the synthesis, antimicrobial activity against Gram(+), Gram(-) bacteria and hemotoxicity of a new type of dendrimers with symmetrical and unsymmetrical interior, containing 3,5-dihydroxybenzoic acid (DHBA) as a branching element. They are designed for effective interactions with biological membranes and therefore, are characterized by relatively wide distribution of long arms, terminated with Lys or Trp amino acids. Additionally their antiproliferative activity against human melanoma cells is given.

Financial support from the National Center for Research and Development, grant NR13-0153-10/2010 and Mazovian PhD Scholarship funded by Mazovia Voivodeship, is acknovledged.

17:00 Poster 98

Development and validation of GCMS method for the control of ethyl and izopropyl methanesulphonates in pharmaceutical substance in a form of mesilate

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Alkyl esters of methanesulfonic acid are known to be involved in reaction with DNA. Pharmaceutical active substance in the form of mesilate and medicinal product containing such API needs control on the presence of alkyl mesilates.

In PRI the method for detection of trace of ethyl and isopropyl methanesulphonate has been elaborated for this purpose. Gas chromatography coupled with mass spectrometry is applied. Reference materials – ethyl and isopropyl mesilates were synthesised in PRI. The analysis is performed by spiking test samples with reference standards and using methyl caprylate as internal standard. Acceptable amount of any alkyl mesilate in the API was set as 1 ppm.

In the first version of the method analytes were extracted from aqueous solution of API to dichloromethane and extract was ten times concentrated, then it needed 2 steps of the sample operating. In the final version of the method analytes were extracted to ethyl acetate without additional concentration. In both method extract was injected to the GCMS and analysed by selected ions monitoring mode.

LOD's in the first version were 0.24 ppm for ethyl mesilate and 0.013 ppm for isopropyl mesilate. In the final method LOD's were 0.012 and 0.008 ppm respectively. The latter method was then more sensitive and simpler.

The method is much simpler than pharmacopoeial one which needs derivatising of ethyl and isopropyl mesilates into iodides and using of headspace GCMS.

17:00 Poster 99

New isothiazolopyridines and their in vitro antibacterial activity

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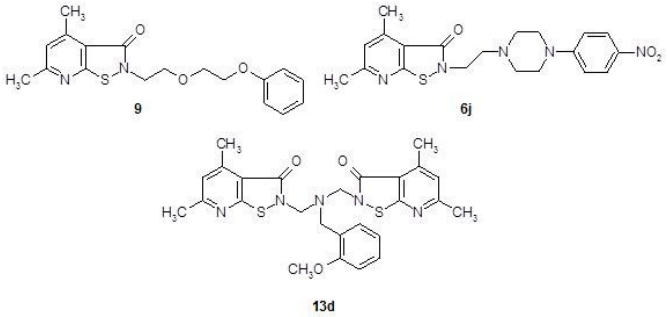
Our previous investigation revealed that some isothiazolo[5,4-*b*]pyridines substituted differently into isothiazole ring exhibited significant antimycobacterial effect under preliminary screening [1]. To study the above observation in more depth and to widen our knowledge of the structure-activity relationship (SAR), several classes of new related compounds were prepared and evaluated microbiologically.

Initially new isothiazolopyridines were screened against *Mycobacterium tuberculosis* H37Rv strain. The most active compound **9** was less active than reference drug (Rifampicin) and showed an inhibition activity of 100% at a concentration of 6.25 mg/mL. At the same time its 3-O-substituted isomer was completely inactive under preliminary screening. Compound **9** was also tested in VERO cells for determination cytotoxicity (IC_{50}) and the selectivity index (SI), defined as the IC_{50}/MIC. Unfortunately, compound **9** exhibited significant cytotoxic activity values of 4.1 μg/mL and low selectivity index (SI=0.68). The new prepared isothiazolopyridines were also evaluated against *Mycobacterium fortuitum* PMC 672 and *Propionibacterium acnes* PCM 2400. Only Mannich base **13d** significantly reduced growth of *M. fortuitum* and this effect was observed for concentration below 1 mg/mL, similar as for the reference drug (Isoniazid). Isothiazolopyridine **13d** was also active at MIC_{90} > 1 mg/mL level. The results of an initial *in vitro* microbiological evaluation against *Propionibacterium acnes* appeared that only compound **6j** showed

strong activity (MIC_{90} > 1 mg/mL) and demonstrated better activity at the concentration range of 1- 0.25 mg/mL when compared of that to the reference drug (Erythromycin).



[1] Malinka W., Sieklucka-Dziuba M., Rajtar G., Zgodziński W., Kleinrok Z., *Pharmazie* 2000, *55,* 416.

17:00	Poster	100

The interaction of new fluphenazine analogues with lipid membrane

Katarzyna Cieślik-Boczula[1], Piotr Świątek[2], Agata Z. Jaszczyszyn3, Wiesław Malinka[2], Kazimierz Gąsiorowski[3]

1. *University of Wrocław, Faculty of Chemistry, Joliot-Curie 14, Wrocław 50-383, Poland* **2.** *Wroclaw Medical University, Department of Chemistry of Drugs, Tamka 1, Wrocław 50-137, Poland* **3.** *Wrocław Medical University, Department of Basic Medical Sciences, Kochanowskiego 14, Wrocław 51-601, Poland*

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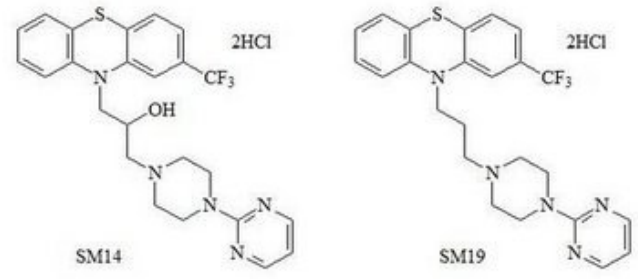
The fluphenazine (FPh) related compounds are active late chemoprevention substances. They are inhibitors of Pgp transport function and may act chemo-sensitizing trough several mechanizms like: 1. by binding directly to the Pgp and lowering the affinity of the outward transport activity of the protein molecules, 2. by impairing the function of Pgp through the disruption of the cell membrane lipid environment in the immediate vicinity of the protein.

As the changes of membrane structure and physical properties seems to be a crucial point of multidrug resistance (MDR) reversion, the interaction of two new analogues of FPh **SM14** and **SM19** with DPPC (1,2-dipalmitoyl-sn-glycero-3-phosphocholine) membranes studied by means of the IR-ATR (Infrared Spectroscopy-Attenuated Total Reflectance) method will be presented.



Both compounds penetrate deeply into the DPPC membranes, causing the greatest changes in the *trans-gauche* isomerization of the hydrocarbon lipid chains, which leads to a decrease in the tmperature of the chain-melting phase transition in dpoed lipid bilayers. Howeever, the character of that alterations strongly dependent on the presence of the OH group in aminoalkyl side-chain. In order to maximalize the information from infrared spectroscopy experiments, the MCR-ALS (Multivariate curve resolution-alternating least squares) analysis was applied to the IR-ATR spectra.

Acknowledgment: This work was supported by the Polish Ministry of Sciences and Higher Education (grant no. N N204150338) and by European Union within European Social Fund, Human Capital.

17:00	Poster	101

Synthesis and in vitro screening of antiproliferative activity of C-glycosides, the derivatives of genistein and daidzein in selected cancer cell lines

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1. *Silesian University of Technology, Department of Organic Chem., Bioorganic Chem. and Biotechnol., Krzywoustego 4, Gliwice 44-100, Poland* **2.** *Centrum Onkologii-Instytut im. M.Skłodowskiej-Curie Oddział w Gliwicach (I.O.), Al. Wybrzeża AK15, Gliwice 44-101, Poland*

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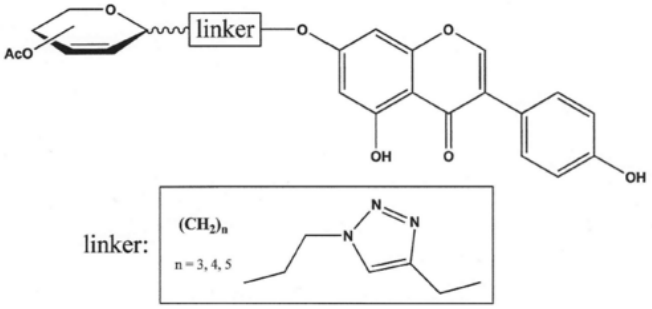
Isoflavones genistein and daidzein show beneficial activity on human health. They help to reduce moderate menopause symptoms, prevent cardiovascular disease and cancer progression. However, their low bioavailability is basic limitation in clinical use of these compounds. Many examples shows, that functionalization of isoflavones can significantly change their bioavailability and ability to interact with defined molecular targets.



We created a library of chemical compounds, in which sugar and isoflavon (Y = OH - genistein, Y = H – daidzein) are connected together by C-glycosidic bond via linkers of various structure. Synthesis of these compounds occurs through the stage of 2,3-unsaturaded C-glycosides, the derivatives of *para*-methoxyphenol or allyl C-glycosides. In result of simple chemical transformations we obtained corresponding C-glycosides with Br or N_3 group on the end of carbon chain in the aglycone. Finally, in result of reactions of genistein or daidzein derivatives we received final compounds shown on upper scheme.

The series of genistein and daidzein derivatives were tested in the *in vitro* assays for their antiproliferative activity, influence on the cell cycle and apoptosis induction. We found some compounds more active than the parent compounds and exhibiting different mode of action.

Our results indicate that both the sugar and a linker structure plays important role for the biological activity of the derivatives.

[1] Dixon R. A., Ferreira D. *Phytochemistry*. **2002**, 60, 205-211.

[2] A. Rusin, Z. Krawczyk, G. Grynkiewicz, A. Gogler, J. Zawisza-Puchałka, W. Szeja, *Acta Biochimica Polonica*, **2010**, 57, 23-34 and adduced literature.

Acknowledgement: This project was supported by National Science Center, Poland (N N204 203340)

17:00 Poster 102

New 1-aryl-6-benzyl imidazo[1,2-a][1,3,5]triazines with potential pharmacological activity.

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1-Aryl-2-aminoimidazoline were used to synthesize many chain and fused imidazoline derivatives exhibiting pharmacological activity. Compounds bearing the 1-aryl substituted were found to process significant central activity, especially antinociceptive and serotonergic [1-3].

When reserching new compounds with potential pharmacological activity 1-aryl-6-(4-chlorbenzy)-5,7(1H0-2,3-dihydroimidazo[1,2-a][1,3,5]trizines were received. This heterocyclic system was obtained in two-step reaction.

Novel cyclic derivatives of dihydroimidazo[1,2-a][1,3,5]trizines were receicved by condensation of 1-(1-arylimidazolidyn-2-ylideno)-3-(4-chlrbenzyl)urea with CDI.

The structure of all new compounds was confirmed by elementar analysis, as well by the 1H NMR.

[1] D. Matosiuk, S. Fidecka, L. Antkiewicz-Michaluk, I. Dybała, A.E.Kozioł, Eur. J. Med.. Chem. 2001, 36 783

[2] K.Sztanke, S. Fidecka, E. Kędzierska, Z. Karczmarzyk, K. Pihlaja, D. Matosiuk, Eur. J. Med.. Chem. 2005, 40 127

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17:00 Poster 103

Synthesis of new 1-(1-arylimidazolin-2-ylo)-3-(4-methoxyphenyl)urea derivatives.

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In the recent years at the Department of Synthesis and Chemical Technology of Pharmaceutical Substances, Faculty of Pharmacy, a number of 1-(1-arylimidazolidine-2-ylidene)-3-arylurea was synthesised. In many cases they exhibited analgesic activity. To find out the part of their structure that can be a possible analgesic 'pharmacophor', a series of new chain carbonyl derivatives of 1-aryl-2-iminoimidazolidine were synthesised.

Based on the reports on the CNS (Central Nervous System) activity of some derivatives of 1-(aryl-2-iminoimidazolin-2-ylo)-3-arylnurea new derivatives were synthesised.

Compounds with potential pharmacological activity 1-(1-arylimidazolidine-2-ylidene)-3-arylurea were obtained by condensation reaction of N-benzylcarbamic acid ethyl ester with appropriate 1-aryl-2-iminoimidazolidine.

The structure of all new compounds was confirmed by elementar analysis, as well by the ^{1}H NMR.

17:00 Poster 104

Distinguishing between polymorphic forms of linezolid by solid-phase electronic and vibrational circular dichroism

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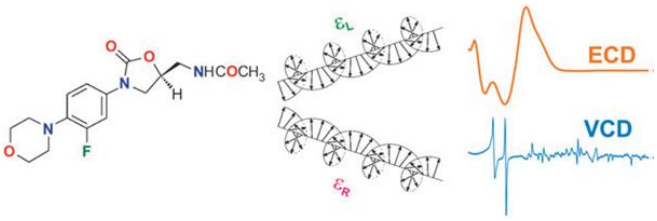

For the first time two crystalline forms of the same compound (linezolid polymorphs) were investigated by means of the solid-phase ECD and VCD spectra [1]. The ECD spectra show distinct differences and the band at 221 nm serves as a diagnostic one because it is present in form II but absent in form III. The VCD spectra strongly differ in the diagnostic carbonyl absorption range exhibiting two relatively strong bands of opposite signs. The results obtained for linezolid clearly demonstrate the usefulness of solid-phase circular dichroism spectroscopy as a new and valuable tool for distinguishing polymorphic forms of chiral compounds.

[1] J. Frelek, M. Górecki, M. Łaszcz, A. Suszczyńska, E. Vass, WJ. Szczepek, *Chem. Commun*., DOI: 10.1039/C2CC31207G

17:00 Poster 105

Synthesis of N-substituted benzo-1,2-thiazine 1,1-dioxide derivatives with potential anti-inflammatory activity

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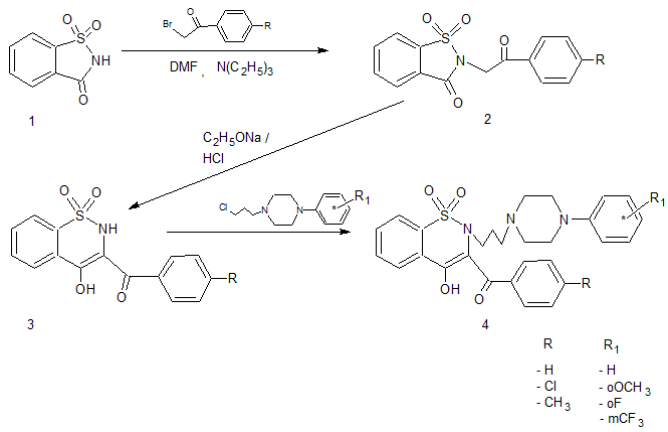
We are searching for new anti-inflammatory agents devoid of the limitations and side effects of the classical non steroidal anti-inflammatory drugs. Oxicams, like piroxicam, meloxicam or tenoxicam, represent a newer chemical category of the enolic acids, used in the treatment of chronic rheumatic diseases. In previous communications we reported the synthesis and anti-inflammatory evaluation of their pyridine-analogues. Some of them exhibited, depending on the substituents in position 2 and 3 pyridothiazines ring, anti-inflammatory, analgesic, antitumor and antioxidant action [1-5]. The pronounced biological action of the pyridothiazines stimulated us to continue our search and prepare their analogues, modified at the pyridine ring, in order to evaluate the influence of this structural change on biological activity.

A series of new 4-hydroxy-3-(4-substituted-benzoil)-2-[3-(4-(R_1-substituted-phenyl)-1-piperazinyl)propyl]-2H-benzo-1,2-thiazine 1,1-dioxides was synthesized starting from commercially available saccharine. Saccharine 1 was reacted with 4'-substituted-2-bromoacetophenone in N,N-dimethylformamide (DMF) to provide the N-alkylated product 2. Compound 2 on refluxing in sodium–ethanol resulted in ring expansion and formed cyclic sulfonamide 3. It was further alkylated with 4-(R_1-substituted-phenyl)-1-(3-chloropropyl)piperazine resulting in the corresponding coupled product 4.



The structure of the final products 4 was confirmed by elemental analysis and spectral analytical methods (IR, H^1NMR).

The newly obtained compounds were qualified for pharmacological tests in Jagiellonian University Medical College, Kraków, Poland.

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17:00 Poster 106

New Mannich bases of N-substituted 1H-pyrrolo[3,4-c]pyridine-1,3(2H)-diones and their biological properties

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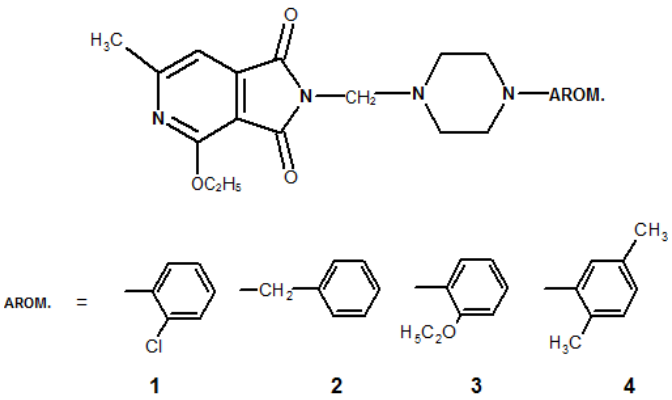
Today, despite numerous analgesic drugs available, many pain syndromes are still unsatisfactorily treated. For this reason great number of the N-aminoalkyl derivatives of 3,4-pyridinedicarboximides were synthesized by H. Śladowska's research group, because our previous investigations revealed significant analgesic activity of that chemical group. Some of them were tested in a pharmacological screening test and they have shown both analgesic and sedative activities and were non-toxic (LD_{50} > 2000 mg/ kg) [1-4]. In order to obtain further information concerning structure-activity relationships (SAR) in this group of compounds we modified the structure of 1H-pyrrolo[3,4-c]pyridine-1,3(2H)-diones by shortening of the side–alkyl chain. We obtained several new Mannich base type compounds. In the "writhing syndrome" test all of the studied imides exhibited strong analgesic activity (ED_{50} 1-4 = 0,72-2,82 mg/kg), which was superior to that of ASA (ED_{50} = 39,15 mg/ kg). Imides 2 and 3 were more potent than morphine (ED_{50} =2,44 mg/kg). In the "hot plate" test all of the abovementioned derivatives showed potent analgesic activity. Furthermore, two compounds tested (3,4) significantly suppressed the spontaneous locomotor activity of mice but two (1,2) increased this activity in mice, acting like stimulants. It may suggested that the analgesic properties of these imides are not consequences of their sedative activity, but the explanation of a precise mechanism of action will demand further pharmacological investigations.



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17:00	Poster	107

Preparation of endotoxin-free bacteriophages from bacterial lysate

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Bacteriophages are viruses that infect bacterial cells. They are widely spread in nature occupying different environmental niches. Phage lysate, which is received by multiplication in Gram-negative bacteria include bacteria cell ingredients such as lipopolysaccharides (endotoxins). Lipopolysaccharides are released from bacteria during cell lyses or multiplication. Released endotoxins induce a strong response of immune system influencing on activation of monocytes and macrophages, they also play a central role in the pathogenesis of septic shock. Biological preparations used in therapy should be endotoxin-free. The threshold level of endotoxin for intravenous application is 5 endotoxin units (EU) per kg body weight and hour (European Pharmacopoeia 1997) and for breath ≈ 200 EU m^{-3} [1].
The present study proposes a new method for endotoxin removal from bacterial lysates. The heterophase extraction technique to higher alcohols such as 1-octanole and 1-buthanole went after addition Mg^{2+} ions to bacteriophage lysate. *Escherichia coli* B were cultured in medium based on casein acid hydrolysate. The preparation of bacteriophage lysate was done in fermentor New Brunswick BIOFLO 415. Phage lysate after multiplication had 33 600 EU/ml and at the end of the purification process 1 EU/ml. The yield of endotoxin removal process was over 99% and the phage lytic activity loss was limited to one range. These method is scalable to technological use.
These work was supported by NCBiR 13-0089-06.
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17:00	Poster	108

Exposure to ionizing radiation of workers in Poland

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In 2011 in Central Laboratory for Radiological Protection about 4500 of employees exposed to ionizing radiation were monitored (standard monitoring cycle lasts 4 months). The group was divided into four subgroups: medical, scientific, industrial and others. Monitoring service was based on two doses assessment methods: Kodak films and MCP-N thermoluminescent detectors.

The results revealed that the average annual doses for employees in each particular group were 0.51 mSv (medical), 0.62 mSv (scientific), 0.48 mSv (industria l) and 0.44 mSv (others). The average annual dose for the entire monitored population was about 0.51 mSv. Comparing to the results from 1999, when about 6200 radiation workers were monitored, the average annual doses for industrial group decreased by 40%. Annual average dose in scientific and others groups increased by about 50%, whereas and in medicine by 40%.

Twelve cases of dose equivalents over the limits of 20 mSv (0.1% of all read outs) have been registered. Six of them occurred in the medical, three in the industrial and three in the scientific group. Almost 97% of all registered dose equivalents were less than 1mSv.

To sum up, in 2011 the laboratory carried out almost 10000 doses estimations from TLD rings dosimeters. There were no cases of doses exceeding of the acceptable limits for extremities.

17:00	Poster	109

Phytic acid down-regulates IL-8 secretion from IL-1β stimulated colonic epithelial cells by influencing mitogen-activated protein kinase signaling pathway.

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Intestinal epithelial cells produce cytokines that play an important role in mucosal immune and inflammatory responses. Some natural

agents can reduce inflammation inter alia by influencing on secretion of proinflammatory cytokines and chemokines. Phytic acid (IP6) is one of the bioactive compound that is present in cereals, legumes, oilseeds and nuts. It is, recognized to posses various significant health benefits including anticancer effects. Previous studies showed that it can also modulate immune functions of intestinal epithelium through regulation of the secretion of some interleukins, but mechanisms underlying that cellular response to IP6 have weakly been examined, as yet. One of the signal transduction pathways involved in a variety of inflammatory responses is p38 mitogen-activated protein kinase (MAPK) pathway.

The aim of this study was to examine the effect of IP6 on the p38 MAPK activity as well as to evaluate its influence on the expression of gene encoding p38 MAPK in unstimulated and IL-1β-stimulated Caco-2 cells. The effect of IP6 on IL-8 secretion by these cells and the interaction of IP6 with p38 MAPK signal transduction pathway were also studied. Cells were preincubated with p38 MAPK activator (anisomycin) and inhibitor (SB203580) followed by incubation with IP6. IL-8 levels were measured in the culture supernatants by ELISA.

In this study, the *in vitro* inhibitory effect of IP6 on recombinant p38 MAPK activity was demonstrated for the first time. Treatment of cells with IP6 for 3h resulted in a decreased p38 MAPK expression in both unstimulated and IL-1β-stimulated cells. Incubation of cells with anisomycin resulted in up-regulation of IL-8 secretion. Pretreatment of cells with anisomycin prior IP6 addition showed down-regulation of IL-8 secretion compared to the cells treated with anisomycin alone.

The study suggests that p38 MAPK could be one of the molecular targets for IP6 effects in the intestinal epithelial cells. These results also suggest that the effect of IP6 on IL-8 secretion by Caco-2 cells could be mediated by its inhibition of p38 MAPK activity.

17:00 Poster 110

Effect of phytic acid on the expression of IL-6, IL-8 and NFkB in human colonic epithelial cells treated with IL-1β

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Intestinal epithelial cells play an important role in the mucosal immune and inflammatory reactions via the expression and secretion of proinflammatory cytokines such as interleukin-6 (IL-6) and interleukin-8 (IL-8). The expression of both interleukins is regulated by nuclear factor kB (NFkB). Phytic acid (IP6) is an essential component of high fiber diet. It is physiologically present in the human large gut at concentrations reaching 4 mM. It exhibits pleiotropic health beneficial effects including anti-oxidant and anti-tumor activities. Recent studies showed that IP6 can modulate immune functions of intestinal epithelium through regulation of proinflammatory cytokines secretion.

The aim of this study was to examine the role of IP6 in immunological function of intestinal epithelial cells by analyzing its effect on the expression of IL-6 and IL-8 genes by IL-1β-stimulated colonocytes Caco-2. It was also evaluate the influence of IP6 on the expression of genes encoding p50 and p65 subunits of NFkB and that of its inhibitor IkBα in by cells stimulated with IL-1β. A kinetic study of mRNAs expression in cells was performed after their treatment with 1 and 2,5 mM IP6 for 3, 6 and 12h. Quantification of the genes expression was carried out using real time QRT-PCR technique.

Treatment of cells with IP6 resulted in a strong decrease in both IL-6 expression (at 3h and 6 h) and IL-8 expression (3h). IP6 at all used concentrations had no influence on transcription of p65 and p50 genes in Caco-2 cells stimulated with IL-1β, however, they expressed higher level of the inhibitor IkBα in response to 2,5 mM IP6 treatment .

The results of these studies suggest that IP6 may exert immunoregulatory effects on intestinal epithelium by influencing transcriptional activity of genes encoding inhibitor IkBα and proinflammatory cytokines IL-6 and IL-8.

17:00 Poster 111

The transport of active antineoplastic complexes of Pt(II) by human serum albumin.

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Cisplatin (cis-diaminedichloroplatinum (II)) is an alkylating agent used in different types of cancers, for example ovarian, testicular, lung, bladder (Fig. 1). The success of cisplatin has aroused great interest in the development of new metal complexes to diagnose and therapy. The important aspect in this field was the discovery of the cisplatin's mechanism of action and pharmacokinetic parameters.

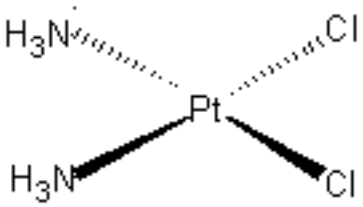

Fig. 1. The structure of cisplatin.

This paper presents the interaction of the cisplatin with the main transporting protein in plasma -albumin, and it's modified by glycation and high level fatty acids forms. The problem is regarding the initial stage of fates of the drug in the body (LADME) - binding protein-drug.

In this work cisplatin binding were studied using atomic emission spectrometry with inductively coupled plasma (ICP-AES) and differential pulse stripping voltammetry (DPSV). The results suggest that one mole of the protein binds four moles of cisplatin. The investigations were also conducted on the glycation modified albumin and in the presence of fatty acids excess. During hyperglycemia and/

or high levels of fatty acids is observed a strong reduction in the affinity of cisplatin to albumin (one mole of protein binds about the half less of the complex of cisplatin). It can be a result of global structural changes associated with the fatty acids binding and local changes in subdomain IIA caused by glycation. Studies have shown that modifying the structure of albumin may increase the amount of free, pharmacologically active fraction of the drug, which could have an impact on therapy.

17:00 Poster 112

Formation of Caco-2 monolayer - model for the prediction of intestinal drug absorption; method validation.

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Today more and more important in research on the drugs bioavailability are cell cultures, which allow for the study of transepithelial transport of various substances. The most commonly used cell line applied in the study of the intestinal drug absorption, due to its spontaneous differentiation to the enterocyte-like structure and function, is a colon cancer line Caco-2. In order to obtain the reliable drug permeability results the monolayer must be integral and free from defects. Hence the cultivation method validation is necessary.

The validation of the method of monolayer cultures included the assay of monolayer consistency and integrity on the basis of cell morphology. The cell nuclei and tight junctions protein (ZO-1) were stained and localized by means of confocal microscopy. The membrane integrity was also assessed by the Lucifer Yellow permeability assay. These parameters were evaluated continuously during 30 day-long growth of the Caco-2 cells as well as the transepithelial electrical resistance (TEER) – the quality control check. Resulting monolayer was also assessed for the presence or absence of areas characterized by a multilayered cell growth. In order to assess the influence of the cell origin the cells from different passages and lots were cultivated.

It has been shown that between 18 and 21 day of growth the Caco-2 monolayer was obtained. The cells shapes were regular, the tight junctions were developed and the Lucifer Yellow permeability was low. The origin of the cells and the passage number however were the crucial factors influencing the time required for monolayer development.

This study was supported by the National Centre for Research and Development (NCBiR), Grant No. R13009706

17:00 Poster 113

Comparison of dissolution profiles of theophylline extended-release dosage forms

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Dissolution testing has emerged in the pharmaceutical field as a very important tool to characterize drug product performance, for example, to demonstrate similarity between different formulations. Because the rate of release of theophylline could differ between the products, the patient health may be endangered, especially that this drug has a narrow therapeutic range.

The investigation of theophylline release from tablets was performed by the basket apparatus type DT700 (Erweka). The f1 (difference factor) and f2 (similarity factor), calculated according to FDA guidelines, were used to compare the obtained dissolution profiles, using Euphyllin long 300 as a reference product.
Obtained values showed that dissolution profiles of investigated formulations were not equivalent to each other. The tablets differed by the mechanism of drug release also. Therefore, these formulations should not be used alternatively, especially that theophylline has a narrow therapeutic range.

17:00 Poster 114

The effects of 300 mT static magnetic field on IL-6 secretion in normal human colon myofibroblasts

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Intestinal subepithelial myofibroblasts play crucial role in the growth and development of the intestine. Colitis, small bowel injury, gastric ulcer disease and inflammatory bowel disease (IBD) accompany the increase of number of activated myofibroblasts [1]. EMF therapy because of its anti-inflammatory properties may be used in medicine in IBD treatment [2]. 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 h incubation TNF-α – dependent IL-6 secretion was determined with ELISA kit (R&D Systems).The influence of magnetic field and its effect on cell proliferation were determined with TOX-2 (In Vitro Toxicology Assay Kit XTT Based, TOX-2, Sigma) and CyQUANT® NF cell proliferation assay kit (Molecular Probes).

It was shown that SMF inhibited TNF-α – dependent IL-6 secretion. The observed effects were statistically significant and depended on the time of incubation. Moreover, SMF triggered cell proliferation whereas it did not alter cell viability.

IL-6 belongs to pro-inflammatory cytokines family and plays a crucial role in IBD [3].

Inhibition of IL-6 secretion by SMF and lack of its cytotoxic effect seems to be advantageous whilst SMF is implicated in the treatment of inflammatory diseases associated by increase in number of activated myofibroblasts.

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17:00 Poster 115

Contribution of ALDH1A1 isozyme to detoxification of aldehydes present in food products.

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Even though food awareness is so developed and more and more people pay attention to what their diet is composed of, it is not possible to exclude all potentially dangerous substances present in our diet. One group of such compounds are aldehydes as several studies in animals indicate that they can be mutagenic, carcinogenic, cytotoxic and genotoxic. These relatively reactive organic molecules are natural constituents of food. They are also extensively used by food industry as additives giving aroma and taste. Fortunately many enzyme systems were developed to protect us against these potentially toxic compounds, one of which is aldehyde dehydrogenase enzyme superfamily.
As mouth is the first part of the digestive system it seems crucial for detoxifying toxic substances introduced with our diet. The only ALDH isozyme present in saliva is ALDH3A1, which has very high affinity for aromatic aldehydes commonly found in food. However, because of hyposalvation, which is not uncommon nowadays, the effectiveness of this barrier can be drastically diminished. As another member of this enzyme family, isozyme ALDH1A1 is also present in digestive system its possible contribution to detoxification of 'food' aldehydes was addressed.
Kinetic parameters (Km,Vmax) of recombinant ALDH1A1 towards several aliphatic and aromatic aldehydes occuring in food products (vanillin, citral, furfural, cinnamaldehyde, anisaldehyde, benzaldehyde and hexenal) were determined by measuring the increase of NADH fluorescence after adding different concentrations of aldehyde substrates. Rates were used to construct the Lineweaver - Burk plot form which Km and Vmax values were calculated.
It turned out that this ubiquitous member of ALDHs superfamily, has very good affinity for examined aldehydes. The resulting Michaelis - Menten constant values are even lower than the corresponding values for ALDH3A1 enzyme. Thus supporting role of ALDH1A1 in the protection of organisms against these potentially dangerous compounds from food can be suggested.

17:00 Poster 116

Inactivation of salivary aldehyde dehydrogenase-influence of antioxidant status in saliva collected in the morning and in the afternoon from smokers and no-smokers.

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Salivary aldehyde dehydrogenase (ALDH3A1) is the only dehydrogenase detected in saliva. The enzyme oxidizes aromatic and long chain aliphatic aldehydes preserving oral cavity from aldehydes derived from food, air pollution. Therefore, its activity may also be an important factor in prevention of chemical carcinogenesis. ALDH3A1 due to sulphydryl groups in its active site is exposed to oxidation even by oxygen from air. The average inactivation of the enzyme in healthy group is about 80% in morning saliva and depends on coffee, drug consumption and age. The inactivation degree is very variable during a day as well as both intra- and interpersonally. Causes of that inactivation have been still examining.
The aim of the present study was to determine influence of salivary antioxidant status (salivary peroxidase (SPO), superoxide dismutase (SOD) activity, uric acid (UA) concentration and total antioxidant capacity (ORAC)) on ALDH3A1 inactivation degree in smoking (SG, N=38) and no-smoking group (NSG, N=42). Fasting saliva samples were collected in the morning or during a day.
ALDH3A1 inactivation, ORAC value and SPO activity were determined fluorometrically. SOD activity and UA concentration was measured photometrically.
Average inactivation degree in SG was higher both in the morning ($p=0.0054$) and during a day ($p=0.00189$) in comparison to NSG. Inactivation degree in morning saliva correlated with SPO ($r=-0.28$, $p=0.0317$), which level was slightly higher in SG. In smokers the correlation was more prominent ($r=-0.62$, $p=0.00121$), whereas in NSG was close to zero and non significant. In saliva collected during a day inactivation degree correlated with UA ($r=-0.30$, $p=0.02820$) and SOD ($r=-0.35$, $p=0.00598$). Correlation with UA in no smokers was more prominent ($r=-0.46$, $p=0.00847$) whereas in smoking group was not visible. Moreover, SOD activity in SG was higher than in NSG. No correlations of ALDH inactivation and ORAC value were observed.
To conclude, ALDH3A1 inactivation degree depends on smoking and antioxidant status. In SG depends stronger to antioxidant enzymes than in NSG. Since smoking results in activation of e.g. Nrf2/ARE pathway the inactivation degree in that group is lower than NSG.

17:00 Poster 117

Microbiologically active Mannich bases derived from 1,2,4-triazoles. The effect of C-5 substituent on antibacterial activity.

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In the recent years, there has been a significant tendency in organic chemistry to imitate chemical phenomena occurring in the nature. The so-called biomimetic reactions consist in transformation of simple substrates into complex products, usually at mild conditions [1]. One of such reactions is the aminomethylation reaction, discovered and described by Carl Mannich [2]. This reaction makes it possible to introduce amine fragment into the different chemical scaffolds which can increase the affinity of the obtained molecule towards appropriate molecular target. 1,2,4-Triazole-3-thione derivatives known for their antibacterial activity [3,4] were used by many researchers as substrates for the Mannich reaction.

Our research proved that chemical character of the C-5 substituent significantly determines the antibacterial activity of the Mannich bases derived from 4,5-disubstituted 1,2,4-triazole-3-thiones. This activity was considerably increased by an introduction of a chlorine atom to the phenyl ring. Furthermore, the disparities in the activity of appropriate *ortho*-, *meta*-, and *para*- derivatives were analysed. The obtained compounds were particularly active against opportunistic bacteria (*Bacillus subtilis*, *Bacilluscereus*). The antibacterial activity of some Mannich bases was similar or higher than the activity of commonly used antibiotics such as ampicillin and cefuroxime.

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17:00 Poster 118

The multiobjective based design, synthesis and evaluation of the arylsulfonamide/amide derivatives of arylxyethyl- and arylthioethyl- piperidines and pyrrolidines as a novel class of potent 5-HT$_7$ receptor antagonists

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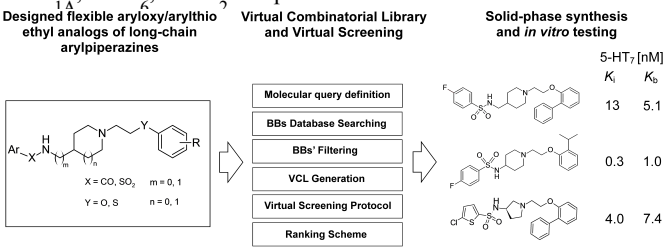
An analysis of the virtual combinatorial library was used for refining a pilot set of 34 derivatives and designing a targeted 38-member library of the arylamide and arylsulfonamide derivatives of aryloxyethyl- and arylthioethyl- piperidines and pyrrolidines. All compounds were synthesized according to an elaborated parallel solid-phase method and were biologically evaluated for their affinity for 5-HT$_7$. Additionally, the targeted library members were tested for 5-HT$_{1A}$, 5-HT$_6$, and D$_2$ receptors.



Selected compounds of particular interest were examined for their intrinsic activity at 5-HT$_7$R in vitro employing a cAMP assay. The study allowed us to identify compound **68** (4-fluoro-*N*-(1-{2-[(propan-2-yl)phenoxy]ethyl}piperidin-4-yl) benzenesulfonamide) as a potent 5-HT$_7$R ligand (K_i = 0.3 nM) with strong antagonistic properties (K_b = 1 nM) and a 1450-fold selectivity over 5-HT$_{1A}$Rs.

This study was partly supported by the Polish Ministry of Science and Higher Education (MNiSW), Grant No. N N405 671540 and Funds for Statutory Activity of Jagiellonian University Medical College. Radioligand binding experiments were financially supported by the Norwegian Financial Mechanism as part of the Polish-Norwegian Research Fund, Grant No. PNRF–103–AI-1/07.

17:00 Poster 119

The highly versatile handle for synthesis of secondary amine derivatives with potential CNS activity: The Pipecolic Linker

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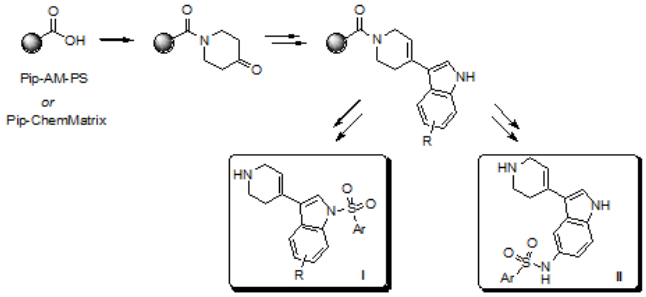
The Pipecolic linker (Pip) was designed on a basis of side reaction observed on BAL linkers while synthetizing a focused library of long-chain arylpiperazines [1]. It represents new strategy for immobilization and structural modifications on solid support of primary, secondary and aromatic amines as well as alcohols, phenols, thiols and hydrazides [2,3].

Herein we present new application for Pipecolic linker for the synthesis of N_1-arylsulfonyl-(1,2,3,6-tetra-hydropyridin-4-yl)-1*H*-indole (**I**) and 5-arylsulfonyl-(1,2,3,6-tetra-hydropyridin-4-yl)-1*H*-indole (**II**) derivatives.



The synthesis of sets **I** and **II** was performed on polystyrene and ChemMatrix resins, respectively. The strategy employed attachment of the 4-piperidone to the Pip resin, followed by condensation with respective indole derivatives. Then, the key stage in the synthesis of set **I** analogs was sulfonylation of the resin-bound indole derivative in basic conditions of BTPP (phosphazene base P_1-t-Bu-tris(tetramethylene)). The critical for synthesis of set **II** compounds was reduction of 5-nitro function. This was accomplished using ChemMatrix resin and sodium dithionite treatment in basic conditions.

Presented solid-phase synthetic routes opens possibility for generation of combinatorial variations extending the variety of substituted sulfonamides available in the development of CNS acting agents.

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17:00 Poster 120

3-(egzo)-ene-Derivatives of oleanolic acid as perspective compounds with valuable pharmacological activities

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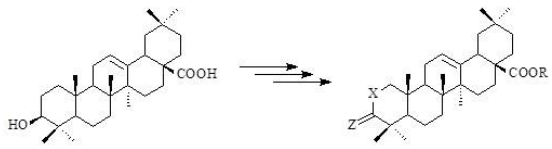
Oleanolic acid (OA) is a compound widely distributed in plant kingdom. It occurs in a form of free acid or numerous glycosides. As it is known from numerous publications, oleanolic acid and another triterpenes, mostly from ursane, oleanane and lupane group, show great variety of pharmacological actions: they have been shown in experimental and clinical studies to demonstrate promising anticancer [1], gastroprotective [2], analgesic, anti-inflammatory [3] and many other important activities.

In our researches oleanolic acid was transformed into miscellaneous derivatives within A or C ring as well as hydroxyl or carboxylic group. New compounds were obtained on the basis of multistep set of transformations with the usage of reactions such as: methylation of carboxylic group, reduction of free or esterified carboxylic group, transformation of carboxylic group into amide, acylation in pyridine or dioxane, hydrolysis, different types of oxidation (Jones oxidation, allylic oxidation, *m*-CPBA oxidation), transformation of ketone group into oxime function, Beckmann rearrangement, thionation of C=O group into C=S function.



The new-obtained products were tested against many tumorous MDR cell lines, as percutaneous penetration enhancers, and as analgesic, anti-inflammatory or antituberculosis agents. It is known from biological tests that the derivatives of OA that possesses double-bounded group at C-3 position (C=O, C=NOH, C=NOAcyl) belong to a class of the most active species. These compounds exhibited cytotoxic activity towards cancer lines, which were resist to many known anticancer drugs [4–6] and some of this derivatives turned to be active analgesic and anti-inflammatory agents [7]. Some derivatives of oleanolic acid have anti-inflammatory effects influencing TNFα, IL-1or IL-12 production [8]. Azaderivatives of oleanolic acid with A- or C-ring lactam system are effective transdermal penetration enhancers [9].

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17:00 Poster 121

The new azole connections as potential biologically active agents

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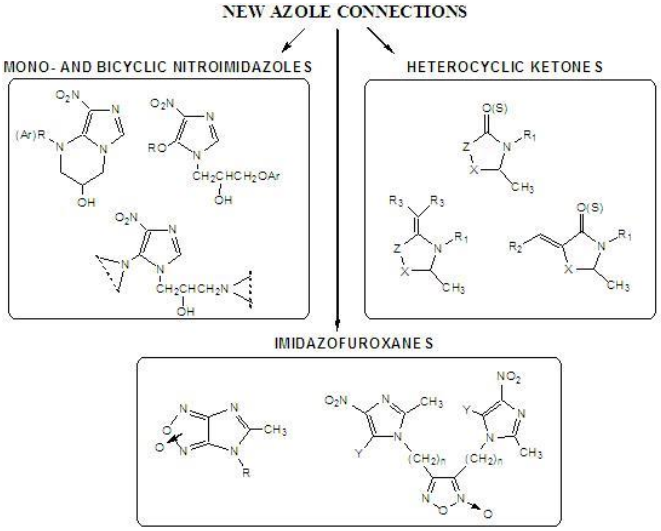
Nowadays, there is the growing interest in new active heterocyclic stuctures with different biologically and pharmacologically activity. Our synthetic research focuses on preparation of new series of active compounds and their structure and potential pharmacological properties elucidations. The main objectives are as follows:

- **bicyclic and monocyclic derivatives of nitroimidazoles as potential tuberculostatic agents** - it was taking the atempt of the nucleophilic substitution of chlorine atom in 2-chloromethylnitroimidazodihydrooxazoles with different nucleophiles e.g. secondary cyclic amines. Unstability of dihydrooxazole ring was observed and it was used for bulding of new, six-membered tetrahydropyrimidyne ring, condensed with nitroimidazole by the reaction with compounds having primary amino-group,
- **heterocyclic ketones as a kind of natural jasmone analogues - based on pyrrolidinone, oxazolidinone, thiazolidinone systems - as fragrant compounds** that have the ability to stimulate the same receptors as jasmone and exhibit intresting and persistent fragrant properties. It has been decided to subject its to further chemical modifications, for example with the use of reactive carbonyl group for the nucleophilic substitution reactions resulting in appropriate thionated or malonilydene derivatives. Presence of carbonyl group also affect the increase in acidity of the hydrogen atoms linked to carbons atoms in *alfa* position and it creates favorable conditions for numerous reaction of condensations e.g. with selected aldehydes leading to new fragrant adducts,
- **active imidazole derivatives containing furoxan moiety and evaluation of their ability to relase nitric oxide** with use medicinal chemical hybridization, which involves the combination of two complementary biological activities by joining appropriate groups directly or via linker. Broad biological significance of furoxanes and their fused derivatives led to attempt a synthesis of such systems. The furoxan ring is formed by the oxidative cyclization of appropriate *alfa*-dioximes. It is planed to obtained series furoxan systems conjugated directly or through alkyl chain with two imidazolyl moieties. Preliminary experiences have showed that fused furoxanes which will combine elements of the known biologically active structures e.g. imidazoles were obtained in oxidative cyclization of respective o-aminonitro compounds under the influence of sodium hypochlorite. *In situ* test imidazofuroxanes obtained showed different biological activity, mainly as vasodilator, antianginal and antiischemic agents.

Most of the above mentioned compounds were tested in many biological activity directions.



17:00 Poster 122

"Eye Drops - requirements for the product and specifics of production"

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The main purpose of this poster is to present the requirements for the eye drops like: pH, isotonia, viscosity, strerylity, surface tension, particles size distribution and some aspects of their manufacture, which have a great influence on the quality of manufactured product. The poster also goes into the subject of sterile production with a special emphasis on the process of steralisation itself: heat sterilisation, steam sterilisation, ethylne oxide sterilisation, radiation sterilisation. It also tacles the problem of different types of prepacks for the eye drops.

17:00 Poster 123

Effects D-tryptophan substitutions on the activity and toxicity of antimicrobial dendrimers

Paulina Zielińska[1], Jolanta Solecka[2], Aleksandra M. Rajnisz[2], Marta Bochyńska[3], Andrzej W. Lipkowski[3], Zofia Lipkowska[1]

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The increasing resistance of bacteria to currently available antibiotics necessitates every effort to develop novel antibacterial agents with lower rates of resistance. Amphipathic cationic dendrimers have been proposed as potential new class of antimicrobial agents, which are analogs of natural antimicrobial peptides (AMPs).

According to literature, incorporation of D-isomers alongside L-isomers in previously toxic AMPs reduce their toxicity without reducing their antimicrobial activity. The aim of this study was to examine the influence of introducing D- amino acid residues into dendrimeric molecules on their biological properties**.**

Therefore, we synthesized a series of dendrimers based on highly tetrabranched amino acid core with lipophilic C-terminus and arms terminated by L- or D-Trp residues. Their potency against Gram(-) and Gram(+) bacteria and their hemolytic activity was studied. The designed compounds were also evaluated for their antiproliferative activity against human melanoma cells.

Financial support from the National Center for Research and Development, grant NR13-0153-10/2010 and in part grant N204 239436 from the Ministry of Sciences and Higher Education, is acknowledged.

17:00 Poster 124

Synthesis of a new series of heteroaryl 4-chloro-2-mercaptophenylsulfonylisothiourea derivatives with potential antitumor activity

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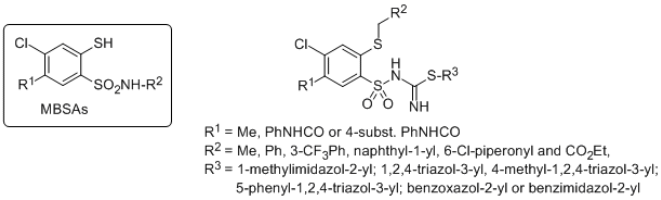
In continuation of our effort to get new chemotherapeutic agents [1-5], we herein report the synthesis of the heteroaryl 2-mercaptophenylsulfonylisothiourea derivatives and their antiproliferative activity. Till now there are no patterns of biological active 2-mercaptobenzenesulfonamides bearing sulfonamide nitrogen atom substituted with heteroaryl carbimidothioate moieties. The aim of the present investigations was to extend our studies in this class of compounds. Therefore, we elaborated the synthesis and anticancer evaluations *in vitro* of novel *N*-substituted analogues of the lead structure (MBSAs), being thus the heteroaryl 4-chloro-2-(R^2-methylthio)phenylsulfonylcarbamimidothioate derivatives as depicted in scheme below.

The synthesis of the desired heteroaryl 4-chloro-2- (R^2-methylthio)-R^1-phenylsulfonylisothiourea derivatives were achieved by reacting of the corresponding *N*-(2-alkylthio-benzenesulfonyl)cyanamide potassium salts with suitable mercapto-azoles/benzazoles in dry toluene in the presence of *p*-toluenesulfonic acid (PTSA) at reflux.



R^1 = Me, PhNHCO or 4-subst. PhNHCO
R^2 = Me, Ph, 3-CF_3Ph, naphthyl-1-yl, 6-Cl-piperonyl and CO_2Et,
R^3 = 1-methylimidazol-2-yl; 1,2,4-triazol-3-yl, 4-methyl-1,2,4-triazol-3-yl; 5-phenyl-1,2,4-triazol-3-yl; benzoxazol-2-yl or benzimidazol-2-yl

Anticancer *in vitro* screening performed at the NCI (Bethesda MD, USA) using 60 cell lines derived from 9 types of human tumors revealed moderate or reasonable anticancer activity of tested compounds. The distinctive compound *e.i.* 1-methyl-1*H*-imidazol-2-yl 4-chloro-2-[(6-chlorobenzo-1,3-dioxol-5-yl)methylthio]-5-methylphenylsulfonylcarbamimidothioate showed remarkable activity against 14 human tumor cell lines representing leukemia, lung, colon,CNS, melanoma, ovarian, renal, prostate and breast at the low micromolar GI_{50} level in the range of 0.38-4.93 μM.

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17:00 Poster 129

Practical advantages of Microflow-UHPLC

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Modern HPLC equipment have much lower extra-column band broadening contributions than conventional instruments of the late last century. Modern columns provide low extra-column band dispersion in the flow rate range between 0.1 and 5 mL/min but with that it is impossible to avoid a certain loss of the efficiency. In practice, particularly under isocratic conditions, extra-column band broadening contributions and the heat friction effects are not negligible with 2.1 mm I.D. columns.

An easy solution to the heat problem is a further decrease of the column diameter, down less than so that the heat power generated per unit column length is sufficiently low at high linear velocities. The performance of the new UHPLC instruments not being consistent with the requirements of a generation of still narrower columns, 0.5 or 1 mm in diameter, Microflow UHPLC instruments are becoming popular among those interested in fast, high resolution separation

methods for complex mixtures, especially available in small size samples.

There is little data available on the role of devices that are of primary importance in the measurements of the peak variances of sub-1 mm I.D. columns. Besides the obvious reduced solvent usage, there are several inherent advantages performing chromatography in smaller ID columns. Less required mixing volume as a fraction of column volume allows for faster gradient separations. A lower flow resistance factor means the same separation can be run at lower backpressure.

In this paper we report on theoretical calculations and experimental results to demonstrate these advantages with columns of 0.5 mm ID. Particular attention will be given to the issue of frictional heating. A simplified model that provides a basis to evaluate the effects of frictional heating in UHPLC will be presented.

Czas wolny
Thursday evening, 31 May, 18:30

Piknik
Thursday evening, 31 May, 19:30

Friday, 1 June

Śniadanie
Friday morning, 1 June, 7:00

Sesja wykładowa VII
Friday morning, 1 June, 8:40
Chair: A. Mazurek, D. Maciejewska

8:40 Oral

Studies toward Novel Peptidomimetc Inhibitors of Thioredoxin-Thiredoxin Reductase System

Ryszard Ostaszewski[1,2], Szymon Kłossowski[2], Angelika Muchowicz[3], Jakub Gołąb[3]

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Thioredoxins (Trx) are ubiquitous multifunctional low-molecular weight proteins that together with thioredoxin reductases (TrxR) participate in the maintenance of protein thiol homeostasis in NADPH-dependent reactions. An increasing number of data reveal that the Trx−TrxR system is an attractive target for anticancer therapies. Based on our studies on synthesis on the successful combination of mutlicomponent reactions with enzymatic transformations to the synthesis of bioactive tripeptide mimetics [1,2,3], we have elaborated a new and simple synthetic approach employing Ugi reaction to synthesize several new inhibitors of this system. The influence of various electrophilic fragments of this new class of compounds on the inhibition of the Trx−TrxR system was evaluated. As a result, a new compound SK053, which inhibits the activity of the Trx−TrxR system and exhibits antitumor activity, was obtained. Biologic analyses revealed that SK053 inhibits induction of NF-κB and AP-1 and decreases H_2O_2 scavenging capacity in tumor cells.

The results of our studies on the successful application of mutlicomponent Ugi reactions to the synthesis of bioactive tripeptide mimetics, will be presented [4].

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9:00 Invited oral

Sugars in new drug design. Sugar moiety structure as a principal determinant of antitumor glycoconjugates biological activity.

Wiesław Szeja

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Approximately half of the existing drugs are derived from natural compounds. Among natural products which found therapeutic applications, glycosides constitute a considerable proportion, but the role of glycon/aglycon parts are seldom clearly defined in terms of molecular pharmacology [1].

Genistein, isoflavone isolated from soy is a compound of particular significance in chemoprevention of many cancers [2]. Although numerous glycosides of genistein are known as secondary metabolites of higher plants, studies of biological activity are sharply focused on the aglycon, supported by general belief that glycosidic bond like the one present in widespread genistin cannot withstand typical biodistribution along the pathway of an orally administered xenobiotic. Carbohydrate scaffolds, adept constructs and other pro – drugs will be discussed. Biological activity of genistein may be enhanced by chemical synthesis of lipophilic glycosides[3].Based on literature data with a new synthetic flavonoid 2,3-unsaturated sugar glycoconjugates are singled out as a class of reasonably available derivatives for useful modification of pharmacological properties of structurally complex and multifunctional drug lead compounds [4]. An example of cell growth phase selective switch in mechanism of action upon isoflavone glycosylation illustrates this point.

A number of synthetic genistein glycoconjugates have been obtained in our laboratories, using various chemical glycosidation methods, in order to study their biological activity in comparison with

underivatized aglycon.. In the presented lecture we describe methods of the synthesis and properties of a new synthetic genistein derivatives, which shows much more potent cytostatic and cytotoxic effect than genistein. The results clearly demonstrated that glycone split off is not necessarily favorite biotransformation of such derivatives. Moreover, some synthetic glycoconjugates exhibited distinctly different mechanism of antiproliferative action that the one observed for the aglycon. Effects of unsaturated genistein glycoconjugates on cycloskeleton and cell cycle in selected tumor cell lines will be discussed in some detail.

The tested compound does not undergo rapid biodegradation in cells or culture media and exerts its biological effects primarily as intact molecule. Our data show the mechanism of cytotoxicity of genistein and its new derivative is significantly different. In conclusion, we postulate that glycodiversification of drug leads by application of glycal chemistry [5] offers new opportunities in drug design and discovery. Examples of the structure – function relationship of glycons will be drawn.

References

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The study supported by the Ministry of Science and Education of Poland (Grant No. N N209 186338).

9:45 Invited oral

Legal protection of borderline products in reference to food supplements and pharmaceuticals.

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The food supplement's market is increasing steadily over the past years and the legal issues of delimitation between food and pharmaceuticals are arising simultaneously. Although, certain attempts are made to resolve this situation, both at national and Community stage, still many similarly composed products have a status of a pharmaceutical and food supplement. A parallel situation occurs with other categories of so called – border line products – like cosmetics or medical devices. Therefore the open question is how to define the legal boundaries between different categories of products, so that their assignment to the relevant category would not create further concerns. Unfortunately, even the Community regulations, that set legal standards for most of these products in the UE, are not sufficiently helpful in this matter. The jurisdiction of Court of Justice of the European Union creates even greater uncertainty. A certain solution of this matter appears in the Community regulation on nutrition and health claims, which to some extent straightens the issues of labeling and the use on food products of claims suggesting health beneficial effects. Increasingly, the disputes on the classification of products are transferred to the judicial level.

Rynek suplementów diety systematycznie przez ostatnie lata wzrasta, a wraz z nim problemy prawne granicy między żywnością a lekami. Jakkolwiek podejmowane są pewne próby uporządkowania sytuacji, zarówno na poziomie krajowym jak i wspólnotowym, to ciągle wiele podobnych w składzie produktów ma status leku i suplementu diety. Podobne sytuacje mają miejsce z innymi kategoriami tzw. Produktów granicznych, tj. kosmetykami, wyrobami medycznymi. Pytaniem otwartym jest jak zdefiniować prawnie granicę pomiędzy różnymi kategoriami produktów, by przyporządkowanie produktów do odpowiedniej kategorii nie rodziło problemów. Niewiele pomocne jest w tej kwestii prawo unijne, które dla większości tego typu produktów wyznacza standardy prawne w Unii Europejskiej. Orzeczenia Trybunału Sprawiedliwości Unii Europejskiej kreują jeszcze większą niepewność. Pewnym rozwiązaniem okazuje się być rozporządzenie unijne w sprawie oświadczeń zdrowotnych, które w pewnym stopniu przynajmniej porządkuje kwestie znakowania i posługiwania się zwrotami prozdrowotnymi przez żywność. Coraz częściej spory w zakresie kwalifikacji produktów przenoszą się na poziom sądowy.

Przerwa na kawę

Friday morning, 1 June, 10:30

Sesja wykładowa VIII

Friday morning, 1 June, 10:50

Chair: P. Zajdel, K. Sidoryk

10:50 Invited oral

Molecular complexes of topo I and II inhibitors from camptothecin and flavonoid families with model DNA oligomers. Search for sequence specific binding. Verification of biological assays on atomic coordinates level.

Lech Kozerski[1,2], Wojciech Bocian[1,2], Elżbieta Bednarek[1], Jerzy Sitkowski[1,2], Robert Kawęcki[2], Karolina Hyz[2], Beata Naumczuk[2]

1. *Narodowy Instytut Lekow (NIL), Chełmska 30/34, Warszawa 00-725, Poland* **2.** *Polish Academy of Science, Institute of Organic Chemistry, Kasprzaka 44/52, Warszawa 01-224, Poland*

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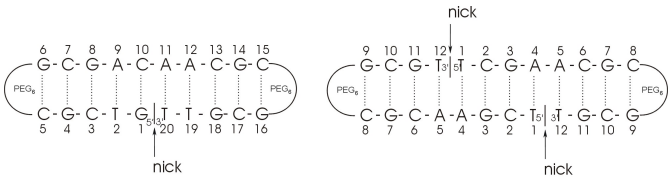
The role of topoisomerases I and II inhibitors involves the ternary complexes composed of enzyme/DNA/Inhibitor. Topoisomerases are essentially enzymes which, *inter alia*, relax the torsional strain in supercoiled DNA generated in replication process. The enzyme cleaves one strand, creating a nick, and thus enables the rotation around the uncleaved strand followed by ligation of the broken strand and restoring DNA duplex.

Human topo I involves Tyr 723 , of which OH group attacks phosphate in the main chain forming 3'-phosphotyrosine. After relaxa-

tion, strain free DNA duplex is restored in nucleophilic attack of free 5'-OH group on phosphorous atom in 3'-phosphotyrosine. Dumbbell DNA (NICK I) mimics nicked DNA in a ternary complex and was designed by us as a general model of DNA duplex for screening its interactions with topo I inhibitors from camtothecin group.

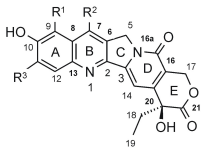
Similarly to topo I , topo II generates nicks separated by four base pairs in both strands forming doubly broken duplex covalently linked to topo II through 5'-phosphotyrosines. Dumbbell DNA (NICK II) mimics doubly nicked DNA and was designed by us as an adequate general model of DNA duplex for screening its interactions with topo II inhibitors from flavonoid group of derivatives.



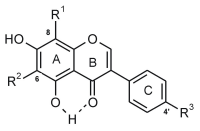
NICK I NICK II

The essential role of inhibitor in a ternary complex is specific binding in a nick, either covalently or noncovalently, and preventing its ligation and further proliferation of cytotoxic DNA.

The chemical basis of selected compounds from both classes of inhibitors is shown below.



R^1= H, CH_2OH, CH_2OMe,CH_2NMe_2,$CH_2N^+HMe_2$ X^-,
R^2 = H, C_2H_5
R^3 = H
X^- = Cl^-, $CF_3SO_3^-$



R^1 = H, alkylamino
R^2 = H ,alkylamino
R^3 = OH

In a lecture there will be presented selected topics of tautomerism and chemistry of topotecan (TPT) from campthotecin family administered clinically as Hycamtin ™. The characteristics of molecular complex of NICK I and parent TPT inhibitor from camptothecin family will be discussed.

The design of NICK II model dumbbell DNA will be presented and molecular complexes of parent genistein and derivatives will be discussed .

11:35 Invited oral

Diastereoselective Synthesis of β-Lactams via Kinugasa Reaction

Bartłomiej Furman

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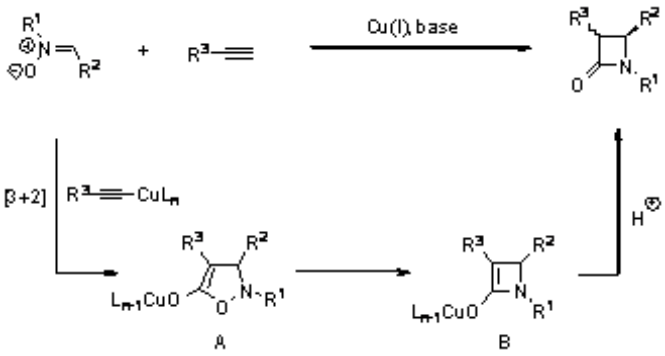
In 1972 Kinugasa and Hashimoto reported a convergent route to β-lactams through the reaction between copper phenyl acetylides and nitrones.??? Four years later, Ding and Irwin??? proposed the mechanism of the Kinugasa reaction, which involved the 1,3-dipolar cycloaddition and subsequent rearrangement of the intermediate isoxazoline. In following years, the extended asymmetric versions of the Kinugasa reaction have been proposed by groups of Basak??? and Fu.???



Very recently, we reported a diastereoselective version of the Kinugasa reaction involving nonracemic cyclic nitrones and simple achiral/chiral acetylenes.??? Such reactions displayed high diastereoselectivity leading to one dominant product. On the basis of the proposed stereochemical model of the reaction, it has been shown that the stereochemical outcome of the Kinugasa reaction depends on the first step, 1,3-dipolar cycloaddition. The addition is controlled by the substituent present in the nitrone, whereas the protonation of intermediate enolate in the second step occurs from the less shielded side of carbapenam skeleton.

The lecture will be devoted to a detailed discussion of this methodology and practical application of developed methodology will also be presented.

??? Kinugasa, M.; Hashimoto, S. *J. Chem. Soc., Chem. Commun.***1972**, 466.

??? Ding, L. K.; Irwin, W. J. *J. Chem. Soc., Perkin Trans. 1***1976**, 2382.

??? Basak, A.; Mahato, T.; Bhattacharya, G.; Mukherje, B. *Tetrahedron Lett.***1997**, *38*, 643.

??? Lo, M.; Fu, G. C. *J. Am. Chem. Soc*. **2002**, *124*, 4572.

???(a) Stecko, S.; Mames, A.; Furman, B.; Chmielewski, M. *J. Org.Chem.***2008**, *73*, 7402. (b) Mames, A.; Stecko, S.; Mikołajczyk, P.; Soluch, M.; Furman, B.; Chmielewski, M. *J.Org. Chem*. **2010**, *75*, 7580. (c) Michalak, M.; Stodulski, M.; Stecko, S.; Mames, A.; Soluch, M.; Panfil, I.; Furman, B.; Chmielewski, M. *J. Org.Chem.***2011**, *76*, 6931.

12:20 Oral

Synthesis of trans-4-cyclohexyl-(S)-proline - an intermediate in Fosinopril process

Michał Fedoryński[2], Barbara Kąkol[1], Magdalena Jezierska-Zięba[1], Jacek Cybulski[1], Bożenna Obukowicz[1]

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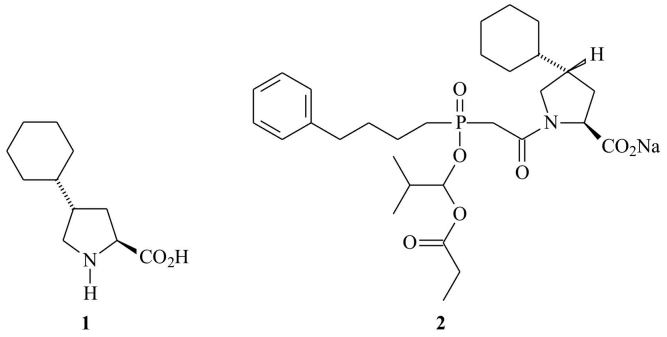
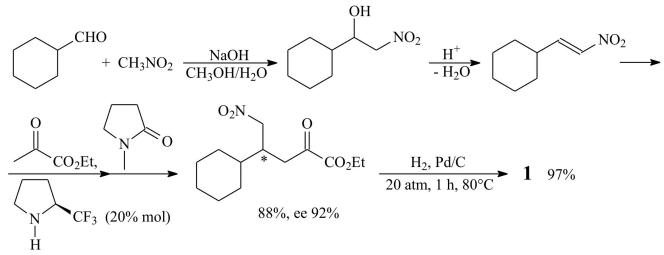
trans-4-Cyclohexyl-(*S*)-proline, **1** is a crucial intermediate in the synthesis of Fosinopril **2** - prodrug used in the treatment of hypertension and acute or chronic congestive heart failure.



1 **2**

In a patent literature [1-3] three methods of synthesis of **1** are described, all of them are multistep process, requiring strictly anhydrous conditions, low temperature and tedious separation procedures.

We proposed and elaborated and original scheme of synthesis or **1**, based on available cyclohexane carboaldehyde and ethyl 2-oxopropanoate [4].



Details of this synthesis will be discussed.

1. Patent USA 4 316 905 (1982).
2. Patent USA 4 588 819 (1986).
3. Patent USA 4 734 508 (1988).
4. Zgł. pat. P-392 283 (2010).

Praca realizowana w ramach projektu badawczego własnego nr N N209 237336 finansowanego przez Ministerstwo Nauki i Szkolnictwa Wyższego.

12:40 Oral

Differential aspects of molecular interactions fenoterol stereoisomers and derivatives with the β_2-adrenergic receptor

Anita Płazińska[1], Karolina Pająk[1], Krzysztof Jóźwiak[1], Irving W. Wainer[2]

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The b_2 adrenergic receptor system is one of the best characterized among G-protein coupled receptors. In the current project the stereoisomers of fenoterol and a series of fenoterol analogues were synthesized and tested for β_2-AR activity and selectivity [1,2].

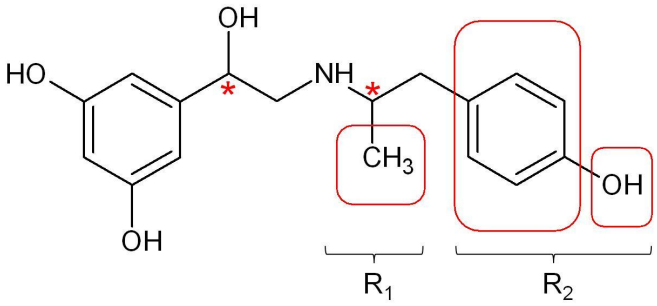

Fig. 1. The structure of (*R*,*R*)-fenoterol in which the sites on the molecule probed in this study are circled in red. Chiral centers are indicated with asterisks.

The data demonstrate that the stereochemistry of the fenoterol molecule influences the magnitude of binding affinity, the thermodynamics of local interaction of ligand within the binding site and the global mechanism of activation of the β_2-AR as evidenced by the G_s/G_i selectivity observation [3, 4].

In the present study we used stereoisomers of fenoterol and some of its derivatives as a molecular probe to identify the role of $Y308^{7.35}$ in their binding process and G_s selectivity. Molecular modeling suggested two alternative binding modes of these molecules interacting within the binding site: (i) compounds having 4-hydroxy-/4-methoxy-/amino-phenyl moiety create hydrogen bond (HB) with $Y308^{7.35}$ (group A), (ii) compounds containing 4-methoxy-1-naphtyl/1-naphtyl moiety do not form HB with $Y308^{7.35}$ and in fact are bended deeper allowing their naphtyl moieties to occupy the cleft formed by a network of the aromatic residues (group B). Our study showed that the interaction with $Y308^{7.35}$ is essential to observe β_2-AR/β_1-AR selectivity for compounds of group A (G_s selective), while it has a little effect on binding derivatives containing the naphthyl moiety (group B, which is not G_s selective). Y308A mutant showed significantly reduced affinities for compounds belonged to group A as compared with the wild type data. This trend was not observed for the analogues belonged to B group. This suggest that the naphthyl moiety plays more significant role in stabilization of the agonist–β_2-AR complex.

Grant information: NIH/NIA contract N01AG-3-1009 and Foundation for Polish Science grant TEAM 2009-4/5.

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2. Jóźwiak K, Woo YH, et al., Bioorg Med Chem 2010;18(2):728-736.
3. Woo YH, Wang TB, et al., Mol. Pharmacol. 2009;75(1):158-165.
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Przerwa obiadowa
Friday afternoon, 1 June, 13:00

Sesja wykładowa IX

Friday afternoon, 1 June, 14:30

Chair: B.Furman, M. Lamparska-Przybysz

14:30 Invited oral

Application of the Silylative Coupling Reaction in Organic Synthesis

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The catalytic silylative coupling reaction of olefins with vinylsilanes allows selective synthesis of stereochemically defined unsaturated organosilicon compounds from commercially available substrates [1]. The products can be further used in stereoselective organic synthesis and the methods of their synthesis can help develop of the original synthetic strategies using the designed and defined organosilicon monomers as intermediates in the synthesis of desired organic products by sequential or tandem process [2].

Intensive study of sequential silylative coupling – desilylation reactions (catalytic Hiyama coupling acylation, halodesilylation) resulted in the development of original methods of synthesis of a series of di- or trisubstituted alkenes such as alkenyl halides, styryl ketones, stilbenes, styrylcarbazoles, styryl alkyl ethers and other systems containing π-conjugated double bonds [3].

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[3] (a) P. Pawluć, J. Szudkowska, G. Hreczycho, B. Marciniec, J. Org. Chem., **2011**, *76*, 6438; (b) P. Pawluć, A. Franczyk, J. Walkowiak, G. Hreczycho, M. Kubicki, B. Marciniec, Org. Lett., **2011**, *13*, 1976; (c) P. Pawluć, G. Hreczycho, J. Szudkowska, M. Kubicki, B. Marciniec*, Org. Lett*. **2009**, 11, 3390; (d) P. Pawluć, G. Hreczycho, A. Suchecki, M. Kubicki, B. Marciniec, *Tetrahedron***2009**, 65, 5497; (e) P. Pawluć, G. Hreczycho, J. Walkowiak, B. Marciniec, *Synlett***2007**, 13, 2061.

15:15 Oral

Synthesis and anticancer properties of coumarin gallates

Elżbieta Hejchman, Przemysław Taciak, Sebastian Kowalski, Dorota Maciejewska

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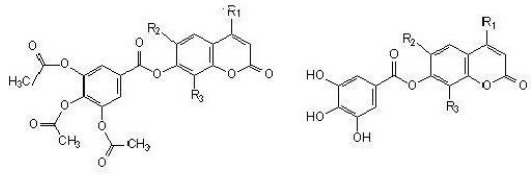
Gallic acid is widely distributed in plants, fruits and food and has a range of biological activities. Many of the foods containing gallic acid have been used for years as natural remedies, and were relied upon by various cultures for their medicinal properties. Studies have shows that gallic acid has anti-cancer properties against leukemia, certain prostate, colon and lung cancer cells. Gallic acid has been shown to prevent cellular mutations and to be toxic to cancer cells, while having no negative effect on healthy cells. Similarly, coumarin derivatives display broad spectrum of biological activity, among them *in vitro* antiproliferative activity against human neoplasms is notable. Therefore, the multistep synthesis of gallic acid derivatives with coumarin components was designed to obtain substances potentially inhibiting growth of cancer cells.

We report the synthesis and anticancer assay of eight new esters – derivatives of substituted 7-hydroxycoumarins and gallic acid:



Compounds **1-4** Compounds **5-8**

R_1 = H, CH_3; R_2, R_3 = H, $COCH_3$

The esters **1-8** were assayed for antiproliferative activity against human leukemia HL-60 and prostate cancer DU-145 cell lines. The antiproliferative effects were estimated in terms of growth inhibition percentage. They were shown to be higher or comparable to the antiproliferative effect of gallic acid. No *harmful effect* was observed for NIH 3T3 mouse embryonic fibroblast cells.

15:35 Oral

Sulforaphane - a modulator of detoxifying enzymes can alter medicines effectiveness.

Katarzyna Wiktorska, Katarzyna Lubelska, Małgorzata Milczarek

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e-mail: wiktorska@il.waw.pl

Sulforaphane (SFN) belongs to the group of isothiocyanates which occurs naturally in vegetables from Brassica family. Due to its chemopreventive properties it is now available also in dietary supplement form. SFN chemoprevention mechanism include the stimulation of the detoxifying cellular system - the enzymes of II and III metabolism phases. However considered beneficial, it can also alter the medicines metabolism and absorption or lead to development of multidrug resistance (MDR). Due to increasing popularity of dietary supplements the study on SFN influence on drug action is of the increasing importance.

The aim of our study was to determine the influence of SFN on the enzymes of II and III metabolism phases and to evaluate SFN effect on the drug absorption and MDR phenomena.

The SFN influence on activity of quinine reductase (QR) – a surrogate of global II metabolizing phase enzymes was studied in colon cancer Caco-2 cells. Additionally SFN ability to modify the expression of glutathione–S–transferase two isoforms GSTA3, GSTM1 and drug transporters MRP1 and PgP was evaluated by qPCR method . In order to quantify the SFN effect on the intestinal drug absorption a Caco-2 model was developed. SFN effect on the perme-

ability of the three drugs belonging to different BCS group: ketoprofen, verapamil and furosemide was evaluated. Also SFN effect on cytotoxicity of anticancer therapeutic 5-Fluorouracil (5-Fu) was studied. The type of interactions in prostate cell line PC-3 was assessed by Chou-Talaly method.

It was shown that SFN induces QR activity in time and concentration dependent manner. Also II and III metabolism phase enzymes gene expression was altered. We have also shown that the drug permeability was modified by SFN especially in case of furosemide. The effectiveness of 5-Fu was altered in concurrent application with SFN. The study indicates that dietary supplements can interact with cellular metabolizing system and in effect modify the drug action.

15:55 Oral

Studies on anticancer iron chelators

Anna Mrozek-Wilczkiewicz[1,2], Robert Musiol[2], Agnieszka Szurko[1,3], Maciej Serda[2], Des R. Richardson[4], Jaroslaw Polanski[2], Alicja Ratuszna[1]

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The subject of interest of this presentation is a group of derivatives of thiosemicarbazones, which are tridentate active iron chelators with high Fe mobilization efficacy and low toxicity [1]. Iron is a common factor participates in a variety of important processes such DNA synthesis, electron transport, oxygen delivery and erythropoiesis. This element is a component of numerous enzymes catalyzing redox processes and taking part in cellural respiration. The mechanism of action of thiosemicarbazone derivatives includes generation reactive oxygen species (ROS) and iron chelation, which is required to rapidly proliferating cancer cells [2]. Cancer cells, when compared with normal, have increased demand for iron which makes them more susceptible to the effects of its depletion. As a consequence the cancer cells treating with compounds of high affinity to iron are arrested at the G1/S interface [3]. However these compounds have other possible mechanisms of action as inhibition of ribonucleotide reductase - mammalian enzyme which playing crucial role in the DNA replication and repair. In addition, depletion of iron affects the regulation of important genes such as BNIP3 and NDRG1, which are crucial for triggering apoptosis of cancer cells. Thiosemicarbazones are an attractive material for the study of anticancer therapy because of their multi-targeted mechanism of action [4]. Photochemical studies included the implementation of the absorbance and fluorescence spectra measurements of thiosemicarbazones and biophysical investigation of the effect of iron chelators on the generation of ROS. This was achieved by flash photolysis of the studied compounds. Antiproliferative activity was estimated on HCT 116 cell line (human colon carcinoma) with normal p53+/+ and suppresed p53 protein functionality (p53-/-) to examined whether the p53 status of this cell line altered their response to the thiosemicarbazone analogues. Protein p53 is the important tumor supresor involved various mechanisms of defence against tumor in healthy cells. Anticancer activity was evaluated using MTS - reduction colorimetric survival assay. Additionally we checked an effectiveness of thiosemicarbazones on the survival and proliferation of cells using clonogenic assay. Fluorescence microscopy was used to record images the morphology of cells after incubation with the investigated compounds. We examined penetration into the cell and changes in cytoskeletal structure and cell organelles. An innovative approach may be the use of thiosemicarbazone derivatives as anciliary drug in photodynamic therapy. PDT is a method characterized by low invasive treatment, and high selectivity relative to normal cells. Nowadays it's an attractive strategy for treating various ailments, including skin cancer. The essence of PDT is to produce singlet oxygen and free radicals in the reaction, of photosensitizer with light of appropriate wavelength. The condition of photodynamic reaction is the presence of oxygen. Formed reactive oxygen species trigger chain reactions in the cell, causing various types of damage leading to the destruction of the affected tissue. As photosensitizer may be used (Photofrin®) unfortunately its use is difficult due to unfavorable pharmacokinetic parameters. As remedy ALA-PDT therapy has been proposed which involves the administration of 5-aminolevulinic acid, which is an endogenic precursor of protoporfirin IX [5]. The accumulation of photosensitizer in diseased tissue is extremely important, because the exposure does not cause damage to healthy tissue. The difference in accumulation of PpIX in diseased and healthy tissue is caused by differences in activities of enzymes regulating the formation of PpIX (porphobilinogen deaminase) and heme (ferrochelatase) [6]. The use of chelators allow to increase the intensity of PpIX accumulation in the tumor, by blocking the last steps of heme formation. In our approach novel highly active against proliferating cells iron chelators are combined with ALA-PDT and tested as multitargeted anticancer therapy in vitro. This simple strategy based on drugs already in use or under clinical trials as well as novel compounds is appealingly effective during this early step study.

References:

[1] D. S. Kalinowski, P. C. Sharpe, P. V. Bernhardt, and D. R. Richardson, "Structure-activity relationships of novel iron chelators for the treatment of iron overload disease: the methyl pyrazinylketone isonicotinoyl hydrazone series.," Journal of Medicinal Chemistry, 51, 2, 331-44, 2008.

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[4] Y. Yu et al., "Thiosemicarbazones from the old to new: iron chelators that are more than just ribonucleotide reductase inhibitors.," Journal of Medicinal Chemistry, 52, 17, 5271-94, 2009.

[5] S. Langer et al., "Active and higher intracellular uptake of 5-aminolevulinic acid in tumors may be inhibited by glycine," Jour-

nal of Investigative Dermatology, 112, 5, 723–728, 1999.

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Anna Mrozek-Wilczkiewicz appreciates the support of UPGOW fellowship and NCN grant N405/068440 Maciej Serda was supported by a TWING fellowship and NCN grant DEC-2011/01/N/NZ4/01166

16:15 Oral

Validation of bioanalytical methods according to new European Medicines Agency guideline

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Results of pharmacokinetic studies are an important background to make critical decisions supporting therapeutic efficacy of drugs and safety of patients. Therefore, reliability of bioanalytical methods must be proven by detailed validation. Novel European Medicines Agency (EMA) guideline [1] describes mostly known and widely applied requirements, but also introduces some novel recommendations, e.g. incurred sample reanalysis.

EMA guideline is dedicated to toxicokinetic and pharmacokinetic studies in all phases of clinical trials and specifies requirements for determination of both macromolecules and small molecular weight drugs. In the latter case, liquid chromatography coupled to mass spectrometry (LC/MS) is a method of choice. Specific recommendations concerning LC/MS-based bioanalytical methods include matrix effect evaluation, internal standard selection (preferably stable isotope-labeled) and assessment of back-conversion of analyte to its metabolite in ionization source.

Currently, the guideline is the most complete and up-to-date document on bioanalytical issues. EMA and U.S. Food and Drug Administration (FDA) [2] requirements in this field are compliant to a large extent, which is very important for pharmaceutical industry.

[1] Guideline on bioanalytical method validation. European Medicines Agency (EMEA/CHMP/EWP/192217/2009). London, 21 July 2011.

[2] Guidance for Industry. Bioanalytical Method Validation. U.S. Department of Health and Human Services. Food and Drug Administration. Center for Drug Evaluation
and Research (CDER). Center for Veterinary Medicine (CVM). May 2001.

16:35 Oral

A novel convergent synthesis of the prostaglandin $F_{2\alpha}$ analogues

Iwona Dams, Michał Chodyński[2], Małgorzata Krupa, Anita Pietraszek[2], Andrzej Kutner[1]

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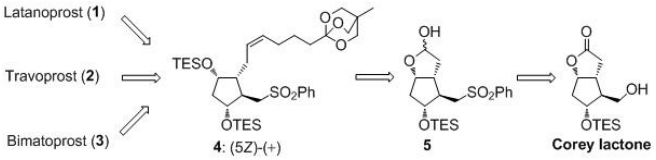

The prostaglandin $F_{2\alpha}$ analogues travoprost (**2**) and bimatoprost (**3**) have potent topical ocular hypotensive activity. A novel convergent synthesis of the title class of prostanoids was developed employing alkylation of the structurally advanced phenylsulfone (5*Z*)-(+)-**4** with an enantiomerically pure (*S*)-4-phenyl-1-iodo-2-(triethylsilyloxy)butane, providing a route suitable for commercial scale production of latanoprost (**1**).[1] As a result of growing demand for hypotensive $PGF_{2\alpha}$ analogues in the pharmaceutical market, the new convergent strategy has recently been applied for the syntheses of travoprost (**2**) and bimatoprost (**3**).[2] The Julia-Lythgoe olefination of the phenylsulfone (5*Z*)-(+)-**4** with novel enantiomerically pure aldehyde ω-chain synthons, followed by hydrolysis of protecting groups and final esterification or amidation yielded travoprost (**2**) or bimatoprost (**3**).

The new procedure overcame most disadvantages of the classic Corey synthesis and ensured the absence of an appreciable quantity of the undesired 15-*epi* isomer of travoprost (**2**) or bimatoprost (**3**). The novel convergent strategy allows the synthesis of a whole series of $PGF_{2\alpha}$ analogues and related compounds from a common and structurally advanced prostaglandin phenylsulfone (5*Z*)-(+)-**4**. The main advantages are the preparation of high purity prostanoids and the application of comparatively inexpensive reagents.

1. (a). J. G. Martynow, J. Jóźwik, W. Szelejewski, O. Achmatowicz, A. Kutner, K. Wiśniewski, J. Winiarski, O. Zegrocka-Stendel, P. Gołębiewski. *Eur. J. Org. Chem.***2007**, 689. (b) J. G. Martynow, J. Jóźwik, W. Szelejewski, O. Achmatowicz, A. Kutner, K. Wiśniewski, J. Winiarski, O. Zegrocka-Stendel, P. Gołębiewski. US Patent no. 7,897,793 B2; Mar. 1, **2011**.

2. I. Dams, M. Chodyński, M. Krupa, A. Pietraszek. M. Zezula, P. Cmoch, M. Kosińska and A. Kutner. Polish patent applications P.398388 and P.398389.

Czas wolny

Friday afternoon, 1 June, 16:55

Kolacja

Friday evening, 1 June, 19:00

Saturday, 2 June

Śniadanie
Saturday morning, 2 June, 7:00

Odjazd autokarów do Warszawy
Saturday morning, 2 June, 9:00

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